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Feeding and management strategies to improve livestock productivity, welfare and product quality under climate change

Edited by:
H. Ben Salem and A. López-Francos

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Most of world's rural population depend on livestock for their livelihood, especially in the Mediterranean, African and many Asian countries. To reduce poverty, fight hunger and ensure global food security, there is an urgent need to increase livestock production in sustainable ways. However, livestock production systems in those areas are constrained mainly by low genetic potential, feed shortage, nutrient deficiency, inappropriate husbandry, and zoonotic and other emerging infectious diseases. Climate change is also expected to exacerbate the vulnerability of livestock systems and to reinforce existing factors that simultaneously challenge livestock production such as rapid population and economic growth, increased demand for food and products, and increased conflicts over scarce resources (e.g. land, water, and feed). Scientific advances in animal nutrition and new strategies in livestock feeding may undoubtedly contribute to this aim, as nutrition is one of the main factors driving the functioning, efficacy, efficiency and evolution of livestock systems.

This volume presents 42 articles selected among the contributions to the 14th Seminar of the FAO-CIHEAM Sub-Network on Sheep and Goat Nutrition, organised by The National Institute of Agricultural Research of Tunisia (INRAT) and the Mediterranean Agronomic Institute of Zaragoza (IAMZ-CIHEAM) in Hammamet (Tunisia) in June 2012. The Seminar provided the opportunity to present and discuss scientific advances and approaches in animal nutrition and feeding sciences, covering the topics of nutritional efficiency, use of local feeding resources, rumen manipulation, animal welfare, products quality, and proposing potential alternatives for coping with climate change and mitigation its effects on the farming systems.

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Foreword

Most of world's rural population depend on livestock for their livelihood, especially in the Mediterranean, African and many Asian countries. To reduce poverty, fight hunger and ensure global food security, there is an urgent need to increase livestock production in sustainable ways. However, livestock production systems in those areas are constrained mainly by low genetic potential, feed shortage, nutrient deficiency, inappropriate husbandry, and zoonotic and other emerging infectious diseases. Climate change is also expected to exacerbate the vulnerability of livestock systems and to reinforce existing factors that simultaneously challenge livestock production such as rapid population and economic growth, increased demand for food and products, and increased conflicts over scarce resources (e.g. land, water, and feed). The challenge is to improve livestock productivity and to produce enough quantities of safe and nutritious meat and milk under these constraints. Scientific advances in animal nutrition and new strategies in livestock feeding may undoubtedly contribute to this aim, as nutrition is one of the main factors driving the functioning, efficacy, efficiency and evolution of livestock systems.

The FAO-CIHEAM Subnetwork on Sheep and Goat Nutrition contributed to the scientific debate and to the exchange of ideas and experiences in an effort to propose solutions to the above mentioned challenges, by the organisation of its 14th Seminar, entitled "Feeding and management strategies for improved livestock productivity, welfare and product quality under climate change", held in Hammamet (Tunisia) from 15 to 17 May 2012. The objective of the Seminar was to encourage participation and interaction between scientists and technicians involved in animal production systems, especially in animal nutrition, with a view to introduce methodologies and experiences in nutrition of small ruminants and other species that may help to improve the sustainability of livestock production by yielding safe and quality products more efficiently and by increasing the capacity of production systems to adapt to current environment and socio-economic changes.

The Seminar was structured into four scientific sessions, devoted to (1) Production and overall nutrition efficiency, (2) Local feeding resource based systems, (3) Animal welfare and Product quality, and (4) Options for mitigating and coping with climate change. A one day field trip was also organised where the participants visited two different production systems with sheep and cattle, and the ancient roman ruins of "Temple des eaux" (Temple of water), the beginning of the aqueduct from the Zaghouan mountain to the city of Carthage on the northern coast of Tunisia.

The National Institute for Agricultural Research of Tunisia (INRAT) and the Mediterranean Agronomic Institute of Zaragoza (IAMZ-CIHEAM) were the organisers of this Seminar, with the collaboration of the Food and Agriculture Organization of the United Nations (FAO), the Office de l'Elevage et des Pâturages (Office of Livestock and Pastures, OEP, Tunisia) and the Institution of Agricultural Research and Higher Education from Tunisia (IRESA). Together with the FAO-CIHEAM Subnetwork Seminar, the LowInputBreeds, an EC-collaborative project (*Development of integrated livestock breeding and management strategies to improve animal health, product quality and performance in European organic and 'low input' milk, meat and egg production*) held its 2nd Symposium on 18th May 2012, facilitating scientific exchanges with the members of this project Consortium. More than a hundred scientists and livestock technicians attended the seminar, coming from 16 countries, mainly from the Mediterranean basin, but also from northern Europe, Africa and Asia.

The current issue of *Options Méditerranéennes* presents 42 articles selected among the contributions to the 14th Seminar of the FAO-CIHEAM Sub-Network on Sheep and Goat

Nutrition. We thank the articles authors and in particular the panel of reviewers [S. Giger-Reverdin (France), D. Yañez-Ruiz (Spain), A. González-Bulnes (Spain), H. Ben Salem (Tunisia), M. Rekik (Tunisia), N. Moujahed (Tunisia), P. Morand-Fehr (France), E. Molina-Alcaide (Spain), G. Luciano (Italy), H.P.S. Makkar (FAO Rome), L. Ferreira (Portugal), M. Gharbi (Tunisia), A. Priolo (Italy), P. Frutos (Spain), L. Biondi (Italy), S. Prache (France), K. Dahlborn (Sweeden), D. Morgavi (France) and N. Mathlouthi (Tunisia)] for their effort and commitment to the publication of this volume. We also thank all the institutions which, together with the organisers, contributed to the success of the Seminar with their generous support: Office de l'Elevage et des Pâturages de Tunisie (OEP), the National Institute of Field Crops of Tunisia (INGC), Aliments Composés du Nord (CAN), and Groupement Interprofessionnel des Viandes Rouges et du Lait (GIVLAIT).

Hichem Ben Salem
Director of INRAT

Ignacio Romagosa
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Session 1
Production and overall nutrition efficiency

Effects of forage type on diversity in bacterial pellets isolated from liquid and solid phases of the rumen content in sheep and goats

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Abstract. Two sheep and two goats, fitted with a ruminal cannula, received two diets composed of 30% concentrate and 70% of either alfalfa hay (AL) or grass hay (GR) as forage in a two-period crossover design. Solid and liquid phases of the rumen were sampled from each animal immediately before feeding and 4 h post-feeding. Pellets containing solid associated bacteria (SAB) and liquid associated bacteria (LAB) were isolated from the corresponding ruminal phase and composited by time to obtain 2 pellets per animal (one SAB and one LAB) before DNA extraction. Denaturing gradient gel electrophoresis (DGGE) analysis of *16S* ribosomal DNA was used to analyze bacterial diversity. A total of 78 and 77 bands were detected in the DGGE gel from sheep and goats samples, respectively. There were 18 bands only found in the pellets from sheep fed AL-fed sheep and 7 found exclusively in samples from sheep fed the GR diet. In goats, 21 bands were found only in animals fed the AL diet and 17 were found exclusively in GR-fed ones. In all animals, feeding AL diet tended ($P < 0.10$) to promote greater NB and SI in LAB and SAB pellets compared with the GR diet. The dendrogram generated by the cluster analysis showed that in both animal species all samples can be included in two major clusters. The four SAB pellets within each animal species clustered together and the four LAB pellets grouped in a different cluster. Moreover, SAB and LAB clusters contained two clear subclusters according to forage type. Results show that in all animals bacterial diversity was more markedly affected by the ruminal phase (solid *vs.* liquid) than by the type of forage in the diet.

Keywords. DGGE – Forage – Goats – Ruminal bacteria – Sheep.

Effets du type de fourrage sur la diversité bactérienne mesurée sur des pellets obtenus à partir des phases liquide et solide du contenu de rumen chez des ovins et des caprins

Resumé. *L'objectif de cette étude était d'analyser la diversité des communautés bactériennes à partir d'extraits isolés dans les phases liquide (LAB) et solide (SAB) du contenu de rumen d'ovins et de caprins nourris avec des régimes différant par le type de fourrage. Les deux régimes expérimentaux consistaient (base matière sèche) en 30% de concentré et 70% de foin de luzerne (AL) ou de foin de graminées (GR). Deux brebis et deux chèvres, porteuses de canule ruminale, ont reçu le régime alimentaire selon un schéma en cross-over avec deux périodes. Les bactéries SAB et LAB ont été isolées à partir de chaque animal immédiatement avant la distribution de l'alimentation (0 h) ou 4 h après cette distribution. A chaque période, les extraits bactériens ont été échantillonnés afin d'avoir 2 extraits bactériens par animal (un SAB et LAB) avant extraction de l'ADN. L'analyse de l'ADN ribosomal 16S par la technique d'electrophorèse sur gel en gradient dénaturant (DGGE) a été utilisée pour établir la diversité bactérienne. Deux gels DGGE différents ont été réalisés, l'un pour les échantillons des brebis et l'autre pour ceux des chèvres. Au total, 78 et 77 bandes ont été détectées dans le gel des échantillons, respectivement, de brebis et de chèvres. Parmi elles, 18 bandes ne se sont retrouvées que chez les brebis recevant le régime AL, et 7, que dans des échantillons provenant de brebis nourries avec le régime GR. Pour les caprins, 21 bandes ne se trouvaient que chez les animaux nourris avec le régime AL et 17 ont été trouvées exclusivement chez les animaux recevant le régime GR. Il n'y a eu aucune interaction espèce animale x régime alimentaire ($p > 0,05$), que ce soit dans le nombre de bandes (NB) ou pour l'indice de Shannon (IS), ce qui indique que les populations bactériennes des deux espèces animales ont répondu de la même façon aux changements liés au type de fourrage. Il n'y a eu aucune différence ($P > 0,05$) entre les deux espèces animales pour le nombre ou l'indice de Shannon pour les extraits SAB, mais les extraits LAB des brebis avaient un nombre plus élevé ($P < 0,05$) de bandes et un index SI supérieur aux chèvres. Chez tous les animaux, la distribution du régime AL tend ($P < 0,10$) à augmenter NB et SI pour les extraits LAB et SAB par rapport à celle du régime GR. Le dendrogramme généré par l'analyse typologique a montré que, chez les deux espèces animales, tous les*

échantillons peuvent être inclus dans deux grands clusters. Les quatre extraits SAB se sont regroupés dans un cluster différant de celui regroupant les quatre extraits LAB. Par ailleurs, les clusters SAB et LAB contenaient deux sous-groupes bien différenciés correspondant au type de fourrage. Les résultats montrent que, chez tous les animaux, la diversité bactérienne a été plus influencée par l'extrait bactérien analysé (LAB ou SAB) que par le type de fourrage dans le régime.

Mots-clés. *DGGE – Fourrage – Caprin – Bactéries du rumen – Ovin.*

I – Introduction

Differences in chemical composition and metabolic functions between bacteria isolated from the liquid (LAB) and solid (SAB) phases of the rumen are widely demonstrated (Carro and Miller, 2002; Ipharraguerre *et al.*, 2007; Molina-Alcaide *et al.*, 2009), but differences in the bacterial communities isolated in LAB and SAB pellets have received relatively little attention. Additionally, composition of bacterial communities in the rumen cannot be studied with traditional cultivation techniques. In the last years, molecular fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), single strand conformation polymorphism (SSCP) or automated ribosomal intergenic spacer analysis (ARISA) have been used to assess the diversity of ruminal bacterial communities. Some studies have analyzed the changes in bacterial diversity promoted by diet in cattle (Tajima *et al.*, 2001; Welkie *et al.*, 2010; Weimer *et al.*, 2010) and sheep (Kocherginskaya *et al.*, 2001; Martinez *et al.*, 2010), but studies in goats are very scarce (Shi *et al.*, 2007; Cunha *et al.*, 2011). The purpose of this study was to analyze the changes in bacterial diversity in liquid and solid phases of the rumen of sheep and goats fed diets differing in forage type. The DGGE technique was selected for this study because it has been shown to be a powerful tool for profiling diversity of microbial communities in the gastrointestinal tract of different animal species (Simpson *et al.*, 2002). To our knowledge this is the first comparative study to examine bacterial diversity in sheep and goats fed the same diets, although Belenguer *et al.* (2011) have previously analyzed the structure of rumen bacterial community in sheep, goats and cows grazing in the same plot.

II – Materials and methods

1. Diets, animals and experimental design

This study was conducted with an experiment investigating the nutrient utilization and N balance in sheep and goats fed different diets (Carro *et al.,* 2012). The two experimental diets contained 30% concentrate (dry matter (DM) basis) and 70% of either alfalfa hay (AL) or grass hay (GR) as forage. The concentrate was based on barley, gluten feed, wheat middlings, soybean meal, palmkern meal, wheat, corn and mineral-vitamin premix in the proportions of 215, 204, 200, 135, 115, 50, 50 and 31 g/kg, respectively (fresh matter basis). Crude protein content was 186 and 121 g/kg DM for AL and GR diet, respectively, and neutral-detergent fibre content was 426 and 499 g/kg DM, respectively.

Two Murciano-Granadina goats (44.5 ± 1.00 kg body weight (BW)) and 2 Merino sheep (55.1 ± 2.90 kg BW) were chosen to investigate changes in rumen bacterial diversity promoted by the experimental diets. Animals fitted with ruminal cannulas were used in a trial with 2 periods of 25 days each. In each period, one sheep and one goat were fed AL diet and the other 2 animals were fed the GR diet. Animals were housed in individual pens and had continuous access to fresh water and vitamin/mineral blocks over the experimental period. Animals were cared and handled in accordance with the Spanish Animal Care Regulations (Royal Decree 1201/2005 of October 10th on the protection of animals used for experimentation or other scientific purposes). Diets were offered to the animals twice daily (08:00 and 14:00 h) at a daily rate of 56 g DM/kg

$BW^{0.75}$ to minimize feed selection. On days 23 and 25 of each period, rumen contents (600 g) were withdrawn from each animal at 0 and 4 h after the morning feeding. Rumen contents were squeezed through 4 layers of cheesecloth and the solid digesta was combined with an equal volume of saline solution (0.9% NaCl) at 39ºC, mixed gently, and squeezed again to remove residual non-attached bacteria. The filtrate obtained at each sampling time was kept at 4ºC, pooled per animal(150 mL from each sampling time), and used to isolate liquid-associated bacteria (LAB) by differential centrifugation (Ranilla and Carro, 2003). The solid digestawastreated with saline solution (0.9% NaCl) containing 0.1% methylcellulose as described by Ranilla and Carro (2003) before isolation of solid-associated bacteria (SAB). Bacterial pellets (LAB and SAB) were lyophilized and ground to a fine powder with a mortar and pestle. Bacterial pellets were composited to have 2 pellets (one SAB and one LAB) per animal and period before DNA extraction. In addition, pH of the fluid was immediately measured, and 5 mL of fluid were added to 5 mL of deproteinizing solution (100 g of metaphosphoric acid and 0.6 g of crotonic acid per L) for volatile fatty acid analysis. Samples were analyzed as described by Carro *et al.* (2012).

2. DNA extraction and DGGE analyses

Samples (40 mg DM) of freeze-dried LAB and SAB were homogenized with steel beads in a Mini-Bead-beater (BioSpecInc, Bartlesville, OK, USA). DNA was extracted from homogenized samples with the QIAmp® DNA Stool Mini Kit (Qiagen GmbH, Hilden, Germany) following the manufacturer´s instructions. Purity and yield of the extracted DNA were assessed using a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

Denaturing gradient gel electrophoresis (DGGE) was used to investigate the effects of experimental diets on bacterial diversity. The V3 region of the *16S rRNA* gene was amplified from the extracted DNA by PCR using the bacterial primers 338f forward 5´-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3´ and 534r reverse 5´- ATTACC GCG GCT GCT GG-30 (Muyzer *et al.*, 1993). The PCR amplification was performed using the following steps: one cycle (94 ºC for 4 min), 30 cycles (94 ºC for 1 min, 55 ºC for 1 min, 72 ºC for 1 min), and one cycle (72 ºC for 7 min). The PCR reaction solution contained 50 ng DNA in a 50 µL mix containing 1 mM buffer, 1.25 mM of each primer, 0.8mM of dNTPs, 2.5 mM $MgCl_2$ and 2.5U of Taq DNA polymerase in 10 mMTrisHCl (pH 9.0). The resulting amplicons were visualized on a 2% (w/v) TBE (89mM Tris, 89mM Boric acid, 2mM Na2EDTA; pH 8.3) agarose gel stained with GelRed.

The DGGE was performed using a BDH system from VWR International Ltd (UK), following the manufacturer's guidelines. The PCR products (10 µl) were loaded onto 8% (w/v) TAE polyacrylamide gels (40 mmol /L of Tris base, 20 mmol/L of acetic acid and 1 mmol/L of EDTA, pH 8.3), which contained a 40–60% denaturant gradient (100% denaturant, 7 mol/L of urea and 40% (v/v) of deionized formamide). Electrophoresis was performed at a constant voltage of 100 V and temperature of 60ºC for 16 h. The DNA was visualised by silver staining with a Bio-Rad Silver stain kit, and scanned DGGE images were analysed with the Quantity One Software (BioRad, Madrid, Spain). Each band position present in the gel was binary coded for its presence or absence within a lane and each lane was compared by using a similarity matrix. Two different DGGE gels were run, one including samples from sheep and another with samples from goats. DGGE profiles within the same gel were compared by using Dice coefficient and the unweighted pair group method with arithmetic averages (UPGMA) clustering algorithm, and shown graphically as a dendrogram. The richness of the bacterial community was determined from the number of bands (NB) in each lane. The Shannon index (SI), a measure of diversity, was calculated by the following equation $H = -\sum (pi \cdot \ln pi)$, where pi is the abundance of every species.

3. Statistical analyses

Data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). The effects of animal species (AS), type of forage in the diet, period, and the interaction AS x diet were considered fixed, and animal within species effect was considered random. Within each animal, differences between LAB and SAB in their NB and SI were analysed by a paired Student's *t* test.

III – Results and discussion

A total of 78 and 77 bands were detected in the DGGE gel from sheep and goats samples, respectively. In sheep, 18 bands were only found in samples from AL-fed animals, and 7 were found only in samples from animals fed the GR diet. In goats, 21 bands were found only in AL-fed animals and 17 were found exclusively in GR-fed goats. The mean values of NB and SI in LAB and SAB pellets from sheep and goats fed the two experimental diets are shown in Table 1. There were no AS x diet interactions (P = 0.165 to 0.259) in any of these parameters, indicating that the individuals from the two AS responded similarly to changes in the type of forage. In sheep, the NB ranged from 43 to 50 and from 27 to 35 for AL and GR diets, respectively. In goats, the NB ranged from 30 to 45 and from 28 to 34 for AL and GR diets, respectively. There were no interspecies differences in the NB (P = 0.655) and SI (P = 0.728) in SAB pellets. In contrasts, LAB pellets from sheep had greater NB (P = 0.016) and SI (P = 0.016) compared with goats, which would indicate a greater diversity of LAB communities in the rumen of sheep. In both animal species, AL diet tended to promote a greater NB and SI in LAB (P = 0.072 and 0.071, respectively) and SAB (P = 0.089 and 0.099) pellets compared to the GR diet.

Table 1. Band numbers and Shannon index calculated from the total bacterial DGGE profiles of LAB and SAB samples obtained from sheep and goats fed diets containing 30% of concentrate and 70% of alfalfa hay (AL) or grass hay (GR) as forage

Item	Bacterial pellet	Animal species	Diet		SEM	Significance levels (*P* =)		
			AL	GR		AS[1]	Diet	AS x Diet
Band number	LAB	Sheep	48.0	33.0	1.07	0.016	0.072	0.165
		Goat	32.5	28.0				
	SAB	Sheep	43.5	30.0	2.88	0.655	0.089	0.259
		Goat	37.5	33.0				
Shannon index	LAB	Sheep	3.87	3.50	0.028	0.016	0.071	0.225
		Goat	3.50	3.33				
	SAB	Sheep	3.77	3.40	0.081	0.728	0.099	0.245
		Goat	3.61	3.50				

[1]AS: animal species.

The similarity index between LAB and SAB ranged from 31.6 to 66.7% (data not shown), and they did not differ (P = 0.481) between the two animal species (51.6 and 45.9% for sheep and goats, respectively). Whereas in sheep both NB and SI were greater in LAB compared to SAB (P= 0.050 and 0.036, respectively), no differences between LAB and SAB were found either in NB (P = 0.269) or SI (P = 0.257) in goats. Moreover, in sheep there was a positive correlation between the NB in LAB and that in SAB (r = 0.966; P = 0.034), but no correlation (r= 0.039; P = 0.961) was observed in goats. In accordance with our results, others have reported differences in the bacterial communities found in the fluid and those in the solid phase of the rumen in sheep (Michalet-Doreau *et al*., 2001; Larue *et al.*, 2005; Stiverson *et al.,* 2011) and goats

(Cunha *et al.,* 2011). However, it must be noticed that whereas LAB are relatively easy to isolate, and a great recovery of the bacterial populations should be expected, recovery of SAB from ruminal digesta is usually low, indicating that a pure SAB isolate may not be representative of the total SAB population. The treatment of sheep ruminal digesta with the detachment method used in the present study has been reported to recover less than 40% of the total SAB in sheep fed high-forage diets (Ramos *et al.*, 2009), but no other method has been shown to be more effective in detaching SAB from solid digesta.

Several studies have pointed out large variations of microbial communities between animals (Firkins and Yu, 2006; Weimer *et al*., 2010). In order to illustrate the inter-animal variability in our study, Fig. 1 shows the NB in LAB and SAB pellets in each animal. The NB in LAB pellets varied from 31 to 50, but it was numerically lower for GR compared with AL diet in all animals. The NB in SAB pellets was numerically lower for GR compared with AL diet in the two sheep and goat 1, but goat 2 showed the opposite results. Bacterial communities in the gastrointestinal tract are influenced by numerous host-related factors, such as mastication, rumination, feeding behavior, digesta passage rate, genetics, etc. Interestingly, the two sheep and goat 1 showed numerically lower values in pH andacetate:propionate ratio in ruminal fluid for GR than for AL diet (Fig. 2), but goat 2 showed the opposite response, and this may be related to the higher SAB diversity observed when this animal was fed GR (Fig. 1).

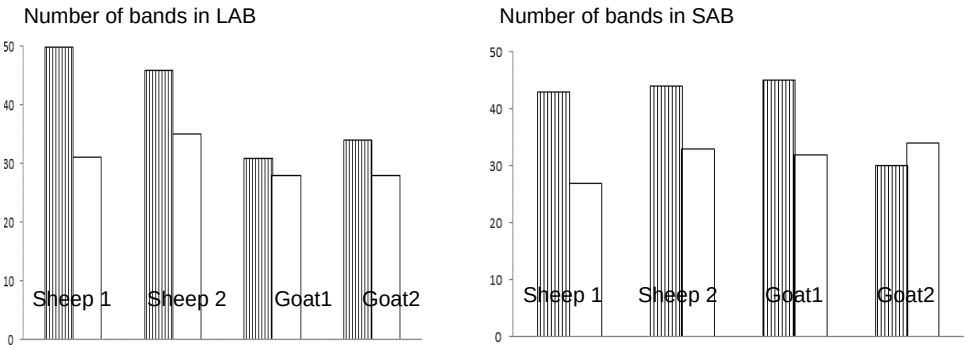

Fig. 1. Total number of bands in bacterial DGGE profiles of liquid-associated (LAB) and solid-associated (SAB) bacterial pellets obtained from sheep and goats fed diets containing 30% concentrate and 70% of either alfalfa hay (striped bars) or grass hay (unfilled bars) as forage. Numbers 1 and 2 correspond to individuals within each animal species.

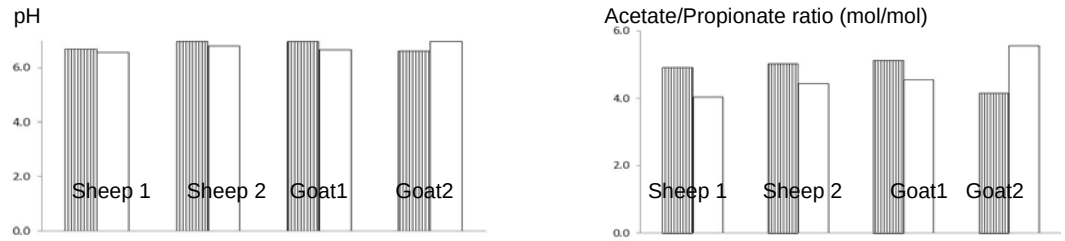

Fig. 2. Ruminal pH and acetate/propionate ratio in ruminal fluid (mean values of samples taken at 0 and 4 h morning post-feeding) from sheep and goats fed diets containing 30% of concentrate and 70% of either alfalfa hay (striped bars) or grass hay (unfilled bars) as forage. Numbers 1 and 2 correspond to individuals within each animal species.

As shown in Fig. 3A, the UPGMA dendrogram generated by the cluster analysis shows that all samples from sheep can be included in two major clusters. The four SAB pellets clustered together and the four LAB pellets grouped in a different cluster. In goats, the four SAB pellets clustered together, but clustering of LAB pellets was not so clear (Fig. 3B). In both animal species, SAB and LAB clusters contained two clear subclusters according to forage type.

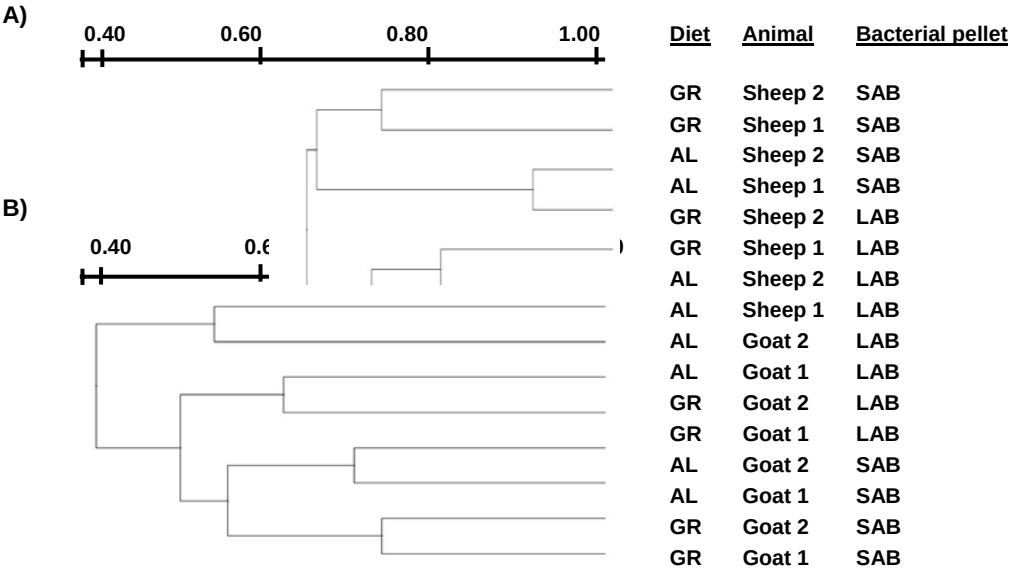

Fig. 3. Dendrograms from DGGE analysis of liquid-associated (LAB) and solid-associated (SAB) bacterial pellets in sheep (A) and goats (B) fed diets containing 30% of concentrate and 70% of alfalfa hay (AL) or grass hay (GR) as forage. Numbers 1 and 2 correspond to individuals within each animal species.

IV – Conclusions

Despite the reduced number of animals used in this study, in all of themLAB and SAB diversity was affected by the type of forage in the diet, and bacterial pellets from AL-fed animals tended to have greater diversity than those from animals fed the GR diet. These results indicate differences in the composition of the bacterial communities in the liquid and solid phases of the rumen. Inter-animal variability was observed, with 3 animals responding similarly to changes in forage type and one animal showing the opposite response in ruminal pH, acetate/propionate ratio and diversity in SAB pellets.

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Use of tannins to modify ruminal biohydrogenation in sheep. 1. Effects on *in vitro* rumen fermentation

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Abstract. Some studies have recommended the use of tannins to modulate ruminal biohydrogenation (BH) of dietary fatty acids and enhance the accumulation of some bioactive metabolites. However, before investigating this use, it is necessary to make sure that tannins will not impair ruminal fermentation and consequently diet utilization. This experiment was conducted in sheep to study the effect of the addition of tannins to a total mixed ration supplemented with 2% DM of sunflower oil on ruminal fermentation. The assay was carried out *in vitro* using batch cultures of rumen microorganisms and the gas production technique. Four commercial extracts of tannins [2 hydrolysable (HT: chestnut and oak) and 2 condensed (CT: quebracho and grape) tannins] × 2 doses of each one (2 and 5% DM) were added to the supplemented diet. Four rumen cannulated ewes were used as donors of ruminal inoculum. According to the kinetics of gas production, only some treatments with tannin (grape5% and oak2%) were able to reduce significantly the rate of gas production, while most of them decreased slightly (1.84% on average) the extent of the degradation in the rumen (ED_{24}). Tannin addition had limited effects on the values of pH and VFA production after 24 h incubation but, as expected, decreased ammonia concentration. This reduction was stronger with HT and at 5%. If these tannin extracts were proved to beneficially modulate ruminal BH, the slight negative effect on the ED_{24} should not prevent their recommendation in doses up to 5% DM.

Keywords. Batch cultures of rumen microorganisms – Condensed tannin – Diet utilization – Hydrolysable tannin.

Utilisation des tanins pour modifier la biohydrogénation ruminale chez les ovins. 1. Effets sur la fermentation in vitro

Résumé. *Quelques études ont préconisé l'usage des tanins pour modifier la biohydrogénation (BH) ruminale des acides gras d'origine alimentaire, et pour accroître la concentration des métabolites bioactifs dans le rumen. Il est préalablement nécessaire de s'assurer que les tanins n'ont pas d'effets négatifs sur la fermentation ruminale et l'utilisation des rations. Nous avons testé l'effet d'un ajout des tanins sur la fermentation ruminale chez des brebis alimentées avec une ration complète supplémentée à 2% par l'huile de tournesol. L'étude a été conduite* in vitro*, en utilisant des cultures non renouvelées de micro-organismes du rumen et la technique de production de gaz. Quatre extraits de tanins, 2 hydrolysables (HT: tanins de châtaignier et de chêne) et 2 condensés (CT: tanins de quebracho et de raisin) ont été ajoutés à deux niveaux (2 et 5% de la matière sèche) à la ration complète supplémentée. Le liquide ruminal était prélevé sur quatre brebis fistulées du rumen. Seuls les traitements raisin5% et chêne2% ont significativement réduit la production de gaz, alors que la plupart des traitements ne diminuaient que modérément (1,84% en moyenne) la mesure de dégradation dans le rumen (ED). L'ajout de tanins n'a eu qu'un léger effet sur le pH et la production d'acides gras volatils au bout de 24 heures, mais a logiquement diminué la concentration d'ammoniac, en particulier avec les TH et au niveau supérieur (5%) de complémentation. Malgré son effet légèrement négatif sur l'ED, une complémentation jusqu'à 5% des rations avec des extraits de tanins serait envisageable à condition qu'un effet positif sur la BH ruminale soit mis en évidence.*

Mots-clés. *Cultures non renouvelées de micro-organismes du rumen – Tanins condensés – Tanins hydrolysables – Utilisation des rations.*

I – Introduction

Some *in vitro* studies (*e.g.*, Khiaosa-ard *et al.*, 2009; Vasta *et al.*, 2009; Buccioni *et al.*, 2011) have recommended the use of tannins to modulate ruminal biohydrogenation (BH) of dietary fatty acids and enhance the accumulation of some bioactive metabolites (*e.g.*, *trans*-11 18:1, a precursor of conjugated linoleic acid; CLA) that are potentially healthy for consumers (Shingfield *et al.*, 2008). However, before investigating this use, it is necessary to make sure that tannins would not impair ruminal fermentation and consequently diet utilization (Makkar, 2003).

The present study was therefore conducted to evaluate, in sheep, the effect of the addition of different types and doses of tannins to a total mixed ration (TMR; forage:concentrate ratio 40:60) rich in linoleic acid on ruminal fermentation.

II – Materials and methods

The experiment was conducted *in vitro* using batch cultures of rumen microorganisms, following a 4 × 2 + 2 (controls) design. Treatments were: 4 types of tannins [2 hydrolysable (HT: chestnut and oak) and 2 condensed (TC: quebracho and grape)] × 2 doses of each one (2% and 5% DM) were added to a diet supplemented with 2% of sunflower oil (on a DM basis; SO). In addition to the positive control (*i.e.*, the supplemented diet), a negative control (*i.e.*, the diet without lipid supplementation) was also included to monitor the effect of the inclusion of lipids in the diet. No tannins were added to the negative control.

In vitro fermentation characteristics were studied using a modification of the gas production technique (Hervás *et al.*, 2005). Two rumen fluid inocula (collected in two different days; one for each replicate) were obtained before the morning feeding from four rumen-cannulated ewes fed a TMR similar to that used in the cultures (forage:concentrate ratio 40:60; 173 g of CP, 260 g of NDF, and 29 g of ether extract/kg DM). Substrates (500 mg) were incubated in 125 ml serum flasks at 39ºC with 10 ml strained rumen fluid and 40 ml medium (Goering and Van Soest, 1970). The pH was adjusted to 6.5 with orthophosphoric acid in order to simulate ruminal conditions in animals fed a concentrate diet. Accumulated head-space gas pressures were measured with a pressure transducer at 2, 4, 6, 9, 12, 15, 19, 24, 30, 36, 48, 60 and 72 h post-inoculation. Pressure values, corrected for the quantity of substrate organic matter (OM) incubated and gas released from controls (*i.e.*, rumen fluid plus buffer medium, without substrate), were used to generate gas volume estimates using a predictive equation derived from earlier simultaneous pressure and volume measurements (Hervás *et al.*, 2005). Organic matter degradation (OMD, %) after 72 h incubation was estimated by filtering residues using sintered glass crucibles (100-160 µm; Pyrex) and ashing at 550ºC for 6 h. Gas production data were fitted to an exponential model to provide parameters describing gas release in terms of cumulative gas production (*A*, mL gas/g OM incubated) and fractional fermentation rate (*c*, /h). Average fermentation rate (AFR, mL gas/h) and extent of degradation in the rumen (ED, %) were estimated assuming a rumen particulate outflow (/h) of 0.042 (Hervás *et al.*, 2005).

Six more samples (3 flasks/replicate) of each treatment were incubated for 24 hours and used to measure pH, ammonia concentration and volatile fatty acid (VFA) production on the supernatant.

All data were analysed as a one-way analysis of variance using the MIXED procedure of SAS (Version 9.2). Treatment means were compared to the positive control using the 'pdiff' option of the 'lsmeans' statement.

III – Results and discussion

The addition of 2% sunflower oil to the diet (positive control) had no significant effects (P>0.10) on the gas production parameters (Table 1) or the other indicators of rumen fermentation

studied (Tables 2 and 3). This is in agreement with previous works proving that the inclusion of SO in the diet of dairy ewes has no apparent detrimental effects on ruminal digestion (Hervás *et al.*, 2008; Toral *et al.*, 2010), even at a much higher dose (6%) than that used in this trial (2%).

Table 1. Gas production parameters (*A*, mL gas/g OM and *c*, /h), average fermentation rate (AFR, mL gas/h), extent of degradation (ED, %) and organic matter disappearance (OMD, %). Mean and standard error of the negative (TMR) and positive (TMR+sunflower oil) controls, and percentage of variation caused by each tannin treatment with respect to the positive control

	A	*c*	AFR	ED	OMD
TMR	337±18.9	0.078±0.0004	19.0±0.97	53.1±0.07	85.4±0.20
TMR+sunflower oil	345±33.4	0.076±0.0002	19.0±1.80	53.1±0.38	85.8±0.67
+ Quebracho 2%	-3.4 ns	0.9 ns	-2.5 ns	-2.2 **	-2.5 **
+ Quebracho 5%	-20.1 *	1.9 ns	-21.7 **	-2.7 **	-2.3 ns
+ Grape 2%	-13.6 ns	-1.5 ns	-15.0 *	-0.5 ns	0.0 ns
+ Grape 5%	-15.1 ns	-4.9 *	-19.2 **	-2.1 *	-0.9 ns
+ Chestnut 2%	-1.6 ns	-2.2 ns	-4.0 ns	-1.4 ns	-0.9 ns
+ Chestnut 5%	-12.5 ns	-4.1 ns	-16.3 *	-3.2 **	-2.8 ns
+ Oak 2%	-5.7 ns	-4.7 *	-10.4 ns	-2.5 **	-1.0 ns
+ Oak 5%	-6.9 ns	-3.8 ns	-10.9 ns	-0.4 ns	1.8 ns

The level of significance of each percentage of variation (tannin treatment *vs.* positive control) appears on its right: ns= non-significant (P>0,10); * = P<0,10 and ** = P<0,05.

Table 2. Final pH, ammonia concentration (mg/L), total VFA production (mmol/L) and acetate/propionate ratio (A/P). Mean and standard error of the negative (TMR) and positive (TMR+sunflower oil) controls, and percentage of variation caused by each tannin treatment with respect to the positive control

	pH	Ammonia	Total VFA	A/P
TMR	6.23±0.060	606±27.8	65.2±4.20	3.14±0.225
TMR+sunflower oil	6.24±0.001	634±10.6	48.5±14.19	3.16±0.195
+ Quebracho 2%	0.7 ns	-6.6 ns	-7.1 ns	0.4 ns
+ Quebracho 5%	1.3 ns	-18.1 *	26.0 ns	-3.5 ns
+ Grape 2%	1.4 ns	-9.6 ns	8.6 ns	-4.0 ns
+ Grape 5%	2.9 *	-13.2 *	7.9 ns	2.3 ns
+ Chestnut 2%	2.7 *	-9.4 ns	18.4 ns	2.6 ns
+ Chestnut 5%	2.3 *	-19.0 *	13.4 ns	-1.8 ns
+ Oak 2%	1.3 ns	-13.4 *	53.8 ns	1.8 ns
+ Oak 5%	1.4 ns	-16.7 *	45.7 ns	3.1 ns

The level of significance of each percentage of variation (tannin treatment *vs.* positive control) appears on its right: ns= non-significant (P>0,10) and * = P<0,05.

However, a number of studies have reported a reduction in the rate and extent of gas production in response to tannin extracts (*e.g.*, Frutos *et al.*, 2004; Vasta *et al.*, 2009). Accordingly, some treatments with tannins were able to reduce the fractional fermentation rate (*c*; grape at 5% and oak at 2%) and the AFR (quebracho at 5%, grape at 2 and 5%, and chestnut at 5%; Table 1). While most of the treatments decreased slightly (–1.84% on average)

the extent of degradation in the rumen (ED_{24}), only the highest dose of quebracho extract tended to reduce the cumulative gas production (–20%). These results would be attributable to the highly variable reactivity of different tannins (Frutos *et al.*, 2004; Mueller-Harvey, 2006) as well as to the dose (Hervás *et al.*, 2003).

Table 3. Molar proportions of acetate, propionate, butyrate and other VFAs (calculated as the sum of isobutyrate, isovalerate, valerate and caproate; mol/100 mol). Mean and standard error of the negative (TMR) and positive (TMR+sunflower oil) controls, and percentage of variation caused by each tannin treatment with respect to the positive control

	Acetate	Propionate	Butyrate	Other
TMR	59.1±0.11	18.6±1.39	17.0±1.40	5.0±0.11
TMR+sunflower oil	58.6±0.46	18.6±1.30	17.6±1.66	5.2±0.11
+ Quebracho 2%	0.5 ns	0.2 ns	-0.5 ns	-5.8 ns
+ Quebracho 5%	1.6 **	5.4 ns	-6.5 ns	-16.1 **
+ Grape 2%	1.2 *	5.5 ns	-5.9 ns	-12.7 *
+ Grape 5%	2.8 **	0.6 ns	-5.1 ns	-16.7 **
+ Chestnut 2%	1.7 **	-0.9 ns	-1.4 ns	-10.5 ns
+ Chestnut 5%	2.3 **	4.3 ns	-4.6 ns	-13.0 **
+ Oak 2%	2.0 **	0.3 ns	-3.7 ns	-12.6 *
+ Oak 5%	3.1 **	0.2 ns	-4.9 ns	-20.8 **

The level of significance of each percentage of variation (tannin treatment *vs.* positive control) appears on its right: ns= non-significant (P>0,10); * = P<0,10 and ** = P<0,05.

With respect to other indicators of rumen fermentation (Table 2), dietary supplementation with tannins had limited effects on the values of pH and VFA production but resulted in a noticeable decrease in ammonia concentration (–16%), in accordance with the well-known inhibition of ruminal proteolysis caused by these phenolics (Makkar, 2003; Frutos *et al.*, 2004; Mueller-Harvey, 2006). The reduction was stronger with HT and with the highest dose (5%), confirming that the effect of tannins depends on the type and concentration used.

It is probably noteworthy the great variability observed between replicates, which was responsible for increases in VFA production as high as about 50% not attaining statistical significance. As reported previously in similar *in vitro* studies, this was likely accounted for by remarkable between-day variations in the rumen bacterial composition of the inoculum (Belenguer *et al.*, 2011).

As shown in Table 3, the addition of tannins to a TMR rich in linoleic acid altered the molar proportions of VFA. Most of the treatments increased (+2%) the proportion of acetic acid and reduced (–15%) the proportion of the so-called 'other' VFA (calculated as the sum of isobutyrate, isovalerate, valerate and caproate). Because the latter are mostly originated from deamination of some amino acids, the decrease would indicate reductions in ruminal proteolisis, which is in agreement with the lower ammonia concentrations. Reports of the effects of tannins on the molar proportions of VFA, as well as on total VFA, are very inconsistent (*e.g.*, Frutos *et al.*, 2004; Tiemann *et al.*, 2008; Toral *et al.*, 2011) and highly dependent, once again, on the type and content of these polyphenolic compounds.

IV – Conclusion

If tannin extracts of quebracho, grape, chestnut or oak were proved to beneficially modulate ruminal BH, their slight negative effect on the extent of degradation in the rumen does not appear to prevent their use at the doses tested in this experiment.

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Use of tannins to modify ruminal biohydrogenation in sheep. 2. Effects on *in vitro* fatty acid composition of rumen digesta

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Abstract. Supplementing the diet of ruminants with tannins has been suggested as a strategy to modulate ruminal biohydrogenation (BH) of dietary polyunsaturated fatty acids (FA) and enhance the accumulation of vaccenic acid (VA) due to an inhibition of the last step of BH. The ultimate goal of this strategy is to increase the content of some bioactive FA (such as CLA or VA) in the ruminant products. This experiment was conducted in sheep to study the effect of the addition of tannins to a total mixed ration supplemented with 2% DM of sunflower oil on ruminal BH. The assay was carried out *in vitro* using batch cultures of rumen microorganisms. Four commercial extracts of tannins [2 hydrolysable (HT: chestnut and oak) and 2 condensed (CT: quebracho and grape) tannins] × 2 doses of each one (2 and 5% DM) were added to the supplemented diet. After 24 h incubations, none of the four tannin extracts and none of the two doses tested were able to modify the rumen contents of total CLA, VA or stearic acid. However, some increases were detected in the concentration of linolenic, linoleic and oleic acids, which may suggest a general inhibition of the ruminal BH rather than a specific inhibition of the last step. Most significant results were observed with HT and at 5%. Overall, the results would not allow to recommend this strategy to modulate ruminal BH and improve the nutritional quality of the ruminant products.

Keywords. CLA – Condensed tannin – Hydrolysable tannin – Lipid supplementation – Vaccenic acid.

***Utilisation des tanins pour modifier la biohydrogénation ruminale chez les ovins. 2. Effets sur la composition en acides gras du digesta ruminal* in vitro**

Résumé. *Afin de modifier la biohydrogénation (BH) ruminale des acides gras polyinsaturés (AG) et d'améliorer l'accumulation de l'acide vaccénique (VA) par une inhibition de la dernière étape de la BH, une des stratégies proposées est d'incorporer des tanins dans les régimes destinés aux ruminants. Le but ultime de cette stratégie est d'augmenter la teneur en certains AG bioactifs (tels que le CLA ou VA) dans les produits animaux. Une expérience a été menée sur le mouton pour étudier l'effet de l'incorporation des tanins dans une ration complète enrichie d'huile de tournesol (2% de la matière sèche) sur la BH ruminale. Le test a été réalisé in vitro en utilisant des cultures non renouvelées de micro-organismes du rumen. Quatre extraits commerciaux de tanins ont été ajoutés à la ration supplémentée [2 extraits de tanins hydrolysables (HT) : châtaignier et chêne et 2 extraits de tanins condensés (CT) : quebracho et raisin] à raison de 2 doses de chacun (2 et 5% de la matière sèche). Après une incubation de 24 h, aucun des 4 extraits de tanins et aucune des deux doses testées n'ont modifié la teneur ruminale en CLA totaux, en VA ou en acide stéarique. Toutefois, une augmentation des concentrations des acides linolénique, linoléique et oléique a été observée, ce qui suggère une inhibition générale de la BH ruminale plutôt qu'une inhibition spécifique de la dernière étape. Les résultats les plus significatifs ont été observés avec les HT et à une dose de 5%. Dans l'ensemble, ces résultats ne permettent pas de recommander cette stratégie pour modifier la BH ruminale et améliorer la qualité nutritionnelle des produits issus de ruminants.*

Mots-clés. *CLA – Tanins condensés – Tanins hydrolysables – Supplémentation lipidique – Acide vaccénique.*

I – Introduction

Recent studies have suggested that supplementing the diet of ruminants with tannins may be a good strategy to modulate the process of ruminal biohydrogenation (BH) of dietary polyunsaturated fatty acids (FA) and enhance the accumulation of vaccenic acid (VA) due to an inhibition of the last step of BH (Khiaosa-ard *et al.*, 2009; Vasta *et al.*, 2009). The ultimate goal of this strategy would be to increase the content of some potencially healthy FA (such as conjugated linoleic acid [CLA] and VA) in ruminant-derived products (Vasta and Luciano, 2011). However, reports in this regard are still limited and inconsistent.

This experiment was therefore conducted to study, in sheep, the effect of the addition of tannins to a total mixed ration (TMR; forage:concentrate ratio 40:60) supplemented with 2% of sunflower oil (as a source of linoleic acid for the synthesis of VA in the rumen; Shingfield *et al.*, 2008; Toral *et al.*, 2012), on ruminal BH, with special attention to the accumulation of some FA of special interest (e.g., linolenic and linoleic acids, CLA and 18:1 metabolites).

II – Materials and methods

The experiment was conducted *in vitro* using batch cultures of rumen microorganisms following a 4 × 2 + 2 (controls) design. Treatments were: 4 types of tannins [2 hydrolysable (HT: chestnut and oak) and 2 condensed (TC: quebracho and grape)] × 2 doses of each one (2% and 5% DM) were added to a diet supplemented with 2% of sunflower oil (on a DM basis; SO). In addition to the positive control (*i.e.*, the supplemented diet), a negative control (*i.e.*, the diet without lipid supplementation) was also included to monitor the effect of the inclusion of lipids in the diet. No tannins were added to the negative control.

Four ewes cannulated in the rumen and fed a TMR similar to that used in the cultures (forage:concentrate ratio 40:60; 173 g of CP, 260 g of NDF, and 29 g of ether extract/kg DM) were used as donors of ruminal inoculum. Rumen fluid was collected before the morning meal. Sixty samples of the diet [10 treatments × 3 flasks/treatment × 2 replicates (*i.e.*, repetition in 2 different days)] ground through a 1-mm screen (≈ 500 mg), plus 6 blanks (3 flasks without substrate/day), were incubated for 24 hours at 39ºC with 10 mL strained rumen fluid and 40 mL phosphate-bicarbonate medium (Goering and Van Soest, 1970).

For FA composition analysis, lipids in 200 mg of freeze dried in vitro ruminal digesta were extracted with 4 mL of a mixture (3:2, v/v) of hexane and isopropanol following the adjustment of digesta pH to 2.0. Lipids were then converted to fatty acid methyl esters (FAME) using a base-acid catalyzed transesterification procedure with freshly prepared 0.5 M sodium methoxide in methanol followed by reaction with 1% sulphuric acid in methanol, as outlined by Toral *et al.* (2010). Tridecanoic acid (Sigma-Aldrich, Madrid, Spain) was used as internal standard. Methyl esters were separated and quantified using a gas chromatograph (Agilent 7890A GC System, Santa Clara, CA, USA) equipped with a flame-ionization detector and a 100 m fused silica capillary column (0.25 mm i.d., 0.2-µm film thickness; CP-SIL 88, Chrompack 7489, Varian Ibérica S.A., Madrid, Spain) and He as the carrier gas. Total FAME profile in a 2 µL sample volume at a split ratio of 1:50 was determined using a temperature gradient programme (Shingfield *et al.*, 2003). Isomers of 18:1 were further resolved in a separate analysis under isothermal conditions at 170ºC (Shingfield *et al.*, 2003). Peaks were identified based on retention time comparisons with authentic standards (from Nu-Chek Prep., Elysian, MN, USA; Sigma-Aldrich, Madrid, Spain; and Larodan Fine Chemicals AB, Malmö, Sweden). Identification of FA was verified based on FAME standard mixtures when available, chromatograms reported in the literature (*e.g.*, Shingfield *et al.*, 2003, 2006) and by comparison with milk samples for which the FA composition was determined based on GC analysis of FAME and GC-MS analysis of corresponding 4,4-dimethyloxazoline derivates (Toral *et al.*, 2010).

All data were analysed as a one-way analysis of variance using the MIXED procedure of SAS (Version 9.2). Means were compared to the positive control using the 'pdiff' option of the 'lsmeans' statement of the MIXED procedure.

III – Results and discussion

Table 1 shows the content of several long-chain FA in the ruminal digesta, and Tables 2 and 3 a partial profile of *cis* 18:1 and *trans* 18:1 isomers, respectively. In all cases, tables show the mean and standard error of the negative (TMR) and positive (TMR+sunflower oil) controls, and the percentage of variation caused by each tannin treatment (*i.e.*, quebracho, grape, chestnut and oak tannin extracts at 2 and 5%) with respect to the positive control. When the inclusion of sunflower oil (*i.e.*, TMR *vs.* TMR+sunflower oil) caused a significant effect, this is indicated on the positive control value.

As expected (Hervás *et al.*, 2008; Shingfield *et al.*, 2008; Toral *et al.*, 2012), diet supplementation with sunflower oil significantly increased (P<0.05) the content of total 18:2 non-conjugated linoleic acid, *cis*-9 *cis*-12 18:2, total CLA, *cis* 18:1 and *trans* 18:1, without effect on stearic acid (P>0.10).

Table 1. Content (mg/g digesta) of several long-chain fatty acids in the ruminal digesta. Mean and standard error of the negative (TMR) and positive (TMR+sunflower oil) controls, and percentage of variation caused by each tannin treatment with respect to the positive control

	18:3*n*-3	**Total 18:2 non-conj.**	***cis*-9 *cis*-12 18:2**	**Total CLA**	**Total 18:1**	**18:0**
TMR	0.19±0.008	1.07±0.115	0.75±0.051	0.19±0.005	4.83±0.809	12.4±0.98
TMR+sunflower oil	0.17±0.009	2.59±0.150**	2.31±0.128**	0.64±0.022**	7.75±0.481**	14.2±0.10
+ Quebracho 2%	5.5 ns	-0.4 ns	-1.2 ns	-3.6 ns	-1.5 ns	1.6 ns
+ Quebracho 5%	27.5 *	17.5 ns	18.2 ns	-9.6 ns	-0.2 ns	-5.8 ns
+ Grape 2%	16.8 ns	8.1 ns	9.0 ns	-3.9 ns	0.5 ns	-11.5 ns
+ Grape 5%	15.9 ns	17.9 ns	18.7 ns	-7.0 ns	0.4 ns	-2.7 ns
+ Chestnut 2%	40.0 **	18.9 *	22.5 *	-10.1 ns	-6.0 ns	3.6 ns
+ Chestnut 5%	40.7 **	47.7 **	54.1 **	-7.9 ns	1.7 ns	-7.1 ns
+ Oak 2%	19.0 ns	17.9 ns	20.7 ns	-6.4 ns	-4.6 ns	-8.6 ns
+ Oak 5%	5.5 ns	-0.4 ns	-1.2 ns	-3.6 ns	-0.4 ns	1.6 ns

The level of significance of each percentage of variation (tannin treatment *vs.* positive control) appears on its right: ns= non-significant (P>0,10); * = P<0,10 and ** = P<0,05.

On the contrary, none of the four tannins at any of the doses used were able to modify the rumen contents of *cis*-9 *trans*-11 18:2, total CLA or *trans*-11 18:1. The levels of *trans*-10 18:1 and 18:0 were not changed either. However, significant increases, due to the action of tannins, were detected in the concentration of some FA that were added with the sunflower oil, such as linoleic, linolenic and oleic acids, which would suggest a general inhibition of the ruminal BH of dietary polyunsaturated FA rather than a specific inhibition of the reduction of 18:1 to 18:0. These *in vitro* results are in line with those observed previously by Kronberg *et al.* (2007) but not with the specific inhibition of the last step of the BH process suggested by Khiaosa-ard *et al.* (2009) and Vasta *et al.* (2009).

The higher content of linoleic acid due to the effect of tannins was accompanied in some cases by increases in non-conjugated 18:2 and decreases in their biohydrogenation products (*e.g.*, *trans*-9 18:1, *cis*-12 18:1 and *cis*-16 18:1; Shingfield *et al.*, 2008). A similar response occurred with other 18:1 deriving mainly from the isomerization of the oleic acid (Jenkins *et al.*, 2008).

Table 2. Content (mg/g digesta) of some *cis* 18:1 fatty acids in the ruminal digesta. Mean and standard error of the negative (TMR) and positive (TMR+sunflower oil) controls, and percentage of variation caused by each tannin treatment with respect to the positive control

	***cis*-9 18:1**	***cis*-11 18:1**	***cis*-12 18:1**	***cis*-16 18:1**
TMR	1.13±0.260	0.21±0.022	0.10±0.016	0.04±0.002
TMR+sunflower oil	2.13±0.162*	0.31±0.013*	0.22±0.007*	0.05±0.003*
+ Quebracho 2%	-1.1 ns	3.3 ns	-10.7 ns	4.6 ns
+ Quebracho 5%	6.9 ns	12.1 ns	-20.4 *	-0.5 ns
+ Grape 2%	6.6 ns	7.7 ns	-2.6 ns	-11.4 ns
+ Grape 5%	9.6 ns	12.5 ns	-16.3 *	-8.3 ns
+ Chestnut 2%	6.6 ns	4.8 ns	-30.3 *	-23.1 *
+ Chestnut 5%	26.0 *	21.5 *	-24.1 *	-21.9 *
+ Oak 2%	6.3 ns	1.4 ns	-7.5 ns	-15.7 ns
+ Oak 5%	18.0 ns	11.4 ns	-11.1 ns	-23.4 *

The level of significance of each percentage of variation (tannin treatment *vs.* positive control) appears on its right: ns= non-significant (P>0,10) and * = P<0,05.

Table 3. Content (mg/g digesta) of some *trans* 18:1 fatty acids in the ruminal digesta. Mean and standard error of the negative (TMR) and positive (TMR+sunflower oil) controls, and percentage of variation caused by each tannin treatment with respect to the positive control

	***trans*-6+7+8 18:1**	***trans*-9 18:1**	***trans*-10 18:1**	***trans*-11 18:1**	***trans*-13 18:1**
TMR	0.14±0.018	0.12±0.012	0.16±0.015	1.80±0.298	0.27±0.028
TMR+sunflower oil	0.20±0.010**	0.21±0.008**	0.34±0.021**	2.63±0.223**	0.36±0.008**
+ Quebracho 2%	-3.4 ns	-2.0 ns	0.4 ns	0.4 ns	-2.0 ns
+ Quebracho 5%	-6.7 ns	-5.6 ns	-2.5 ns	-2.9 ns	-0.9 ns
+ Grape 2%	6.4 ns	-11.5 ns	10.3 ns	-1.5 ns	-7.3 ns
+ Grape 5%	-5.2 ns	-5.6 ns	-2.6 ns	-6.2 ns	9.0 ns
+ Chestnut 2%	-16.7 **	-12.6 *	-16.3 ns	-9.6 ns	-9.8 ns
+ Chestnut 5%	-7.2 ns	-20.0 **	-10.0 ns	-6.1 ns	-11.9 ns
+ Oak 2%	-9.0 ns	-5.6 ns	-12.9 ns	-9.1 ns	-10.3 ns
+ Oak 5%	-1.5 ns	-16.9 **	-7.4 ns	-5.7 ns	-17.3 *

The level of significance of each percentage of variation (tannin treatment vs. positive control) appears on its right: ns= non-significant (P>0,10); * = P<0,10 and ** = P<0,05.

In general, the effects on the ruminal biohydrogenation of dietary polyunsaturated FA varied remarkably depending on the dose and type of tannin, which would probably be accounted for by the great diversity in the structural features, and consequently in the reactivity, of different tannins (Mueller-Harvey, 2006). Most of the significant results were observed in treatments with hydrolysable tannin extracts (chestnut at 2 and 5% and oak at 5%).

These large variations in the effect of different tannins would explain the controversy in the literature surrounding the use of these phenolic compounds to modulate the fatty acid profile of ruminant-derived products (Vasta and Luciano, 2011).

IV – Conclusion

Overall, the results of this study do not allow to recommend the treatment of a diet enriched in linoleic acid (namely, supplemented with 2% of sunflower oil) with tannin extracts when the ultimate goal is to modulate ruminal BH in order to improve the nutritional quality of sheep-derived products.

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Effect of alternate supplementary feeding on semen and sexual behavior traits of Barbarine rams

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Abstract. Alternate supplementation with energy and protein could be an interesting saving option to cut on feeding costs in low input systems. The aim of this trial was to assess the effect of alternate (every 2 days) distribution of a flushing supplement above maintenance requirement on semen and sexual behavior traits of Barbarine rams. Fifteen adult rams were allocated to three groups of five animals. Rams in C group were fed a diet composed of 1.2 kg of hay/ram/day calculated to provide the metabolisable energy for maintenance for the studied breed. Rams in D group and in addition to the basal diet, were each, daily supplemented with 0.450 kg of a concentrate containing 80% barley, 17% faba bean and 3% a mineral and vitamin supplement. Rams in A group received the same diet as D rams but the concentrate was distributed every 2 days. The feeding regimes lasted 8 weeks. Both absence of supplementation and its distribution pattern (daily or alternate) did not affect semen traits such as volume and concentration of ejaculates. However feeding at maintenance requirements negatively affected sexual behavior by impairing anogenital sniffing, flehmen, penis erection, lateral approaches, reaction time and libido score Furthermore, alternate in contrast to the daily distribution of the supplement depressed reaction time, number of lateral approaches and libido score. In conclusion, these results suggest that supplementation above maintenance has not a major effect on sperm quality but enhances sexual behavior expression. Alternate feeding could be an interesting option to costly daily supplementation during 2 months prior to the mating season.

Keywords. Alternate supplementation – Sexual behavior – Semen characteristics – Rams.

Effet d'une supplémentation par intermittence sur les caractéristiques séminales et le comportement sexuel du bélier de race Barbarine

Résumé. *La supplémentation alternée en aliment concentré pourrait être une alternative pour diminuer le coût de l'alimentation dans les systèmes à faible intrant. Le but de cette étude était d'évaluer l'effet de la supplémentation en alternance (tous les 2 jours) par des apports en énergie et protéine au-dessus des besoins de maintenance, sur les paramètres spermatiques et le comportement sexuel des béliers de race Barbarine. Quinze béliers adultes ont été répartis en trois lots de 5 béliers. Les béliers du lot C ont reçu un régime alimentaire calculé pour fournir les besoins d'entretien des béliers, il est composé de 1,2 kg/bélier/jour de foin. Les béliers du groupe D reçoivent, en plus de la même quantité de foin, 0,450 kg de concentré composé de 80% d'orge, 17% de fèverole et 3% de complément minéral et vitaminé. Les béliers du groupe A reçoivent le même régime que le groupe D mais distribué seulement un jour sur deux. L'application du traitement a duré 8 semaines. La supplémentation, quotidienne ou en alternance, n'a pas d'effet significatif sur les paramètres spermatiques tels que le volume et la concentration. Par contre, l'alimentation selon les besoins de maintenance affecte le comportement sexuel des béliers (flairage ano-génital, flehmen, érection du pénis, approche latérale, temps de réaction et score de libido). La supplémentation des béliers un jour sur deux seulement, affecte le nombre d'approches latérales, le temps de réaction et le score de libido. En conclusion, cette étude montre que la complémentation énergétique et protéique des béliers au-dessus des besoins de maintenance, quotidienne ou par intermittence, n'a pas un effet significatif sur les paramètres spermatiques mais améliore le comportement sexuel des béliers. L'alimentation alternée pourrait présenter une alternative intéressante pour diminuer le coût de la supplémentation avant la lutte.*

Mots-clés. *Alimentation par alternance – Comportement sexuel – Caractéristique spermatique – Béliers.*

I – Introduction

Drought is common in arid and semi-arid regions of the world. In the Mediterranean regions. For successful mating of farm animals under these conditions, supplementary feeding has to be targeted to achieve the optimum level of reproduction (Boukhliq and Martin, 1997). Several studies have demonstrated that underfeeding can reduce semen quality and sexual activity (Murray *et al*., 1990; Brown, 1994). Supplementation can be expensive, and the cost of feeding is an important factor influencing profitability of flocks mainly in such system. Research has shown that providing protein supplements in a less frequent pattern to ruminants may reduce costs without negatively affecting performance (Huston *et al*., 1999; Farmer *et al*., 2001; Currier *et al*., (2004). There are few data available concerning the effects of improved diet and infrequent supplementation feed on animal performances, especially with regard to effects on reproduction traits. Therefore, the aim of this study was: (i) to determine whether or not supplementation above maintenance improves semen quality and testicular size (as a direct indicator of sperm production) and sexual behaviour traits; and (ii) to assess if daily or alternate (every 2 days) distribution of the supplement affects the same traits.

II – Materials and methods

The experiment was carried out in the sheep experimental station of Bou Rebiaà of the National Institute of Agricultural Research (INRAT). The station has a semi-arid climate and is located 25 km south of the town of Tunis 36°38' N latitude, 10°07' E longitude.

Fifteen adult Barbarine rams with an average initial body weight of 61.2±7.49 kg were allocated to three groups of five animals each balanced for age and live weight. Experiment was conducted between April and June and the feeding regimes lasted 8 weeks. The rams were housed in individual pens. Rams in C group were fed a diet composed of 1.2 kg of hay/ram/day calculated to provide the metabolisable energy for maintenance for the studied breed. Rams in D group and in addition to the basal diet, were each, daily supplemented with 0.450 kg of a concentrate containing 80% barley, 17% faba bean and 3% a mineral and vitamin supplement. Rams in A group received the same diet as D rams but the concentrate was distributed on an alternate basis every 2 days. Measurements of live weight and body condition score (BCS) were performed every 15 days. Scrotal circumference and semen traits were performed every 20 days and sexual behavior traits were tested once at the end of the experimental period by exposing the rams individually to a group of ewes induced to exhibit estrus in a small enclosure for a predetermined period of time (Price *et al.*, 1987).

III – Results and discussion

Changes in Body Condition Score (BCS) are represented in Fig. 1. No statistical differences were observed between the groups during the study.

Variation of live weight and body condition scores of the rams during the experiment were similar in all groups. Live weights at the end of the experiment were (63.5±10.5; 64.6±8.5 ; 59.7±9.0 kg for respectively D, A and C rams; $p>0.05$).

As shown in Table 1, there was a tendency for SC to increase in D group in comparison to C group, however, this difference was not statistically significant between groups. Throughout the experiment, there was an increase in sperm concentration from the beginning of the trial to the end of the experiment ($p>0.05$). Relationship between SC and sperm production is not established contrary to study of Gherardi *et al.* (1980).

In general, most of the nutritional- induced changes to reproductive function in adult rams are temporary but their severity can vary from little effect on seminal characteristics and/or libido to infertility. Fertility trials were not carried out in the present study. However, fertility in the ram has

been reported as closely related to semen quality. Data obtained in the present study indicated that semen traits (quality) were in general favourably comparable to traits observed in breeds of sheep from temperate climates. Despite the differences recorded for the testicular characteristic, no significant differences were recorded between the 3 nutritional groups regarding the different quantitative and qualitative semen parameters evaluated, namely semen volume, sperm cell concentration, overall motility and individual Motility. Our results are in agreement with those obtained by Bielli *et al. (*1999) and Lindsay *et al.* (1984), who found no significant effect between improved pasture or high dietary protein on testicular dimensions. Although these results are inconsistent with those obtained by Fernandez *et al.* (2004) and Boukhliq *et al.* (1997) or described by Brown (1994) that when rams are not in good body condition, supplementary feeding in the 2 months prior to joining may improve their reproductive performance. However, as the spermatogenic cycle in rams takes about 50 days, an effect on sperm quality could have been expected given a longer treatment period.

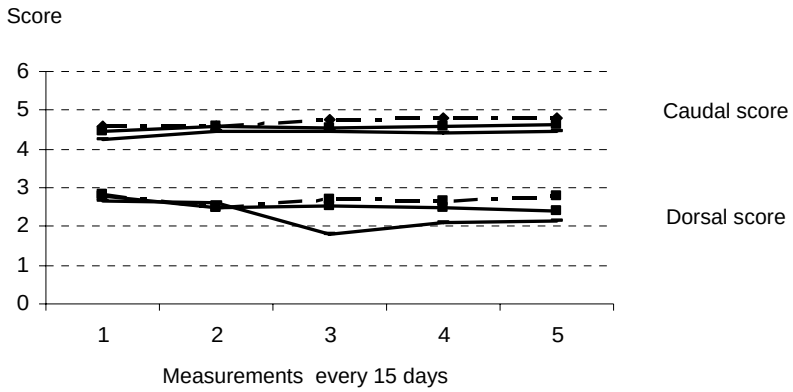

Fig. 1. Changes in Body Condition Score of Rams subjected to daily or alternate supplementation.

Table 1. Effect of regimes on scrotal circumference (SC), ejaculate volume (EV) sperm concentration (SC), Mass Motility (MM) and Individual Motility (IM), Mean (± SEM)

	SC (cm)		EV (ml)		CC (×10^9)		MM		IM	
	Initial	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**
D	27.6 ±1.51^a	28.6 ±2.07	1.15 ±0.25	0.55 ±0.21	5.6 ±2.19	6.22 ± 1.4	3.50 ±0.58	3.5 ±0.64	1.63 ±1.11a	2.85 ±0.57
A	30.4 ±1.51^b	30.4 ±2.41	0.86 ±0.56	0.57 ±0.41	4.63 ± 1.13	6.54 ± 0.90	4.41 ±0.14	4.15 ±0.48	2.75 ±1.39^{ab}	3.19 ±0.55
C	30.2 ±2.28^b	28.6 ±2.07	0.80 ±0.45	0.64 ±0.22	5.27 ±0.4	7.81 ±1.21	4.05 ±0.37	4.75 ±0.31	3.25 ±0.61^b	3.5 ±0.31

Means with different superscripts (a, b) within each column are significantly different (P<0.05).

According to James (1968) semen production appears to be related to live weight and in our study the diets had no effect on body weight which may explain the absence of any observed effect in sperm parameters. As described by many authors, the evidence suggests that when rams are not in good body condition, supplementary feeding in the 2 months prior to joining may improve their reproductive performance (Brown, 1994).

The sexual behaviour in terms of genital sniffing, flehmen reaction intensity, reaction time,

lateral approaches and libido score were positively affected (P<0.05) by supplementation. Therefore the nutrients' supplies provided by concentrate improved sexual behavior traits. These results are not consistent with those reported by Al-Hobby *et al.* (1999) who showed that protein supplementation did not influence sexual activity in Awassi rams. Similarly Fernàndez *et al.* (2004) showed that Assaf rams treated by different levels of protein had similar sexual behavior during the mating season.

Table 2. Effect of supplementation and alternate feeding on sexual behavioural traits

Groups	Genital sniffing	Flehmen reaction intensity	Erection of penis	Reaction time (s)	Lateral approaches	Libido score
D	1.8^{a}	1.6^{a}	2^{a}	6.4^{a}	2.8^{a}	7^{a}
A	1.6^{a}	1.6^{a}	2^{a}	11.2^{b}	2^{b}	4^{b}
C	0.6^{b}	0.6^{b}	0.8^{b}	18.6^{c}	1.2^{c}	1.4^{c}

Means with different superscripts (a, b,c) within each column are significantly different (P<0.05).

The other important result revealed by this work is that the frequency of supplementation is important for sexual behavior since alternate distribution of concentrate slightly depressed the libido score and increased the reaction time when compared to daily distribution of the supplement.

IV – Conclusion

In conclusion, these results suggest that supplementation above maintenance requirements has not a major effect on sperm quantitative and qualitative traits of Barbarine rams raised in a semi arid environment. This finding stresses once again the good reproductive ability of rams of this breed. Nevertheless, supplementation improved expression of sexual behavior and this can be related tot eh overall mating ability of the rams under field conditions. Alternate distribution of the supplement did not affect sperm quality and quantity and despite a slight effect on some behaviour traits, it remains an interesting option to cut on the cost of feed in low input systems of semi arid Tunisia.

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Effects of essential oils from *Rosmarinus officinalis* and *Thymus capitatus* on *in vitro* rumen fermentation in sheep

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Abstract. This study aimed to evaluate the effects of increasing doses (0, 5, 10, 20 and 40µl/50 ml of buffered rumen fluid) of essential oils (EO) extracted by steam distillation from the leaves and twigs of rosemary (*Rosmarinus officinalis*) and thyme (*Thymus capitatus*)on *in vitro*gas production and the fermentation characteristics. Doses of EO were added to 500 mg of a diet composed of 50% of rye-grass hay and 50% of concentrate on dry matter (DM) basis. The medium of incubation consisted on ruminal liquid extracted from 2 cannulated sheep, mixed in equal proportions with a buffer solution introduced in 100 ml glass syringes (39°C). At 24 h of incubation gas production was measured and part of the liquid was collected for analysis of ammonia nitrogen (NH_3-N). Volatile fatty acid (VFA) concentration was calculated on the basis of total gas production. Rosemary EO had no effects, neither on total gas production nor VFA accumulation. However, at 24 h of incubation, rosemary EO increasedNH_3-N concentration ($P<0.001$) when administered at the doses of 20 and 40 µl. Thyme EO decreased ($P<0.0001$) gas production starting from 10 µl dose. An increase ($P<0.001$) of NH_3-N concentration was recorded from the low dose of thyme EO, while the medium and high doses declined ($P<0.001$) NH_3-N concentration. VFA concentration decreased linearly from 5 µl to 40 µl doses of thyme EO. Medium doses from thyme EO might reduce carbohydrate fermentation and protein deamination. It was concluded that the EO from thyme have a more drastic anti-bacterial effect on rumen fermentation than those from rosemary.

Keywords. Essential oil – Rosemary – Thyme – Fermentation – Sheep.

Effets des huiles essentielles de* Rosmarinus officinalis *et* Thymus capitatus *sur les fermentations ruminales* in vitro *chez le mouton

Résumé. *Cette étude avait pour but d'évaluer les effets de doses croissantes (0, 5, 10, 20 et 40 µl/50 ml de fluide du rumen) d'huiles essentielles (HE) extraites par distillation à la vapeur à partir des feuilles et brindilles de romarin (*Rosmarinus officinalis*) et de thym (*Thymus capitatus*) sur la production de gaz et les caractéristiques de fermentation* in vitro*. Les doses d'HE ont été ajoutées à 500 mg d'un régime composé de 50% de foin d'avoine et 50% de concentré sur la base de la matière sèche (MS). Le milieu d'incubation est composé du liquide ruminal extrait à partir de 2 moutons canulés, mélangé dans des proportions égales à une solution tampon dans des seringues en verre de 100 ml (39°C). À 24 h d'incubation, la production de gaz est mesurée et une partie du liquide a été conservée pour l'analyse de l'azote ammoniacal ($N\text{-}NH_3$). La concentration en acide gras volatil (VFA) a été calculée sur la base de la production totale de gaz. Les HE de romarin n'ont eu aucun effet, ni sur la production totale de gaz ni sur la concentration des AGV. Cependant, à 24 h d'incubation une augmentation de la concentration de $N\text{-}NH_3$ ($P<0,001$) a été notée aux doses de 20 et 40 µl d'HE de romarin. Les HE du thym ont réduit ($P<0,0001$) la production de gaz à partir de la dose de 10 µl. Une augmentation de la concentration en N-NH3 a été notée à partir de la faible dose d'HE de thym, alors que les doses intermédiaires et élevées ont réduit ($P<0,001$) la concentration en $N\text{-}NH_3$. La concentration en AGV a diminué linéairement entre les doses de 5 et 40 µl d'HE de thym. Les doses intermédiaires d'HE du thym auraient réduit la fermentation des carbohydrates et la désamination des protéines. Il est à conclure que les HE du thym auraient un effet antimicrobien plus intense sur les fermentations que celles du romarin.*

Mots-clés. *Huiles essentielles – Romarin – Thym – Fermentation – Moutons.*

I – Introduction

Antibiotics are frequently administrated to animals in order to prevent metabolic diseases and troubles and to improve feeds valorisation as well as animal performances. However, during the last years, the use of these substances as an improving growth factor is more and more contested because of emergence of antimicrobial resistance in humans, leading to their ban in European Union since 2006 (Regulation 1831/2003/EC). Consequently considerable efforts were deployed to develop alternatives to substitute antibiotics. Among these alternatives, essential oils (EO) are used as feed additives to improve growth performances of ruminants raised under intensive conditions. These compounds consist of complex mixtures of various aromatic and volatile substances extracted from various plant materials mainly by steam distillation. The antimicrobial effect of EO is mainly related to their capacity to modify the permeability of microbial cells (Conner, 1993; Helander *et al.*, 1998). Consequently, these additives were used at low doses, to modify the fermentation trends in the rumen because of their toxicity for some unfavourable bacterial species in the rumen, such as methanogens (Wallace, 2004). In the current study we aimed to determine the effect of increasing doses of EO from frequent pasture species in the central region of Tunisia (*Thymus capitatus* and *Rosmarinus officinalis)* on *in vitro* rumen fermentation parameters of moderate beef cattle diet.

II – Materials and methods

1. Plant material

Thymus capitatus and *Rosmarinus officinalis* leaves and twigs were collected from the region of Kairouan (central region of Tunisia, arid). Samples from each species were taken from different places and mixed to make an overall sample. Dry matter (DM) was determined at 105°C in a forced-air oven. A subsample was dried at 40°C during 48h and then ground to pass through 1 mm screen and stored for chemical analysis and *in vitro* assays. Fresh samples were stored at –20°C for essential oil extraction using steam distillation methods.

2. Diets, animals and measurements

A composed diet (D: 50% of ray-grass hay and 50% of commercial concentrate) was used to determine the effect of growing doses (0, 10, 20, 40, 80 and 120µl) of essential oil from *Rosmarinus officinalis* and *Thymus capitatus* on *in vitro* rumen fermentation parameters. Diet mixture was made of ground feeds using a mixer.

Two adult local sheep ("Noire de Thibar" breed) with rumen cannula (average age and live weight: 24 months and 48.5 kg, respectively) were used for *in vitro* determinations. They received twice per day a ration (70 g kg-1LW0.75) composed of 70% of oat-vetch hay and 30% of barely concentrate on dry matter (DM) basis. The medium of incubation consisted on ruminal liquid, mixed in equal proportions with a buffer solution introduced in 100 ml glass syringes (39°C). Each dose of EO was dissolved in 200 µl of methanol and added immediately before incubation to 500 mg of experimental diet. Incubation lasted 24h, then gas production and pH were measured and fluid samples were taken for ammonia-N (NH_3-N). Each dose of EO was incubated in triplicate through two successive incubations.

3. Chemical analysis

Feeds were analyzed for dry matter (DM), ash and crude protein (CP) contents (AOAC, 1984). Cell wall fractions (NDF, ADF and ADL) in feeds were determined as described by Van Soest *et al*. (1991).Short Chain Fatty Acids (SCFA) at 24 h of incubation calculated as:

SCFA (mM/syringe) = 0.0239 GP – 0.0601 (Getachew *et al.*, 2000)

4. Statistical analysis

The General Linear Model procedure (GLM) of SAS (1996) with the option of LSMEANS multiple ranges was used to analyze data. The model included effects of dose and incubation.

III – Results and discussion

Chemical composition of feeds is presented in Table 1. Chemical composition of *Thymus capitatus* is relatively equivalent to hay except for total wall content (NDF): Lignocellulose fractions (ADF) were equivalent between the two species but the lignification level of *Thymus capitatus* was higher compared to *Rosmarinus officinalis*. Extracted EO content was higher in *Rosmarinus officinalis* than in *Thymus capitatus*.

Table1. Chemical composition of feeds (g/kg DM)

Feeds	Ash	CP	NDF	ADF	ADL	EO
Ray-grass hay	112	99	689	363	53	-
Concentrate	57	169	342	53	-	-
Thymus capitatus	102	88	409	326	226	2.5
Rosmarinus officinalis	62	58	389	301	167	4.3

Gas production, NH_3-N and SCFA concentrations are reported in Table 2. Rosemary EO had no effects, neither on total gas production nor short chains of VFA accumulation. However, at 24 h of incubation, rosemary EO were associated to an increase of NH_3-N concentration ($P<0.001$) observed at the doses of 20 and 40 µl. This result confirmed the findings of Noirot *et al.* (2007), who classified *Rosmarinus officinalis*, according to its main component (α-pinen), as the lowest bactericide compounds within EO. The authors suggested that component may be responsible of the deamination and /or of the limitation of N bacterial uptake in culture medium.

Table 2. Effects of increasing doses of EO from *Rosmarinus officinalis* on gas production and NH_3-N and SCFA concentrations

Dose (µl/50 ml)	0	5	10	20	40	SEM
Gas 24 (ml)	114.1	100	108.5	112.6	110.2	2.02
NH_3-N (mg/l)***	116^{b}	117^{b}	118.6^{b}	127^{ab}	138^{a}	1.82
SCFA (mM/50ml)	53.3	46.6	50.7	52.6	51.5	1.34

a,b. Values with different letters in the same line are statistically different, *** $P<0.001$, SEM: Standard error of the mean.

Table 3 reports the gas production and NH_3-N and SCFA concentrations generated from the administration of increasing doses of EO from *Thymus capitatus*. Thyme EO decreased ($P<0.0001$) gas production starting from 10 µl dose. An increase ($P<0.001$) of NH_3-N concentration was recorded from the low dose of thyme EO, while the medium and high doses declined ($P<0.001$) NH_3-N concentration. VFA concentration decreased linearly from 5 to 40 µl doses of thyme EO. This is consistent with the results found by Macheboeuf *et al.* (2007) who studied the effect of carvacrol, which we identified as carvacrol chemotype (Moujahed *et al.*, 2011) on *in vitro* fermentation parameters and noted that changes of pH values followed a growing tendency with increased doses. The effect of growing doses of thyme EO on NH_3-N concentration followed a decreasing trend. This result confirmed those observed by

Macheboeuf *et al*. (2007) and Castillejos *et al*. (2007) who studied the effect of several doses of essential oil of *Thymus capitatus* and *Thymus vulgaris* respectively, on in vitro NH_3-N concentration.

Table 3. Effects of increasing doses of EO from *Thymus capitatus* on gas production and, NH_3-N and SCFA concentrations

Dose (µl/50ml)	0	5	10	20	40	ESM
Gas 24 (ml)***	100 [a]	88.9 [b]	67.6 [c]	54 [d]	41[e]	2.03
NH_3-N (g/ml)***	91.5 [b]	110 [a]	110.3 [a]	67.6 [c]	73 [c]	1.31
SCFA (mM/50 ml)***	46.6[a]	41.3[a]	31.2[b]	24.6[c]	18.4[d]	1.01

a,b,c. Values with different letters in the same line are statistically different, *** P<0.001, SEM: Standard error of the mean.

IV – Conclusions

The EO from thyme have a more drastic anti-bacterial effect on rumen fermentation than those from rosemary. *In vivo* trials are currently carried out to investigate the effect of EO on intake, digestion and performances and to study the form of adding EO in diets of sheep.

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Sheep management in the eastern Moroccan area: Livestock breeders' practices and effect of ewes' supplementation on lamb performances

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Abstract. The study was conducted in the eastern Moroccan area and aimed to analyze and describe sheep feeding practices and reproductive performance in the region, and to study the effect of supplementary feeding under extensive system on lamb performances before weaning (at 80 days of age) and after (until 30 kg live-weight). The first objective was carried out by interviews with farmers and the second objective was carried out through an experiment using two treatments: (i) T1-lambs whose dams grazed and receive supplementation during the late gestation and early lactation periods; (ii) T2-lambs whose dams grazed without supplementation. Results obtained from livestock farmers interviews (objective 1) showed that 75% of livestock holders prepare the fattening lambs for *Eid El Adha*. The fattening period is 3.7 months (varying between 2-5 months) and the fattening diet is characterized by the use of mixture of barley and wheat bran (97.5%) used alone (62.5%) or mixed with other concentrates (35%). The reproductive management is characterized by natural mating, early age at lambing, and late weaning. Results obtained from the experiment (objective 2) showed that supplementary feeding had a positive effect on the growth and fattening performances. The weaning weight (at 80 days) of T1 was higher than T2 (20 *vs* 15 kg, respectively). Comparing between the ewe's supplementary feeding cost and the obtained average daily gain, we conclude that the supplementation is recommended for its economic and environmental benefits. Results of the current study recommended the use of supplementary feeding for ewes during late gestation and early lactation to improve lamb's growth and fattening performances and to maintain pasture condition and avoid overgrazing.

Keywords. Eastern Morocco – Supplementation – Sheep – Fattening – Growth – Livestock breeders' practices.

Conduite des élevages ovins dans la zone du Maroc oriental : Pratiques des éleveurs et effet de la supplémentation des brebis sur les performances des agneaux

Résumé. *Les objectifs de ce travail mené dans la zone du Maroc oriental étaient : 1. Analyser et décrire la conduite alimentaire et de reproduction dans la zone, et 2. Etudier l'effet de la supplémentation des brebis, conduites sur parcours, sur les performances de croissance (de la naissance jusqu'à 80 jours) et d'engraissement (du sevrage au poids de 30 kg) de leurs agneaux. Le premier objectif est atteint en réalisant des enquêtes auprès des éleveurs de la zone et le deuxième en conduisant un essai constitué de 2 traitements: T1: agneaux issus des brebis ayant reçu une supplémentation durant la fin de gestation et début de lactation (pâturage et supplémentation quotidienne) et T2 : agneaux issus des brebis qui n'ont reçu aucune supplémentation (pâturage seul). Les résultats obtenus à travers les entretiens auprès des agriculteurs ont montré que 75% des éleveurs pratiquent l'engraissement pour la fête de Aid El Adha, la durée d'engraissement est de 3,7 mois (2-5 mois), les rations d'engraissement sont caractérisées par l'utilisation du mélange orge-son (97,5%) seul (62,5%) ou mélangé avec d'autres aliments concentrés (35%). La conduite de reproduction est caractérisée par la lutte libre, l'âge précoce des antenaises au premier agnelage et le sevrage tardif. Les résultats obtenus lors de l'essai (objectif 2) ont montré que la supplémentation avait un effet positif sur les performances de croissance et d'engraissement. Le poids au sevrage (à 80 jours) de T1 a été supérieur à T2 (20* vs *15 kg). En comparant le coût de supplémentation des brebis, durant la fin de gestation et début de lactation, et le GMQ obtenu on conclut que la supplémentation est rentable du point de vue économique et environnemental. Ces résultats recommandent la supplémentation des brebis en fin de gestation et début de lactation pour améliorer les performances de croissance et d'engraissement des agneaux.*

Mots-clés. *Maroc oriental – Supplémentation – Ovin – Engraissement – Croissance – Pratiques des éleveurs.*

I – Introduction

Sheep farming plays an important socio-economical role in Morocco. It provides about 40% of the red meat production. It has expanded to all the country and is considered as one of the main sources of income to farmers. It allows the exploitation of pasture and marginal zones. The eastern Moroccan region is mainly dominated by extensive sheep production system. It contains vast rangelands around 5 million hectares, and about 2 million sheep heads (USAID, 2006). Rangelands and livestock ecosystem has an important environmental and socioeconomically role as a source of income for rural populations in this region. However, sheep production in the region faces many constraints such as rainfall irregularity, scarcity and shortage of feed resources, and also inadequate health management. This inappropriate management is considered an important obstacle to improve the productivity performance of sheep and the farmer's income. The objective of this work is to analyze and describe sheep feeding practices and reproductive performance in the region and to study the effect of supplementary feeding on the lambs' growth and performances.

II – Materials and methods

This study was conducted in the eastern Moroccan area. It consisted of two parts: the first one is related to the description and analysis of sheep feeding practices and reproductive performance, and the second one investigated the effect of supplementary feeding on lambs' growth and fattening performances. To achieve the first objective, forty interviews were done with farmers who are selected based on their farm activity and level of collaboration. The questionnaire covered aspects related to farm structure (herd and agricultural area), and sheep reproductive performance and feeding practices. For the second objective, 92 ewes were used and divided into 2 groups, grazing with (T1) or without (T2) concentrate supplement (UFL: 0.9, CP: 14%). Seventy-two ewes were employed in T1 and supplemented during the late pregnancy (0.4 kg/ewe/day) and early lactation (0.5 kg/ewe/day) periods, while other twenty ewes were assigned as a control group (T2) without supplement. Before starting the experiment, ewes and then lambs (before fattening) were treated against internal parasites and vaccinated against enterotoxemia.

During the early lactation phase, the grazing forage resources were scarce and ewes of both treatments were supplemented with alfalfa hay (UFL: 0.6, CP: 17%, and 0.5 kg/ewe/day) to cover the shortage of forage in the pasture. After birth, lambs were weighed directly at birth, and at 10, 30 and 80 days (weaning time). In addition, the average daily gain was calculated from 10 to 30 days (ADG 10-30), and from 30 to 80 days (ADG 30-80). Milk was the only feed for lambs during the first six weeks after birth and alfalfa hay was offered afterwards and continued till weaning. After weaning, lambs were employed in a fattening trail for 42-day period or 30 kg of live-weight. All lambs were weighed at 21 days interval and receiving a diet containing alfalfa hay and a starter compound feed (DM: 88%, UFV: 0.9, CP: 16%) during 25 days and a finishing compound feed (DM: 88%, UFV: 0.9, CP: 14%) during 17 days. The diet was administrated twice daily. Free clean water was available all time.

The effect of supplementary feeding was tested through the difference between the average gain for T1 and those for T2 lambs. The cost of kg live-weight gain resulting from the supplementary feeding effect is calculated as the cost of supplementation (Moroccan Dirham: MAD; 1 USD = 8.4 MAD) divided by the average gain. The effect of supplementary feeding for ewes on lambs' growth performance (weaning weight or initial fattening weight is used as

covariable) was analysed by means of ANOVA using the PROC GLM procedure of the SAS statistical package (version 8.01).

III – Results and discussion

1. Livestock farmers' practices

Average parameters describing the sheep farmers interviewed are summarized in Table 1. Sheep production is the main activity of farmers. The fattening lamb was designed by farmers to be the main source of income. Farmers are using the agriculture and livestock together to meet their needs for livelihood level.

The average size of useful arable area (UAA) is 57 ha. The results analysis showed a positive correlation (R^2 = 0.7) between the UAA and the number of sheep and fattening lambs. This correlation is explained by the dependence of sheep to feedstuffs produced in the farm.

The number of sheep per farm ranged from 100 to over 600 with an average of 358 heads. The difference of sheep number among farmers gave an idea to different managements, in terms of fattening and feeding and reproductive management. The number of fattening lambs per year is 160 head as average. Farmers have reported that the number of fattening animals is variable and depending mainly on the environmental conditions, such as raining, available feed resources, etc.

Table 1. Livestock breeders characteristics in the eastern Moroccan region (n=40)

Parameters	Average
Sheep number/farm	358
Useful arable area (ha)/farm	57
Fattened lambs (number/farm)	160
Sex ratio	25
Fertility rate (%)	86.5
Level of prolificacy (number of lambs born/ewes lambed)	1.01
Age at 1st lambing (months)	13
Concentrates-feed/lamb at beginning of fattening (kg/head/day)	0.6
Concentrates-feed/lamb at the end of fattening (kg/head/day)	1.5

Fattening concerns mainly male lambs (80% of fattened animals). The inquisitions reported that the young male sheep within 7-8 months are the most employed for fattening (77.5%), while those having 3-6 months and those more than one year are represent almost 15 and 7.5% of fattening trails, respectively. Most farmers prepared the fattening lambs to be ready for *Eid El Adha.* The fattening period varies from 2 to 5 months with an average of 3.7 months. It depends on drought and dry season and the cost of feedstuffs.

Feeding the fattening lambs is based on grazing and concentrate supplementation. The supplement is mainly consisted of mixture from barley and wheat bran (97.5 %) and used alone (62.5%) or mixed with others concentrates (35%) (maize, dried beet pulp, or compound feed). The fattening diet is administered twice daily. The amount of concentrate distributed at the beginning and at the end of fattening was 0.6 kg/lamb/day and 1.5 kg/lamb/day, respectively.

The energetic supply from concentrate at the beginning of fattening is about 0.5 UFV/kg DM of the supplement. This value is lower than requirements for fattening lambs and leading, therefore, to decrease in lamb growth rate. In contrast, at the end of fattening, the amount used

is much higher than lamb requirements (Jarrige, 1988). Live-weight at the beginning of fattening averages 30 Kg, and at the end of fattening varies from 45 to more than 50 kg.

Reproductive performance: There was an important lack of information on reproduction data in farms. No management mating is implemented. All interviewed farmers practice natural mating, in which males remain permanently with females all year. This leads to a random distribution of lambing and also early mating for young females at approximately 7 to 8 months of age. The average age at first lambing is 13 months. However, most of lambings occur during November and December. The sex ratio adopted is on average 25 ewes per ram with a variation of 15 to 30. The average of fertility rate was 86.5% and level of prolificacy (number of lambs born/ewe lambed) was 1.01.

2. Effect of ewes' supplementation on the growth and fattening performances of lambs

Growth and lamb performances are given in Table 2. Birth weight of lambs born from ewe given supplementation was significantly (P<0.05) higher than those born from not supplemented ewes (4.0 *vs* 3.4 kg for T1 and T2, respectively). This superiority of T1-lambs birth weight is due to the positive effect of ewe's supplementary feeding in late pregnancy (Robinson, 1985).

During the growing period (birth to weaning), lambs in T1 had greater (P <0.05) BW (at 0, 10, and 30 days, and at weaning) and higher ADG (10-30, and 30-weaning) than lambs in T2. At weaning, the difference in live weight between lambs in T1 than those in T2 was almost 5 kg. The marked increase in average gain is attributable to the positive effect of ewes' supplementation during late pregnancy and early lactation that resulted in more milk yield being consumed by the offspring (Muñoz *et al.*, 2008; Robinson, 1985).

Table 2. Effect of ewes' supplementation on growth and fattening performances of their lambs

	Treatment		Significance
	T1-lambs(n=72)	T2-lambs (n=20)	
Growth performances			
Birth weight (kg)	4.0[a]	3.4[b]	<0.0001
Weight at 10 days (kg)	5.9[a]	4.5[b]	<0.0001
Weight at 30 days (kg)	9.7[a]	7.5[b]	<0.0001
Weaning weight (kg)	20.0[a]	15.0[b]	<0.0001
ADG10-30 (g/day)†	191[a]	153[b]	<0.0001
ADG30-80 (g/day) †	207[a]	151[b]	<0.0001
Fattening performances			
Weight at 21 days post-weaning (kg)	24.9[a]	19.3[b]	0.0457
Weight at 42 days post-weaning (kg)	30.7	25.0	0.6858
Fattening ADG0-21 (g/day) †	233[a]	200[b]	0.0011
Fattening ADG21-42 (g/day) †	277[a]	271[b]	0.0457

†ADG: Average daily gain. [a, b] Means with different superscripts are significantly different (P< 0.05).

During the finishing phase (fattening, 42 days), the objective was to produce lambs with live weight of 30Kg. The initial weight at the beginning of fattening was 20.0 and 15.0 kg for lambs in T1 and T2 groups respectively. This initial weight was used as co-variable in the statistical analysis. After 42 days post weaning, the live-weight was 30.7 and 25 kg for lambs in T1 and T2, respectively. The ewes' supplementation didn't have effect on the live-weight at 42 days

(P=0.6858), in contrast, it had a significant effect on live-weight at 21 days post-weaning and ADG21-42 (P=0.0457).

At the end of fattening, lambs were sold at price of 30 MAD/kg LW. In addition, the kg of LW gained by supplementation was 23.6 MAD (Table 3). Considering this price, the supplementation of ewes during late pregnancy and early lactation presented an economical interest.

Table 3. Production cost of kg lamb live-weight generated by ewes' supplementation

	ADG† of lambs (g)	ADG generated by supplementation (g)	Supplementation cost†† (MAD/day)	Production cost of 1 kg of LW generated by supplementation (MAD/Kg LW)
T1-lambs	200	55	1.3	23.6
T2-lambs	145			

†ADG from birth to weaning
††Concentrate price: 3 MAD/kg, selling price of lamb: 30 MAD/1kg LW, MAD: Moroccan dirham, 1 USD=8.4 MAD

IV – Conclusions

Fattening lamb is considered as the main activity for livestock farmers. In addition, animal nutrition is based on grazing and the supplementary feeding is mainly used for growing and fattening lambs. The lack of organized management negatively affects the reproductive performance in the area.

The ewes' supplementation had a positive effect on the offspring's performance mainly during the pre-weaning phase. Supplementation may allow ewes to recover the weight loss during gestation and lactation, and meet their nutrients requirements. Moreover, the late pregnancy and lactation periods coincide with the forage shortage on pastures. Therefore, ewes' supplementation may maintain the sustainable management of pastoral resources and avoid overgrazing and desertification.

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Session 2
Local feeding resources based systems

Strategies to manipulate rumen fermentation for better utilizing feedstuffs in goats

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Abstract. The development and implementation of strategies aiming to optimize rumen fermentation is key to improve feedstuffs utilization and then to optimize ruminant production. Of special importance are strategies that may be applied to unconventional feedstuffs. The main challenges nowadays of ruminant production are to reduce feeding costs, improve products quality and diminish the impact of production on environment. Strategies have to be developed to achieve these objectives. Strategies based on the use of unconventional feedstuffs like by-products and wastes may contribute to both decreased feeding cost and environmental impact of ruminant production thought both recycling by-products and decreasing methane emissions and, improved products quality (i.e. fatty acid profile in milk). Microbial protein synthesis, methanogenesis and biohydrogenation are key processes in ruminal fermentation. The development of efficient strategies is, in a great extent, based on the knowledge of those processes and rumen microorganisms involved. Some strategies based on both the use of feed blocks including by-products and wastes or additives are considered and their effects on microbial protein synthesis, methane emissions and products quality in goats described. Feed blocks including olive cake or waste fruits from greenhouse horticulture have been used to partially replace cereals-based concentrates in goats diet and the effects on feeding cost, ruminal fermentation, microbial protein synthesis, methanogenesis and milk yield and composition were studied. Changes in the abundances of total bacteria and methanogenics archaea were studied as well. A wide range of plant extracts and secondary compounds have been used as additives to manipulate the fermentation in the rumen to both reduce protein degradability and minimize methane emissions. The main challenge is to confirm in vivo the potential that in vitro trials have shown.

Keywords. Dietary strategies – Feedstuffs utilization – Rumen fermentation – Microbiota – Methane – Milk-Goat.

Stratégies pour manipuler les fermentations du rumen vers une utilisation optimale des aliments

Resumé. *Le développement de stratégies pour optimiser la fermentation du rumen est essentiel pour améliorer l'utilisation des aliments et pour optimiser la production des ruminants. Les stratégies appliquées aux aliments non conventionnels comme les sous-produits et les déchets sont particulièrement importantes. Les principaux objectifs de la production des ruminants sont de réduire le coût d'alimentation, d'améliorer la qualité des produits et de diminuer l'impact de la production sur l'environnement. Les aliments non conventionnels peuvent contribuer à diminuer le coût d'alimentation et l'impact environnemental et améliorer la qualité des produits (profil d'acides gras dans le lait par exemple). La synthèse des protéines microbiennes, la méthanogénèse et la biohydrogénation sont des facteurs clés des fermentations ruminales. Des stratégies pour maximiser la protéine microbienne, minimiser la production de méthane et optimiser la biohydrogénation sont présentées. Le développement de stratégies efficientes est basé en grande partie sur la connaissance du macrobiote ruminal. Certaines stratégies basées sur l'utilisation de blocs multinutriments, y compris les sous-produits et déchets agro-industriels, ou additifs ainsi que leurs effets sur la protéine microbienne, la production de méthane et la qualité du lait chez les chèvres seront décrites. Des blocs incluant des grignons d'olive ou déchets des fruits de l'horticulture sous serre ont été utilisés pour remplacer partiellement des concentrés à base de céréales dans les régimes des chèvres en lactation. Leurs effets sur le coût du régime, la fermentation ruminale, la synthèse de protéine microbienne, les productions de méthane et de lait ainsi que la composition du lait ont été étudiés. Les changements de l'abondance des bactéries totales et d'archaea méthanogènes ont été aussi étudiés. Une large gamme d'extraits de plantes et de composés secondaires ont été utilisés comme additifs pour manipuler la fermentation dans le rumen et la dégradabilité des protéines et à la fois réduire et minimiser les émissions de méthane. Le principal défi est de confirmer* in vivo *le potentiel que les essais* in vitro *ont montré.*

Mots-clés. *Stratégies nutritionnelles – Utilisation des aliments – Fermentations du rumen – Microbiote – Méthane – Lait – Chèvre.*

I – Introduction

The development and implementation of strategies aiming to optimize rumen fermentation is key to improve feedstuffs utilization and then to optimize ruminant production. Of special importance are strategies that may be applied to unconventional feedstuffs. The main challenges nowadays in ruminant production are to reduce feeding costs, improve products quality and diminish the impact of production on environment. The use of unconventional feedstuffs may contribute to decrease feeding cost and environmental impact through reduced methane emissions. In addition, improving products quality (i.e. fatty acid profile in milk) has been also shown potentially achievable by using such ingredients in the diet (Vasta *et al.*, 2008). Microbial protein synthesis, methanogenesis and biohydrogenation are key factors in ruminal fermentation. A better knowledge of those processes, driving factors and microorganisms involved and their mechanisms of action may help to develop efficient strategies.

In this work we present some results obtained in our group on the effects of using feed blocks for optimizing the use of agricultural by-products/wastes and also additives (i.e. essential oils, organosulphur compounds and other antimethanogenic chemicals) on rumen function, nutrient utilization and milk yield and composition in goats. Nowadays there is an increased interest on the development of dietary strategies that could reduce methane emissions by ruminants (Martín *et al.*, 2010). Methane emission from the enteric fermentation is of concern worldwide due to its potential as greenhouse gas (Wright and Klieve, 2011). Additionally methane represents an energy loss of around 2 to 12% of the gross energy supply for the animal (Johnson and Johnson, 1995; Hook *et al.*, 2010). Research has been focused on feed additives or diet ingredients that could reduce the formation of saturated FA and increase the concentration of unsaturated FA in ruminant products. As far as we know, few studies have investigated the effect of strategies based on the use of by-products on milk FA composition (Molina-Alcaide *et al.*, 2010; Modaresi *et al*., 2011) and methane emissions by ruminants at the same time.

II – Strategies based on the inclusion of by-products and wastes in feed blocks

Livestock production in Mediterranean countries is constrained by the scarcity of pastures and high prices of feedstuffs such as cereals, the base of most of the concentrates. Feed blocks (FB) based on local resources and by-products (Ben Salem and Nefzaoui, 2003) might be used to overcome this situation. Because olive trees and the derived industries are of great importance in Mediterranean countries, their by-products are of economic and environmental interest. The most important by-product is the crude 2-stage olive cake (CTSOC), composed of olive pulp, skin, and stones as well as water (Hermoso *et al.,* 1995). Production of CTSOC accounts for 2,000,000 t/yr (Molina-Alcaide and Yáñez-Ruiz, 2008) with high pollutant potential, in part because of its high moisture content. Greenhouse horticulture account for 15% of world horticulture production in the Mediterranean area, Spain being the main producer (MARM, 2009). This intensive agriculture generates large amounts of fruit wastes, mainly tomato and cucumber, which have to be stored with economic and environmental troubles. Increased in cereal prices (43% from 2008 to 2011; FAO, 2011) has driven the attention of ruminant nutritionists toward local alternative feedstuffs (Ben Salem, 2010; Molina-Alcaide *et al.,* 2010) in order to reduce production cost. To our knowledge, the use of tomato by-products for ruminant feeding has been explored (Ben Salem and Znaidi, 2008) but no information is available

regarding the inclusion of cucumber in ruminant diets. Either 2-stage crude olive cake (COC) and wastes of tomato (T) and cucumber (C) fruits have been included in feed blocks to state their respective potential to replace concentrate in diets for both unproductive and lactating goats.

1. In vivo trials with unproductive goats

A. Feed blocks based on COC

Six dry non-pregnant, rumen-fistulated Granadina goats (49 ± 2.2 kg of BW) were used to study the effect of three experimental diets on ruminal fermentation and microbial N flow by following a 3 x 3 Latin square design with repetition. Diets were composed (fresh matter basis) of 600 g alfalfa hay and 400 g of cereals-based concentrate (diet AC), 600 g alfalfa hay, 200 g concentrate, and 180 ± 80 g feed block including 120 g/kg of crude olive cake (diet ABCI) and, 600 g alfalfa hay, 200 g concentrate, and 291 ± 61 g feed block including 100 g/kg of crude olive cake (diet ACBII). No effect ($P \geq 0.18$) of diet was found for pH and VFA concentration (Table 1) indicating that partial replacement of concentrate with FB did not compromise the ruminal fermentation of carbohydrates. On the contrary, both microbial N flow and efficiency of microbial protein synthesis in the rumen decreased ($P \leq 0.001$) in animals fed diets including feed blocks compared with the control one. A better supply of available carbohydrates and N utilization in diet AC, compared to those including feed blocks, could occur as previously suggested by Lee *et al.* (2003) and Merry *et al*. (2006). Although the most important factors influencing microbial growth are rumen outflow and energy available to microbes, growth may also be influenced by the substrate and type of microbes growing in the rumen (Van Soest *et al.*, 1988).

Table 1. Effect of the partial replacement of concentrate with feed blocks including crude olive cake on the average values of pH, volatile fatty acid (VFA), and microbial growth and efficiency in the rumen of unproductive goats

Item	Diets†			*SEM*	*P-value*
	AC	ACBI	ACBII		
pH	6.35	6.45	6.51	0.030	0.10
VFA mmol/L	80.5	88.8	95.7	4.3	0.35
Acetate	59.1	64.1	69.1	3.2	0.45
Propionate	13.3	16.3	15.5	0.73	0.24
Isobutyrate	0.38	0.62	0.53	0.052	0.18
Butyrate	7.03	6.89	9.24	0.61	0.23
Isovalerate	0.60	0.54	0.67	0.063	0.68
Valerate	0.74	0.89	1.05	0.071	0.23
Acetate/propionate	4.44	3.93	4.46	0.23	0.38
Microbial N flow, g/d	17.0^{b}	9.82^{a}	11.3^{a}	1.05	0.001
g microbial N/kg DOM	31.4^{c}	18.2^{a}	18.0^{a}	1.4	<0.001

†AC: control diet (alfalfa hay:concentrate, 1:1); ACBI and ACBII: alfalfa hay and concentrate (1:0.5) and feed blocks including 120 and 100 g of crude olive cake/kg feed block fresh matter basis, respectively.

B. Feed blocks based on greenhouse waste fruits

Four adult dry non-pregnant rumen-fistulated Granadina goats (32 ± 5.5 kg BW) were fed four experimental diets formulated with alfalfa hay and concentrate in a 1:1 ratio (diet AC), alfalfa hay, concentrate and feed blocks (1:0.5:ad libitum) including wastes of tomato (diet ACT), cucumber (diet ACC) or barley (diet ACB). The effect of diet on ruminal fermentation, microbial N flow and efficiency of synthesis, methane emission, nutrients utilization and abundances of

total bacteria and methanogens were studied following a 4 x 4 Latin square design. The feed blocks intakes were 203 ± 73, 179 ± 40 and 144 ± 68 g/animal/d for those including wastes of tomato, cucumber and barley grain, respectively. Diets including tomato and cucumber wastes promoted (Table 2) decreased ($P < 0.001$) methane emissions (37 and 13%, respectively) in comparison with diets control and the one including barley-based FB, which showed similar ($P>0.05$) methane emissions. Average pH and ammonia concentration values in the rumen were similar ($P \geq 0.108$) independently of diet supplied to the animals. The VFA content was higher ($P = 0.001$) in goats fed diets ACT and ACC compared to AC and ACB diets. The acetate:propionate ratio decreased ($P < 0.001$) with diets including tomato wastes and barley grain the first promoting the higher decreasing. Both microbial N flow and efficiency of synthesis were modified ($P = 0.047$ and 0.044, respectively) decreasing with diet ACT and increasing with ACC diet. The partial replacement of concentrate with feed blocks modified digestibility of DM ($P = 0.022$), OM ($P = 0.021$) and fat ($P = 0.049$). Total bacteria abundance in the rumen was not affected ($P = 0.072$) by diet while methanogens abundance increased ($P = 0.015$) in animals fed diets including wastes-based FB in comparison with diet AC and ACB. Diet including tomato based feed blocks decreased MNF without affecting the efficiency of synthesis but it decreased methane emission by 33% compared to the other studied diets. This dietary approach involves the use of agricultural by-products and reduced methane emissions, which represents advantages against approaches using antibiotics or other chemicals potentially harmful to the animal or the environment. Feeding cost decreased by 10% with diets including feed blocks compared with control diet. Further research is needed to understand the mechanisms involved in the antimethanogenic effect of greenhouse waste fruits-based feed blocks and in order to improve ruminal protein yield and energy utilization, which may limit the use of this type of blocks in non-productive ruminants practical feeding (Romero-Huelva and Molina-Alcaide, 2013)

Table 2. Effects of the partial replacement of concentrate with feed blocks including wastes of tomato or cucumber and barley grain on average values for methane emissions, pH, concentrations of NH_3-N and VFA, acetate:propionate ratio, microbial N flow and efficiency of synthesis, nutrients apparent digestibility and total bacteria and methanogens abundances in unproductive goats

Item	Diet†				*SEM*	*P-value*
	AC	**ACT**	**ACC**	**ACB**		
CH_4 L / kg DMI	29.3^{c}	18.5^{a}	25.4^{b}	28.2^{c}	0.330	< 0.001
pH	6.94	7.05	7.09	7.09	0.024	0.108
NH_3-N, mg / 100 mL	16.4	15.6	16.3	16.0	0.483	0.865
Total VFA, mmol / L	66.9^{a}	80.3^{b}	82.2^{b}	67.2^{a}	2.011	0.001
Acetate:propionate	5.03^{c}	3.89^{a}	4.58^{bc}	4.12^{b}	0.036	< 0.001
Microbial N flow, g/d	16.2^{b}	13.1^{a}	18.9^{c}	15.4^{b}	1.00	0.047
EMNS, g/kg of OMAFR	24.2^{b}	21.1^{a}	30.8^{c}	24.9^{b}	1.03	0.044
Apparent digestibility (g/g)						
Dry matter	0.68^{b}	0.62^{a}	0.64^{a}	0.67^{ab}	0.003	0.022
Organic matter	0.71^{b}	0.66^{a}	0.67^{a}	0.70^{ab}	0.003	0.021
Fat	0.68^{b}	0.58^{a}	0.63^{ab}	0.55^{a}	0.014	0.049
CP	0.72	0.70	0.72	0.73	0.005	0.802
NDF	0.59	0.60	0.57	0.59	0.006	0.939
ADF	0.56	0.55	0.57	0.58	0.007	0.923
log gen copies / g fresh matter						
Bacteria	11.2	11.4	11.4	11.2	0.058	0.072
Methanogens	8.48^{a}	8.86^{b}	8.85^{b}	8.49^{a}	0.050	0.015

†AC: control diet (alfalfa hay:concentrate, 1:1); ACT and ACC and ACB: alfalfa hay and concentrate (1:0.5) and feeding blocks including tomato and cucumber fruit wastes and barley grain, respectively.

2. *In vivo* trials with lactating goats

Dairy goat farming is of relevant economic, environmental, and sociological importance in the Mediterranean basin (Rancourt *et al.*, 2006) with an increasing demand for gourmet cheeses, yogurt, and milk from sheep and goats (Haenlein, 2001). Milk and dairy products from goats are important foods for man, especially for people with food allergies or for those living in dry areas where cow's milk is scarce (Park, 2006; Sanz Ceballos *et al.*, 2009). The influence of diet on milk production may depend more on ruminal fermentation balance and end products than on the digestible or metabolizable energy content (Sanz- Sampelayo *et al.,* 1998). It is well documented that diet composition affects ruminal fermentation, and hence milk composition (Morand-Fehr *et al.,* 1991). Lipid composition is one of the most important factors determining the technological, nutritional, and health quality of goat's milk (Chilliard *et al.*, 2003).

A. Feed blocks based on COC

Eighteen Granadina goats (39.6 ± 1.89 kg of BW) in the middle of the third lactation were fed four diets made (fresh matter basis) of alfalfa hay and concentrate (1:1), alfalfa hay (diet AC), concentrate and feed block (1:0.5: ad libitum) including 120 or 100 g/kg of crude olive cake (diets ACBI and ACBII, respectively). The effect of diet on nutrients utilization, microbial growth and milk yield and composition was studied by following an experimental design 3 x 3 Latin square with 6 replications. Blocks intake resulted in 209 ± 32 and 346 ± 56 g/animal/d for diets ACBI and ACBII, respectively. The feeding cost was 36% lower with diets including feed blocks compared to the control one. Body weight of animals fed the different diets (Table 3) was similar ($P \geq 0.626$). The N intake was similar ($P = 0.201$) but milk total and protein N and the milk protein N/N balance were lower ($P = 0.012$, 0.023 and 0.050, respectively) for goats fed diets including feed blocks compared with control diet. On the contrary, retained N was higher ($P = 0.050$) for animals fed diets including FB than control diet. Regarding energy the intake and urinary energy were lower ($P = 0.016$ and 0.043, respectively) for diet ACBI in comparison with diets AC and ACBII. Milk energy was lower ($P = 0.049$) for diets including FB compared to control diet. Both microbial N flow and efficiency of synthesis were lower ($P = 0.045$ and 0.036, respectively) in goats fed diet ACBI than in those receiving diets control and ACBII. The presence of soybean meal in the concentrate, with high CP and lysine content (De Blas *et al.*, 1994) could explain the results obtained in the present work.

Milk yield (Table 4) decreased ($P = 0.016$) by 22 and 18%, respectively, with diets ACBI and ACBII, respectively, in comparison to diet AC. The milk contents in casein, whey protein, fat, lactose, total solid and gross energy did not vary ($P \geq 0.144$) with diet. However changes in milk fatty acids profile were promoted by the partial replacement of concentrate with FB: saturated fatty acids decreased ($P = 0.046$) by 3 and 7% in milk from goats fed diets ACBI and ACBII, respectively, in comparison with milk from goats receiving diet AC; mono and polyunsaturated fatty acids content in milk was not ($P \geq 0.164$) modified by diet; medium chain fatty acids content decreased ($P = 0.043$) by 12% in milk from goats fed diet ACBII in comparison with diets AC and ACBI; vaccenic acid content increased ($P = 0.050$) 1.5 and 3.5 folds in milk from goats fed diets ACBI and ACBII, respectively, compared to milk from animals fed diet AC; rumenic acid content was 19% and 35% higher ($P = 0.020$) in milk from goats fed diets ACBI and ACBII, respectively than in milk from those fed diet AC; the C18:3 (6, 9, 12) acid content was 41.5% higher ($P = 0.033$) in average in milk from goats fed diets ACBI and ACBII than from goats fed diet AC. Because CLA is considered healthy for consumers (Pariza, 2004) the inclusion of FB containing crude olive cake in goats feeding could be an option to improve the nutritional and healthy quality of milk. The partial replacement of concentrate with FB promoted decreased feeding cost by 36%. The decrease of milk yield with diet ACBII could be tolerated when considering the healthier quality of milk obtained with this diet compared with diets AC and ACBI, the lower cost of feeding diets including FB, and the environmental advantage of recycling by-products.

Table 3. Effect of the partial replacement of concentrate with feed blocks including crude olive cake on the average values of body weight, N and energy intake and utilization, microbial N flow and efficiency of microbial N synthesis in dairy goats

Item	Diets†			*SEM*	*P – value*
	AC	ACBI	ACBII		
BW, kg	38.3	39.0	39.5	0.523	0.626
	g/kg $BW^{0.75}$				
N intake	2.46	2.20	2.30	0.082	0.201
Faecal N	0.773	0.636	0.657	0.060	0.322
Urine N	0.886	0.819	0.901	0.036	0.455
Milk total N	0.497[b]	0.343[a]	0.343[a]	0.036	0.012
Milk protein N	0.405[b]	0.317[a]	0.317[a]	0.012	0.023
Digestible N	1.68	1.56	1.65	0.069	0.583
N balance	0.798	0.741	0.745	0.065	0.929
Retained N	0.301[a]	0.397[b]	0.402[b]	0.067	0.050
	%				
Digestible N/intake N	69.2	71.4	71.5	1.96	0.452
N balance/digestible N	46.8	46.7	43.1	2.47	0.792
Milk protein N/digestible N	25.1	20.6	19.8	1.77	0.239
Milk protein N/N balance	55.6[b]	48.2[a]	53.1[b]	6.21	0.050
Milk protein N/milk total N	88.6	93.1	92.2	0.433	0.491
Milk protein N/intake N	16.9	14.5	14.0	1.06	0.289
	MJ/kg $BW^{0.75}$				
Energy intake	1.51[b]	1.37[a]	1.52[b]	0.490	0.016
Faecal energy	0.488	0.448	0.449	0.031	0.502
Urine energy	0.056[b]	0.043[a]	0.062[b]	0.003	0.043
Milk energy	0.258[b]	0.218[a]	0.219[a]	0.017	0.049
Digestible energy	1.03	0.92	1.07	0.04	0.116
Methane energy	0.106	0.095	0.111	0.04	0.180
ME	0.863	0.781	0.899	0.035	0.173
	%				
Digestible energy/energy intake	68.3	67.5	70.3	1.61	0.418
Milk energy/digestible energy	25.1	24.0	21.0	1.75	0.136
Milk energy/ME	29.8	28.4	25.2	2.12	0.194
ME/energy intake	57.5	57.3	58.9	1.47	0.676
ME/digestible energy	84.3	84.9	83.7	0.312	0.052
Microbial N flow, g/d	27.6[b]	17.3[a]	23.4[b]	1.26	0.045
EMNS, g/ kg OMAFR	49.1[b]	38.8[a]	44.4[ab]	2.75	0.036

†AC: control diet (alfalfa hay:concentrate, 1:1); ACBI and ACBII: alfalfa hay and concentrate (1:0.5) and feeding blocks including 120 and 100 g/kg of crude olive cake, respectively.

B. Feed blocks based on greenhouse waste fruits

Eight Granadina goats (39.4 ± 5.39 kg BW) in the middle of the third lactation were fed four experimental diets formulated with 1kg alfalfa hay plus 1 kg concentrate (diet AC), and alfalfa hay, concentrate and feed blocks containing greenhouse wastes of tomato (diet ACT) and cucumber (diet ACC) fruits or barley (diet ACB) which replaced 22% of the concentrate in the control diet. Although this substantial reduction in feeding cost, wastes transportation and FB

manufacturing should be considered in each situation in order to estimate the profit margins. The effect of diet on ruminal fermentation, microbial N flow and efficiency, methane emissions, nutrients utilization, milk yield and composition and microbial abundances was studied by following a 4 x 4 Latin square experimental design with repetitions. The feed blocks intakes were 231 ± 69, 238 ± 59 and 223 ± 88 g/animal/d for tomato, cucumber and barley-based feed blocks. Methane emission (Table 5) was reduced ($P < 0.001$) by diets including blocks with tomato and cucumber wastes (38.5% in average) and barley grain (30%) compared to diet AC. Therefore, FB including tomato or cucumber fruit wastes may also have an added value derived from the presence of plant secondary compounds which could act as natural safe antimethanogenics additives, alternative to the chemical ones as suggested by Patra and Saxena (2010). Diets ACT and ACC showed similar antimethanogenic effect, but different fermentation patterns, which could support previous speculations concerning the association between the antimethanogenic effect of plant secondary compounds and their molecular structure and weight together with chemical composition of diets (Guo *et al.,* 2008). Similar values for methane emissions (L / kg DMI) using respiration chambers were also found in goats treated with bromochloromethane with proved antimethanogenic activity (Abecia *et al.,* 2011). An additional advantage of the strategy involving the replacement of 35% of concentrate with feed blocks including tomato or cucumber fruits, rely to the lack of effect on DMI that has been shown to decreased with some antimethanogenic strategies (Beauchemin *et al.*, 2008). In addition, the abundances of total bacteria and methanogens were not affected by diet, suggesting the absence of any relationship between the reduction in methane emissions and abundances of methanogens (Machmüller *et al.,* 2003). It has been hypothesized that rather than the number is the species composition of archaea community what drives the synthesis of methane in the rumen (Morgavi *et al*., 2010) but it still remains unknown which genera or species of archaea are more involved in ruminal methane production.

Table 4. Effect of the partial replacement of concentrate with feed blocks including crude olive cake on the average values of milk yield and composition in dairy goats

Item	Diets†			*SEM*	*P-values*
	AC	**ACBI**	**ACBII**		
Milk, g/d	1255[b]	973[a]	1029[a]	81.4	0.016
g/kg milk Protein	31.8	33.8	31.1	1.20	0.657
Casein	25.3	26.7	23.7	1.12	0.581
Whey protein	6.60	7.12	7.48	0.327	0.294
Fat	45.9	49.7	42.7	2.24	0.547
Lactose	47.6	44.9	43.3	2.11	0.882
Total solid	131	139	127	3.12	0.144
Gross energy, MJ/d	3.25	3.51	3.36	0.113	0.533
FA profile, g/100 g total identified FA					
C18:1 (trans 11)	0.122[a]	0.187[a]	0.432[b]	0.040	0.050
C18:2 (cis 9, trans 11)	0.436[a]	0.522[b]	0.590[b]	0.040	0.020
C18:3 (cis 6, 9, 12)	0.475[a]	0.671[b]	0.676[b]	0.042	0.033
Medium chain	37.5[b]	37.2[b]	32.8[a]	0.913	0.043
Monounsaturated fatty acids	18.6	20.4	23.3	0.835	0.164
Polyunsaturated fatty acids	3.34	3.71	3.68	0.185	0.241
Saturated fatty acids	78.1[b]	75.9[b]	73.1[a]	0.773	0.046

†AC: control diet (alfalfa hay:concentrate, 1:1); ACBI and ACBII: alfalfa hay and concentrate (1:0.5) and feeding blocks including 120 and 100 g/kg of crude olive cake, respectively.

Table 5. Effects of the partial replacement of concentrate with feed blocks including wastes of tomato or cucumber and barley grain on average values for methane emissions, pH, concentrations of ammonia N an VFA, acetate:propionate ratio, abundances (log gene copies/g fresh matter) of total bacteria and methanogens, microbial N flow and efficiency and N and energy balances in dairy goats

Item	Diet†				*SEM*	*P-value*
	AC	ACT	ACC	ACB		
CH_4 L/kg DMI	28.2[c]	17.4[a]	17.2[a]	19.7[b]	0.558	< 0.001
pH	6.94	7.00	6.93	6.91	0.036	0.391
NH_3-N, mg/100 mL	28.1[b]	18.2[a]	18.5[a]	25.3[b]	0.497	0.003
Total VFA, mmol/L	149[c]	109[b]	161[c]	85.6[a]	2.62	< 0.001
Acetate:propionate	5.35	5.01	4.48	5.44	0.152	0.269
Bacteria††	11.5	11.4	11.5	11.4	0.092	0.423
Methanogens††	9.04	8.83	8.91	8.86	0.095	0.441
Microbial N flow, g/d	16.2[b]	13.1[a]	18.9[c]	15.4[b]	1.00	0.047
EMNS, g/kg of OMAFR	24.2[b]	21.1[a]	30.8[c]	24.9[b]	1.03	0.044
Body weight (BW), kg	38.6	38.7	40.3	40.2	1.79	0.411
g/kg of $BW^{0.75}$						
N intake	3.25	3.12	3.01	3.10	0.13	0.384
Fecal N	0.730	0.760	0.737	0.704	0.066	0.922
Urine N	1.51[b]	1.31[a]	1.32[a]	1.40[ab]	0.064	0.037
Milk total N	0.398	0.346	0.386	0.373	0.036	0.728
MJ/kg of $BW^{0.75}$						
Energy intake	1.93[b]	1.80[a]	1.77[a]	1.79[a]	0.060	0.029
Fecal energy	0.567	0.569	0.543	0.532	0.038	0.517
Urine energy	0.080[b]	0.070[a]	0.074[a]	0.075[a]	0.004	0.008
Milk energy	0.251	0.229	0.243	0.233	0.019	0.970
Methane energy	0.110[b]	0.072[a]	0.067[a]	0.080[a]	0.004	< 0.001
ME	1.18	1.09	1.09	1.10	0.033	0.575

†AC: control diet (alfalfa hay:concentrate, 1:1); ACT and ACC and ACB: alfalfa hay and concentrate (1:0.65) and feeding blocks including tomato and cucumber fruit wastes and barley grain, respectively.
††Log gen copies/g wet weight

The pH values were similar (P = 0.391) for all the diets while VFA concentration was affected (P < 0.001) by diet and not (0.269) the acetate to propionate ratio. The lack of correlation between pH values and VFA concentration agrees with observation of other authors (Busquet *et al.*, 2005a; Cantalapiedra *et al.*, 2009) and may be due to the contamination of rumen samples with saliva. The type of carbohydrates present in the concentrate (Ørskov and Fraser, 1975) and the buffer properties attributed to alfalfa (Dixon and Stockdale, 1999) could contribute to the lack of variations in rumen pH with dietary treatments. Ammonia concentration decreased (P = 0.003) with diets including blocks containing tomato and cucumber wastes. Either bacteria or methanogens abundances were not (P = 0.423 and 0.441, respectively) modified by diet. Both microbial N flow (P = 0047) and efficiency (P = 0.044) decreased and increased, respectively, with diet including tomato and cucumber wastes. Body weight was not affected by diet (P = 0.411) and regarding N balance only N in urine was affected by diet (P = 0.037) with lower values for diets including wastes which may have importance from the environmental point of view. Energy intake, energy in urine and in methane decreased (P ≤ 0.029) with diets including feed blocks compared to the control one.

Milk yield (Table 6) was not affected (P = 0.826) by diet. With the exception of lactose (P = 0.037) no effect (P ≥ 0.037) was detected in milk composition. Regarding milk fatty acids profile

no effect of diet was observed both on total saturated (P = 0.578) and monounsaturated (P = 0.762). On the contrary total PUFA increased (P = 0.034) by with diets including FB (15, 14 and 9%, respectively for tomato, cucumber and barley-based FB compared to control diet) and rumenic acid content increased (0.043) with diet including FB containing tomato wastes (9% in comparison to control diet). Feeding cost was reduced by 22% in diets including FB.

Table 6. Effects of the partial replacement of concentrate with feed blocks including wastes of tomato or cucumber and barley grain on average values for milk yield, composition and fatty acids profile

Item	Diet†				*SEM*	*P-value*
	AC	**ACT**	**ACC**	**ACB**		
Milk, g/d	1019	944	1041	1000	83.8	0.826
g/kg milk						
Protein	34.7	33.9	33.9	33.9	1.68	0.935
Fat	55.1	55.7	50.8	54.4	2.00	0.067
Lactose	52.6^{b}	46.0^{a}	55.2^{b}	59.5^{c}	2.55	0.037
Total solid	148	141	145	153	4.94	0.069
Gross energy, MJ/kg	3.76	3.77	3.67	3.72	0.149	0.744
g/100 g of identified FA						
Total SFA	74.7	73.4	73.4	74.4	0.458	0.580
Total MUFA	19.7	20.3	20.4	19.6	0.498	0.762
Total PUFA	3.34^{a}	3.83^{b}	3.80^{b}	3.65^{b}	0.108	0.034
cis-9, trans-11 CLA	0.57^{ab}	0.62^{b}	0.55^{ab}	0.50^{a}	0.048	0.043

†AC: control diet (alfalfa hay:concentrate, 1:1); ACT and ACC and ACB: alfalfa hay and concentrate (1:0.65) and feeding blocks including tomato and cucumber fruit wastes and barley grain, respectively

In addition to FA supply, other factors such as energy supply, proportion of fiber or concentrate and the presence of plant secondary compounds should be considered when the effects of dietary treatments on milk FA profile are assessed (Leiber *et al.,* 2005). Therefore, the higher accumulation of LNA in the milk of goats receiving FB could be associated with changes in the ruminal ecosystem due to energy shortage or specific secondary plant metabolites presence in the diet (Leiber *et al.*, 2005). Moreover, the synchronous and fractionated supply of nutrients allowed when using FB in ruminants feeding (Ben Salem and Nefzaoui, 2003) may have been associated with a better FA absorption in the small intestine (Romero-Huelva *et al.,* 2012).

III – Use of additives to manipulate rumen fermentation

An enormous variety of secondary compounds are produced by plants to provide protection against microbial and insect attack (Levin, 1976, Cowan, 1999, Isman, 2000 and Iason, 2005). Some are toxic to animals, but others may not be, and indeed many have been used to manipulate gut function in both ruminant and non-ruminant animals (Greathead, 2003). Broadly, these compounds fall into four categories: essential oils, organosulphurs, polyphenols and saponins. We will consider the effect of essential oils and organosulphur compounds on rumen fermentation in goats.

Previous studies reported that essential oils and garlic extracts in certain amounts can enhance rumen fermentation (Cardozo *et al.,* 2006; Castillejos *et al.*, 2008; Kamel *et al.*, 2008). However, effects reported in the literature are variable and contradictory, which may be due to the different concentrations, plant and basal diets used (Hart *et al.,* 2008). Among the garlic derived compounds, some thiosulfate compounds have been shown to exhibit methane reduction

potential (Kamel *et al.*, 2008; Benchaar and Greathead, 2011; Soliva *et al.*, 2011). Furthermore, some of the recently developed thiosulfates with high antimicrobial activity (Ruiz *et al.*, 2010) have not been fully tested in ruminants and offer room for further screenings (Martin-Garcia *et al.,* 2011).

In our group several in vitro trials have been performed to screen the effects of some essential oils and organosulphur compounds on rumen fermentation and methane emissions using rumen fluid from goats. Some of the compounds tested in vitro have been further tested in goats fed at maintenance level. Furthermore, an in vivo trial was conducted to evaluate the effect of reducing methane emissions in dairy goats on milk production and milk composition by using bromochloromethane, a synthetic compound with a well known antimethanogenic effect.

1. *In vitro–In vivo* assays

Different experiments have been conducted in our group to investigate the effects of some essential oils and organosulphur compounds on rumen fermentation and methane emissions. Table 7 summarizes the effects observed on some parameters by the addition of carvacrol (CAR), cinnamaldehyde (CIN), propyl-propane-thiosulfinate (PTS), propyl-propane-thiosulfonate (PTSO), diallyl disulfide (DDS) and bromochloromethane (BCM) as antimethanogenic reference (Martinez et al., submitted). We have used two different substrates as diet, which are based on a alfafa hay:concenrrate mix (1:1), in which the concentrate included sources of starch and protein with high (I: barley and faba beans) or low (II: maize and sunflower cake) degradability.

As shown in Table 7, the asymptotic gas production was affected ($P<0.001$) by substrate with all compounds with the exception of PTS ($P=0.386$). The gas production rate only increased ($P<0.001$) with CAR and DDS at the highest dose (320 µl/L) while PTS and PTSO reduced it ($P<0.001$). The highest doses of BCM and CIN did not have any effect ($P>0.100$) on gas production rate. The gas produced per gram of digested DM (Table 7) was not affected ($P=0.225$) by BCM, while it was reduced ($P\leq0.047$) by the rest of compounds at different doses. Substrate had statistical significant effect ($P\leq0.002$) with all compounds, decreasing gas produced per gram DM digested by substrate II compare with substrate I. Truly digested DM after 72 h of incubation (Table 6) was affected ($P\leq0.015$) by all doses of CAR and PTS, while only the highest dose (320 µl/L) of DDS decreased it ($P=0.022$). Finally, BCM tended ($P=0.099$) to decrease truly digested DM while CIN and PTSO showed no effect ($P>0.170$). Methane produced in vitro from 12 to 24 h (Table 6) was reduced by all doses of PTS and DDS ($P<0.001$). The PTS exhibited a methane reduction ($P<0.001$) up to 88% at the highest dose (320 µl/L), while DDS reduced methane ($P<0.001$) with both doses up to 55% compared with the control. Likewise, the addition of both doses of BCM decreased ($P< 0.001$) methane concentration by 95% as compared to the control.

As outlined above, the addition of CAR did not affect ($P >0.05$) methane concentration in vitro suing rumen liquor from goats, which is in contrast to Macheboeuf *et al.* (2008), who reported a linear decrease in CH_4 production with CAR at 225, 300, 450 and 750 mg/L in batch cultures, with a reduction in acetate, propionate and total VFA concentrations. Methane concentration was not affected either by CIN, which disagrees with other authors (Macheboeuf *et al.*, 2008), that reported a reduction of methane production with similar doses to those used in the present work. This difference might have been due to the different origin of the inoculum (ovine vs. caprine), the basal diet (25:75 vs. 50:50 forage:concentrate) used or even the extraction process of the essential oil. PTS and DDS reduced methane emission up to 90% and 60 %, respectively, which are comparable to those reported in other in vitro studies. Busquet *et al.* (2005b) reported a decrease of about 70% in methane emission after 17 h of incubation in batch cultures with a similar dose (300 mg/L) of garlic oil and DDS. Likewise, Soliva *et al.* (2011) showed 90% inhibition of methane production with garlic oil (300 mg/L) in an experiment carried out with Rusitec fermenters. However, Kamel *et al.* (2008) did not find such effect of DDS on methane emission in 24 h incubations using batch cultures, although doses were lower

than in other experiments. The antimicrobial effect of garlic derived compounds has been suggested to be realted to its reaction with thiol groups of some enzymes as acetyl-CoA (Focke *et al.*, 1990) or to the inhibition of HMG-CoA reductase (Busquet *et al*., 2005a; 2005b). PTSO did not affect methane production, although its chemical structure is very similar to that of PTS. Whereas PTS is more active against enterobacteria. PTSO has higher activity on lactobacilli, bifidobacteria, bacteroides and clostridia (Ruiz *et al.,* 2010). This differential inhibition showed by PTS and PTSO may be due to different composition of microbial membranes and their permeability to these compounds as suggested by Miron *et al.* (2000).

Table 7. Effects of the substrate and additives dose on gas production (GP, gas mL/g digested DM), kinetics gas parameters (A: potential gas volume at steady state, mL; c: gas production rate, h-1), truly digested dry matter (tDDM, g/g) after 72 h incubation and on methane (mL/ mL total gas) production over 24 h of incubation in batch cultures

Compound†		Substrate		Dose††			*SEM*	*P-value*		
		I	II	0	I	II		Substrate	Dose	SxDo†††
CAR	GP	254	215	265^{a}	252^{a}	188^{b}	3.40	<0.001	<0.001	0.825
	A	103	87	111^{a}	100^{b}	73^{c}	1.14	<0.001	<0.001	0.998
	c	0.108	0.109	0.089^{b}	0.099^{b}	0.136^{a}	0.00	0.985	<0.001	0.984
	tDDM††††	0.86	0.85	0.89^{a}	0.85^{b}	0.83^{b}	0.01	0.730	0.015	0.781
	CH_4	0.127	0.118	0.130	0.127	0.110	0.00	0.312	0.168	0.977
CIN	GP	275	248	265^{ab}	272^{a}	250^{b}	3.37	0.002	0.047	0.685
	A	118	103	111^{ab}	113^{a}	108^{b}	0.82	<0.001	0.048	0.836
	c	0.084	0.084	0.089	0.085	0.077	0.00	0.957	0.139	0.934
	tDDM††††	0.89	0.88	0.89	0.88	0.89	0.00	0.067	0.405	0.884
	CH_4	0.127	0.122	0.130	0.126	0.117	0.00	0.503	0.307	0.794
PTS	GP	234	201	265^{a}	246^{b}	142^{c}	3.10	<0.001	<0.001	0.654
	A	102	95	111^{a}	98^{a}	87^{b}	4.54	0.386	0.127	0.648
	c	0.063	0.060	0.089^{a}	0.073^{a}	0.019^{b}	0.00	0.674	<0.001	0.847
	tDDM††††	0.86	0.85	0.89^{a}	0.84^{b}	0.83^{b}	0.01	0.231	0.006	0.930
	CH_4	0.086	0.082	0.130^{a}	0.108^{b}	0.014^{c}	0.00	0.451	<0.001	0.697
PTSO	GP	279	251	265^{ab}	274^{a}	256^{b}	2.36	<0.001	0.032	0.603
	A	116	102	111^{a}	112^{a}	105^{b}	0.80	<0.001	0.015	0.467
	C	0.083	0.083	0.089^{a}	0.090^{a}	0.069^{b}	0.00	0.879	0.002	0.906
	tDDM††††	0.89	0.87	0.89	0.87	0.87	0.00	0.088	0.171	0.976
	CH_4	0.124	0.116	0.130	0.119	0.111	0.00	0.210	0.096	0.947
DDS	GP	243	213	265^{a}	229^{b}	190^{c}	2.50	<0.001	<0.001	0.782
	A	100	87	111^{a}	94^{b}	76^{c}	0.88	<0.001	<0.001	0.419
	C	0.109	0.111	0.089^{b}	0.113^{a}	0.130^{a}	0.00	0.755	<0.001	0.991
	tDDM††††	0.88	0.87	0.89^{a}	0.87^{a}	0.86^{b}	0.01	0.540	0.065	0.360
	CH_4	0.077	0.076	0.130^{a}	0.056^{b}	0.044^{b}	0.00	0.912	<0.001	0.533
BCM	GP	280	238	265	252	260	2.76	<0.001	0.225	0.441
	A	117	98	111^{a}	103^{b}	107^{a}	0.85	<0.001	0.010	0.244
	C	0.091	0.099	0.089	0.097	0.098	0.00	0.095	0.160	0.501
	tDDM††††	0.89	0.87	0.89	0.87	0.88	0.00	0.015	0.099	0.935
	CH_4	0.048	0.045	0.130^{a}	0.005^{b}	0.004^{b}	0.00	0.636	<0.001	0.619

†Interaction between Substrate and Dose. ††Dose I for CAR, CIN, PTS and BCM was 160 µl/L, for PTSO was 40 µl/L and for DDS was 80 µl/L; Dose II for CAR, CIN, PTS, DDS and BCM was 320 µl/L and for PTSO was 160 µl/L. †††Compounds: CAR (carvacrol), CIN (cinnamaldehyde), PTS (propyl propane thiosulfinate), PTSO (propyl propane thiosulfonate), DDS (diallyl disulfide) and BCM (Bromochloromethane). ††††Calculated as proposed by van Soest *et al*. (1966): true digested DM = (DM input – FND output)/DM input, FND output being that analyzed in the residue after 72 h incubation. [a-c] Within a row doses means without a common superscript letter differ, P < 0.05. (Multiple comparisons LSD test).

Table 8. Effects of PTS and BCM on body weight (BW), daily dry matter intake (DMI) and methane (CH_4) emissions by goats

Compound†		Doses††				*SEM*	*P-value*	Contrast†††	
		0	I	II	III			L	Q
PTS	BW, kg††††	33.3	34.4	32.5	32.5	1.47	0.968	n.d.	n.d.
	DMI, g/day	601	584	628	478	64.10	0.654	0.426	0.463
	CH_4, L/day	21.8^{a}	19.9^{ab}	18.5ab	11.0^{b}	2.53	0.191	0.050	0.443
	CH_4, L/Kg DMI	34.5^{a}	29.8^{a}	30.4^{a}	23.0^{b}	1.14	0.002	<0.001	0.407
BCM	BW, kg††††	32.3	32.1	34.1	36.2	1.43	0.720	n.d.	n.d.
	DMI, g/day	440	434	580	458	52.85	0.470	0.545	0.440
	CH_4, L/day	17.4^{a}	12.1^{a}	13.4^{a}	6.35^{b}	1.37	0.010	0.003	0.627
	CH_4, L/Kg DMI	43.9^{a}	28.6^{ab}	24.1^{b}	15.7^{b}	1.38	0.027	0.004	0.551

†PTS: propyl propane thiosulfinate; BCM: Bromochloromethane. ††Doses I, II and III in PTS were 5.5, 11 and 22 mg/kg BW respectively. Doses I, II and III in BCM were 5.5, 11 and 17.6 mg/kg BW respectively. †††Linear (L) and quadratic (Q) effect of dose. ††††Average of weighing prior and after measurements. $^{a-b}$ Within a row doses means without a common superscript letter differ, P<0.05. (Multiple comparisons LSD test).

Based on our observations made from in vitro trials, PTS and BCM have been further tested in vivo in our group using goats fed at maintenance level (Martinez *et al.*, submitted). As shown in Table 8, methane emissions, expressed per unit of dry matter intake decreased over 33% with PTS at the highest dose (22 mg/kg BW per day). This is equivalent to the reduction (17%) observed in vitro with 160 µl/L dose and far less than the reduction (87%) achieved with a higher dose (320 µl/L) in vitro. On the other hand, the reduction observed with BCM in vivo (34 to 64 %) was not as high as obtained in vitro (96%); however, it was similar to the decrease (30%) achieved in our lab with dairy goats treated with the same compound and similar dosage over two months (Abecia *et al.*, 2012). The disagreement observed between the same doses in vitro and in vivo data strongly supports the need of testing in vivo what it is previously observed in the lab and may be explained by a number of factors: the compounds used in this study had very low solubility in water and therefore the homogenous distribution across the rumen compartments might have not been fully achieved. In addition, the direct extrapolation of concentrations from in vitro to in vivo did not take into account the rumen outflow, which in our conditions is around 3%/h (Yáñez Ruiz *et al*., 2004). This would require an increase of the daily dosage of about 80% in vivo and would explain the proportionally lower reduction achieved in vivo as compared to in vitro.

2. Antimethanogenic additives in dairy goats

As pointed above, several technologies have been tested to reduce enteric methanogenesis, but very few have been successfully used in practical conditions for livestock. Furthermore, the consequences of reduced rumen methane production on animal performance and milk quality are poorly understood.

As reported in Abecia *et al.* (2012), an in vivo trial was conducted in our group to investigate the effect of feeding bromochloromethane (BCM), a halogenated aliphatic hydrocarbon with potential antimethanogenic activity, to dairy goats on rumen methane production, fermentation pattern, the abundance of major microbial groups, and on animal performance and milk composition. Eighteen goats were allocated to 2 experimental groups of 9 animals each: treated (BCM+) or not (BCM−) with 0.30 g of BCM/100 kg of body weight per day. The BCM was administered in 2 equal doses per day from parturition to 2 wk postweaning (10 wk). As shown in table 9, the treatment with BCM reduced methane production by 47% (19.3 vs 10.1 L/kg DMI), although it did not affect the abundance of rumen bacteria, protozoa and total

methanogenic archaea. The observed improvement in the efficiency of digestive processes was accompanied by a 45% increase in milk yield, probably due to the more propionic type of rumen fermentation. Positively, the increase in milk yield did not affect its concentration of fat, protein or lactose. Moreover, there were only minor changes in milk fatty acid profile, which suggests that the substantial decrease in methane production reported here did not seem to alter ruminal biohydrogenation pathways either. Compounds with similar mode of action deserve therefore further development for future application in the dairy sector.

Table 9. Methane emissions, total VFA, microbial numbers in the rumen and milk production by goats treated (BCM+) or not (BCM-) with bromochloromethane (n = 9 per treatment)

	BCM-	BCM+	*SED*	*P-value*
Methane emissions				
CH_4 L/kg DMI	19.3	10.1	3.30	0.013
CH_4 L/kg of milk	24.1	13.7	4.86	0.051
VFA concentration, mmol/L	58.5	74.4	8.79	0.216
Acetate:propionate	5.6	3.9	0.26	<0.001
log gene copies/g fresh matter				
Bacteria	10.3	10.02	0.116	0.124
Protozoa	9.57	9.54	0.077	0.810
Methanogens	7.88	7.96	0.161	0.753
Milk, g/d	901	1324	164	0.021
g/100 g milk				
Fat	5.46	4.98	0.701	0.505
Protein	3.73	3,52	0.335	0.551
Casein	3.18	3.05	0.252	0.606
Lactose	4.63	4.73	0.131	0.476
Total solids	14.4	13.9	0.909	0.570

Conclusions

The results obtained showed that replacement of concentrate with feed blocks based on by-products decreased (22-36%) feeding cost and methane production (37-39%) both in nonproductive and lactating goats and improved the fatty acids profile in milk. However, it compromises N metabolism which could be overcome by improving the formulation of the block;On other hand, some essential oils and organosulphur compounds show in vitro antimethanogenic effect without affecting overall ruminal fermentation; In vivo studies confirm the antimethanogenic effect of PTS in the short term (7 days) and a significant reduction of methane emissions by BCM in lactating goats implies an increase in milk yield production and no effect on milk composition. The use of either feed blocks with by-products/wastes or plant extracts does not essentially modify the numbers of the main microbial groups in the rumen.

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Nutritive evaluation of foliage from some Acacia trees characteristic of Algerian arid and semi-arid areas

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Abstract. Chemical composition and digestibility of foliage from five Acacia species (*Acacia nilotica, Acacia horrida, Acacia saligna, Acacia albida and Albizia julibrissin*) from arid and semi-arid areas of Algeria were evaluated. Feed components of proximate analysis were determined, whereas phenolic and tannin compounds were analysed by colorimetric procedures and their activity tested using a biological assay. Digestibility was assessed by conventional gravimetric *in vitro* and *in situ* methods, and rumen fermentation kinetics were measured using the *in vitro* gas production technique. Results showed that all browses contained high levels of CP (157-252 g/kg DM). The content of neutral detergent fiber was highest in *A. horrida* (551 g/kg DM) and lowest in *A. nilotica* (290 g/kg DM). The content of lignin was highest in *A. saligna* (147 g/kg DM). The tannin concentrations varied considerably between species, but in general the plants investigated in this study had high tannin contents. *A. nilotica* had the highest levels of TP and TET (213 and 205 g/kg DM, respectively). The TCT content ranged from 60 g/kg DM in *A. albida* to 726 g/ kg DM in *A. nilotica.* The leguminous fodder tree *A. julibrissin* shows high protein content and its foliage is highly digestible probably due to its low tannin content. It was concluded that foliage from Acacia species is a protein-rich fodder for ruminants, although the high lignin and tannin content of some species represents an important constraint that could limit digestive utilization in the gastro-intestinal tract of these species.

Keywords. Nutritive value – Forage – Acacia – Tannins.

Valeur nutritive du feuillage de quelques Acacia prépondérants des régions arides et semi-arides d'Algérie

Résumé. *La composition chimique et la digestibilité du feuillage de cinq espèces d'Acacia (*Acacia nilotica, Acacia horrida, Acacia saligna, Faidherbia albida *et* Albizia julibrissin), *récoltées de zones arides et semi-arides d'Algérie, sont étudiées. Leurs composants organiques majeurs sont déterminés, tandis que leurs teneurs en polyphénols et tannins sont analysées par colorimétrie. Leur digestibilité est évaluée par les méthodes gravimétriques* in vitro *et* in situ*. La technique dite* in vitro gas production *est utilisée pour le suivi cinétique des fermentations ruminales. Les échantillons étudiés montrent donc des taux élevés en protéines (157-252 g/kg MS), le contenu le plus élevé en FND étant noté chez* A. horrida *(551 g/kg MS) et le plus faible chez* A. nilotica *(290 g/kg MS). La valeur la plus élevée en lignine est enregistrée chez* A. saligna *(147 g/kg MS). Les concentrations en tannins varient considérablement entre les différentes espèces étudiées mais, de manière générale, elles sont élevées. Les taux les plus hauts en polyphénols totaux et en tannins totaux extractibles sont enregistrés chez* A. nilotica *(213 et 205 g/kg MS, respectivement). Pour les tannins condensés totaux, les valeurs varient de 60 g/kg MS chez* F. albida *à 726 g/kg MS chez* A. nilotica*. La légumineuse* A. julibrissin *présente un taux protéique élevé et son feuillage est hautement digestible, probablement en raison de son faible contenu en tannins. En conclusion, les résultats obtenus montrent que le feuillage issu des espèces d'Acacia est un fourrage globalement riche en protéines, alors que des taux élevés en lignine et tannins sont observés, ce qui peut représenter un facteur limitant de leur assimilation dans le tractus gastro-intestinal.*

Mots-clés. *Valeur nutritive – Fourrage – Acacia -– Tannins.*

I – Introduction

Algerian steppe covers more than 30 million ha of land and constitutes a buffer zone between the Sahara Desert and the green belt in the North. Sheep breeding is the main agricultural activity. Sheep has become adapted, showing a particular productive performance. But the steppe rangelands are in a process of degradation and this situation is attributed to several factors, mainly the fragility of the physical environment and changes in nomadic pastoral. This area is very intensively exploited by livestock, and it feeds, approximately, 20 millions of sheep and goat population of Algeria and about 80% of the national herd (FAOSTAT, 2009). The processes of desertification are thus omnipresent and affect vast surfaces primarily confined in the arid, semi-arid and sub-humid dry areas. Acacias and other shrubby plants can be used to combat desertification, mitigating the effects of droughts, allowing soil fixation and enhancing the restoration of the vegetation and the rehabilitation of rangelands. Browsing tree foliage plays an important role in ruminant feeding systems in many tropical and Mediterranean environments around the world (Kumara Mahipala *et al*., 2009). The rational use of fodder and browse requires accurate information about the nutritive value of these fibrous resources and the presence of antinutritional compounds, as high concentrations of tannins may be found in many fodder trees (Reed, 1986; Dube *et al.*, 2001), thus impairing their utilization.

The objective of this study was to investigate the nutritive value of selected browse Acacia species (i.e., *A. albida, A. nilotica*, *A. horrida*, *A. saligna, A. julibrissin*) grown in arid and semi-arid areas of Algeria.

II – Material and methods

Leaves, without twigs, from Acacia species (*Acacia albida, Acacia saligna* [syn. *Acacia cyanophylla], Acacia nilotica, Acacia horrida, Albizia julibrissin)* from Bousaada and Constantine districts were clipped with scissors from the aerial part of the plants, and taken inmediately to the laboratory where the samples from the different specimens were pooled, oven-dried at 50ºC (Makkar, 2003), and subsequently ground to pass a 1 mm screen for chemical analysis and 3 mm for in situ degradability. Dry matter (DM), ash and crude protein (CP) contents were determined following the methods of AOAC (2000). Neutral and acid detergent fibre (NDF and ADF, respectively) and sulphuric acid detergent lignin (ADL) were determined as described by Van Soest *et al.* (1991). Phenolic compounds and total extractable phenols were determined according to Makkar (2003) and Julkunen-Tiitto (1985), respectively. Free condensed tannins (FCT) were measured in the extract using the butanol–HCl assay (Porter *et al.*, 1986), with the modifications of Makkar (2003) and using purified quebracho tannin as standard. Concentration of total condensed tannins (TCT) as calculated as follows: TCT = FCT + BCT (Bound condensed tannins).

Four mature Merino sheep (body weight 49.4 ± 4.23 kg) fitted with a permanent ruminal cannula were used for the extraction of rumen fluid or for *in situ* incubation of nylon bags. Animals were fed lucerne hay *ad libitum* (167 g CP, 502 g NDF, 355 g ADF and 71 g ADL/kg DM). *In vitro* dry matter digestibility was determined using the ANKOM-DAISY procedure (Ammar *et al.*, 1999) following two different approaches, Tilley and Terry (1963) and Van Soest *et al.* (1966), separately in different incubations. Gas production profiles were obtained using an adaptation of the technique described by Theodorou *et al.* (1994). The procedure to measure *in situ* disappearance has been described in detail by López *et al.* (1991, 1999).

One way analysis of variance (Steel and Torrie, 1980) was performed on *in vitro* digestibility, gas production fermentation kinetics and *in situ* degradability data, with browse species as the only source of variation (fixed effect) with source of inoculum for *in vitro* and animal for *in situ* as a blocking factor (random effect). Analysis of variance was performed using the GLM procedure of the SAS software package (SAS Institute, 2008).

III – Results and discussion

The forages used in the present study substantially varied in chemical composition and tannin composition (Table 1). Condensed tannins varied widely among species, being highest in *A. nilotica* (726 g/kg) and lowest for *A. albida* (60 g/kg). Their CP was relatively high and fit with those reported by other authors (Al-Soqeer, 2008) for similar species, which justifies their use to supplement poor quality natural pastures and crop residues such as straw and stover (Osuga *et al.* 2006). The low to moderate fibre content of browse foliage would positively influence their voluntary intake and digestibility by small ruminants (Bakshi and Wadhwa, 2004). However, high lignin in some *Acacia* spp., for example *A. saligna* (148 g/kg DM) and *A. albida* (139 g/kg DM), could be associated with low digestibility (Rubanza *et al.*, 2005).

Table 1. Chemical composition (g/kg DM), phenolic compound contents (g/kg DM, equivalent standard) and tannin biological activity[a] of forages

	Plant species				
	A. nilotica	***A. horrida***	***A. saligna***	***A. albida***	***A. julibrissin***
DM	900	904	913	918	904
OM	920	895	899	936	872
CP	243	217	157	252	186
NDF	290	551	447	430	264
ADF	198	200	255	269	92
ADL	126	74	148	140	50
TEP	213	99	205	31	105
TET	206	92	198	28	101
FCT	609	387	451	26	502
TCT	726	476	631	60	587
Tannin bioassay[†]					
6h	2.27	0.95	1.55	1.16	1.20
12h	2.18	1.15	1.7	1.12	1.21
24h	1.73	1.08	1.64	1.11	1.17
48h	1.43	1.08	1.58	1.06	1.10

[†]Tannin bioassay as the ratio between gas production measured at different incubation times adding PEG *vs* control (i.e., Gas PEG / Gas control)

In vitro digestibility and *in situ* DM disappearance were variable ($P<0.05$) across the examined forages (Table 2). The highest IVD values (*in vitro* and *in situ* DM digestibilities) were observed in *A. julibrissin.* An intermediate value was found for leaves of *A. horrida.* The lowest IVD value was observed for *A. saligna.* Differences among browse species in digestibility and gas production parameters may be partly attributed to the variations in chemical composition.

Kinetic fermentation parameters from gas production curves were variable ($P<0.05$) across the acacia species examined (Table 3). Asymptotic gas production (A) and G24 were low in *A. albida* (71.6 and 56.2 ml/g DM, respectively). High extent of degradation (E) and fast average fermentation rate were noted in *A. julibrissin* (48.1 % and 6.05 ml/g/h). Values of the fermentation parameters (c, E and L) were lowest for *A. saligna*.

Gas production values indicate large differences in the fermentation parameters for the substrates studied. Cumulative gas production at 24 h of incubation was lowest ($P<0.05$) in some of the samples with the highest CP content, such as *A. albida*, *A. saligna* and *A. nilotica.* For the last two Acacia spp. this can be attributed to their high TCT concentrations. As for *A.*

albida, its high protein content may have interfered in the gas production results. As protein is degraded, ammonia released will combine with CO_2, so that the amount of gas measured in the headspace is considerably reduced. This effect is more pronounced with protein-rich substrates (Cone and Van Gelder, 1999). Nevertheless, the ranking of the fodder species according to rate and extent of degradation estimated from the gas production curves was similar to that observed for digestibility determined by *in vitro* and *in situ* gravimetric techniques.

For tree foliage from *A. nilotica, A. horrida, and A. saligna,* where total phenolics constitute an appreciable proportion of DM, an overestimation of IVD can be observed, this results is in agreement with that reported by Mlambo *et al.* (2008) with tannin-rich tree fruits from *A. nilotica*, *Acacia erubescens*, *Acacia sieberiana*, and *Acacia erioloba*.

Table 2. *In vitro* dry matter (g g^{-1} DM) digestibility and *in situ* dry matter disappearance (g g^{-1} DM) at different forage incubation times

Species	ivDMloss	TIVD	IVD-TT	*In situ* DM disappearance after incubation times		
				0 h	24 h	96 h
A. nilotica	0.396 [c]	0.643 [b]	0.497 [c]	0.183 [c]	0.508 [b]	0.696[b]
A. horrida	0.571 [a]	0.703 [a]	0.657 [a]	0.195 [c]	0.536 [b]	0.628[b]
A. saligna	0.307 [d]	0.480 [d]	0.457 [c]	0.205[c]	0.386 [c]	0.639[b]
A. albida	0.430 [b]	0.583 [c]	0.567 [b]	0.242 [b]	0.561 [b]	0.661[b]
A. julibrissin	0.451[b]	0.723 [a]	0.634 [a]	0.340 [a]	0.741 [a]	0.830[a]
SEM	0.0198	0.0203	0.0183	0.0190	0.0309	0.0210

ivDMloss: *in vitro* dry matter loss; IVD-TT: *in vitro* digestibility of Tilley & Terry; TIVD: true *in vitro* digestibility; [a, b, c, d, e, f, g] Means in a column with different superscripts are significantly different ($P < 0.05$); SEM: standard error of the mean.

Table 3. *In vitro* fermentation kinetics (estimated from gas production curves) of Algerian forages

Species	A (ml/g)	c (/h)	L (h)	G24 (ml/g)	E (%)	Average rate (ml/g/h)
A. nilotica	165.9 [b]	0.0333 [b]	0.05 [b]	90.5 [b]	33.4[c]	3.97 [b]
A. horrida	200.9 [a]	0.0425 [b]	1.88 [a]	121.9 [a]	42.4[b]	5.56 [a]
A. saligna	136.9 [c]	0.0313 [b]	0.00 [b]	71.7 [c]	25.9 [d]	3.07 [b]
A. albida	71.6 [d]	0.0690 [a]	0.00 [b]	56.2 [c]	43.1[a b]	3.55 [b]
A. julibrissin	188.6 [a]	0.0431 [b]	0.00 [b]	124.3 [a]	48.1[a]	6.05 [a]
SEM	8.51	0.0030	0.169	5.27	1.56	0.25

A: asymptotic gas production, c: fractional rate of fermentation; G24: gas production at 24 h of incubation; E: extent of degradation for a fractional passage rate of 0.03 h^{-1}; [a, b, c, d, e, f, g] Means in a column with different superscripts are significantly different ($P < 0.05$).

In conclusion, foliage from Acacia tree species found in arid areas of Algeria can be considered as protein-rich roughage for ruminants, although their high lignin and tannin contents constitute an important constraint limiting their digestive utilization and likely nutrient absorption in the gastro-intestinal tract. The leguminous fodder tree *A. julibrissin* showed high protein contents and its foliage is more digestible than that from other *Acacia* spp. owing to its lower tannin content. Alternatives and practices to alleviate adverse effects of the studied secondary compounds, mainly condensed tannins, may be investigated.

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Effect of partial replacement of concentrate with feed blocks including tomato wastes from greenhouse horticulture on methane and milk production and milk composition in goats

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Abstract. The aim of the present work was to study, in dairy goats, the effect of replacing 35% of concentrate in the diet with feed blocks including tomato fruits wastes on CH_4 emission, milk yield and fatty acid and amino acid profiles. Eight Granadina goats (39.6 ± 1.89 kg BW) in the middle of the third lactation were used, and a two-periods trial was carried out following a crossover design. In each period, 4 animals randomly received 1.0 kg of alfalfa hay (AH) plus 1 kg of concentrate (diet AC) and the other 4 received 1.0 kg AH plus 0.65 kg of concentrate plus tomato feed blocks *ad libitum* (diet ACB). The amounts of AH and concentrate supplied to the animals fed diet AC were sufficient to allow daily milk production of up to 2 kg per goat (Aguilera et al., 1990). The feed block was supplied *ad libitum* resulting in average intakes of 208 ± 31.8 g of DM/animal/d. Each period included 25 d for adaptation to diet and 8 d for sampling the 3 last for CH_4 measurement in chambers. The ACB diet resulted in a 38.3% reduction (P = 0.001) in CH_4 emissions, and increased (P ≤ 0.048) average proportions of linolenic, linoleic and total polyunsaturated fatty acids in milk. The amino acid profile and concentration were unaffected by diet, but the proportion of phenylalanine was lower (P = 0.039) in ACB milk compared to the control diet. It is concluded that feed blocks based on tomato fruits wastes could replace 35% of the concentrate in dairy goats diet without detrimental effects on milk production and fatty acid or amino acid composition. Overall, diet including tomato feed blocks promoted the production of milk with healthier fatty acid profile and reduced feeding cost and the environmental damage caused by CH_4 emissions, compared with a conventional diet.

Keywords. Goat – Feed blocks – Tomato – Methane – Milk – Fatty acids – Amino acids.

Effet du remplacement partiel du concentré par des blocs alimentaires contenant des sous-produits de la tomate issus de cultures sous serre, sur la production de méthane et la production et la composition du lait chez des chèvres

Resumé. *Ce travail avait pour objectif l'étude, chez des chèvres laitières, du remplacement de 35% de la fraction concentrée de la ration par des blocs alimentaires contenant des sous-produits de tomate sur les émissions de CH_4, la production laitière et la composition du lait (acides gras et acides aminés). Huit chèvres de la race Granadina (39,6 ± 1,89 kg poids vif) en milieu de troisième lactation ont participé à deux essais selon un schéma en cross-over. Dans chaque essai, 4 animaux tirés au hasard ont reçu 1,0 kg de foin de luzerne (AH), plus 1 kg de concentré (régime AC), alors que les 4 autres ont reçu 1,0 kg AH plus 0,65 kg de concentrés et des blocs alimentaires contenant des tomates offerts en quantité ad libitum (régime ACB). Le régime ACB a entraîné une réduction de 38,3% (P = 0,001) des émissions de CH_4, et une augmentation dans le lait (P ≤ 0,048) des proportions des acides linolénique et linoléique et des acides gras polyinsaturés totaux. Le profil en acides aminés et leur concentration n'ont pas été affectés par le régime, mais la proportion de la phénylalanine a été plus faible (P = 0,039) dans le lait des animaux recevant le régime ACB par rapport au témoin. En conclusion, le régime ACB distribué aux chèvres favorise la production d'un lait avec un profil en acides gras de meilleure valeur nutritionnelle tout en réduisant le coût de l'alimentation et les impacts environnementaux négatifs de la production caprine liés aux émissions de méthane.*

Mots-clés. *Chèvre – Blocs alimentaires – Tomate – Méthane – Lait – Acides gras – Acides aminés.*

I – Introduction

Ruminant production in the Mediterranean area is limited by the poor quality and scarcity of pasture. Thus concentrates based on cereals are frequently used, but increase in cereal prices has driven the attention of ruminant nutritionists toward local alternatives (Ben Salem and Znaidi, 2008). Greenhouse horticulture is very important in the area, producing large amount of wastes, which could be an alternative to cereals for ruminants. Feed blocks manufacturing allows the inclusion of high-moisture wastes in animal feeding (Ben Salem and Nefzaoui, 2003). Milk fatty acid profile can be manipulated by the inclusion of some agro-industrial by-products in the diet depending on their energy value, fatty acid composition and fibre content (Vasta *et al.*, 2008). Nevertheless, few studies have investigated the use of by-products in dairy goats, analysing milk fatty acid composition (Molina-Alcaide *et al*., 2010a; Modaresi *et al.*, 2011). As for conventional protein sources, the success of the utilization of alternative protein sources in dairy goat feeding depends on the ability to formulate diets balanced in essential amino acids (Vasta *et al.,* 2008). Moreover the presence of plant secondary and other unknown compounds in greenhouse wastes could modify rumen fermentation and thus, methane emission (Patra and Saxena, 2010). The aim of the present experiment was to study, in dairy goats, the effect of replacing 35% of concentrate in the diet with feed blocks including wastes from tomato fruits on CH_4 emission and on milk yield and fatty acid and amino acid profiles.

II – Materials and methods

Eight Granadina goats (39.6±1.89 kg BW) in the middle of the third lactation were used, and 2 periods were carried out following a crossover design. In each trial, 4 animals randomly received 1.0 kg of alfalfa hay (AH) plus 1 kg of concentrate (diet AC) and the other 4 received 1.0 kg AH plus 0.65 kg of concentrate plus feed blocks (B) including greenhouse wastes of tomato (diet ACB) with 8 replications per diet at the end of the trials. Animals were individually kept in boxes with free access to food and water. Ingredient composition of concentrate and tomato FB is shown in Table 1. The amounts of AH and concentrate supplied to the animals fed diet AC were sufficient to allow daily milk production of up to 2 kg per goat (Aguilera *et al.*, 1990). The feed block was supplied *ad libitum*, resulting in average intakes of 208±31.8 g of DM/animal/d for ACB diet. Each trial consisted of 25-d for adaptation and 8-d for sampling. Individual intakes of diet ingredients were registered through the whole trial. Goats were hand-milked once a day before feeding and milk yield recorded and milk density was measured and aliquots were stored at −30°C without preservatives until analyzed. The last 3 days of each trial animals were individually placed into square polycarbonate chambers (1.8×1.8×1.5 m) to measure CH_4 emissions. Ground (1-mm) samples of ingredients were analyzed for dry matter (DM), organic matter (OM), ether extract (EE) and total N (Table 2) according to the AOAC (2005). The NDF (neutral detergent fibre) and ADF (acid detergent fibre) were analyzed according to van Soest *et al.* (1991) using an ANKOM Model 220 Fiber Analyzer (Macedon, NY, USA) with α-amylase for NDF analysis in concentrate samples and both NDF and ADF contents referred to ash-free weight. The ADL (acid detergent lignin) was determined by solubilisation of cellulose with 72% sulphuric acid. The energy content was analysed using an oxygen bomb calorimeter (PARR 1356, Biometer). Total N content in feedstuffs and milk was determined as described by (Molina-Alcaide *et al.,* 2010a). Extraction of total fatty acids in feedstuffs was based on the method of Folch *et al.* (1957), with modifications (Devillard *et al.,* 2006). Total fatty acids in milk were extracted as described by Toral *et al.* (2011). The amino acid N (AA-N) content in samples of feeds and milk was determined by HPLC using the Waters® (Waters Corporation, Mildford, MA, USA) following the procedures described by Molina-Alcaide *et al.* (2010b). Methane emission was calculated from CH_4 concentration analysed using a gas analyzer ADM MGA3000 (Spurling Works, Herts, UK) and airflows into and out of each chamber. Data were analyzed by GLM (general linear models) using repeated measures of ANOVA. Diet was considered as a fix effect, and trial and animal as random effects. When a significant effect of diet was found, post hoc comparison of means was made using the LSD

test. Differences were considered significant at P<0.05 and P<0.10 values were declared as trends and discussed.

Table 1. Ingredients composition of concentrate and feed block (g/kg fresh matter)

Ingredient	Concentrate	Feed block
Wheat shorts	350	—
Corn grain	50	—
Barley	160	—
Tomato waste	—	585
Sunflower meal	115.5	36
Soybean hulls	90	—
Corn middlings	90	—
Soybean meal	90	—
Wheat straw	—	221
Beet molasses	—	100
Fatty acid salts	4.5	—
Quicklime	22	27
NaCl	3.0	16
Vitamin-mineral mixture†	25	3.3
Urea	—	11.7

†Formulated (per kg) with NaCl, 277 g; ashes from the two-stage dried olive cake combustion, 270 g; $(PO_4)_2H_4Ca$, 250 g; MgSO4, 200 g; CuO, 184 mg; I, 25 mg; CoO, 8.5 mg; Se, 4 mg; ZnO, 2.28 mg; and 83,500 and 16,700 IU of vitamins A and D, respectively.

III – Results and discussion

The composition of diets in ingredients is shown in Table 1. The chemical composition of diet components is shown in Table 2.

Table 2. Chemical composition (g/kg DM) and gross energy (GE) of alfalfa hay, concentrate and feed blocks (n=3)

Item	Alfalfa hay	Concentrate	Feed block
DM, g/kg fresh matter	906	926	907
OM	881	893	814
CP	212	170	165
NDF	417	338	466
ADF	251	143	273
ADL	59	25	44.7
Ether extract	13.8	34.1	7.29
GE, MJ/kg DM	18.2	18.2	16.0

Diet did not affect total dry matter intake or animals live weight (data not shown). The ACB diet resulted in a 38.3% reduction (P=0.001) of CH_4 emission (Table 3). It has been reported that plant secondary compounds, like tannins or saponins, could modify rumen fermentation,

inhibiting enteric methanogenesis (Guo *et al.*, 2008; Patra and Saxena, 2010). The presence of these compounds in tomato byproducts may have contributed to the methane reduction by (i) decreasing OM fermentation in the rumen and increasing OM fermentation in the intestine; (ii) diverting hydrogen away from CH_4 production; and (iii) and inhibition of microbial activity or optimization of rumen fermentation, decreasing methane emission per unit of OM digested. However, the association between the antimethanogenic effect of plant secondary compounds and their molecular structure and weight together with chemical composition of diets, should be taken into account (Newbold *et al.*, 2004).

Table 3. Methane emissions, milk production and fatty acid composition (g/100 g of identified FA), and milk recovery rate (g of FA excreted in milk/g of FA intake) of selected fatty acids of goats fed with different experimental diets

Item	Diet†		SEM	*P*-value
	AC	ACB		
CH_4 emission, L/kg DMI	28.2[b]	17.4[a]	0.819	0.001
Milk yield, ml/d	997	922	94.16	0.372
Total fat, g/L milk	56.2	56.8	3.589	0.846
Total saturated FA	74.7	73.4	0.650	0.319
Total Monounsatuared FA	19.7	20.3	0.672	0.565
Poly unsaturated FA				
cis-9, *cis*-12, 18:2	1.94[a]	2.17[b]	0.069	0.048
cis-9, *trans*-11, CLA	0.57	0.62	0.061	0.487
trans-11, *cis*-15 18:2	0.037[a]	0.053[b]	0.006	0.033
cis-6, *cis*-9, *cis*-12, 18:3	0.017	0.025	0.002	0.503
cis-9, *cis*-12, *cis*-15, 18:3	0.39[a]	0.54[b]	0.035	0.028
20:3 n-6	0.020	0.027	0.005	0.244
20:4 n-6	0.20	0.23	0.012	0.082
Total Polyunsaturated FA	3.34[a]	3.83[b]	0.149	0.012
FA recovery rate				
cis-9, *cis*-12, *cis*-15, 18:3	0.090	0.12	0.011	0.063
cis-9, *cis*-12, 18:2	0.13[a]	0.17[b]	0.020	0.021
[3]*cis*-9 18:1	1.16[a]	1.48[b]	0.194	0.012
18:0	1.57	1.63	0.204	0.569

†AC = Alfalfa hay and concentrate (1:1); ACB = alfalfa hay, concentrate (1:0.65) and tomato feed block. *About 2.4 of non identified fatty acids.

Milk yield, as well as fat and protein concentrations, were not affected ($P \leq 0.907$) by the inclusion of feed block in the diet, which may be due to the similar energy concentration and rumen protein degradability of both diets. Dietary intakes of linoleic (LA; *cis*-9, *cis*-12, 18:2) and linolenic (LNA; *cis*-9, *cis*-12, *cis*-15, 18:3) acids (data not shown) showed that goats feeding the diet including tomato fed block consumed less unsaturated fatty acids than those under the control diet. However, milk from goats fed the diet including block showed higher content of LA ($P=0.048$), LNA ($P=0.028$) and total polyunsaturated fatty acids (PUFA) ($P=0.012$); a concomitant tendency ($P=0.054$) to decrease 18:0 (SA) concentration (11%) was also observed when tomato feed block was fed. Regarding *cis*-9, *trans*-11CLA concentrations, milk fat of goats on tomato feed block diet remained unchanged ($P=0.487$) in comparison to milk from animals fed diet AC. The higher ($P<0.033$) accumulation of *trans*-11, *cis*-15, 18:2 in milk from goats fed diet including tomato fed block would suggest an incomplete biohydrogenation of LNA. In addition to the fatty acid supply, other factors such as energy supply, the proportion of fibre or

concentrate as well as the presence of plant secondary compounds (Leiber *et al*., 2005; Vasta *et al.*, 2008) should be considered when dietary effects on milk fatty acid profile are assessed. Since the fatty acid supply was lower in the ACB diet than in the AC diet, and taking into account that energy and fiber intakes were similar for goats fed both diets (data not shown), it might be that the observed effect in milk fatty acid composition could have been due to the administration of the ingredients in the feed block. The synchronous and fractionated supply of nutrients allowed when using feed blocks in ruminants feeding (Ben Salem and Nefzaoui, 2003) may have been associated to a better fatty acid absorption in the small intestine. Additionally, it could be speculated that during the manufacture of the block tomato seeds were partially crushed so one fraction of fatty acids may have been accessible to biohydrogenating bacteria leading to accumulation of biohydrogenation intermediates, whereas another fraction could have been still protected and therefore higher amounts of PUFA would have been available in the small intestine for absorption. Fatty acid recovery rates were higher for LA and oleic acid (OA; *cis*-9, 18:1) when feeding diet ACB which would support the previous speculation. This finding was in agreement with those of Abbeddou *et al.* (2011) and Khiaosa-Ard *et al.* (2010) who showed increased linoleic acid recovery rates in milk following decreased linoleic acid intakes. Also, other components of the tomato by-products might have been involved in the observed effect; for example, tannins and saponins have been shown to be of potential usefulness in controlling biohydrogenation (reviewed by Lourenço *et al.,* 2010). Overall, milk of goats fed tomato block diet could be considered of healthier fatty acid composition as increased amounts of LA and LNA (1.12 and 1.38 fold, respectively) in the milk would be important due to their beneficial effects in the prevention of cardiovascular diseases and hypertension in humans.

Total N and amino acid profile of milk (Table 4) were unaffected by diet ($P \geq 0.215$) with the exception of phenylalanine that was lower ($P=0.039$) in the milk of goats fed diet ACB compared to AC. Taking into account the relative proportions of cereals in concentrate and in feed blocks, their average intakes in the current study, and the cost of cereals and milk (MARM, 2012), a reduction in feeding cost of 22% may be achieved with diets containing feed blocks compared to the control diet.

IV – Conclusions

Feed blocks based on tomato fruits wastes could replace 35% of the concentrate in dairy goats diet without detrimental effects on milk production and fatty acid or amino acid composition. Overall, diet including tomato feed blocks promoted the production of milk with healthier fatty acid profile and reduced feeding cost and the environmental damage associated with by-products accumulation and CH_4 emissions compared with a conventional diet.

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Table 4. Total N and amino acids profile of goats milk fed with different experimental diets.

Item	Diet†		SEM	*P*-value
	AC	ACB		
Total N, mg/g DM	40.6	40.4	1.21	0.907
Total amino acids, g/L milk	5.10	4.91	0.234	0.451
Total amino acids††, g/100g total N	84.0	84.6	2.78	0.900
AA, g/100 g total AA				
Aspartic acid	6.38	6.69	0.254	0.244
Glutamic acid	18.5	18.6	0.331	0.867
Serine	4.47	4.64	0.091	0.332
Glycine	1.72	1.75	0.042	0.667
Histidine	3.57	3.59	0.044	0.753
Arginine	2.50	2.79	0.113	0.237
Threonine	4.62	4.63	0.096	0.962
Alanine	3.10	3.15	0.131	0.687
Proline	8.20	8.28	0.189	0.791
Tyrosine	4.61	4.70	0.168	0.528
Valine	5.54	5.49	0.141	0.843
Methionine	8.15	7.65	0.571	0.744
Cysteine	4.14	4.23	0.419	0.902
Isoleucine	4.38	4.31	0.041	0.460
Leucine	7.53	7.43	0.123	0.592
Phenylalanine	4.28[b]	4.12[a]	0.073	0.039
Lysine	8.31	7.94	0.104	0.215
EAA†††	48.9	48.0	0.489	0.290
NEAA††††	51.1	52.0	0.480	0.285

†AC = Alfalfa hay and concentrate (1:1); ACB = alfalfa hay, concentrate (1:0.65) and tomato feed block. ††Without tryptophane. †††EAA: threonine, arginine, valine, metionine, isoleucine, leucine, phenylalanine, lysine and histidine. ††††NEAA: alanine, aspartic acid, glutamic acid, glycine, proline, serine, tyrosine and cysteine.

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Reproductive response of female and male sheep to targeted introduction of cactus (*Opuntia ficus-indica f. inermis*) cladodes in the diet

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Abstract. At some critical stages, reproduction in sheep is an energy demanding function and when additional feed is provided at an appropriate timing of the animal physiological stage, it can boost sperm production, increase ovulation rate and improve offspring survival through a better colostrum synthesis. A series of trials are reported to test effects of supplementation with cactus cladodes on reproductive response of Barbarine sheep. In most trials, cactus is compared to conventional concentrates generally referring to isonitrogenous and isoenergetic supplements. When incorporated in the diet of late pregnant-early suckling ewes, colostrum immunoglobulin G concentrations averaged 160 and 149 g/l (S.E.M. = 12.9) in the barley and cactus groups, respectively. Milk yield at 30 days was 1030 and 1041 g/day (S.E.M. = 96.9; P>0.05) for barley and cactus, respectively. We tested effects of supplementation with cactus on prolificacy components. Ewes receiving cactus had higher number of large preovulatory follicles (≥ 6 mm; 1.08 ±0.05), between Days 14 and 19 after introduction of rams, than females supplemented with concentrate or soybean meal (0.64 ±0.06; P<0.05). In another study, cactus ewes had 1.6 ± 0.2 and concentrate sheep had 1.2 ± 0.2 large follicles on estrus day (P < 0.05). Ovulation rate was highest in sheep fed with cactus for 6–10 days (1.7 ± 0.1) than in ewes supplied with cactus for more than 11 days (1.3 ± 0.1; P < 0.05), in sheep fed with concentrate for 6–10 days (1.2 ± 0.1; P< 0.01) and even than in individuals subjected to classical flushing with concentrate (1.3 ± 0.1; P < 0.05). During the mating season, maiden ewes were allowed to graze natural pastures and received per female per day either 0.45 kg of a commercial concentrate (CC), a mixture of 3.5 kg of cactus cladodes and 70 g of soybean meal (CAS) or ad-libitum access to cactus and olive cake-based feed blocks (FB). The percentage of lambing ewes differed (P<0.05) being 73%, 90% and 70% for CC, CAS and FB groups respectively. In rams and at the end of a 75-day period of supplementation with cactus or concentrate, total number of sperm/ejaculate averaged 5.9±2.2 109 and 4.9±2.9 109 for respectively cactus and concentrate rams. Over an 8-hour sampling period, cactus rams had a mean number of 1.83±0.408 pulse of testosterone in comparison to 1.33±0.516 pulse for concentrate rams (P=0.07). Concomitant figures of LH for the same sampling interval were 0.22 and 0.12 ng/ml for cactus and concentrate rams respectively (P>0.05). Real, practical applications for the inclusion of cactus at critical points of the reproductive calendar emerge from these results.

Keywords. Reproduction – Sheep – Supplementation – Cactus cladodes.

Réponse reproductive des ovins mâles et femelles à l'introduction ciblée des raquettes de cactus (*Opuntia ficus-indica f. inermis*) dans la ration

Résumé. *À des stades critiques, la reproduction chez les ovins est une fonction exigeante en apports énergétiques et quand de l'aliment complémentaire est apporté, il peut promouvoir la production spermatique, augmenter le taux d'ovulation et améliorer la survie néonatale au travers d'une meilleure synthèse colostrale. Ce papier rapporte une série d'essais qui testent les effets d'une complémentation à base de raquettes de cactus sur la réponse reproductive des ovins de race Barbarine. Dans la plupart des essais, le cactus est comparé à des concentrés et les deux types de compléments sont calculés pour être iso-énergétiques et iso-azotés. Suite à l'incorporation dans la ration de brebis en fin de gestation – début d'allaitement, les concentrations colostrales en immunoglobulines G étaient en moyenne de 160 et 149 g/l (E.S.M. = 12,9) pour les brebis recevant de l'orge ou du cactus respectivement. Le rendement en lait à 30 jours était de 1030 et 1041 g/jour (E.S.M. = 96,9; P>0,05). Nous avons aussi testé la complémentation avec*

le cactus sur les différentes composantes de la prolificité. Entre les jours 14 et 19 après introduction des béliers, les femelles recevant le cactus avaient un plus grand nombre de larges follicules (≥ 6 mm) que celles recevant du concentré ou du tourteau de soja (1,08 ± 0,05 vs. 0,64 ±0,06; P<0,05). Dans une seconde étude, les brebis au cactus avaient 1,6 ± 0,2 et celles au concentré 1,2 ± 0,2 follicules larges le jour de l'oestrus (P<0,05). Le taux d'ovulation était plus élevé pour les brebis complémentées pour 6-10 jours avec du cactus (1,7 ± 0,1) que celles recevant le même complément pour plus de 11 jours (1,3 ± 0,1; P<0,05), celles recevant du concentré pour 6-10 jours (1,2 ± 0,1; P<0,01) ou même celles soumises à un flushing classique avec du concentré (1,3 ± 0,1; P<0,05). Durant la saison de lutte, les antenaises étaient mises sur parcours naturels et recevaient par femelle et par jour soit 0,45 kg de concentré (CC), soit un mélange de 3,5 kg de raquettes de cactus et 70 g de tourteau de soja (CAS) ou bien un accès à volonté à des blocs multi-nutritionnels à base de cactus et grignons d'olive (FB). Le % de femelles mettant bas était de 73%, 90% et 70% pour les traitements CC, CAS et FB respectivement. Au terme d'une phase de 75 jours de complémentation avec du cactus ou du concentré chez des béliers, le nombre total de spermatozoïdes/éjaculat a atteint 5,9±2,2 109 et 4,9±2,9 109 chez respectivement le lot cactus et concentré. Sur une période de 8 heures de prélèvements, les béliers complémentés au cactus avaient une moyenne de 1,83±0,408 pulses de testostérone en comparaison à seulement 1,33±0,516 pulses pour les béliers recevant du concentré (P=0,07). Les concentrations de base de LH pour la même période étaient de 0,22 et 0,12 ng/ml pour les béliers recevant le cactus et le concentré respectivement (P>0,05). Des possibilités pratiques et réelles pour l'inclusion du cactus à des stades critiques du calendrier reproductif des ovins de race Barbarine émergent des résultats présentés.

Mots-clés. *Reproduction – Ovins – Complémentation – Raquettes de cactus.*

I – Introduction

It is widely accepted that nutrition exerts major effect on reproductive responses in males and females. Nutrition status influences virtually all aspects of female reproductive performance starting at the beginning of fetal life to their oocyte and embryo quality (Ferguson *et al.*, 2003, Adamiak *et al.*, 2005). The nutritional needs are critical for each stage of the reproductive process, from conception to puberty to the start of productive life (Blache and Martin, 2009). Nutritional levels before mating are particularly important to subsequent reproductive success in sheep and high quality food supplies are needed. Synchronization of food supply and the physiological events is critical and it has been shown that reproductive performance of sheep and goats can be improved by using short, targeted feeding regimes: "focussed feeding" (Martin *et al*., 2004).

In low-input systems, concentrate feeds and/or high-quality pastures can be a limiting resource for a sustainable animal production. Concentrates are expensive and not always available to farmers in remote rural areas (Table 1). Thus, resorting to alternative feed supplements (e.g. agro-industrial by-products, feed blocks, fodder trees and shrubs), less expensive than conventional supplements (e.g. barley) and locally available is recommended (Ben Salem and Smith, 2008).

A possible option for sheep reared under harsh arid climatic and nutritional conditions (most of the countries of North Africa, Latin America and West Asia) is the abundant native cacti; specifically, the spineless-cactus or nopal (*Opuntia ficus-indica f. inermis*). Feeding with cactus cladodes is an economic supplement for sheep having very low quality diets (Ben Salem *et al*., 2004); being mainly used as an emergency feed supplement for ruminants in periods of severe drought (Ben Salem and Smith, 2008). Cactus cladodes have a high proportion of water (850–900 g/kg) but, at the same time, have a high energy-content, providing up to 700 g/kg dry matter (DM) of carbohydrates (Nefzaoui and Ben Salem, 2002).

To our knowledge, there are no previous data from other countries, on the use of energetic supplementation with cactus for enhancement of reproductive traits in sheep. This paper aims to summarize a series of trials that were designed in central semi-arid Tunisia to investigate the effects of supplementation with cactus cladodes on reproductive response of sheep and confirm

the hypothesis that locally available cactus can be incorporated in the diets with no adverse effects on the performances. In most trials, cactus is compared to conventional concentrates generally referring to isonitrogenous and isoenergetic supplements and in all the reported trials, sheep of the Barbarine breed were used.

Table 1. Import prices in Tunisia of major animal feed ingredients (US $ and Tunisian Dinar TD/Ton)

Feed ingredient		2005	2006	2007	2008	2009	2010	2011
Corn	$/Ton	136.18	150.09	238.96	296.17	191.59	227.35	315.61
	TD/T	170.713	198.918	302.995	360.540	256.880	325.303	444.721
Soybean meal	$/T	258.65	245.76	309.61	449.34	445.73	395.09	424.91
	TD/T	329.528	327.47	396.183	551.899	606.222	583.212	583.671
Barley	$/T	149.92	165.24	264.27	303.71	176.56	205.31	295.31
	TD /T	191.374	219.347	338.121	374.615	238.792	298.050	419.798

Source: Direction Générale de la Production Agricole, Ministère de l'Agriculture de Tunisie.

II – Cactus incorporation in the diet of ewes prior to mating

The effects of mid or short-term nutritional supply with cactus cladodes prior to mating on fertility, follicle development and ovulation rate in sheep in comparison to other conventional feeding sources were undertaken.

1. Lambing rate of maiden ewes

During spring-time mating season, 90 maiden aged 18 months old (average live weight 29.7±2.53 kg) of the Barbarine breed were allowed to graze natural pastures and received per female per day either 0.45 kg of a commercial concentrate (CC), a mixture of 3.5 kg of cactus cladodes and 70 g of soybean meal (CAS) or ad-libitum access to cactus and olive cake-based feed blocks (FB). Feed blocks were mainly composed of 44% olive cake, 30% wheat bran, 8% cactus cladodes, 4% urea and 5% salt. Further details of the experimental protocol are reported by Sakly *et al*. (2012). Throughout the experimental period, the animals were allowed to graze available vegetation cover of native *Medicago* spp. Supplementation lasted during the entire mating period that lasted 60 days. Lambing date and the number of lambs born per female were recorded. A proportion of 93% females displayed oestrus at least once, with no differences between feeding regimes. However, the percentage of lambing ewes differed ($P<0.05$) being 73%, 90% and 70% for CC, CAS and FB groups respectively. This experiment which is more a field trial, revealed improvement of fertility of cactus-supplemented ewes over concentrate or feed block counterparts. This result is important for extensive systems of semi arid and arid regions where fertility is the most important productive trait, ensuring birth of a lamb.

2. Follicular growth of cactus or concentrate-supplemented ewes synchronised with FGA intra-vaginal sponges

Preliminary work to investigate the effect of cactus feeding on follicular growth of sheep was undertaken using 30 adult ewes of the Queue Fine de l'Ouest that were synchronized with vaginal sponges (40 mg Flurogestone Acetate). Ewes were divided into two groups balanced for body condition score and were either flushed for 14 days (duration of sponges insertion) with 300 g per ewe/day of a concentrate composed of barley, soybean meal and a mineral and vitamin supplement or were supplemented with 3000 g of shopped cactus pads and 70 g of soybean meal per ewe/day for the last 6 days before sponges' removal. At the time of sponges removal (Day 0) and 48 hours later (Day 2) expected to be day of estrus, number of follicles according to their size were determined by transrectal ultrasonographic assessment. Three

rams of the QFO breed were introduced in each group of 15 ewes at the time of sponges' removal. Ewes supplemented with cactus for only 6 days prior to sponges removal had a higher number of large follicles on their ovaries on day 0 ($P<0.01$) but also on the day of estrus ($P<0.05$) (Table 2).

Table 2. Total number of follicles (average/ewe) on the day of estrus for synchronized Queue Fine de l'Ouest ewes supplemented with concentrate or cactus

Feeding regime	Number of ewes	Follicle diameter		
		4 mm	5 mm	≥ 6 mm
Concentrate (14 days)	15	30a (2)	15a (1)	18a (1.2)
Cactus (6days)	15	30a (2)	16a (1.06)	28b (1.86)

3. Follicular growth of cactus or concentrate-supplemented ewes induced to breed with the ram effect

In this experiment, a total of 120 seasonally anoestrous ewes, grazing natural pastures were distributed in 4 equal groups supplemented with either cactus cladodes (CA; 3.5 kg/ewe/day), cactus cladodes and soybean meal (CAS; 3.5 kg cactus and 70 g soybean meal/ewe/day), concentrate (CC; 0.45 kg/ewe/day) or soybean meal only (S; 70 g/ewe/day). The ewes were induced to breed with the ram effect (Day 0) and supplementation was initiated day 10 and lasted until day 30 after the introduction of rams. The appearance and growth of preovulatory follicles (≥ 6 mm) was evaluated in 60 ewes randomly chosen (n = 15 in each experimental group). Screening was performed daily by the same operator, starting at Day 14 after introduction of rams and lasting until onset of oestrus behaviour or until Day 19 in those ewes not displaying oestrus. The choice of this time interval is based upon oestrous distribution of Barbarine flocks in the same station during 5 consecutive years showing an important peak of oestrus between days 14 and 19 following start of the mating season (M. Rekik, unpublished data). Observations of the ovaries were done by 7.5 MHz transrectal ultrasonography (Aloka SSD-500; Ecotron, Madrid, Spain), as described previously and validated in sheep (González-Bulnes *et al*., 1994). Cactus fed ewes tended to have more large follicles as depicted in Fig. 1 where representation of the variation of the number of large follicles is restricted to those animals that displayed oestrus during the 6 day observation period (n =11 for animals of both CA and CAS groups and n = 9 for animals of both CC and S groups). For animals supplemented with cactus (CA and CAS) and those supplemented with concentrate or soybean meal (CC and S), there was an increase in the number of large follicles prior to oestrus (Fig. 1) that was not different 2 and 1 days prior to oestrus but tended ($P = 0.1$) to be higher for ewes receiving cactus at the day of oestrus (1.62 ± 0.20 for CA and CAS versus 1.25 ± 0.20 for CC and S ewes).

4. Ovulation rate of cactus or concentrate-supplemented ewes induced to breed with the ram effect

In this experiment (for full details, please refer to Rekik *et al*., 2012), 76 non-lactating adult Barbarine sheep during anoestrous season are used. The ewes grazed natural pasture and were induced to breed with the ram effect by the introduction of 10 harnessed rams (Day 0). Starting day 10 after introduction of ram and until day 30, ewes in the Concentrate feeding regime were supplemented with a soybean meal and barley based concentrate (0.45 kg per ewe and day). Each ewe in group Cactus received, daily, 3.5 kg of fresh cactus cladodes; for

feeding the animals, terminal and sub-terminal cactus cladodes were regularly harvested and cut into small slices using a manual chopper. To balance energy and crude protein provision between Cactus and Concentrate ewes, a little quantity (0.075 kg) of soybean meal was added to Cactus ewes. The nutritional necessities were calculated according to Agricultural and Food Research Council (AFRC) Manual (1993) for daily maintenance requirement (Mm) based on ME [(Mm (mJ/day)) = (FCA)/Km, where F is the fasting metabolism and A the activity allowances]. In response to the male effect, 69 ewes came into estrus (90.8%) without differences between Cactus and Concentrate groups (34 and 35 females respectively). Results on ovulation rate, determined by transrectal ultrasonography, indicate, first, that a short-term supplementation with cactus cladodes increases ovulation rate when compared to supplementation with concentrate (1.5 ± 0.1 in Cactus ewes and 1.3 ± 0.1 in Concentrate sheep ($p < 0.01$)). Second, duration of cactus supplementation was found to have a significant effect on ovulation rate; ovulation rate was highest in sheep fed with cactus for 6–10 days (1.7 ± 0.1) than in ewes supplied with cactus for more than 11 days (1.3 ± 0.1; $P < 0.05$), in sheep fed with concentrate for 6–10 days (1.2 ± 0.1; $P< 0.01$) and even than in individuals subjected to classical flushing with concentrate (1.3 ± 0.1; $P < 0.05$).

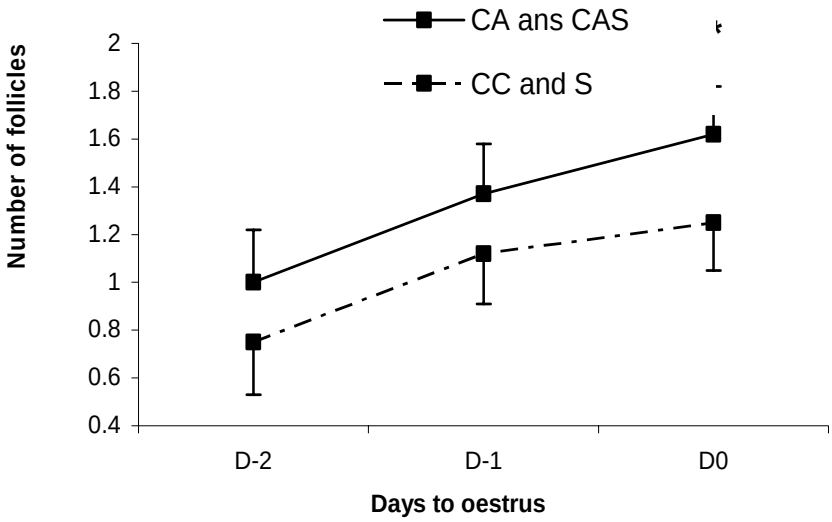

Fig. 1. Variation of the number of large follicle (≥6 mm) of ewes supplemented with cactus or cactus-soybean meal (CA & CAS) and concentrate or soybean meal (CC & S).

III – Cactus incorporation in the diet of ewes during end of pregnancy – early suckling

The investigation of the effects of cactus incorporation in the diet of the late pregnant-early suckling ewe was undertaken. The objective of this study was to investigate effects of total replacement of barley grain by cactus on mammary secretions, growth of lambs, blood metabolite levels and resumption of postpartum ovarian activity in singleton-bearing ewes of the Barbarine breed (Rekik *et al*., 2010). Thirty-four single bearing ewes of the Barbarine breed that were oestrus synchronised were selected. Animals were allocated to either barley treatment or

to a cactus treatment. The trial lasted approximately 60 days and started 4 weeks before lambing and continued until 30 days postpartum. Cactus-fed ewes tended to accumulate more colostrum at birth and yielded more colostrum to 24 h than barley ewes but differences were not statistically significant (P > 0.05). There were no differences between both treatments in IgG concentration in accumulated colostrum at lambing, which averaged 160 and 149 g/l (S.E.M. = 12.9) for barley and cactus, respectively. Milk yield at day 10 and 30 from birth was not affected by treatment (P > 0.05). Milk yield at 30 days was 1030 and 1041 g/day (S.E.M. = 96.9) for barley and cactus, respectively. At 10, 20 and 30 days postpartum, the number of ewes having resumed their ovarian activity was not different (P > 0.05). At 30 days after lambing, respectively, 9 and 6 ewes in the cactus and barley groups had ovulated.

Most of the measured physiological and productive traits in this experiment were unchanged or non significantly improved when barley grain was totally replaced by cactus cladodes in the diet of late pregnant-early suckling ewes of the Barbarine breed. It is concluded that cactus can totally replace barley grain in the diet of late pregnant-early suckling Barbarine ewes without affecting mammary secretions, resumption of ovarian activity or lamb growth.

IV – Cactus incorporation in the diet of straw-fed rams

The effect of barley substitution by cactus pads on testicular traits and blood metabolites of rams was studied. A total of 12 Barbarine rams fed straw (1.2 kg/head/day) were used and they were allocated to two groups supplemented with concentrate made of barley and soybean meal (0.6 kg/head/day) or cactus (6 kg of fresh pads and 110 g of soybean meal per head per day). The feeding regimes were applied for a period of 75 days. Throughout the trial, little differences were depicted between animals in the two groups with regards levels of proteins, urea and glucose in plasma. Apparent digestibility of dry matter (P <0.05) and the organic matter (P <0.001) in the concentrate regime was higher than for the cactus regime. From day 50 of application of the feeding regimes and until the end of the trial, live weight increase of rams receiving concentrate was higher (P<0.05) than those supplemented with cactus and this trend was paralleled by plasma leptin levels (Fig. 2). Scrotal diameter increased for animals in the two groups but was similar (P>0.05) during all the trial. Similarly, the volume of the ejaculate, sperm concentration and the total number of sperms produced did not differ between animals in the two groups. Throughout the experiment, the total number of sperms tended to increase in both groups varying from 2.6±1.3 10^9 to 5.9±2.2 10^9 sperms in the cactus group and from 2.3±0.6 10^9 to 4.9±2.9 10^9 Spz in the concentrate treatment group. Plasma testosterone concentrations measured every 20 minutes during a sampling period of 8 hours in the beginning and the end of the trial were also similar (P>0.05) in the two experimental groups. However, the average number of pulses/animal at the end of the trial tended (P=0.07) to be more elevated for animals in the cactus regime in comparison to rams receiving concentrate (1.83±0.408 vs. 1.33±0.516). It is concluded that for a supplementation period up to 75 days, cactus can totally replace barley in the diet of Barbarine rams with no adverse effects on the studied testicular traits.

V – Conclusion

At any stage of the reproductive function in Barbarine sheep, did cactus incorporation in the diet depress measured reproductive traits. Even when total substitution of concentrate by cactus was associated with a decline of live weight such as with the rams, sperm output was not affected and endocrine signals (here testosterone pulses) were improved. Improvements were very often observed with those traits very sensitive to nutrients flow like follicular growth and ovulation rate. Best results are obtained when the supply is very short (< 10 days) and this would classify supplementation with cactus cladodes as an immediate, acute effect. One possible pathway to explain some of the observed positive reproductive response may be the fermentable starch content; in agreement with the hypothesis of Vinõles *et al*. (2009), for whom

the best results of short-term food supplementation on reproduction in sheep are obtained when the feeding supplement has high levels of fermentable sugars. According to Ayadi *et al*. (2009), spineless cactus cladodes originating from central Tunisia have high soluble sugars content (over 60 g/kg DM of which 90.33% is represented by fructose) which is responsible for improving rumen fermentation (Ben Salem *et al*., 2004). A possible synergy between sugars from cactus and the small protein supply from soybean meal, that has always been added to balance nitrogen supply, should not be discarded.

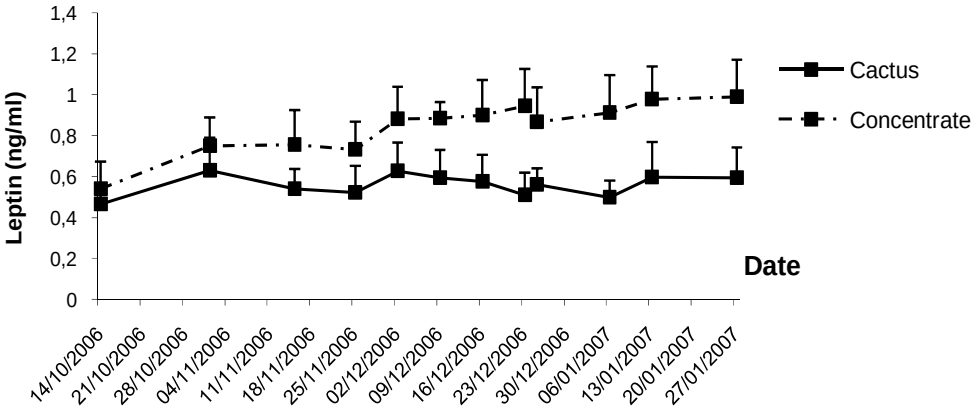

Fig. 2. Plasma leptin concentration for concentrate or cactus supplemented Barbarine rams.

In conclusion, spineless cactus cladodes may be considered as a less-expensive alternative to conventional concentrate supplements for preserving or even improving the reproductive efficiency in semi-arid regions. This technical option would fit the global concept of developing nutritional strategies that are based on locally available, culturally accepted by sheep owners, cheap and sustainable feeding resources.

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Tables of nutritive values for farm animals in tropical and Mediterranean regions: An important asset for improving the use of local feed resources

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Abstract. The demand for livestock products has been growing steadily in emerging and developing countries, and this requires a better knowledge of available animal feeds. However, in these countries, available information is either obsolete or about temperate feedstuffs. The project "Tables of nutritive values for farm animals in tropical and Mediterranean regions" led by INRA, CIRAD and AFZ (and supported by FAO) aims to create an updated and comprehensive set of datasheets for more than 700 fodders and raw materials. The datasheets provide information on physical descriptions, feed availability and environmental impact, as well as feeding recommendations and nutritional values of local feedstuffs for the main farm animal species. The first goal of the project is to better identify and characterize local feed resources in order to improve the technical and economic performance of farms. Nutrition modelling, collaboration between research teams and identification of gaps in knowledge are part of the scientific objectives. The datasheets are created by a group of 20 scientists and engineers, who rely on a massive collection of scientific literature and experimental data to write qualitative and quantitative syntheses (via methods such as meta-analysis) and build representative and consistent tables of composition and nutritional values. The publication of the datasheets is due in 2013, first as a website (www.feedipedia.org) and later as a book.

Keywords. Feed evaluation – Tables – Farm animals – Tropical and Mediterranean areas.

Tables de la valeur nutritive des aliments pour les animaux domestiques dans les régions tropicales et méditerranéennes : Un important atout pour améliorer l'utilisation des ressources alimentaires locales

Résumé. *La demande en produits animaux augmente dans les pays émergents et en développement avec comme corollaire un besoin d'information pour les aliments locaux destinés aux animaux. Cependant, les tables d'aliments disponibles dans ces pays sont obsolètes ou issues des aliments des pays tempérés. Le projet "Tables de la valeur nutritive des aliments pour les animaux domestiques dans les régions tropicales et méditerranéennes" conduit par l'INRA, le CIRAD et l'AFZ (et soutenu par la FAO) a pour but la création d'un ensemble de 700 fiches à jour et cohérentes concernant des matières premières ou des fourrages. Les fiches donnent des informations, comme la description physique, la disponibilité et l'impact environnemental, les recommandations alimentaires et la valeur nutritive pour les principales espèces d'animaux domestiques. Ce projet a d'abord pour objectif de mieux identifier et caractériser les ressources locales afin d'augmenter les performances techniques et économiques des fermes. La modélisation, les collaborations entre les équipes de chercheurs et l'identification des trous dans la connaissance font partie des objectifs scientifiques. Les fiches sont créées par un groupe d'une vingtaine de chercheurs et ingénieurs qui se basent sur une importante masse de données issues de la littérature ou de résultats expérimentaux pour écrire les synthèses quantitatives et qualitatives (en utilisant des méthodes comme les méta-analyses) et construire des tables de composition chimique et de valeur nutritive. La publication des fiches est prévue pour 2013, d'abord sur un site web (www.feedipedia.org), puis sous forme de livre.*

Mots-clés. *Valeur nutritive – Tables – Animaux domestiques – Régions tropicales et méditerranéennes.*

I – Introduction

There is an abundance of studies concerning the nutritive value of feeds. However, these studies are often difficult to synthesize due to their dispersion, heterogeneity and to the various languages used (Morand-Fehr and Lebbie, 2004). In this context, the approach chosen for the Feedipedia project is valuable (Devendra and Leng, 2011).

II – Historical perspective

Profitable and sustainable animal production can only be achieved by the rational use of feed resources, which requires comprehensive and updated information about both the chemical composition and nutritional values of feeds and feeding recommendations. These data are usually collected in feed tables that allow farmers to formulate diets that meet animal requirements at the lowest cost. From the late 19th century up to the 1980s, spurred by breakthroughs in animal nutrition and chemistry and by the establishment of agricultural experimental stations, feed tables have accompanied the rise of animal productions, particularly in developed countries (Europe and United States). Numerous landmark books have been written. In the USA, *Feeds and feeding* was published by Henry in 1898 and revised several times by Morrison until 1956. Another important compendium was *Feeds of the World* (Schneider, 1947). In Germany, Becker and Nehring published *Handbuch der Futtermittel* in 1965. In North America, NRC published the *Atlas of nutritional data on United States and Canada feeds* in 1971. In France, INRA published *Alimentation des ruminants* (Jarrige, 1978) and other tables. Tables for non-Western countries were also published: *Latin American tables of feed composition* (McDowell *et al.*, 1974), *Nutrient composition of some Philippine feedstuffs* (Castillo and Gerpacio, 1976), *Malaysian feedingstuffs* (Devendra, 1979), *Middle East feed composition tables* (Kearl *et al.*, 1979), and the fundamental FAO book *Tropical feeds* (Göhl, 1975), which summarized one century of worldwide research. One of the latest global efforts was the CIHEAM book: *Tables of the nutritive value for ruminants of Mediterranean forages and by-products* (Tisserand and Alibes, 1991).

In the past decades, attempts at summarizing feed information at international level have been less successful. FAO's International Network of Feed Information Centres (INFIC), which provided data to some of the tables cited above, stopped its activities in the 1980s and a similar project by the European Union (ENFIC) failed to take off in 1996. Today, initiatives are mostly local: feed tables targeting specific animal production systems are published in several countries and regions, including the USA, Brazil, China, India, France, the Netherlands, Germany, Spain and Scandinavia. Their content varies: some tables are built on top of comprehensive database systems (France, Netherlands) while others are compilations of previous tables mixed with local data. Some of them are based on research reports from agricultural stations and are thus purely regional.

Local tables are highly relevant since animal production systems, including animals, feeds, agricultural practices and even some scientific concepts are generally specific to a geographical area. Still, the current situation of feed information is not fully satisfying. During the past two decades, human consumption of animal products increased in developing countries whereas global animal production was shifting from industrial to developing regions (Rae and Nayga, 2010). However, only a few countries have been able to develop national feed tables including accurate and updated feed information. Outside developed countries, and even in some developed ones, users must rely on data obtained in other agronomic conditions or use obsolete or incomplete sources. New productions (such as aquaculture) are still poorly represented in feed tables. Information about non-conventional feeds – which may be locally significant – is still relatively difficult to obtain and many of these feeds are often badly known and described in the literature. The latter point is of particular importance in emerging and developing countries where feed resources available locally are often under-utilized due to a lack of information. Moreover, feed efficiency is no longer the sole criteria of performance and

new concerns have arisen. The impact of feeding on the sustainability and environmental footprint of animal productions, its effect on the quality and safety of animal products as well as on animal health and welfare need to be taken into account in livestock farming practices. As a consequence, the global animal response has become more complex and must include new criteria besides the usual physiological and economic performance parameters. Feed tables will have to adapt their content to this rapidly evolving multi-criteria approach in animal feeding.

Paradoxically, feed information has never been so abundant. The widespread implementation of quality control in feed laboratories, notably in the private sector, generates large amounts of data. Also, feed data, which were once produced almost exclusively in Western countries (or by Western organizations established in developing countries), are now being produced massively outside Europe and North America, for the benefit of the local animal production sectors. This information, however, remains largely scattered in papers, books, reports and dissertations.

III – The Feedipedia project

The Feedipedia project results from the merging of two projects. The first one was launched by INRA, CIRAD (two French public research institutes in agriculture) and AFZ (French association for animal production, in charge of the French Feed Database). INRA and AFZ collaborated to publish in 2002 the French feed tables (Sauvant *et al.*, 2002). After this book was translated into other languages, it emerged that there was a strong interest in other countries, especially in Mediterranean and tropical regions, for a similar work that would contain information on local feeds. INRA and AFZ then collaborated with CIRAD (specialized in tropical agriculture) to start a project on international feed tables for tropical and Mediterranean regions. The second project originated from FAO and aimed at renovating the Animal Feed Resources Information System (AFRIS) website, which was the on-line version of the *Tropical feeds* book cited previously. While being an important resource for feed information, particularly for non-conventional feeds, the AFRIS website needed to be updated: the median year of publication of the papers originally reviewed for *Tropical feeds* was 1960. Moreover, AFRIS data mostly concerned ruminants, with limited information on pigs, poultry and other species. In 2009, FAO, INRA, CIRAD and AFZ agreed to collaborate for the creation of an updated version of AFRIS, named Feedipedia, using the methodology developed in 2002 by INRA and AFZ.

Feedipedia is an open access information system on animal feed resources that provides information on the nature, occurrence, chemical composition, nutritional value and safe use of more than 1,300 worldwide livestock feeds. Its main objective is to provide extension workers, planners, project formulators, feed manufacturers, farmers, science managers, policy makers, students and researchers with the latest scientific information. Two series of benefits are expected. Feedipedia should help feed users to better identify, qualify and quantify local feed resources, resulting in improved technical, economic and environmental performance in the livestock sector, better opportunities for livestock in sustainable integrated farming systems and a better use of local feeding practices with less reliance on imported feeds and techniques. In a near future, it will also be possible to include quantitative environmental data in the tables. Feedipedia should also help to promote collaboration between research teams working on tropical and Mediterranean animal feeding to identify areas of incomplete knowledge, thereby stimulating needed research. Feedipedia is on-line at www.feedipedia.org.

It is important to note that Feedipedia is not meant to replace local feeding guides when they do exist, as it would be impractical to provide detailed recommendations and nutritive values for every system where a feed may be used. Instead, the reader will find global estimates, examples provided in the literature and links to papers that may be more directly applicable to a specific production system.

Feedipedia consists of about 700 datasheets containing the following information (in English):

(i) Feed names, including vernacular and scientific names.

(ii) Description of the plants or plant parts/products used as feed.

(iii) Feeding recommendations for the main livestock species: cattle, sheep, goats, camels, poultry, pigs, rabbits, horses, fish and crustaceans.

(iv) Tables of composition and nutritive value. There may be several tables per datasheet.

(v) These tables include averages, standard deviation, minimum and maximum values and the number of observations, as well as the list of references used to build the tables.

(vi) Illustraions, including photos and process charts.

(vii) Distribution and basic agronomic information.

(viii) Forage management.

(ix) Processes for improving nutritional value.

(x) Potential concerns such as presence of anti-nutritional factors.

(xi) Environmental impact of the production and use of feeds.

(xii) The list of references used to write the texts of the datasheets.

For the users, Feedipedia will be mainly perceived as an on-line encyclopedia. However, the project leaders will be able to leverage the underlying database and methodology to create other informational products, such as regional tables or monographs.

IV – Methodology

The following methodology is used for each feed or family of feeds. Two tasks are carried out:

The first task consists in creating texts, based on a comprehensive literature search in order to identify the scientific papers, reports, literature reviews, books etc. relevant to the feed. This task is easier nowadays due the growing availability of on-line material: both historical papers and recent research can be included in the literature survey. The AFZ staff and INRA or CIRAD researchers write a summary of the literature for the parts of the datasheet that correspond to their area of expertise. Once all the contributions are collected, they are edited and corrected to create a single consistent text. An important feature of the system is that, like a regular scientific paper, the literature used is cited in the text. When the text is uploaded on the website, those inline citations become hyperlinks that go to short bibliographical notices which, in many cases, contain a link – whenever possible a Digital Object Identifier – to the original document.

The second task consists in creating the tables themselves. Data on the composition and nutritional value of the feed are taken from the literature found during the previous task and inserted in a central database. Other data come from the databases of the project leaders or of other contributing organizations. Once these data are collected and validated, representative and consistent profiles of chemical and nutritive values are established. The main difficulty is that raw feed data are rarely ideal from a statistical point of view. For instance, crude protein value are often more numerous than fibre values. Among fibre values, crude fibre measurements are still often more numerous than Van Soest analysis. Data related to energy and nutrient availability (digestibility, degradability) are much rarer than chemical values. Creating profiles from data with different number of observations may result in inconsistent tables. Moreover, due to the natural variability of the feeds (genetics, processes, environment), analytical variations, imprecision in feed naming and actual mistakes, outliers are often quite numerous. Depending on the situation, different methods are used to create the profiles. In some cases, either because there are not enough available data to create representative profiles or because the original data are already consistent with each other, it may be enough to use simple statistics. In other cases, a more or less complex statistical approach may be necessary, and the methods of meta-analysis are used for large families of feeds. Finally, equations derived from the literature or calculated from the database itself are used to predict or correct the final values.

An innovative aspect of the development of Feedipedia is its reliance on the website as a tool for collaborative writing: writers and editors can edit the site in real time.

V – Results

In October 2013, 242 datasheets have been completed and uploaded on the Feedipedia website. The remaining datasheets are under progress and marked as such (most of them are based on the AFRIS texts). The central database used to create tables contains 2.3 million raw data about 6000 feeds, which means that there is a large reserve of data for additional datasheets and tables. The literature database now contains more than 10,000 papers.

Feedipedia is an ongoing project and a number of tasks remain to be done. The main goal is to complete the datasheets, which will take at least a couple of years. The equation system that has been implemented is fully functional in some areas but certain equations are still missing and need to be established. Particularly, many non-conventional feeds produced in tropical countries contain anti-nutritional factors that make it difficult to use generic equations obtained on conventional feeds. For that reason, the equations are not yet published on the website. Some important points are also still under discussion, notably the complex issue of the energy and protein systems to be used in ruminant feeding. As a website open to the public, Feedipedia will also have to take into account the demands of its visitors, both in terms of form (interface, features) and content (feeds, nutritional parameters, animal species).

VI – Conclusion

By providing global knowledge on feed resources, including on unconventional and lesser known ones, the Feedipedia encyclopedia contributes to the development and use of innovative and appropriate feeding options.

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Analysis of ruminant's feeding systems in some Algerian farms: Obstacle to achieve autonomy

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Abstract. A survey of 30 farms belonging to five departments in east Algeria was carried out. The study of their feeding systems, demonstrated the low development of fodder production with 60% of farms reserving less than 50% of their agricultural area for these crops. Most of the land is reserved to cereal production which provides their feeding system with straw, concentrate and fallow. Forage productions are dominated by winter-growing grasses. Leguminosae is limited to the use of vetch grown in association with common oats. The feeding system is characterized by a low degree of autonomy with excessive use of dry forage at the expense of green fodder and silage. 90% of the surveyed farms have never used treatments to improve the nutritional value of their low quality forage and straw. According to 63.33% of farmers, the lack of outreach programs is the major impediment to the development of these treatments.

Keywords. Ruminants – Feeding systems – Forage production – Algeria.

Analyse des systèmes d'alimentation des ruminants dans certaines exploitations algériennes: les obstacles qui entravent l'autonomie

Résumé. *Une enquête portant sur 30 élevages appartenant à cinq wilayas de l'Est algérien a été réalisée. L'étude des systèmes d'alimentation pratiqués, a démontré le faible développement des cultures fourragères avec 60% des exploitations réservant moins de 50% de leur SAU pour ces cultures. La majeure partie des SAU exploitées était réservée à la céréaliculture qui fournit paille, concentré et jachère pour l'alimentation des animaux. Les cultures fourragères sont dominées par les graminées à croissance hivernale. La culture des légumineuses se limite à l'apport des vesces cultivées en association fréquente avec l'avoine. La conduite de l'alimentation est caractérisée par un faible degré d'autonomie avec utilisation excessive des fourrages secs au détriment des fourrages verts et de l'ensilage. 90% des élevages enquêtés n'ont jamais utilisé de traitements d'amélioration de la valeur nutritive de leurs fourrages. Selon 63,33% des éleveurs le manque de vulgarisation constitue l'entrave majeure au développement de ces traitements.*

Mots-clés. *Ruminants – Systèmes d'alimentation – Production fourragère – Algérie*.

I – Introduction

In a ruminant's production system, feeding the herd is the farmer's main concern. To daily feed his animals, the farmer is called to establish an effective feeding system through making strategic choices, from forage production to ration formulation (Agabriel, 2007). In Algeria, low forage production remains the major problem hindering livestock production (Benazzouz, 2001) Bad climate conditions and water shortages are not the only responsible of this situation, the incapacity of Algerian farmers to adopt new strategies to improve their feeding systems is also incriminated (Abdeldjelil, 2005) The survey conducted in our study will help to analyse ruminant's feeding systems in some Algerian farms, through evaluating the existing potentials and identifying obstacles to achieve autonomy.

II – Materials and methods

1. Farms

The studied sample includes 30 farms of mixed crop–livestock (sheep and cattle) farming system. The surveyed Farms belong to five provinces of eastern Algeria (Bordj Bou Arreridj, Sétif, Jijel, Mila, Constantine).The choice of the surveyed regions within each province was based on the importance of agriculture and ruminants breeding in the economic activity of the region.

2. Methodology

The survey was conducted during the months of February and March 2008. Data collection was based upon a detailed questionnaire of 22 questions of qualitative and quantitative nature. The questions cover two main subjects: forage cultures and distributed feed (nature, origin, quantities). Data collection was based on the responses of the farmers, our own observations and farm records when they exist.

III – Results and discussion

1. Predominance of small sized farms

The UAA (utilized agricultural area) which determines agricultural growth is small for most farms (Table 1). Half of them have less than 30 ha of UAA area and 23.33% of farmers are exploiting less than 10 ha. The small farmland size is a prominent feature of the Algerian agricultural landscape, making Algerian farms the smallest ones in the Maghreb region (Abaab *et al*., 1995). This situation results of : the successive land reform policies that had limited private property (Chehat, 1994), land dispersal by multiple successions that reduce the size of exploited land through generations (Abdelguerfi and Laour 1997) and the urban growth that consumes yearly large areas of agricultural land (Saidi, 2000). The size of farmed land being small, the farmers are condemned to activate within the limits of their farms; with two major consequences: the reduction of their herd size and a change in their agricultural practices, promoting the most remunerative crops (cereals, vegetables) at the expense of forage culture which occupies lesser surfaces.

Table 1. Distribution of farms according to their UAA (ha)

Surface (ha)	[1-10 [	[10-30 [	[30-50 [	[50-70 [	Over 70
Percentage of farms	23.33%	26.66%	16.66%	13.33%	20%

2. Competition between cereal culture and forage crops

60% of farms reserve less than half of their UAA for forage crops. The most important part of the UAA is devoted to other profitable crops, mainly cereals which are present in 80% of farms and occupy more surfaces than forage cultures. Instead of forage, it's the association between cereal and livestock that represents the basis of agricultural activity. Cereals cultivated for human consumption contribute largely to the animals' feeding system providing it with straw, stubble, concentrate and fallow. According to Boulberhane's (1996) estimations nearly 60% of consumed UF (Unit forage) are supplied by cereal culture, 30% for concentrates and 29% for straw and stubble (Benazzouz, 2001).

3. Poor forage diversification

Due to the combined effect of forage monoculture and selective use of some species over others, forage crops are poorly diversified; with domination of two winter growing grasses, oat and barley, while summer-growing grasses are limited to corn. Legumunosae is limited to vetch grown in association with oats.

The association oats-common vetch culture is practiced by 83.32% of farms, a choice motivated by oat's low selectivity to soil, its modest requirements for fertilizer, and its adaptation to Mediterranean climate conditions (Figueireido Nunes 1989). Barley, the second most grown cereal after wheat in Algeria (Boumati, 2000) is practiced by 60% of farmers. Although some of its grain production is destined to human alimentation, barley provides the feeding system with green forage in early harvesting, a good quality silage and concentrate after grain maturation. Corn crop, is met in only 10% of the farms, like the other summer growing forages their weak presence is related to their water requirements, that a lack of effective irrigation systems curb the development.

4. Limited natural feed resources

Despite their low productivity and their variable nutritional value, fallows, remain the most used natural feed resource. Part of the agricultural rotation, fallow is practiced by all cereal growers and leasing fallow is a commonly used practice. The cereal- fallow rotation system remains the most common practice, in the absence of more productive systems such as ley farming, a system based on replacing unproductive fallow, by self-generating annual legumenusae (*Trifolium subterraneum* and *Medicago* spp.). This system practiced for several years in the South Australian wheat belt has proved to be effective in a region with a semi-arid, Mediterranean climate and an agricultural activity similar to ours, based on cereal culture and sheep breeding (Puckridge and French, 1983). This system (cereal - legume) is more beneficial compared to the cereal - fallow rotation, regarding, pasture, soil fertility, fight against weeds and insects, fight against erosion and a better integration of cereal culture and livestock enterprises.

Concerning the other natural forage resources, the contribution of rangelands is also low and often variable; many of them were replaced by profitable crops (Laour *et al*., 1997) or consumed by the urban growth. The remaining areas have seen their productivity significantly reduced by overgrazing and successive years of drought.

We note finally, the complete absence of artificial grassland, a phenomenon considered by Mohguen *et al*. (1999), as a particular feature of the Algerian forage system. The introduction of artificial grasslands based on perennial grasses would enrich considerably the forage system and allow the replacement of unproductive spontaneous vegetation by high nutritional value plants.

5. Excessive use of dry forage at the expense of green fodder and silage

Aside from the small seasonal contribution of natural forage resources or the first cuts of cultivated forage (mainly barley), green fodder use, is limited to a short period of the year. The poor development of green forage results not only from water resource shortages; but also from the unavailability of appropriate seed (Abdeldjelil, 2005). The use of silage is also limited (only 1.87% of the surveyed farmers) this technique, although traditional, is very little developed, because of its poor mastery by farmers and lack of appropriate materials.

Animal feeding through most of the year is based on dry forages (hay and straw), mainly on vetch-oat hay, this traditional forage of cereal producing areas (Kayouli *et al*., 1989) can give a fairly good quality hay (about 0.70 UF / kg DM) if stored under proper conditions (Abdelguerfi, 1987). Unfortunately, little attention is given while using it (exceeded cutting stage, bad storage conditions), which results in the production of a low quality hay (Benazzouz, 2001).

Cereal straw is used regularly by 54% of farmers; this crop residue occupies an important place in the feeding system. Like the case in other Mediterranean regions, the use of straw contributes on a small or large scale to feed animals, depending on the situation and degree of drought of the year (Nefzaoui, 1994). In good conditions straw is used to feed animals with low needs, but in extreme circumstances, it could be the only feed source with a possible addition of small quantities of barley or bran as a complement. This excessive use of dry forage induces two main consequences: the excessive use of concentrates with digestive or metabolic risks and high production costs.

6. Poor development of forage treatments

Low quality forage and the large amounts of straw provided by cereal culture are distributed without any treatment to improve their low nutritive value. 90% of the surveyed farmers have never used such treatments. Many farmers ignore their positive impact and those wishing to apply them do not master the technique. The majority of farmers (73.33%) believe that the lack of outreach programs is the major impediment to the development of these treatments. Other obstacles cited are purely of material nature, such as the unavailability of the treatment products and their excessive price when available.

7. Excessive use of concentrate

To compensate the low nutritional value of their dry fodder, farmers use large amounts of concentrate; this practice inherited from the period during which the state subsidized animal feed (Ferrah, 2000), induce high production costs, because the concentrate UF is generally more expensive than the forage one (Martial and Copin, 1987). Since cereals and their by-products are the main source of concentrate, their use for animal feed is in competition with the human alimentation. To reduce the tension on cereals, and reduce production costs the integration of agro- industrial byproducts would be an economic solution.

8. Low degree of autonomy

Since most farms grow cereals, 73.33% of them had total autonomy concerning straw supply. For hay supply, 40% of farmers turn to the market to cover all (13%) or part (26.66%) of their animals needs of hay. A high degree of dependency is recorded for concentrate supply; with 70% of farmers purchase all their needs of concentrate. This low degree of autonomy is a common situation in our farms, where in some cases; the rate of fodder self-sufficiency could cover only 27% of animal's needs (Farrah, 2000).

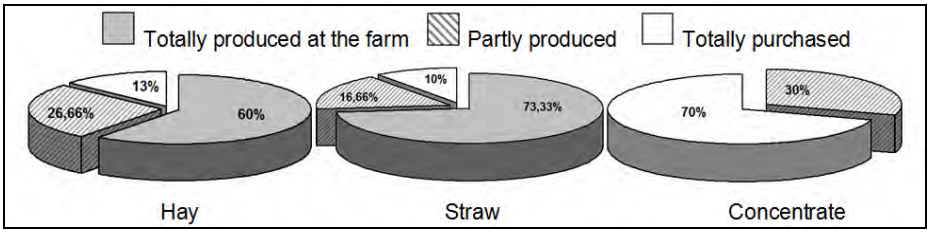

Fig. 1. Degree of autonomy for hay, straw and concentrate supply.

This dependency is explained, not only by the poor development of forage crops, but also by their average yields, their variable production from year to year, and their low nutritional value (Abdeldjelil, 2005). A situation due to the use of traditional farming techniques, wrong choice of cultivated species and bad climate conditions. Two major consequence of this state of

dependency is an increase in production costs and instability in animal's feed supply. Under such conditions, the farmer's main concern is how to ensure his herd's daily feed, quality and even quantity, become secondary to discontinuity which he tries to avoid.

IV – Conclusions

The study of the feeding systems in the surveyed farms showed a poor development of fodder crops, in terms of diversification and devoted areas. The limits of the forage system are responsible of a low degree of autonomy and excessive production costs. While some obstacles to achieve autonomy are insurmountable (small land size, bad climate conditions, water shortages); some simple solutions can be proposed to improve the situation such as abandonment of unproductive fallow in favor of ley farming, the development of forage nutritional value treatments and integration of agro- industrial byproducts into animal's feed.

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Effects of feed resources in arid lands on growth performance of local goat kids in southern Tunisia

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Abstract. In southern Tunisia, breeders have developed several strategies to ensure the nutritional requirements of their herds. They profit of the favourable season to establish reserves by harvesting and drying range species and weeds as natural hay called "*khortane*". Other dried resources are commonly used such as *Stipa tenacissima* (*gueddim*) and olive leaves. The objective of this study was to evaluate the effects of local feed resources on intake and growth performances in goat kids. Twenty seven goat kids of local breed (average body weight and age: 15.85 kg and 4.5 months, respectively) were equitably divided in three homogenous groups and used for 90 days. Groups received respectively oat hay (Diet 1), dried olive leaves+ dried *gueddim* (D2) and *khortane* (D3). There were supplemented with 350 g of concentrate and received water twice a day. During the experimental period, animals were weighed every week until slaughter day. Live body weight (LBW) was higher ($p<0.001$) in D 2 (17.65 ± 1.61) compared to D1 and D3 (16.69 ±1.67 and 17.1 ± 1.56 kg respectively). The average dry matter intake was 488.82 ± 21.92; 619.23 ± 16.70 and 591.51 ± 23.08 respectively for D1, D2 and D3. Daily body gain (DBG) in D2 and D3 groups was comparable (35.2 and 34.4 g, respectively), but lower ($P<0.0001$) in D1 group (24.8 g). In conclusion, *khortane*, *gueddim* and dried olive leaves could efficiently substitute oat hay during fattening period of local goat kids.

Keywords. Arid land – Local feed resources – Goats – Growth performance.

Effet des ressources fourragères locales dans les zones arides sur la croissance des chevreaux de la population locale du sud tunisien

Résumé. *Dans les régions arides tunisiennes, pour gérer les sécheresses saisonnières et prolongées, les éleveurs du sud tunisien ont recours à diverses stratégies d'adaptation pour assurer les besoins alimentaires de leurs troupeaux. Ils profitent des saisons favorables pour produire des réserves alimentaires notamment par la fauche et le séchage d'espèces végétales pastorales comme le type de foin naturel connu sous l'appellation populaire « khortane ». D'autres ressources sont également utilisées comme le gueddim (*Stipa tenacissima*) sec et les feuilles d'olivier séchées. L'objectif de ce travail est d'évaluer l'effet de ces ressources fourragères locales sur les performances de croissance des chevreaux de la population caprine locale. Trois groupes homogènes de 9 chevreaux chacun (poids et âge moyen : 15,85 Kg ;et 4,5 mois, respectivement) ont été utilisés durant l'expérience (90 j). Les 3 groupes ont reçu respectivement 400 g de foin d'avoine, 250 g de feuilles d'olivier séchées +200 g de gueddim et 500 g de khortane. Dans tous les groupes, les chevreaux ont été complémentés par 350 g de concentré et ont reçu l'eau deux fois par jour. Au cours de la période expérimentale, les animaux ont été pesés chaque semaine. Les résultats ont montré que le poids vif moyen a été supérieur chez le groupe 2 (*17,65 ±1,61*) comparativement aux groupes 1 et 3 (*16,69 ±1,67 kg et 17,1 ±1,56 kg respectivement). *L'ingestion moyenne de la matière sèche a été de l'ordre de* 488,82 ±21,92; 619,23 ±16,70 *et* 591,51 ±23,08 *respectivement pour le foin d'avoine, le gueddim + feuilles d'olivier séchées et le khortane. Le gain moyen quotidien (GMQ) dans les groupes 2 et 3 était comparable (35,2 et 34,4 g, respectivement) alors qu'il était plus faible ($p<0,0001$) dans le groupe 1 (24,8 g). En conclusion : nos résultats indiquent que l'utilisation traditionnelle du khortane, du gueddim et des feuilles d'olivier séchées a montré son efficacité pour substituer les fourrages commercialisés durant la période d'engraissement des chevreaux de la race locale.*

Mots-clés. *Régions arides – Ressources fourragères locales – Chevreaux de la population locale – Performances de croissance.*

I – Introduction

In Tunisian arid lands, small ruminant feeding is based on natural resources, range land and crop residues. The availability of such resources is uncertain (Elloumi *et al.,* 2001). In addition, the arid regions aremarked by a long dry season (6-9 months) and grazing is available only during a short period of about 3 to 4 months, mainly in the spring (Le Houérou, 1962). During favourable years, the pastoral species may provide excellent food for animals from autumn to spring. These ressources are used, either green by direct grazing during the growth of grass or collected to be preserved as natural forage (range species and weeds hay, called *khortane*, and *Stipa tenacissima*, called *gueddim*) and used during dry periods (Ayeb *et al.*, 2010; Genin, 2005; Visser *et al.*, 2002). The use of natural resources is a standard practice in southern Tunisia, therefore it's useful to study their impact on animal performances, particularly for goats, which are considered as an important source of meat by a large class of local population.

II – Materials and methods

1. Animals and diets

Twenty seven local goats kids ((body weight and age averaged: 15.85 kg and 4.5 months respectively) belonging to the experimental heard of Laboratory of Livestock and Wildlife in Arid Land Institute (Medenine, Tunisia), were used in this study. Animals were treated against internal and external parasites. Kids were housed in individual boxes (1.5×1 m) in a stable covered with metal roof. Kids were divided in 3 groups of 9 animals each and used during 90 days (from 10 july to 10 october 2011, mean temperature ranged from 20.97 to 34.00 and 0.6 mm of precipitation). Groups received: 400 g oat hay (Diet 1, which was the control group), 250 g dried olive leaves+ 200 g dried *gueddim* (D2) and 500 g *khortane* (D3). All animals were supplemented by 350 g of concentrate (which included barley; wheat and corn bran; calcium carbonate and vitamin mineral supplement) and received water twice a day.

Stipa tenacissima L and *khortane* were harvested during the spring and placed in open air to be dried during 20 and 12 days respectively for *gueddim* and *khortane*. *S. tenacissima* was gathered in the forms of small balls from 3 to 4 kg but *khortane* collected was placed in a pile. *Khortane* used in this study was mainly composed of 90% of *Lolium multiflorum* and *Launea residifolia*. Floristic composition of *khortane* is presented in Table 1.

Forage samples were dried at 65°C for 48 hours to determine the chemical composition. The feed samples were dried at 105ºC to determine dry matter (DM); ash was determined by incinerating samples in a furnace at 600°C for 6 h and crude protein (CP) was determined by Kjeldahl method (AOAC, 19…). Analysis of neutral and acid detergent fiber (NDF, ADF) was done according to the method described by Van Soest *et al.,* (1991). Experimental feeds characteristics are reported in Table 2.

2. Measurements during the experimental period

After three weeks of adaptation period, feeding began at 09.00 a.m. daily, and collection of feed refusals was done 24 h later. Feed offered and refusals from each group were weighed individually. Intake was calculated as the difference between feed offered and refused corrected for dry matter content. Each kid's was weighed at the beginning of the experiment and weekly thereafter. Average daily gains (g/d) were calculated as differences between final and initial body weights divided by number of days of feeding experiment.

3. Statistical analysis

Data were analysed statistically by ANOVA (SPSS (11.5)) to determine the effects of feeds on growth performance. Means and standard deviations (s.d.) were calculated. Significance of

difference ($p<0.05$) between means was determined by Duncan test. Chemical composition was analysed by Kruskal-Wallis.

Table1. Floristic composition of the *Khortane* used in the experiment

N.	Identification	BT	SC (%)	IP
1	*Anacyclis cyrtoploïdes*	a	4.12	2
2	*Argyrolobium uniflorum*	p	0.00	5
3	*Avena sterelis*	a	0.00	4
4	*Brassica tourneforti*	a	1.37	3
5	*Chenopodium murale*	a	0.00	0
6	*Chrysanthemum coronarium*	a	6.60	1
7	*Cutandia dichotoma*	p	0.00	4
8	*Cynodon dactylon*	a	1.19	5
9	*Dactylis glomerata*	p	0.00	5
10	*Daucus bisutorta*		0.00	3
11	*Deverra tortuosa*	a	0.27	
12	*Diplotaxis harra*	a	0.92	2
13	*Emex spinosa*	p	0.00	2
14	*Erodium glaucophyllum*	a	2.47	1
15	*Erodium triangulaire*	a	0.09	3
16	*Hordeum mirunum*	p	0.18	3
17	*Hedysarum spinosissimum*	a	0.00	3
18	*Launaea resedifolia*	a	44.00	5
19	*Lolium multiflorum*	a	29.79	5
20	*Malva aegyptiaca*	a	0.64	3
21	*Mathiola longipetala*	a	3.39	2
22	*Medicago minima*	p	0.00	3
23	*Medicago truncatula*	p	0.00	5
24	*Phalaris minor*	a	2.29	3
25	*Plantago albicans*	p	1.37	5
26	*Plantago ovata*	a	0.00	3
27	*Shismis barbatus*	a	1.28	4
28	*Stipa retorta (capensis)*	a	0.00	2

BT: Biological type. a: annual species; p:perennial species.
IP: Index of palatability reported by Le Héourou and Inesco (1987).
SC: specific contribution.

III – Results and discussion

1. Chemical composition of the diets

Table 2 lists results of the analyses for dry matter, crude protein, crude fiber (NDF, ADF), ash, for the samples using in experimental period. The lowest DM content was observed in *khortane* (86.63 %). The highest value was observed in the *gueddim* and dried olive leaves (92.87 and 92.33 % respectivey). The value of dry matter obtained for *khortane* was similar to that reported by Ayeb *et al.* (2010) who have found a content of 90.4% in samples of *khortane* after three ecological zones (mountains, plains and coast). The average DM content of oat hay (89.3%) is lower than that reported by Selmi *et al.* (2011) (92%). The DM in *S. tenacissima* was similar to

that reported by Genin *et al.* (2007) who found 92.7%. The DM content was high, which can be explained by the late stage of maturity of the foliages at the time of collection as the DM increase with maturity of forage (Moore and Jung, 2001).

Table2. Chemical composition of the experimental feeds (% DM)

Samples	Oat hay	Dried olive leaves	*Gueddim*	*Khortane*	p
DM*	89.35	92.33	92.87	86.63	<0.027
Ash	3.09	8.74	4.91	7.99	<0.016
CP	6.34	10.16	6.33	9.44	<0.207
NDF_{om}	60.06	34.59	85	42.18	<0.019
ADF_{om}	42.72	30.35	55.51	29.32	<0.024

DM*: % fresh matter.

For the forage using, the levels of crude protein were slightly higher in dried olive leaves and *khortane* (10.16 ad 9.44% DM respectively). In the case of *khortane*, floristic composition is an important role in the variation of the chemical composition of which the increase in the proportion of species of the leguminosae and compositae family causes an increase in the level of nitrogen. In our experience, the CP content of *khortane* is close to that mentioned by Ayeb *et al.* (2009) for *khortane* and Longo Hammouda *et al.* (2007) for a mixture of annual species and small perennial that recorded levels of about 9.5 and 12% DM respectively for the two authors. The CP content obtained for dried olive leaves is higher than that reported by Alibes and Berge (1983) where CP content is of the order of 7.7% DM. The content CP was lower for *S. tenacissima* and oat hay. CP content of oat hay is higher to that reported by Selmi *et al.* (2011), which was about 4.9% DM. But, it is comparable to that obtained by Jarrige (1988) (6.0% DM). CP content of *S. tenacissima* is similar to that reported by Genin (2005, 2007), which varies from 5.0 to 7.0% DM and similar to the value (6.02% DM) reported bay Laudadio *et al.* (2009). By comparing between feeds, we can see that the analysis of fibers showed that *S. tenacissima* and oat hay were significantly higher in NDF compared to other feeds studied (Table 2).

We can conclude that the *khortane* and the dried olive leaves have the advantage of providing a richer animal ration (higher content in nitrogen), so they may be associated with forage poor in nitrogen as straw; *S. tenacissima* and oat hay to improve the rate of protein in the ration.

2. Intake and growth performance

Average Intake and growth performance are shown in Table 3. Total dry matter intake was significantly higher ($p<0.001$) in diet 2 and 3 (*gueddim* + olive leaves and *khortane*) compared to diet 1 (619.23 ± 16.70 and 591.51 ± 23.08 *vs* 488.82 ± 21.92 g DM /day respectively). This can be explained by the fact that goats prefer shrubs like *gueddim* and that *khortane* has higher palatability due to its rich floristic composition where the majority of species have a high index of palatability (Table 1). Values of oat hay intake (containing 59 g/kg DM of crude protein) recorded are slightly lower than those found by Atti *et al.*, (2009) (500g DM /day) for indigenous goat kids (aged 7 months) reared in Northern Tunisia. The low value of DMI for all groups may illustrate the adaptation of this breed to the specific natural factors and production system (Mahjoub *et al.*, 2005).

DM intake per metabolic weight is affected ($P<0.0001$) by the type of diets. The mean is 59.31 ± 6.73; 71.68 ± 2.76 and 70.56 ± 4.52 g/kg $W^{0.75}$ for diets 1, 2 and 3 respectively. Crude protein intake (g/d) was higher ($p<0.001$) in diet 2 and 3. This is related to the level protein in *khortane* and dried olive leaves compared to the oat hay.

Table 3. Mean values for growth rate and intake in kids receiving *S. tenacissima* + dried olive leaves (diet 2) and khortane (diet 3) comparison of oat hay (diet 1)

	Diet 1	Diet 2	Diet 3	SEM	P
Initial live weight (kg)	15.77 ± 1.46	15.85 ± 1.17	15.93 ± 1.37	0.24	<0.9657
Final live weight (kg)	18.00 ± 2.29	18.94 ± 1.51	18.74 ± 2.02	0.37	<0.5720
Average daily gain (g/d)	24.8 ± 34.38	34.3 ± 20.27	35.2 ± 20.63		
DM intake (g/d)					
Concentrate	304.5	304.5	304.5		
Oat hay	184.32 ± 21.92	-	-		
Gueddim	-	103.97 ±10.83	-		
Dried olive leaves	-	210.76 ±7.52	-		
Khortane	-	-	286.01± 23. 08		
Total	488.82 ± 21.92[b]	619.23 ± 16.70[a]	591.51 ± 23.08[a]	73.08	<0.0001
DM intake (g/ kg live weight /d)	29.36 ± 2.04[b]	34.93 ± 1.82[a]	34.77 ± 2.68 [a]	4.06	<0.0001
DM intake (g/kg0.75/d)	59.31 ± 6.73 [b]	71.68 ± 2.76 [a]	70.56 ± 4.52 [a]	7.90	<0.0001
Crude protein intake (g/d)	52.53 ± 2.97[b]	69.75 ± 2.21 [a]	71.79 ± 3.75[a]	9.74	<0.0001

Values on the same line with different letters are significantly different (P <0.05).

Body weight progress by each group is presented in Table 3. Initial body weight was similar for all groups; the mean for three diets was 15.85± 1.28. In fact, body weight increases during the experimental period, the average final weight is comparable for all three regimes (18.56±1.93 kg). Growth responses were related to intake.

Although there were no significant differences among the three groups, average daily gain appeared to be higher for *khortane* and *gueddim* + olive leaves (35.20 and 34.38 g respectively) than the oat hay group (24.81 g). This change in values is due to large deviations explained by the presence of negative values during the experiment, due to the loss of some weight of kid's

The results obtained were close to those found by Bunyeth and Preston (2006) in which the initial and final body weight and average daily gain of goats receiving grass hay were respectively 14.1 and 17.4 kg (Wildeus *et al.*, 2007), and Bunyeth and Preston (2006) reported similar average daily gain (37 g/day) for kid's goats receiving grass hay. Najari *et al.* (2007) showed that the weight of the kids attained an average weight of 15.65 kg at age = 8 months and their average daily gain was 14.71 g/day with a maximum of 34.83 g/day.

The low performances in our experience are explained by the low of protein content of the local feed resources (Table 2). Negesse *et al.* (2001) have shown that increasing crude protein level (80 to155 CP/kg DM) in the diet of male Saanen kids (initial weight = 12.1± 0.18 kg), the feed intake increased from 448 to 608 g DM/day and the weight gain from 94 to 181g/day. Indeed, these forages are used to develop local resources in southern Tunisia and their use by agro-pastoralists in animal feed in order to minimize the cost of purchasing hay in the market.

IV – Conclusions

This work has allowed us to have a clear idea about the chemical composition of the most used livestock feed in southern Tunisian. In this area, local resource forage harvesting aims at making available low-cost forage for periods of scarcity. Crude protein was 10.16; 9.44 and 6.33 % DM respectively for dried olive leaves *khortane* and *S. tenacissim*a. However, using only *S. tenacissima* is not recommended, because this forage is a poor due to its chemical structure and cannot on its own meet nutritional requirements of even dry ruminants. It is better to mix the feeds to get a balanced ration.

The average body weight was equal in three groups, but there was a trend to be higher in the groups receiving *gueddim*+olive leaves and *khortane*. The DM intake was higher for animals receiving the local resources (*gueddim* + olive leaves dried*, khortane*) than in those receiving commercial oat hay (means where 619.23 ± 16.70 and 591.51 ± 23.08 *vs* 488.82 ± 21.92 g DM /day respectively). These local resources present an effective mean of drought management. They can be produced with little equipment, at a low cost and they are distributed almost without waste. So raising kids under local resources feeding regimes could be economically more efficient than using the classic regime.

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Evaluation of the nutritive value of the diet of Ouled Djellal breed's sheep in semi-arid zone

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Abstract. This study was undertaken to investigate the nutritive value and mineral components of breeding ewes' diet composed of forage from natural pastures. Physico-chemical analyzes were carried according to the methods by AOAC. Major and minor minerals were measured by atomic absorption spectrophotometry with flame. Green fodders are the richest in crude protein and total ash. The concentrate is high in energy because of its high content in organic matter and parietal carbohydrates. Hay despite its timber, contains the highest rate of Ca and P. It is suggested to improve the diet by a supply of minerals by the use of licks.

Keywords. Ewes – Feed – Analysis – Nutritional status.

Évaluation de la valeur nutritive de l'alimentation des brebis reproductrices de race Ouled Djellal en zone semi-aride

Résumé. *Cette étude a été entreprise pour étudier la valeur nutritive et les composants en minéraux de la ration des brebis reproductrices composée de plantes fourragères issues de pâturages naturels. Des analyses physico-chimiques ont été effectuées selon les méthodes de l'AOAC. Les minéraux majeurs et mineurs ont été dosés par Spectrophotométrie d'Absorption Atomique à flamme. Les fourrages verts sont les plus riches en protéines brutes et en cendres totales. Le concentré est le plus énergétique par sa teneur en matière organique et en glucides pariétaux. Le foin malgré son caractère ligneux, renferme le taux le plus élevé en Ca et P. Il serait nécessaire d'amener une correction de l'alimentation par des apports nutritifs qui s'accordent avec les périodes critiques de la brebis palliant aux exigences métaboliques imposées. Il est suggéré d'améliorer l'alimentation par un apport de minéraux par l'utilisation de pierres à lécher.*

Mots-clés. *Brebis – Alimentation – Analyses – Statut nutritionnel*.

I – Introduction

Sheep breeding in Algeria is concentrated mainly in the steppe with approximately 60% of the total number of heads, estimated at more than 19 million head. The arab white breed (Ouled Djellal) represents around 63% of animals (MADR, 2006). Today, farming intensification is handicapped by environmental problems of pollution and space maintaining. This intensification resulted in a weak equilibrium between feeding in one hand and animal production and reproduction in the other one (Brunet, 2002). The development of animal production is becoming a priority but there are many problems, being animal feeding one of the most important. The study conducted in the wilaya of Constantine, has allowed the investigation of the influence of nutritional level of the diet of breeding ewes. Food samples were analyzed in order to assess the nutritional value through their mineral and chemical compositions.

II – Materials and methods

This work was conducted in the pilot farm of El-Baaraouia, City of El Khroub located 12 km southwest of Constantine. In order to determine intakes of nutrients and mineral elements in this

exploitation, a survey was conducted among shepherds to determine the routes most commonly used. Plant uptake was determined using the equal area blocks along a diagonal (Maach *et al.,* 2000). The identification of forage species is predominated by *Trifolium stellatum* (Clover star) associated with *Sinapis arvensis* (wild mustard) and *Hordeum murinum* (Barley rats).

The dietary supplement is served to the trough-based coarse food (oats vetch hay, wheat straw) and concentrates (barley and bran). Chemical analysis of foods, as recommended by AOAC (1999), is carried out in the laboratories of Animal Science and Soil Science, University of Batna. It focused on the determination of dry matter, organic matter and ash; total proteins by the Kjeldahl method; parietal carbohydrates (NDF, ADF, ADL) by the method of Van Soest; the crude method Weende; the total fat by the method of ether extract or continuous Soxhlet extraction. At environmental health laboratory and animal production, mineral extraction is performed using nitro-perchloric digestion (Kamoun, 2008), for assaying Ca, P, Mg, Na, K, Fe, Mn, Cu, Zn by atomic absorption spectrophotometry flame.

III – Results and discussion

Figures 1, 2, 3 and 4 below represent the dry matter (DM), analytical dry matter analysis (aDM), total ash (TA), organic matter (OM), insoluble ash (IA), nitrogen content (Crude protein), fat (MG), crude fiber (CF), neutral detergent fiber in (NDF), acid detergent fiber (ADF), cellulose, hemicellulose and lignin contained in the food eaten by the sheep (expressed as% DM).

The physico-chemical analysis revealed that the green fodder are the richest in nitrogenous matter and total ash ($p < 0.05$). The concentrate feed is the richest in energy due to its high content of organic matter ($p < 0.01$), in addition, it contains the largest concentration in the cell walls. In contrast, it has the highest rate in crude fiber ($p<0.01$).

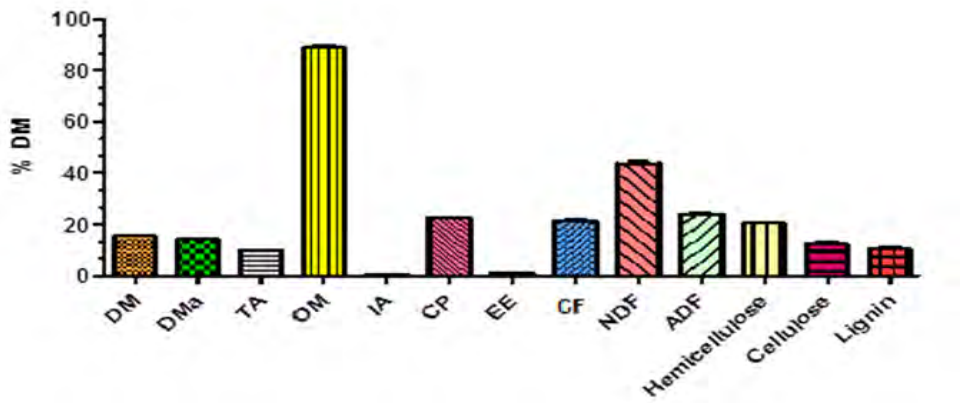

Fig. 1. Chemical composition of green fodder.

Green forage contains a greater value of nitrogenous matter than the results obtained by Arab *et al.* (2009) (12.75 -15% DM) but near the values of Lemnouar-Haddadi (2001) (17% DM). Indeed, the nitrogen sources are particularly important since in addition to pasture, the concentrate should cover the nitrogen requirements during lactation.

For the extraction of crude fiber, green fodder values are comparable to those found by Jarrige (1988) (16-28% of DM). In contrast, this content in the concentrate is lower (2-14% DM), but remained higher in the hay (28.1 to 37.6%) compared to results of Jarrige (1988).

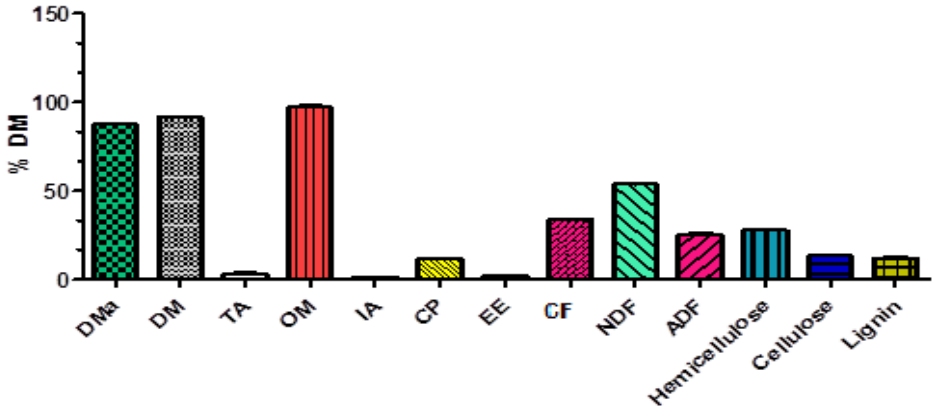

Fig. 2. Chemical composition of oat hay.

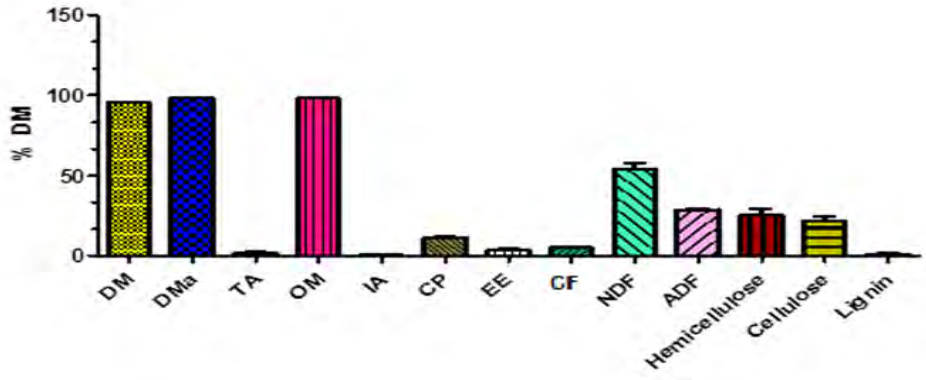

Fig. 3. Chemical composition of concentrate.

Concentrations in cell walls of concentrate are significantly higher compared to those recorded by Dønnem *et al.* (2010) (18.1%), Dias *et al.* (2010) (31.6%) and Rekik *et al.* (2010) (33%). Contrary to our results, the rate of NDF concentrate prepared by Abbeddou *et al.* (2010) is higher (67.2%). According to Arab *et al.* (2009), the proportions of fibers ADF and NDF of forage plants studied are indexes of their nutritional value; NDF give a fairly accurate estimation of the total fiber of foods and a prediction of the amount of DM intake. When NDF increase, the voluntary consumption of DM decreases. For ADF fibers, they are usually inversely related to the digestibility and energetic feed value.

Hay contains largest value in CT due to the presence of the highest rates of Mg (3.92 g/kg DM), Na (5.09 g/kg DM), K (34.14 g/kg DM), Cu (12.25 mg/kg DM) and Mn (35.10 mg/kg DM). Meanwhile, the hay has high levels of Ca (4.57 g/kg DM), P (7.36 g/kg DM) and Fe (157.68 mg/kg DM), while rates in major minerals and miners for concentrate are lowest. For zinc, green fodder and concentrate respective contents have very close (63.31 ± 1.14 and 63.31 ± 12.94 ppm) as opposed to hay (40.42 ± 4.88 ppm). According to Meziane (2001), leaching of plants by rain and the time of storing food (hay and concentrate) are all factors that influence their low levels of nitrogen content, fat, and total ash.

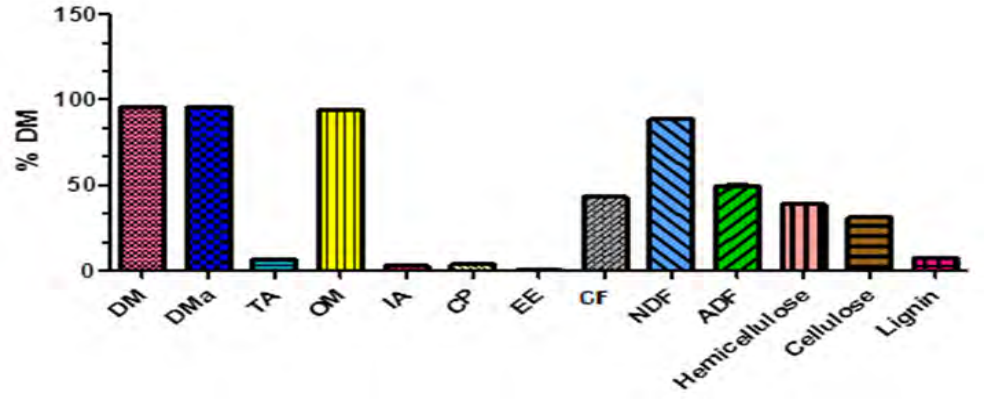

Fig. 4. Chemical composition of wheat straw.

IV – Conclusions

The nutritive values of diets distributed to the sheep are in accordance of standards recommended by literature. Green fodder is the richest in crude protein and ash especially Mg, Na, K, Cu and Zn. The concentrate is richer in energy and that by its high content of organic matter. In contrast, hay despite its timber character, contains the highest levels of Ca and P. It is recommended to correct the ration by digestible dietary fibers and minerals (salt lick).

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Effect of faba bean intake on growth and carcass characteristics of lambs from three breeds

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Abstract. The lambs fattening regimen in Tunisia is based on barley. The aim of this study was to assess the effect of feeding faba beans on lamb's growth and carcass characteristics. Forty two lambs from Barbarine, Queue Fine de l'Ouest and Noire de Thibar breeds were used. Animals of each breed were divided into two groups receiving oat hay (7 % crude protein, 34 % crude fiber) ad libitum and 1 kg of concentrates. The concentrates were: 100 % barley for Control (CG) and 50 % barley 50 % faba beans for the experimental group (FG). At the end of the experiment, all animals were slaughtered. Oat hay intake was similar for all groups (527g). The diet and breed did not significantly affect slaughter weight and average daily gain, although a trend toward higher values of these parameters was observed for FG than CG (51.8 *vs.* 49.60 kg and 178 *vs* 166 g, respectively). Carcass yield was significantly higher for Barbarine breed (P= 0.01). The gut was significantly more developed for thin tailed breeds than for Barbarine, but was not affected by the concentrate type. Cold carcass and lean weights were higher for FG (25.2 and 11.8 kg) than CG (23.3 and 10.9 kg). Faba bean incorporation tends to improve lamb's growth and carcass composition. Applying this regimen to lighter lambs may lead to more significant results.

Keywords. Faba bean – Lambs – Growth – Carcass composition – Barley.

Effet de l'incorporation de la féverole sur la croissance et les caractéristiques de carcasses des agneaux de trois races

Résumé. *Le but de cette étude était d'étudier l'effet de l'incorporation de la féverole sur la croissance et les caractéristiques de carcasses des agneaux. Quarante-deux agneaux des races Barbarine, Queue fine de l'Ouest et Noire de Thibar ont été utilisés. Les agneaux de chaque race ont été divisés en lot témoin (CG) recevant 1 kg d'orge et lot expérimental (FG) recevant 1 kg de concentré fermier (50 % d'orge 50 % de fèves). Tous les animaux ont reçu du foin d'avoine et de l'eau à volonté. À la fin de l'expérience, tous les animaux ont été abattus. Le régime et la race n'ont pas affecté de manière significative le gain moyen quotidien et le poids à l'abattage, bien que ces paramètres aient été plus élevés pour FG que pour CG (51,8* vs. *49,60 kg et 178* vs *166 g). Le rendement en carcasse était significativement plus élevé pour la race Barbarine (P = 0,01). Le tube digestif était significativement plus développé pour les races à queue fine que pour la Barbarine, mais le type de concentré n'avait pas d'effet significatif sur ce paramètre. Le poids de la carcasse froide et celui de la masse musculaire étaient plus importants pour FG (25,2 et 11,8 kg) que ceux du lot CG (23,3 et 10,9 kg), mais les différences n'étaient pas significatives. En conclusion, l'incorporation de fèverole tend à améliorer la croissance de l'agneau et la composition en carcasse. L'application de ce régime à des agneaux plus légers peut mener à des résultats plus signifiants.*

Mots-clés. *Féverole – Agneaux – Composition de croissance – Orge.*

I – Introduction

Most of concentrate feedstuffs for livestock is based on soya bean cake and corn. The rise in prices of these materials in 2007 leads to search other solutions in order to provide alternative feed resources. In Tunisia, barely is often used as concentrate in ovine fattening diets, but this energetic source is known for its low protein content. Adding a protein source such as locally produced faba bean, with high protein (30% MAT) and starch (42%) contents (Yu *et al.*, 2002)

to the ovine feeds, became essential especially in growing and finishing steps of meat lambs. The objective of this work was to study the effect of using both barely and faba bean in finishing diets on growth and carcass characteristics of Tunisian lambs belonging to three breeds.

II – Materials and methods

The experiment was carried out with a total of 42 lambs from Barbarine, Queue Fine de l'Ouest (QFO) and Noire de Thibar (NT) breeds. Animals were 11 months old and 32.7 kg body weight (BW). Lambs of each breed were divided into two groups. All groups had free access to water and they were offered oat hay ad-libitum and an average amount of 1 kg of concentrate. For each breed, the control group (CG) received barely as a concentrate; the experimental group (FG) received a farmer concentrate composed by 50 % barely and 50 % faba bean (Table 1). Animals were allowed 120 days in this growth trial and then were slaughtered.

Table1. Chemical composition (g/kg DM) of ingredients of concentrate and roughages

	Hay	Barely	Faba bean
Dry matter g/kg	933	965	954
Organic matter	97.31	99.71	98.06
Ash, g/kg DM	5.82	2.65	2.57
Crude protein g/kg	6.2	13.2	27.6

Feed intake was recorded daily and lambs BW weekly. At slaughter, external and internal organs were weighed. All fractions of the digestive tract were weighed full then empty, in order to determine the empty body weight (EBW). Carcasses were weighed cold (CCW) after storage 24 h at 4°C. After removing the tail, each carcass was split longitudinally into two halves; the left sides were dissected into fat, muscles and bones.

The statistical effects of dietary treatment and breed on growth and carcass composition were performed by analysis of variance using the GLM procedure of SAS (1989). Differences between groups were evaluated by t-test; significance was declared at $p<0.05$.

III – Results and discussion

1. Food intake, growth performance and slaughter parameters

During the whole trial period, oat hay intake was similar for both diet treatments and had not exceeded an average daily consumption of 550g/day for all groups (Fig.1). The FG group was in phase lead by one week compared to CG group, which can be explained by the improvement of fermentative facieses ensured by the nitrogen contribution and by the variation of the rumen pH which value decreases by the intake of a large quantity of concentrate. At the end of the trail, both diets reached the same quantity of concentrate 1400g/day (Fig. 1). Lambs of all groups had a similar slaughter BW (50.15 kg). The average total body gain was 17.7 kg, which could be considered as an important result for animal having 11 months old at experiment start. Neither regimen nor breed affected average daily gain (ADG) which was similar for all groups. This ADG (171 g) was lower (Table 2) than gains (214 g) achieved by lighter lambs (15 kg) from Rasa Aragonesa breed receiving diets based on faba bean (Purroy *et al*., 1992). FG group had higher body weight, carcass weights and dressing percentages than CG group with tendency to significant effect (p=0.1). The advantage of faba bean diet was found by other authors (Lanza *et al.*, 1999), who detected significant difference in these parameters when using post weaned

lambs. EBW, carcass weights and consequently the dressing percentage were higher for Barbarine lambs than both other breeds (Table 2) this result is in accordance with other finding concerning the same breeds (Atti and Khaldi, 1989).

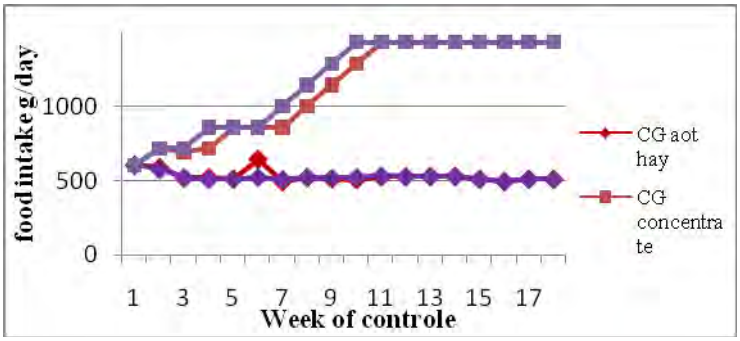

Fig.1. Evolution of food intake.

Table 2. Growth performances parameters, carcass weight (CCW) and dressing percentage (DP, %)

	Barbarine	QFO	NT	CG†	FG†	Bd†	Rg†	MES
Initial BW	32.14	33.24	32.19	32.27	32.70	0.83	0.79	4.97
SBW	50.05	51.72	49.28	48.75	51.56	0.56	0.15	6.45
ADG (g)	163	190	163	165	178	0.18	0.33	42.26
EBW (kg)	43.30	44.72	40.54	41.32	44.20	0.17	0.11	5.69
CCW (kg)	24.89	24.49	23.36	23.30	25.17	0.52	0.11	3.63
DP	49.87	48.05	47.95	48.38	48.93	0.01	0.16	1.58

†CG: Group receiving barely; FG : group receiving barely and faba bean; Bd: breed effect ; Rg: regimen effect

2. Non carcass components

For all non carcass components lambs from FG groups had slightly better development than CG groups, but only the head weight was significantly affected by faba bean incorporation (Table 3). According to Morbidini *et al.*, (2005), using light lambs, faba bean increase significantly the development of both pelt and head. The weight of most offal components was not different between groups slaughtered at a similar BW, despite the difference in feed level and quality (Atti *et al.*, 2003). This suggests that the weight of most offal components depends more on weight at slaughter rather than on the intake level or diet composition as mentioned by several authors (Kamalzadeh et al 1998). Breed had significant effect on head and skin weights. Head was heavier in QFO breed (2.88 *vs* 2.65 kg); this is in relation with its developed skeleton (Table 3). Red cut downs (heart and lungs) and rumen were significantly more important in thin tailed breeds than in fat tailed one ($P< 0.05$).

3. Carcass composition

Faba bean incorporation tended to have a positive effect on tissues' weight and proportion (Table 4). This trend may be due to the heavy initial weight of lambs (32.7 kg) whilst, with light lambs, there was no significant effect of faba bean on carcass composition (Morbidini *et al.*,

2005). Although faba bean incorporation as protein source has a positive effect on nitrogen intake there was no increase on muscle amount. The barely protein content seems to be sufficient to produce the same amount of muscle as barley plus faba bean. For heavy and old (11 months) lambs, the use of barely alone or its mixture with faba bean resulted in the same carcass composition. Muscle weight was similar for all breeds (11.33 kg) but its percentage was higher for NT lambs (Table 4). Fat was significantly more important for Barbarine than both other breeds (36 *vs* 29 %).

Table 3. Non carcass components (kg)

	Barbarine	QFO	NT	CG†	FG†	Bd†	Rg†	mse
Head	2.46	2.88	2.84	2.60	2.84	0.001	0.01	0.281
Skin	7.09	6.98	5.73	6.30	6.86	0.01	0.18	1.267
Liver	0.753	0.872	0.835	0.81	0.82	0.13	0.91	0.154
Heart	0.190	0.208	0.227	0.199	0.217	0.02	0.11	0.034
Rumen	1.03	1.17	1.19	1.114	1.147	0.01	0.45	0.138

†CG: group receiving barely; FG: group receiving barely and faba bean; Bd: breed effect; Rg: regimen effect.

Table 4. Weight of different carcass tissues

	Barbarine	QFO	NT	CG†	FG†	Bd†	Rg†	mse
Muscle, kg	11.1	11.6	11.3	11.0	11.7	0.76	0.18	1.67
Muscle %	48.3	49.1	51.8	49.1	50.4	0.01	0.23	3.22
Fat, kg	8.5	7.6	5.9	6.9	7.7	0.001	0.18	1.79
Fat %	36.3	30.9	26.8	30.7	32.0	0.0001	0.30	4.15

†CG: group receiving barely; FG: group receiving barely and faba bean; Bd: breed effect; Rg: regimen effect.

IV – Conclusions

Faba bean incorporation in finishing diet for heavy lambs had a minor positive effect on growth and carcass components allowed to achieve an important weight in spite of their advanced age. However, this effect of faba bean intake was not significant with regards to carcass composition traits because of the high initial weight of lambs. Studying the effect of this protein source on meat quality and testing its effect using lighter lambs may have an important interest.

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Sexual behaviour of Ile-de-France rams receiving a short term flushing with lupins

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Abstract. This study investigated the effect of a short term (2 weeks) supplementary feeding on sexual behavior traits of Ile-de-France rams during late spring. Ten adult rams were fed a basal diet composed of 0.5 kg of straw, 0.8 kg of hay, 0.27 kg of barley, 0.05 kg of molasses and 0.01 kg of a mineral and vitamins supplement per day providing approximately the neat energy for maintenance according to INRA tables. During two weeks prior to joining, control rams (C) were offered the basal diet, while Supplemented (S) rams received the basal diet and were given 1 kg of lupins per ram per day. The rams were selected based on their sexual motivation and allocated to treatment groups balanced for live weight and testicular volume. Sexual activity of the rams was tested at the end of the supplementary period and following joining with a flock of Ile-de-France ewes. Rams were tested 3 times over a 2-day period. Supplementation did not affect changes in live weight or the testicular volume being 214±57.2 and 215±30 g for C and S rams for the latter trait. Latency to first activity, sniffing investigations, flehmen reaction and lateral approaches were not different between rams of the two groups. Nevertheless, total activity time tended to be longer for supplemented than control rams being 6.8±1.09 and 6.2±0.83 min respectively. Following joining, there was an overall tendency for supplementation to affect negatively sexual behavior traits. Sniffing investigations during the first 30 min of the test were 29.8 and 22 for C and S rams ($P<0.05$). Average numbers of lateral approaches during the first 180 minutes of the test reached 19 and 11 for C and S rams ($P<0.05$). Control rams also attempted more mounts without ejaculation than supplemented males. Preliminary conclusions point out to a possible relationship between short-term food supplementation and expression of sexual behavior of male sheep.

Keywords. Rams – Supplementation – Sexual behavior – Sniffing investigations – Flehmen.

Comportement sexuel des béliers Ile-de-France recevant une supplémentation de courte durée avec du lupin

Résumé. *Cette étude a investigué l'effet d'une complémentation à court terme (2 semaines) sur les caractéristiques du comportement sexuel des béliers Ile-de-France à la fin du printemps. Dix béliers adultes ont été nourris avec un régime de base composé de 0,5 kg de paille, 0,8 kg de foin, 0,27 kg d'orge, 0,05 kg de mélasse et 0,01 kg d'un minéral et un supplément de vitamines par jour fournissant approximativement l'énergie propre pour l'entretien en fonction des tables de l'INRA. Pendant deux semaines avant de la lutte, les béliers témoins (C) ont été soumis au régime de base, tandis que les béliers complétés (S) ont reçu en plus du régime alimentaire de base, 1 kg de lupin par bélier par jour. Les béliers ont été sélectionnés en fonction de leur motivation sexuelle et divisés en deux groupes équilibrés pour le poids vif et le volume testiculaire. L'activité sexuelle des béliers a été testée à la fin de la supplémentation et au cours de la lutte dans un troupeau de femelles Ile-de-France. Les béliers ont été testés 3 fois sur une période de 2 jours. La supplémentation n'a pas entraîné de variation directe du poids ou de volume des testicules qui est de 214 ± 57,2 et 215 ± 30 g pour les béliers du groupe C et S. Le temps de latence à la première réaction, le nombre de flairages et de flehmen et le nombre d'approches latérales ne sont pas différents entre les béliers des deux groupes. Néanmoins, le temps d'activité totale a tendance à être plus long pour le lot complété que pour les béliers du lot témoin 6,8 ± 1,09 et 6,2 ± 0,83 min respectivement. Lors de la lutte, il y avait une tendance générale à faire que la supplémentation puisse affecter négativement les comportements sexuels. Le nombre de flairages pendant les 30 premières minutes de l'essai était de 29,8 pour le lot témoin et 22 pour le groupe C ($P<0,05$). Le nombre moyen d'approches latérales pendant les 180 premières minutes de l'épreuve a atteint 19 et 11 pour le groupe S et le groupe C ($P<0,05$). Les béliers du groupe S ont également tenté plus de montes sans éjaculation que les béliers supplémentés. Les premières conclusions semblent indiquer une relation possible entre une supplémentation à court terme et l'expression du comportement sexuel chez le bélier.*

Mots-clés. *Béliers – Supplémentation – Comportement sexuel – Flairage – Flehmen.*

I – Introduction

The control of sexual behavior is a key element for the improvement and/or management of reproduction in domestic species. Several factors interact over sexual behavior of the males explaining large individual variations in sexual efficiency.

Reproductive activity of the ram is affected by several external factors that can be socio-sexual, photoperiodic and nutritional (Blache *et al.*, 2000). In general, depressive effects of nutrition on sexual activity of the rams are only observed if prolonged restriction of feed supply occurs and loss of weight ensues. Salamon (1964) has reported that sexual drive was more intense in animals fed supplements rich in proteins than supplements with low protein content. Several others workers have demonstrated that rams receiving feed supplies less than maintenance requirements display a diminution of sexual activity (Parker and Thwaites, 1972; Mattner and Braden, 1975). Overall, studies targeting interactions between nutrition and reproduction in rams have targeted testicular growth, sperm production traits and related endocrine changes. Very few studies dealt with sexual behavior and this study aims to investigate the effect of a short term supplementation with lupin grains on sexual behavior traits of rams in preparation to mating.

II – Materials and methods

The experiment was carried out at the INRA Nouzilly station (Tours, France) during Mai-June. Ten adult rams of the Ile-de-France breed were selected based on their sexual motivation and were divided into two groups (n=5) balanced for live weight, body condition score and testicular volume. Rams received every day on an individual basis a basal diet composed of 0.5 kg of wheat straw, 0.8 kg of hay, 0.27 kg of molasses and 0.01 kg of a mineral and vitamin supplement. Rams in group C received only the basal diet calculated to provide the neat energy for maintenance according to INRA tables. Rams in the supplemented group (S) and in addition to the basal diet, received each 1 kg of lupin grains equally divided into a morning and an evening meal. First day of supplementation is indicated D0 and supplementation continued for 15 days. Care was taken to introduce lupin progressively in the diet of S rams.

During the supplementation period, rams in both groups were sexually stimulated for two consecutive days. Each ram was placed in a box of 4 m² with two ewes induced in estrus. In order to assess the effect of supplementation on libido of the rams, a behavior test was undertaken at the end of the two-week period of supplementation by placing each of the rams in the box for 10 minutes. Afterwards, the rams were introduced in a flock of anoestrous females for a spring mating. A second behavior test was then carried out during the 3 hours that followed the introduction of the rams. During each of the previous two tests, the following parameters were observed and recorded: latency time to reaction in seconds, number of sniffing, number of flehmen reactions, number of lateral approaches, number of mounting attempts and total time of activity (minutes).

In addition, live weight and body condition score were measured at the start and the end of the supplementation period while testicular volume was measured weekly using an orchidometer.

III – Results and discussion

Throughout the experiment, live weight and body condition score did not markedly change in both experimental groups. Supplementation with lupin did not cause any change in live weight

and body condition score when compared to C rams. Similarly, no differences occurred in testicular volume between C and S rams (Figure 1). Testicular volume reached close values at the end of the supplementation period being 214±57.2 ml and 215±30 ml for respectively C and S rams.

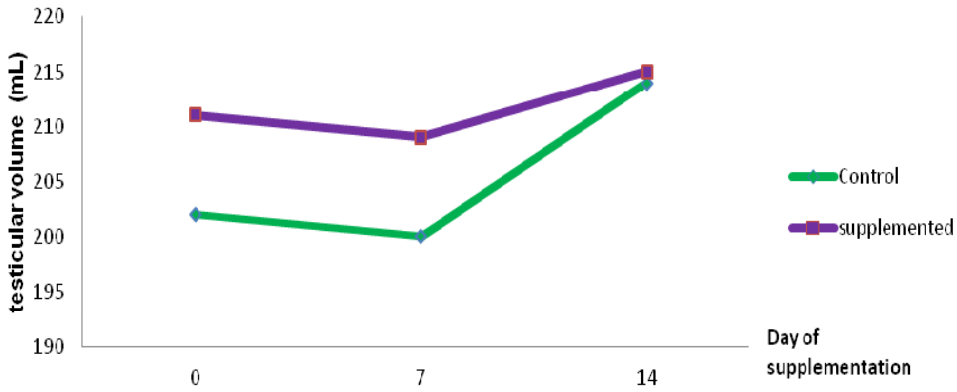

Fig. 1. Changes in testicular volume for control and lupin-supplemented rams.

These first results differ with those reported by Martin and Walkden-Brown, (1995) who recorded an increase in both live weight and volume of the testicles after 8 weeks of supplementation. This difference could be explained by the duration of the supplementation period and as concluded by Boukhliq, (1993); changes in testicular size are rarely observed before 2 weeks of supplementation but continue up to week 5. According to Oldham (1978), testicular volume is more sensitive to changes in the diet than live weight hypothesizing the existence of a preferential allocation of nutriments to the reproductive system.

Results of the behavior test at the end of the period of supplementation are depicted in Table 1. Latency time, number of sniffings and Flehmen, number of lateral approaches and the total activity time of S rams were not much different from those recorded for C rams. S rams tended to have a longer total activity when compared to C counterparts (6.8±1.09 vs 6.2±0.83 minutes)

Table 1. Sexual behavior traits at the end of supplementation period (2 weeks)

Parameter	Control	Supplemented
Latency (s)	1	1
Sniffing investigation	21.2	19.8
Flehmen	3.4	2.8
Lateral approach	10	12
Total activity time (min)	6.2	6.8

Following introduction of the rams in the flock of the ewes at the start of the mating season, results of the sexual behavior test are presented in Table 2. Number of sniffings during the first 30 minutes after introduction of the rams was higher ($P<0.05$) for C in comparison to S rams. Number of lateral approaches during the first 180 minutes, averaged 19 and 11 for respectively C and S rams ($P<0.05$). C rams also attempted more mounts than S ones. The results seem to point out that lupin addition to the diet of Ile de France rams did not improve expression of their sexual behavior. These findings corroborate what has been reported by Kara *et al.* (2010) that

nutritional supplements in the form of vitamins and minerals had no effect on the sexual behavior of the ewes. They are not consistent with results by Mahouachi *et al.* (2011) who observed an increase in the libido of rams as a result of an increase in the feeding level. Nevertheless, Martin *et al*. (2004) have suggested that sexual behavior in sheep is not much linked to nutrition pointing out that when extreme changes in weight and body reserves are induced by severe under nutrition, this may affect the motor activity of the animals and full expression of libido.

Table 2. Sexual behavior traits during mating

Parameter	Control	Supplemented
Sniffing (0-10mn)	14a	11.4a
Sniffing (10-30mn)	15.8a	10.6a
Total sniffing 1st 30 mn	29.8a	22b
Sniffing (3h)	11.2a	11.4
Lateral approach (0-10mn)	7.4a	7.8a
Lateral approach (10-30mn)	9.2a	8.2a
Lateral approach (180mn)	19a	11b
Total mount 1st 30mn	0.8a	0.6a
Attempted mount (120mn)	0.4a	0a
Attempted mount (180mn)	1a	0a

Means of the same line followed by two separate letters are significantly different (P <0.05).

IV – Conclusions

The study of the effect of a short term supplementation with lupin grain for two weeks in Ile de France rams prior to mating was not associated to changes in live weight or testicular volume but slightly depressed sexual behavior. Offering for short time a novel food could be the cause opening new prospects to investigate the relationship between nutrition and sexual behavior in sheep.

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Chemical composition and *in vitro* fermentation characteristics of range species growing in Central Tunisia

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Abstract. Chemical composition and *in vitro* fermentation characteristics were determined on 15range(herbaceous and shrubby) species collected in a postural area from the region of Kairouan (Central Tunisia) during the grazing period (end of winter and spring). The *in vitro* fermentation parameters were determined using 100 ml glass syringes containing the plant material and inoculum and incubated for 96 hours. Correlations between chemical parameters, calculated *in vitro* organic matter digestibility ($IVOMD_{24h}$) and metabolisable energy (ME) were determined. Crude protein (CP) content was highest in *Rosedaalba* (184 g/kg DM) and *Medicago minima* (175 g/kg DM) and the lowest in *Rosemarinus officinalis* (58 g/kg DM) and *Globularia alypum* (61 g/kg DM). The NDF contents varied widely between the studied species and ranged from 273 g/kg DM in *Rosedaalba* to 607 g/kg DM in *Marrubium vulgare*. The same wide variation was observed for ADF since it varied from 169 to 502 g/kg DM respectively in *Rhus tripartita* and *Artemisia herba alba*. Lignin content (ADL) was highest in *Pistacia lentiscus* (239 g/kg DM) and lowest in *Calendula arvensis* (49 g/kg DM). Asymptotic gas production (A) varied from 82.7 ml in *Chrysanthemum coronarium* to 26.7 ml in *Artemisia herba alba*. Positive correlations were found between CP content and $IVOMD_{24h}$ (r= 0.74, P<0.0001) and also ME (r= 0.76, P<0.0001). While, negative correlations were found between $IVOMD_{24h}$ and NDF (r= -0.58, P<0.0001), ADF (r= -0.65, P<0.0001) and ADL (r= -0.72, P<0.0001) contents. The same trend was noted with ME (r= -0.58, -0.63, -0.73, P<0.0001, respectively for NDF, ADF and ADL). It was concluded that the range species available in the studied area presented a wide nutritional variability, thus they could have a complementary role for small ruminants grazing. Considering the laborious *in vivo* approach in pasture conditions, the in vitro technique may considerably contribute into evaluating such diversity of resources. Secondary compounds should be considered in order to improve accuracy of predictive equations.

Keywords. Nutritive value – *In vitro* fermentation – Pasture – Small ruminants.

Composition chimique et paramètres de fermentation* in vitro *des espèces pastorales de la Tunisie Centrale

Resumé. *La composition chimique et les caractéristiques de la fermentation* in vitro *ont été déterminées pour 18 espèces pastorales (herbacées et arbustives) collectées dans une zone pastorale de la région de Kairouan (Tunisie centrale) au cours de la période de pâturage (fin d'hiver et printemps). La composition chimique a été déterminée et les paramètres de fermentation* in vitro *ont été mesurés dans des seringues en verre de 100 ml pendant 96 heures. Nous avons établi des corrélations considérant, d'une part,les valeurs calculées de la digestibilité de la matière organique* in vitro *($DIVMO_{24h}$) et de l'énergie métabolisable (EM) et d'autre part la composition chimique des espèces étudiées. Les teneurs en matières azotées totales (MAT) les plus élevées ont été enregistrées pour* Roseda alba *(184 g/kg MS) et* Medicago minima *(175 g/kg MS) et les plus basses pour* Rosmarinus officinalis *(58 g/kg MS) et* Globularia alypum *(61 g/kg MS). La teneur en NDF variait considérablement entre 273 g/kg MS pour* Roseda alba *et 607 g/kg MS pour* Marrubium vulgare*. La même variation a été observée pour la teneur en lignocellulose (ADF), qui est passée de 169 à 502 g/kg MS, respectivement pour* Rhustripartita *et* Artemisia herba alba*. La teneur en lignine (ADL) était la plus élevée pour* Pistacia lentiscus *(239 g/kg MS) et la plus basse pour* Calendula arvensis *(49 g/kg MS). L'asymptote (a) de la production de gaz varie de 82,7 ml pour* Chrysanthemum coronarium *à 26,7 ml pour* Artemisia herba alba*. Des corrélations positives significatives ont été trouvées entre la teneur en MAT et la $DIVMO_{24h}$ (r = 0,74 ; P <0,0001) et également avec l'EM (r = 0,76 ; P <0,0001). Cependant, des corrélations négatives ont été trouvées entre la $DIVMO_{24h}$ et la NDF (r = -0,58; p <0,0001), l'ADF (r = -0,65, p <0,0001) et l'ADL (r = -0,72, p <0,0001). La même tendance a été notée avec l'EM (r = -0,58 ; -0,63 et -0,73 ; p <0,0001, respectivement pour NDF, ADF et ADL). Il a été conclu que les espèces*

disponibles dans la zone étudiée présentent une grande variabilité nutritionnelle qui pourrait représenter une certaine complémentarité pour le pâturage des petits ruminants. Compte tenu des difficultés et du coût des approches in vivo *dans les conditions de pâturage, la technique* in vitro *peut considérablement contribuer à l'évaluation de la diversité des ressources. Les composés secondaires devraient être considérés pour améliorer la précision des éventuelles équations prédictives.*

Mots-clés. *Fermentation* in vitro *– Valeur alimentaire – Espèces pastorales – Petits ruminants.*

I – Introduction

In Tunisia, grazing pasture represents the major feeding system for small ruminants, mainly in the central regions. A large biomass diversity composed of native species is available in these regions. Rangeland improvement and rehabilitation and grazing management inquire the nutritive characterization of grass and shrubs species (Ben Salem *et al*., 1994; Nefzaoui *et al*., 1995).The study aimed to determine the nutritional characteristics of range species from the region of Kairouan (central Tunisia),and to contribute into the establishment of feeding value database in this specific feeding system.

II – Materials and methods

1. Plant material

Fifteen range specieswere collected from the region of Kairouan (semi-arid, central region of Tunisia) in March 2008. Samples from each species were taken from different sites and pooled to make a global sample. The dry matter (DM) was determined at 105°C in a forced-air oven and a part of each sample was dried at 40°C during 48h and then ground to pass through 1 mm screen and stored for chemical analysis and *in vitro* determinations.

2. Animals and measurements

Two adult "Noire de Thibar breed" sheep (average age and live weight respectively 24 months and 48.5 kg) with rumen cannula were used for *in vitro* determinations. They were housed in individual pens and received twice per day 70 g DM $kg^{-0.75}$of a diet composed of 70% oat-vetch hay and 30% of barely grains on dry matter (DM) basis. Samples (300 mg DM) of each species were incubated in 100 ml glass syringes according to the technique of Menke and Steingass (1988).The incubation medium (30 ml) was a mixture of rumen fluid and Menke buffer solution (1:1). Gas production was measured at 2, 4, 6, 12, 24, 36, 48, 72 and 96 h of incubation. Diets were incubated in triplicate and two successive incubations were carried out.

3. Chemical analysis

Feeds were analyzed for dry matter (DM), ash and crude protein (CP) contents (AOAC, 1984). Cellwall composition (NDF, ADF and ADL) in feeds were determined as described by Van Soest *et al*. (1991).

4. Calculation and statistical analysis

Gas production kinetic was fitted using the non-linear model of France (2000):

$$G=b*(1-e^{-k(t-L)})$$

where: "G" is the gas production at time t; "b" asymptotic gas production, "k" the fractional rate

of gas production and "L" lag-phase. Parameters were calculated using NLIN procedure of SAS (SAS, 1996). *In vitro* organic matter digestibility at 24h ($IVOMD_{24h}$) and metabolizable energy (ME) were calculated according to the specific equations of Menke and Steingass (1988).

III – Results and discussion

Chemical composition of the range species is presented in Table 1. *Reseda alba* showed the highest CP content (184 g/kg DM), whereas the lowest concentrations were observed in *Rosmarinus officinalis* (58 g/kg DM). *Artemisia herba alba* and *Marrubium vulgare* L. were highest in NDF (637 and 607 g/kg DM, respectively). The same trend was observed for ADF contents (502 and 421 g/kg DM, respectively in *Artemisia herba alba* and *Marrubium vulgare* L.). The range species evaluated in the current work exhibited a wide nutritional variability, suggesting that they could have a complementary role for goat feeding and grazing. Our results were close to those found by several authors (e.g. Ben Salem *et al*., 1994, 2000; Gasmi-Boubaker *et al*. (2009).

Table1. Chemical composition of feeds (g/kg DM)

	Ash	CP	NDF	ADF	ADL
Artemisia campestris L.	58	98	561	452	126
Artemisia herba alba	67	99	637	502	216
Calendula arvensis	156	125	306	222	49
Chrysanthemum coronarium	81	140	335	257	62
Globularia alypum	53	61	425	328	125
Marrubium vulgare L.	86	121	607	421	135
Medicago minima	149	175	334	234	86
Olea europea	59	89	390	238	142
Picris echioides	220	161	435	267	141
Pistacia lentiscus	50	93	378	315	239
Reseda alba	114	184	273	178	56
Rhamnus lycioides	177	164	289	179	59
Rhus tripartita	134	140	321	169	199
Rosmarinus officinalis	62	58	389	301	167
Thymus capitatus	102	88	409	326	226

In vitro fermentation parameters of feeds are presented in Table 2. Asymptotic gas production (b) ranged from 26.7 and 82.7 ml respectively in *Artemisia herba alba* and *Chrysanthemum coronarium.* The Lag time (L) was shortest and the rate of gas production was highest in *Reseda alba*. The Metabolizable energy ranged between 1122 kcal/kg DM in *Rosmarinus officinalis* and 3099 kcal/kg DM in *Rhamnus lycioides.*

As an example, Figure 1 shows the *in vitro* gas fermentation of *Calendula arvensis.* Gas productions increased with increased incubation time and tended to be stabilized from 48 h of incubation.

The main correlations between *in vitro* parameters and chemical composition are presented in Table 3. Negative correlations were found between EM and NDF (r=-0.58, P<0.01), ADF (r=-0.65, P<0.01), ADL (r=-0.72, P<0.01) and L (r=-0.24, P<0.01). The same trend is observed between $IVOMD_{24h}$and NDF (r=-0.58, P<0.01), ADF (r=-0.63, P<0.01), ADL (r=-0.72, P<0.01) and L (r=-0.29, P<0.01). Positive correlation was detected between $IVOMD_{24h}$ and k (r=0.55, P<0.01).These results are in line with those reported by Ammar *et al.* (2005).

Table 2. Parameters of *in vitro* fermentation

	b (ml)	k ($10^{-3}h^{-1}$)	L(h)	$IVOMD_{24h}$ (%)	ME (kcal/kg DM)
Artemisia campestris L.	37.1	85	0.3	42.6	1801
Artemisia herba alba	26.7	55	2.58	36.3	1179
Calendula arvensis	62.5	85	0.46	63.6	2886
Chrysanthemum coronarium	82.7	95	0.18	70.5	2908
Globularia alypum	48.6	57	0.4	43	1801
Marrubium vulgare L.	36.4	72	0.73	44.6	1427
Medicago minima	61.8	91	0.48	65.5	2935
Olea europea	55.9	59	0.06	48.6	2027
Picris echioides	41.3	72	0.97	58.5	2895
Pistacia lentiscus	34.1	40	1.80	36.2	1227
Reseda alba	73.6	136	0	72.1	3071
Rhamnus lycioides	58.4	130	0.30	67.6	3099
Rhus tripartita	49.6	55	0.98	53.4	1606
Rosmarinus officinalis	27.7	90	1.01	34.4	1122
Thymus capitatus	54.3	76	1.83	53.4	1704

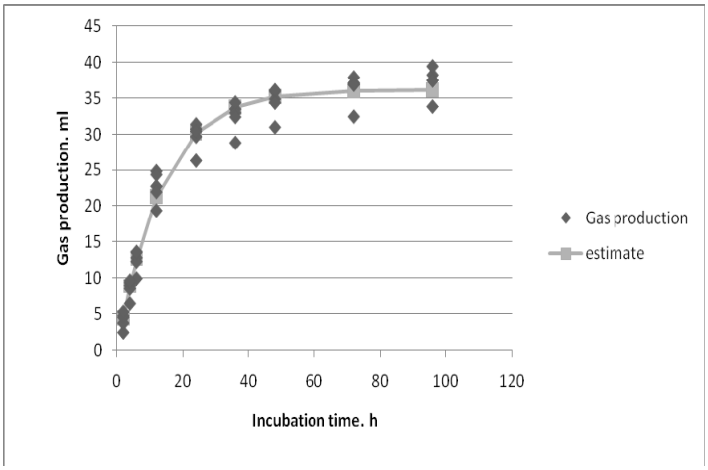

Fig. 1. Gas production from *in vitro* fermentation of *Artemisia campestris* L.

Table 3. Main correlation coefficients between chemical composition and *in vitro* parameters.

	CP	NDF	ADF	ADL	b	k	L
ME	0.76**	-0.58**	-0.65**	-0.72**	0.64**	0.55**	-0.24**
$IV_{24h}OMD$	0.74**	-0.58**	-0.63**	-0.72**	0.81**	0.55**	-0.29**

**P<0.01

IV – Conclusions

It was concluded that the range species evaluated in the current work presented a wide nutritional diversity, thus they could exhibit a complementary role for small ruminants grazing.

Considering the laborious *in vivo* approach in pasture conditions, the *in vitro* procedure may considerably contribute into evaluating such diversity of resources. However, the secondary compounds should be considered in order to improve accuracy of predictive equations.

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Chemical composition, *in vitro* digestibility and fermentation kinetics of arboricultural and agro-industrial by-products in the north of Morocco

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Abstract. Main agricultural by-products in the North of Morocco were evaluated for chemical composition, phenolic compounds, in vitro dry and organic matter digestibility (IVDMD, IVOMD), fermentation kinetics, microbial biomass production (MBP) and partitioning factor (PF). Olive by-products contained highest crude protein (CP), ether extract (EE), ADL and NDF contents (69.8, 178, 526and 691 g/kg DM, respectively). Cactus cladodes of the season (YC), destoned olive cake (OC) preserved by silage (EOC) and OC obtained from olive oil extraction by three-phase centrifugation (EC3P) presented the highest IVDMD (84.10, 69.8 and 65. 1, respectively). However, EC3P and EOC promoted more MBP than YC (395 and 341 vs 122 mg/g DM incubated, respectively), and showed good PF (4.39 and 5.02 mg IVOMD/ml gas, respectively). Fig leaves (FL) showed the lowest MBP (30.3 mg/g of DM incubated) because of its low protein and energy content. Among studied by-products, EOC could be beneficial for ruminant feed, while YC could be an interesting minerals source.

Keywords. By-products – Chemical composition – *In vitro* gas production – *In vitro* digestibility.

Composition chimique, digestibilité* in vitro *et cinétique de fermentation des sous-produits de l'arboriculture et de l'agro-industrie du nord du Maroc

Résumé. *Les principaux sous-produits de l'agriculture du nord du Maroc sont évalués en termes de composition chimique, composés phénoliques, digestibilité* in vitro *de la matière sèche et matière organique (IVDMD, IVOMD), cinétique de fermentation, biomasse microbienne (MBP) et facteur de partition (PF). Les sous-produits d'olivier contiennent la teneur la plus élevée en protéine (CP), extrait éthéré (EE), ADL et NDF (69,8, 178, 526 et 691 g/kg MS, respectivement). Les jeunes raquettes de cactus (YC), les grignons d'olive dénoyautés et ensilés (EOC) et ceux obtenus par centrifugation à 3 phases (EC3P) ont montré une IVDMD élevée (84,1, 69,8 et 65,1, respectivement). Cependant, EC3P et EOC favorisent plus de MBP que YC (395 et 341* vs *122 mg/g de MS incubée, respectivement), et montrent un bon PF (4,39 et 5,02 mg IVOMD/ml gaz, respectivement). Les feuilles de figuier (FL) qui contiennent moins de protéines et d'énergie, ont présenté des MBP faibles (30,3 mg/g de MS incubée). Parmi les sous-produits étudiés, EOC est plus recommandable pour l'alimentation des ruminants, alors que YC constitue une source intéressante de minéraux.*

Mots-clés. *Sous-produits – Composition chimique – Production de gaz – Digestibilité* in vitro*.*

I – Introduction

In the northern region of Morocco, agricultural by-products are largely available. Several studies showed the possibility of exploiting agricultural by-products as alternative feed resources (Makkar, 2003; Ben Salem and Smith, 2008). However, by-products are not yet extensively used because of lacking information on their nutritional value. Chemical analysis, particularly in vitro digestibility can help in the preliminary evaluation of by-products nutritive value in order to identify the suitable feeds (nutrient content and digestibility) for livestock. The objective of this

study was to assess the potential nutritive value of main agricultural by-products based on their chemical composition, and in vitro gas production kinetics.

II – Materials and methods

This work has concerned olive leaves (*Olea europaea,*OL), fig leaves (*Ficus carica,*FL) and cactus (*Opuntia ficus indica*) cladodes of the season (YC) and mature (MC), destoned olive cake (OC) obtained from mechanical pressure (EMP), two-phase (EC2P), three-phases (EC3P) centrifugation extraction procedures of olive oil, and destoned olive cake preserved by silage (EOC). By-products were oven-dried (60°C) and milled using a 1-mm sieve for their later chemical, in vitro digestibility and kinetics analysis.

Dry matter (DM) was determined by drying at 135°C for 4 h (AOAC, 1997). Crude protein (CP) content was determined using the Kjeldahl method (AOAC, 1997). Ether extract (EE) was determined using di-ethylic ether extraction in a Soxhlet system (AOAC, 1997). Ash content was obtained after incineration at 600°C for 8 hours (AOAC, 1997). The NDF, ADF and ADL were determined using fiber extractor (FiberTech) as described by Van Soest *et al.* (1991).

Fermentation kinetics and in vitro digestibility were estimated by the in vitro method of Menke and Steingass (1989). The rumen fluid used for incubation was taken from three slaughtered goats grazing on forest pasture. The inoculum was prepared as described by Goering and Van Soest (1975). The volume of gas was recorded at 0, 2, 4, 8, 12, 24, 48, 62 and 72 hours of incubation using 100 ml gradual glass syringe plunger. At the end of the incubation, contents of each syringe were used to estimate the potential in vitro dry matter (DM) and organic matter (OM) disappearance (IVDMD and IVOMD, respectively). In order to estimate parameters of gas production kinetics, data of the cumulative gas volume produced was fitted to the exponential equation $P=a+b(1-e^{-ct})$ (Ørskov and Mc Donald, 1979), where P (ml) represents the cumulative gas volume at time t; "a" the gas production from soluble fraction; "b" the gas production from insoluble fraction; "a+b": the potential gas production and, "c": the constant rate of gas production during incubation. Microbial biomass production MBP (mg/g of incubated DM) = IVOMD - (Vgas x SF) is measured according to Blümmel (2000); where Vgas is the gas volume produced in ml per g of DM, and (SF) is the stoichiometric factor. The partitioning factor at 24 h of incubation (PF24; a measure of fermentation efficiency) was calculated as the ratio of truly degraded substrate in vitro (mg) to the volume of gas (ml) produced at 24 h (Blümmel *et al.,* 1997).

The in vitro gas production parameters (a, b and c) were estimated using Proc NLIN (SAS, 2002). Data on chemical composition, in vitro digestibility parameters (IVDMD, IVOMD, MBP and PF), gas volume production at time t, in vitro gas production constants (a, b, a+b, c) were subjected to analysis in completely randomized design using GLM procedure (SAS, 2002). Differences between mean values were tested using LSD's test.

III – Results and discussion

1. Chemical Composition

Olive by-products, in particular OC, contain the highest rate of CP (60.4 to 69.8 g/kg DM), ether extract (113 to 178 g/kg DM) and dry matter (519 to 731 g/kg DM). Parietal constituents (ADF, ADL and NDF) are more presented in olive by-products contrary to FL and cactus cladodes (Table 1). Centrifugation mode of oil extraction gets more EE than mechanical pressure (177 and 157 g/kg *vs* 114 g/kg DM), but accuses more ash losses (31.6 and 35.3 *vs* 85.9 g/kg DM, respectively for EC3P, EC2P and EMP). However, this technique seems to give an olive cake with more content of lignin (456 and 526 vs 251 g/kg, respectively for EC2P, EC3P, EMP). Among all by-products, the highest contents of parietal components were obtained in the olive

residues particularly EC3P (691; 526 and 519 g/kg DM, respectively for NDF, ADF and ADL). The FL have less lignin than OL (230 vs 377 g/kg DM), but the cactus cladodes showed the lowest content in lignin among all by-products (114 g/kg DM).

Table 1. Chemical composition of by-products (g/kg DM)

By-products	DM	CP	EE	Ash	ADF	ADL	NDF
OC EMP	731^{a}	60.4^{c}	114^{c}	85.9^{d}	477^{b}	251^{e}	604^{b}
OC EC2P	652^{b}	69.8^{a}	157^{b}	35.3^{f}	450^{c}	456^{b}	579^{c}
OC EC3P	573^{c}	64.6^{b}	178^{a}	31.6^{f}	519^{a}	526^{a}	691^{a}
Ensiled OC	519^{d}	67.0^{ab}	113^{c}	94.9^{c}	229^{e}	344^{d}	620^{b}
Olive leaves	571^{c}	56.8^{c}	89.5^{d}	63.3^{e}	378^{d}	377^{c}	419^{d}
Mature cladodes	52.6^{f}	29.8^{e}	18.9^{e}	129^{a}	148^{f}	149^{g}	341^{f}
Young cladodes	47.9^{f}	48.6^{d}	29.5^{e}	110^{b}	112^{g}	113^{h}	355^{ef}
Fig leaves	296^{e}	35.5^{de}	61.8^{d}	92.8^{cd}	229^{e}	230^{f}	362^{e}
SEM	8.73	1.45	6.95	1.21	2.85	0.18	0.25

Among studied by-products, olive cake obtained by centrifugation has interesting protein and energy values. But, nutritive value of by-products is globally low. In fact, total protein content not exceed 70 g/kg DM. However, ether extract reached 177 g/kg DM. Martín García *et al.* (2003) reported similar CP contents of OC (72.6 g/kg DM) but with lower EE contents (54,5 g/kg DM). Also, lower EE content of OL (56.4 g/kg DM) have been obtained by Molina *et al*. (2003b). Molina and Yáñez-Ruiz (2008) explained that the variation in composition depends on the plant variety, climatic conditions and moisture content. Also, variable amounts in EE depend mainly on residual oil that comes from the crushing of olives during cleaning prior to oil extraction. The NDF and ADF levels are similar to those reported by Martín García *et al.* (2003), Molina *et al.* (2003b), and Al-Masri (2003) with 676 and 406 g NDF/kg DM, 544 and 302g ADF/kg DM, respectively for OC and OL. However, the lignin content of OC (289 g/kg DM) and OL (199 g/ kg DM) reported by these authors are largely lower than ours values. In fact, cell wall constituents vary widely depending on the proportion of stones in OC. Rodriguez-Filex and Cantwell (1988) reported lower contents in CP, EE and ash of cactus cladodes (10.1, 2, and 13 g/kg DM, respectively). Soil and climatic conditions of the cactus plantation and mainly the *Opuntia* species, may be the cause of this difference.

2. Degradation kinetics and in vitro digestibility

During the first 12 hours of incubation, gas production has been quicker and reaches an asymptotic speed faster with fig and cactus by-products than olive by-products (Fig. 1). In fact, FL and YC produce more fermentation gas in a shorter time than olive by-products (Fig.1). Within the OC, ensiled olive cake (EOC) is the by-product which presents more gas production. The highest digestibility is recorded with YC, EOC and EC3P (84.1%, 69.8% and 65.0% respectively; Table 2). In fact, compared to others OC, EOC promoted the highest MBP (395 mg/g of incubated DM) with good fermentation efficiency (PF: 5.02) and presents satisfactory degradation rate (0.15 h^{-1}) of the insoluble fraction. Also, high degradability has been obtained with YC and OL (0.15 and 0.15 h^{-1}). But, these resources did not promote good microbial biomass production (122 and 154 mg, respectively) and showed low fermentation efficiency (3.23 and 3.30 mg IVOMD/ml gas, respectively) than EOC.

Globally, digestibility of most studied by-products is low, except for cactus cladodes. The Anti-nutritionals substances, especially lignin, and also the low content in protein and energy which influence microbial proliferation in rumen, cause digestibility decrease. EOC, which contains

less lignin and NDF, has the highest digestibility coefficient among olive by-products. Indeed, ensiled olive cake (EOC) shows good fermentation at 72 h producing a high amount of gas. Bendaou (2003) explains that the pre-degradation in cell walls during silage fermentation facilitates micro-organisms access in the rumen contents cell, which improves the digestibility. Therefore, EOC shows clearly a nutritional advantage. Vaccarino *et al.* (1982) obtained with stoned OC very low in vitro digestibility values of dry and organic matter (15.8% and 9.7%). This difference is due to the oil extraction mode and preservation by silage. However, Molina *et al.* (2003a) obtained, with using goats juice, similar values of in vitro dry and organic matter digestibility (49% and 46%, respectively). Delgado Pertiñez *et al.* (2000), Martín García *et al*. (2003) and Molina *et al. (*2003a) reported similar in vitro dry matter digestibility of OL (46%).

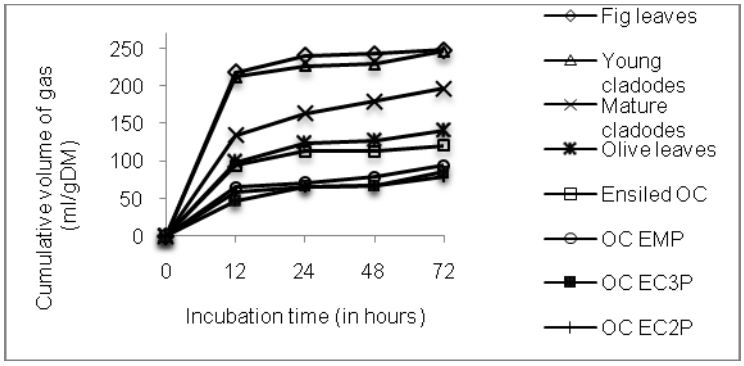

Fig. 1. Cumulative gas production (ml/g DM) of by-products at different incubation times.

Young cactus cladodes present some satisfactory nutritive parameters. Nevertheless, their forage use requires necessarily protein and energy supplementation to improve its MBP. In fact, the low microbial production observed with cactus cladodes may due to the poor quality feeds specially lack of soluble carbohydrates in cladodes which decrease protozoa concentrations in the rumen (cited in Molina and Yáñez Ruiz (2008).

Table 2. In vitro digestibility (%), microbial biomass production (mg), the partitioning factor (PF, in mg IVOMD/ml gas 24h) of by-products and dry matter degradation constants (a,b,c)

By-Product	IVDMD	IVOMD	MBP	PF	a	b	c	a+b
OC EMP	48.4^{f}	41.4^{ef}	206^{d}	4.3^{b}	6.20^{dc}	76.5^{e}	0.14^{ab}	82.7^{d}
OC EC2P	35.1^{g}	38.8^{f}	215^{cd}	4.3^{b}	3.35^{d}	67.8^{e}	0.17^{a}	71.2^{d}
OC EC3P	65.0^{c}	58.3^{cd}	341^{b}	4.9^{ab}	6.61^{c}	75.1^{e}	0.08^{b}	81.7^{d}
Ensiled OC	69.8^{b}	60.7^{c}	395^{a}	5.0^{a}	13.3^{a}	119^{d}	0.15^{ab}	132^{c}
Olive leaves	46.7^{f}	46.2^{e}	154^{e}	3.3^{c}	11.2^{ab}	120^{d}	0.14^{ab}	131^{c}
Mature cladodes	83.6^{a}	63.4^{b}	202^{d}	2.7^{d}	13.9^{a}	167^{c}	0.13^{b}	181^{b}
Young cladodes	84.1^{a}	66.5^{a}	122^{e}	3.2^{c}	8.36^{bc}	229^{b}	0.15^{ab}	237^{a}
Fig leaves	62.3^{d}	57.5^{d}	30.3^{f}	2.3^{d}	-1.01^{e}	251^{a}	0.12^{b}	250^{a}
SEM	0.20	0.20	0.02	0.02	1.02	5.73	0.01	6.12

$^{a-g}$ Means within the same column with different superscript are significantly different (P<0.05).
SEM: standard error of the mean.

IV – Conclusions

The studied by-products constitute a source of medium-to-low quality feed. On the basis of their nutritives parameters, olive cake EC3P especially when it is ensiled is better classified as a local food resource. However, the improvement of their effective digestibility is necessary by mean of totally stoning and increasing protein value. Considering their satisfactory in vitro digestibility and minerals contents, young cactus cladodes could fill the mineral deficiency in the ruminant diet. However, to optimize by-products use in ruminant diet, their anti-nutritional composition has to be identified.

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Effect of oxalic acid on rumen function and microbiota in sheep fed a low quality diet

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Abstract. Oxalic acid is a potentially toxic compound present in many plants that can be consumed by ruminants in some less-favoured areas. However, consequences of its consumption on the rumen function and microbiota remain unclear. Five ewes receiving low quality grass hay were daily dosed 0.6 mmol of oxalic acid/kg body weight through a rumen cannula for 14 days. On days 0 (before starting the dosing), 4, 7 and 14 of administration, alfalfa hay and barley straw were in situ incubated, and samples of rumen digesta were collected throughout the day (0, 3, 6 and 9 h after morning meal). The rumen bacterial community was studied using the terminal restriction fragment length polymorphism (T-RFLP) technique. Oxalic acid administration reduced the dry matter disappearance of alfalfa hay on days 7 and 14 and of barley straw on day 7. Neither pH values nor total volatile fatty acid concentrations were affected. Nevertheless, ammonia and butyrate concentrations were reduced and molar proportions of acetic and propionic acids were increased. Although oxalic acid did not modify rumen bacterial diversity, it altered the structure of the community and the relative frequencies of a number of TR-fragments over the total peak area. Most of these changes were reversed at the end of the experiment (on day 14). Therefore, despite the slight negative effect on ruminal degradation, the lack of a clear detrimental effect on rumen fermentation and the recovery of the initial values in some parameters suggest an adaptation of the ruminal microbiota within 2 weeks.

Keywords. Oxalate – Rumen fermentation – Secondary compound – T-RFLP.

Effets de l'acide oxalique sur le fonctionnement du rumen et le microbiote chez les ovins alimentés avec un régime de faible qualité

Résumé. *L'acide oxalique est un composé potentiellement toxique présent dans de nombreuses plantes qui peuvent être consommées par les ruminants dans des zones défavorisées. Cependant, ses effets sur la fermentation ruminale et le microbiote restent inconnus. Dans l'objectif d'étudier ces effets, cinq brebis canulées au niveau du rumen et alimentées avec une ration de faible qualité recevaient quotidiennement et durant 14 jours, 0,6 mmol d'acide oxalique/kg de poids vif administré à travers la fistule. Avant l'ajout de l'acide oxalique (jour 0) et durant la période d'administration (jours 4, 7 et 14), 3 aliments (foin de luzerne, paille d'orge et épinards) ont été incubés* in situ*, et une collecte* in vivo *des échantillons du contenu ruminal a été réalisée tout au long de la journée (0, 3, 6 et 9 heures après l'administration d'acide oxalique). La communauté bactérienne du rumen a été étudiée en utilisant la technique moléculaire d'étude du polymorphisme de longueur de fragments de restriction terminaux (T-RFLP). L'administration de l'acide oxalique a réduit la disparition de la matière sèche du foin de luzerne les jours 7 et 14, et celle de la paille le jour 7. Toutefois, ni le pH ni la concentration des AGV totaux ont varié significativement, bien que les concentrations en ammoniac et butyrate aient été réduites et les proportions molaires des acides acétique et propionique augmentées. Malgré que la diversité bactérienne n'ait pas varié significativement, l'administration de l'acide oxalique a causé une altération de la structure de la communauté bactérienne du rumen et des fréquences relatives de nombreux pics de fragments de restriction terminaux. Cependant, la plupart de ces changements n'ont pas été révélés en fin d'expérimentation. Ainsi, malgré le faible effet négatif sur la dégradation ruminale, l'absence d'un effet négatif clair sur les fermentations du rumen et le retour aux valeurs initiales de certains paramètres suggèrent que la communauté bactérienne du rumen serait capable de s'adapter à la présence d'acide oxalique en moins de 14 jours.*

Mots-clés. *Acide oxalique – Fermentation ruminale – Composé secondaire – T-RFLP.*

I – Introduction

Oxalic acid is a simple organic acid that is present in a range of plant species commonly consumed by ruminants in some less-favoured areas (Ben Salem *et al.*, 2010). Renal toxicity and hypocalcaemia may occur after the consumption of a substantial quantity of oxalic acid-containing plants (Von Burg, 1994). However, this plant secondary compound is known to be degraded in the rumen by microorganisms to yield formic acid and CO_2 (Allison *et al.*, 1985). *Oxalobacter formigenes* is considered as the main oxalate-degrading bacteria in the gastrointestinal tract, although other digestive microbes are also able to metabolise this compound (Sahin, 2003; Abratt and Reid, 2010). Gradual exposure to increasing levels of oxalic acid leads to an adaptation of the rumen microbiota, the breakdown of this compound preventing its detrimental consequences (Duncan *et al.*, 1997; Duncan *et al.*, 2000). Nevertheless, the action of oxalic acid on the ruminal function and microbial community composition remains unclear. Therefore, this work was carried out to study, in sheep, the effects of oxalic acid consumption on rumen fermentation, utilization of feedstuff and rumen bacterial structure.

II – Materials and methods

Five adult ewes (80 ± 13.9 kg body weight; BW), fitted with a rumen cannula, were individually penned and fed a low quality grass hay for a 2.5 week adaptation period. Then, animals received daily a dose of 0.6 mmol of oxalic acid (Sigma-Aldrich, Germany)/kg BW through the cannula for 14 days. The administration was gradually increased (from 20 to 100%) during the first 5 days. On days 0 (before starting the dosing; Control), 4 (Oxa4), 7 (Oxa7) and 14 (Oxa14) of administration, two different feeds (alfalfa hay and barley straw) were *in situ* incubated in nylon bags, for 12 and 24 h, to assess ruminal degradation, and samples of rumen digesta were collected throughout the day (*i.e.*, immediately before morning meal and 3, 6 and 9 hours later) to study *in vivo* ruminal fermentation parameters (pH, and concentrations of ammonia and volatile fatty acids, VFA) and the bacterial community structure. Samples were strained through two layers of muslin cloth and rumen fluid was used to measure the pH and for ammonia (4 mL, acidified with 4 mL 0.2 M HCl) and VFA (0.8 mL, deproteinized with 0.5 mL of 20 g/L metaphosphoric and 4 g/L crotonic acids in 0.5 M HCl) determinations. All these samples were stored at -30 ºC until analysis. For microbial studies, the same volume of rumen contents were taken at the indicated times, composited daily for each animal and immediately frozen at -80 ºC.

Dry matter (DM) in feeds and in *in situ* incubation residues was determined at 100ºC to constant weight. Ammonia concentration was analysed by a spectrophotometric method and VFA by gas chromatography, using crotonic acid as an internal standard, as reported in Toral *et al.* (2009). For microbial studies, stored rumen samples were freeze-dried and thoroughly mixed before DNA extraction (Belenguer *et al.*, 2010). Duplicate DNA samples were combined and used as templates for terminal restriction fragment length polymorphism (T-RFLP) analysis, which was performed using a universal bacteria-specific primer pair set and the enzymes *HhaI* and *MspI*, as described in Belenguer *et al.* (2010). The fluorescently labelled terminal restriction fragments (T-RF) were analyzed by capillary electrophoresis on an automatic sequence analyzer (MegaBace 500, GE Healthcare Life Sciences, Buckinghamshire, UK). Determination of the sizes of T-RF was performed with the size standard ET 900-R (GE Healthcare Life Sciences, Buckinghamshire, UK) and using the GeneMarker Analysis software (SoftGenetics, USA). Sample data, which consisted of size (base pair, bp) and peak area for each T-RF, were standardized and used to determine the number of T-RF (richness) and the Shannon-Weiner index (Hill *et al.*, 2003). In order to infer the potential bacterial composition in the samples, *in silico* restriction for the major rumen bacteria with the primers and the enzymes used were obtained from the Ribosomal Database Project (http://rdp.cme.msu.edu/; Cole *et al.*, 2009).

From the T-RFLP results, the relative abundance of each fragment over the total peak area was calculated. These data, the diversity indices, and the rumen degradation and fermentation parameters (pH, VFA and ammonia) were analysed by one-way ANOVA using the SAS software package version 9.2 (SAS Institute Inc., USA). The bacterial profiles obtained from T-RFLP were analysed by hierarchical clustering with the Ward's method based on Jaccard distances to build a dendrogram. This was performed with the Community Analysis Package 4 software (Pisces Conservation Ltd., UK).

III – Results and discussion

The effect of the administration of oxalic acid on DM disappearance of alfalfa hay and barley straw, and on the rumen fermentation parameters (daily means) are presented in Table 1. Dry matter disappearance of alfalfa hay, after 12 h of incubation, was reduced (P<0.01) on days 7 and 14, but this effect was not significant after 24 h incubation. The degradation of straw was significantly decreased (P<0.01) on day 7 in 24 h incubation and the initial values were recovered by the end of the study (day 14). The reduction in the degradation of both feeds might be related to an effect of oxalic acid on rumen microbiota (James *et al.*, 1967), although fibrolytic bacteria seemed to have adapted to the presence of oxalic acid at the end of the experiment, as reflected by the recovery of the initial values of DM disappearance of straw.

Table 1. Dry matter disappearance (DMD) of alfalfa hay and barley straw incubated *in situ* for 12 and 24 h, and rumen fermentation parameters (pH, ammonia and VFA) in cannulated sheep fed a low quality diet before (day 0; Control) and after 4 (Oxa4), 7 (Oxa7) and 14 (Oxa14) days of administration of 0.6 mmol oxalic acid/kg BW

		Treatment				SED†	P
		Control	Oxa4	Oxa7	Oxa14		
DMD Alfalfa hay (%)	12 h	63.8^{a}	63.6^{a}	61.2^{b}	62.2^{b}	0.68	**
	24 h	67.0	66.8	65.9	66.3	0.63	NS
DMD Barley straw (%)	12 h	39.6	39.0	35.8	37.9	1.56	NS
	24 h	56.4^{a}	55.7^{a}	51.2^{b}	55.0^{a}	1.33	**
pH		7.01	6.91	6.99	6.95	0.083	NS
Ammonia (mg/L)		108.2^{a}	78.6^{b}	67.1^{b}	80.2^{b}	15.27	***
Total VFA (mmol/L)		89.0	89.8	89.0	85.6	6.52	NS
Molar proportions (%)							
Acetic		66.9^{b}	67.5^{b}	68.3^{a}	68.6^{a}	0.65	***
Propionic		18.9^{c}	20.7^{a}	19.6^{b}	19.4^{bc}	0.59	***
Butyric		11.0^{a}	9.2^{c}	9.6^{b}	9.4^{bc}	0.39	***
Minor VFA††		2.81^{a}	2.36^{b}	2.12^{b}	2.13^{b}	0.176	***

†Standard error of the difference.
††Calculated as the sum of valeric, isobutyric, isovaleric and caproic acids.
NS, non significant (P<0.10); **, P<0.01; ***, P<0.001. Values in a row with different superscripts are significantly different (P<0.05).

Oxalate reduced the ammonia concentrations (P<0.001), whereas the variations in pH and total VFA concentrations were not significant (P>0.05). However, some specific VFA were significantly altered, with increases in the molar ratios of acetic and propionic acids and reductions in the concentrations (data not shown) and molar proportions of butyric and minor VFA (sum of valeric, isobutyric, isovaleric and caproic, P<0.001). These minor VFA originate mostly from deamination of specific amino acids, so their decrease, together with the lower ammonia concentration, is consistent with an inhibitory effect of oxalic acid on rumen

proteolysis. The lack of effects on the total VFA would indicate that rumen fermentation was not negatively affected, although the changes in molar proportions of VFA reflects divergences in fermentation pathways, probably due to stimulation of specific microbial groups to the detriment of others or to a shift in microbial degradation pathways (Russel and Wallace, 1997).

Even though diversity indices (richness and Shannon-Weiner) were not altered significantly (P>0.10), the administration of oxalic acid modified the bacterial structure in the rumen, as observed in Fig. 1. T-RFLP profiles in samples from days 0 and 14 were grouped together and separated from those from days 4 and 7, indicating that the bacterial community was able to adapt to the presence of oxalic acid within 14 days.

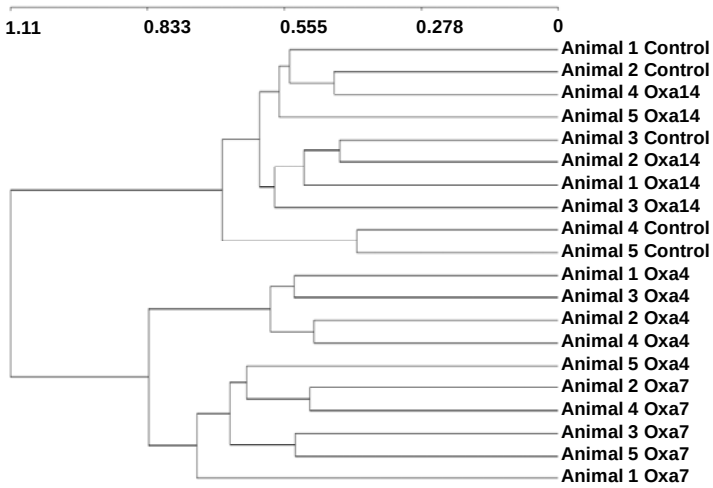

Fig. 1. Cluster analysis of the T-RFLP profiles of total bacteria present in the rumen contents of cannulated sheep fed a low quality diet before (day 0; Control) and after 4 (Oxa4), 7 (Oxa7) and 14 (Oxa14) days of administration of 0.6 mmol oxalic acid/kg BW.

The relative frequencies over the total peak area of several T-RF were significantly affected by the treatment (Table 2). Surprisingly, the T-RF compatible with *O. formigenes* obtained with *HhaI* (567 bp) showed no significant variations and those obtained with *MspI* (486 + 489 bp) decreased their relative abundance on days 4 and 7. Nonetheless, it is important to consider that other bacteria belonging to the orders *Bacteroidales* and *Clostridiales,* which are abundant in the rumen (Edwards *et al.*, 2004), may also match those fragments. Therefore, concomitant variations in these microorganisms might have masked the potential variations in *O. formigenes*. Although the latter is the only functional oxalotrophic bacteria isolated from the gastrointestinal tract (Sahin, 2003), other digestive bacteria are also able to degrade oxalates (Abratt and Reid, 2010). Unfortunately, the methodology used does not allow discerning which bacterial populations may or may not be responsible for the degradation of the oxalic acid. The initial abundance of some other detected T-RF (*e.g.*, 579 bp with *HhaI* and 91 bp with *MspI*) was recovered by the end of the experiment, supporting the hypothesis that the bacterial community would be adapted to oxalic acid consumption within 14 days.

IV – Conclusion

Overall, the results show that oxalic acid alters the pattern of ruminal fermentation and the bacterial community structure. Nonetheless, despite the slight negative effect on feed

degradation, the lack of detrimental consequences on ruminal fermentation and the recovery of the initial values in some parameters suggest that the rumen microbiota was able to adapt to the presence of this secondary compound in less than 14 days.

Table 2. Relative abundances (%) over the total peak area of several fragments obtained by T-RFLP analysis of microbial DNA samples extracted from the rumen contents of cannulated sheep fed a low quality diet before (day 0; Control) and after 4 (Oxa4), 7 (Oxa7) and 14 (Oxa14) days of administration of 0.6 mmol oxalic acid/kg BW

Enzyme	Length (bp)	Treatment				SED†	P
		Control	Oxa4	Oxa7	Oxa14		
HhaI	321	1.61^{a}	0.19^{b}	0.00^{b}	0.16^{b}	0.200	***
	567	5.63	5.38	5.60	6.12	1.239	NS
	577	5.97	1.12	3.88	6.00	2.000	T
	579	3.94^{b}	12.83^{a}	4.13^{b}	1.53^{b}	2.938	**
MspI	91	3.87^{a}	0.78^{c}	2.04^{bc}	3.34^{ab}	0.767	**
	95	15.01	4.26	12.5	11.76	3.760	T
	261	2.31	7.09	4.25	2.36	1.908	T
	486+489	3.92^{ab}	2.54^{b}	2.53^{b}	5.45^{a}	0.806	**

†Standard error of the difference.
NS, non significant (P>0.10); T, P<0.10; **, P<0.01; ***, P<0.001. Values in a row with different superscripts are significantly different (P<0.05).

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Effect of the nature of the concentrate feed on the end products of digestion in the Sicilo-Sarde sheep

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Abstract. The pH, VFA concentration, total gas and methane production were determined in the rumen of four Sicilo- Sarde rams fitted with permanent canulas. Rams received a ration that included 1.5 kg DM of oat hay and were supplemented with one of four concentrates: CC (10% barley, 43.3% corn, 25% wheat bran, 17.7% soybean meal, 4% sheep Vitamin and Mineral Mixture (VMM)), SC (66% white sorghum, 30% faba, 4% sheep VMM); TC (71% triticale, 18% faba, 7%, soybean meal, 4% VMM) or BC (71.5% barley, 17.5% faba, 7% soybean meal and 4% VMM). 50 ml samples were taken before, 2, 5 and 8 hours after the morning meal. Total gas was determined on rumen content before the morning meal. The rumen pH was statistically different (P<0.05) before and 2 hours after the morning meal among concentrates feed. It was in favour of TC and BC (P<0.05) concentrates but was comparable at the end of the day. The concentration of VFA was significantly higher (P<0.05) for diets TC and BC following the meal and became comparable among concentrates thereafter. The proportion of acetate and butyrate acids evolved in the same way during the day regardless of the regimen. The total volume of gas was different (P<0.05) among diets, the BC showed the highest value (87.00±17.29 ml) while the lowest value was found in the TC concentrate (56.58±13.06 ml). The CH_4 production for the BC was significantly different (P<0.05) from that of TC. Quantities produced by the CC and SC were similar (22.08±4.18 *vs*. 21.16±3.21).

Keywords. Acetate – Butyrate – Propionate – Rams – Rumen – Total gas.

Effet de la nature des aliments concentrés sur les produits terminaux de la digestion des ovins de race Sicilo-Sarde

Résumé. *Le pH, la concentration des AGV, la production de gaz total et de méthane sont déterminés dans le rumen de quatre béliers Sicilo-Sardes porteurs de canules ruminales permanentes. Les béliers ont reçu une ration de base de foin d'avoine à raison de 1,5 kg MS/j complémentée par l'un des quatre aliments concentrés: CC (10% orge, 43,3% maïs, 25% son de blé, 17,7% tourteau de soja, 4% CMV), SC (66% sorgho blanc, 30% fèverole, 4% CMV); TC (71% triticale, 18% fèverole, 7%, tourteau de soja, 4% CMV) ou BC (71,5% orge, 17,5% fèverole, 7% tourteau de soja, 4% CMV). 50 ml de jus de rumen étaient prélevés avant, 2, 5 et 8 heures après la distribution du repas du matin. Le gaz total a été déterminé sur le contenu de rumen prélevé avant le repas du matin. Le pH ruminal était statistiquement différent (p<0,05) avant et 2 heures après le repas en fonction des aliments concentrés. Il a été en faveur de TC et BC (p<0,05), puis il a été comparable pour tous les aliments concentrés à la fin de la journée. La concentration des AGV était statistiquement élevée (p<0,05) pour les aliments concentrés TC et BC et statistiquement comparable pour les autres aliments concentrés. La proportion d'acétate et de butyrate évolue de façon similaire durant la journée quel que soit le régime alimentaire. Le volume total de gaz a été différent (p<0,05) entre les régimes, l'aliment BC présente la valeur la plus élevée (87,00±17,29 ml) alors que l'aliment TC affiche la valeur la plus faible (56,58±13,06 ml). La production de* CH_4 *a été significativement différente avec TC (p<0,05). La quantité produite par les aliments concentrés CC et SC était similaire (22,08±4,18* vs. *21,16±3,21).*

Mots-clés. *Acétate – Béliers – Butyrate – Gaz total – Propionate – Rumen.*

I – Introduction

The development of ruminant livestock in needs the parallel use of different sciences (nutrition, reproduction, genetics, health), in an integrated way with the agriculture systems. The conditions of the rearing environment (temperature, humidity, conditions, and quality of fodder, etc.) in sheep farms are frequently difficult and limit individual performances (milk and meat). The feeding of ruminant livestock , the sustainability of the farming system and income of the farmer remain the main areas of research in nutrition.

Supplementation with concentrated feed rations remains at high costs, which is not a stimulating factor for high production (Poncet *et al*., 2003).

Indeed, in the current global economic environment characterized by rising prices of these ingredients, using local raw materials (barley, triticale, sorghum and faba bean white) in total or partial replacement of imported raw materials (corn meal soybeans) is an alternative to be tested. It is within this framework that fits our work which aims to measure the effect of nature of supplementation on ruminal fermentation parameters.

II – Materials and methods

Animals and diets

Four rams of Sicilo-Sarde breed with an average live weight at the beginning of the trial of 45.25±3.5 kg and aged 4.8±0.5 years, fitted with permanent canulas in the rumen, were used in this experiment. Animals had a common basal diet at 1.5 kg DM/head/day of oat hay supplemented by four concentrates differing by the nature of protein and energy ingredients they contained: CC (10% barley, 43.3% corn, 25% wheat bran, 17.7% soybean meal, 4% sheep Vitamin and Mineral Mixture (VMM)), SC (66% white sorghum, 30% faba, 4% sheep VMM); TC (71% triticale, 18% faba, 7%, soybean meal, VMM) or BC (71.5% barley, faba 17.5%, 7% soybean meal and VMM) at 500 g / head / day. Food values were 0.98, 0.99, 0.98 and 1.01 UFL / kg DM respectively and similar protein (104.9; 95; 103; 103 g PDIE/kg DM, respectively) and (99; 96; 102; 100 g PDIE/kg DM, respectively).

Rumen fermentation

The samples for the determination of various parameters of rumen fermentation took place just before serving meals in the morning (before, 2, 5 and 8 hours after the meal). The pH of the inoculum was measured just after each sampling. The concentrations of VFA were measured by gas chromatpgraphy. Determining the total gas (CO_2 and CH_4) was performed on the filtered rumen contents, collected before the distribution of the morning meal. 0.5 g of substrate (oat hay milled at 1 mm), 10 ml of rumen fluid and 40 ml of artificial saliva (Menke and Steingass, 1988) were put in syringes, and at the end of each incubation, 5 ml of NAOH (10 N) were injected in each syringe to determine the amount of methane.

Statistical analysis

The results of the effects of diets on the parameters measured were subjected to analysis of variance by GLM procedure of SAS (1989) and compared by the Duncan test (1955). The kinetics of gas production used is analyzed using nonlinear regression on the model by Orskov and MacDonald (1979): $Gas = b (1 - e^{-ct})$.

III – Results and discussion

The pH of the rumen before the morning meal distribution was statistically comparable ($p > 0.05$)

for diets CC, TC and BC (6.67±0.34; 6.60±0.27 and 6.71±0.27, respectively) and significantly lower ($p<0.05$) for the SC system (6.28 ± 0.22). This result is similar to those of Rouissi (1994) and Hammami *et al*. (2009) and below the range of pH in the rumen of sheep receiving hay alone (Giger *et al*. 1988). Two hours postprandial, pH decreases for the four diets, but the decrease is larger for BC and TC (0.44 and 0.49 points respectively), while in the other two concentrates this decrease is minimal (0.19 to 0.1 for CC and SC). After 5 hours the pH continues to decrease: 6.22±0.42; 5.97±0.22; 6.06±0.29 and 6.01±0.12, respectively for concentrates CC, SC, BC and TC. Statistical analysis reveals that there is no difference between the pH of the different diets ($p>0.05$). At the end of the day, the pH increased significantly ($p<0.05$), being more stable in buffered diets TC and BC thanin CC and SC.

Volatile fatty acids are the end products of rumen digestion of carbohydrate foods that include various compounds which are derived from either plant cell walls such as cellulose, hemicelluloses and pectin, or the cell contents, such as the starch and soluble sugars (Jarrige *et al*,. 1995; Sauvant, 1997), their concentration depends on the amount of energy provided by the food and the quality of starch and its speed of degradation (Sauvant *et al*., 1994; Cuvelier *et al.*, 2005).

The study of the effect of the nature of the energy source at the complementation showed that the concentration of total VFA in the rumen just before the distribution of the morning meal is lower compared to other periods of control during the day with a minimum value observed for the concentrate SC ($p<0.05$). This can be explained by the absorption of VFA across the rumen wall on one side and use the bacteria to produce their own proteins. Two hours after the distribution of the morning meal, the concentration increases with an intense speed ($p<0.05$) for diets TC and BC (86.5±1.76 and 85.45±0.69 mmol/l respectively) compared to diets CC and SC. This result is consistent with that of Chikagwa-Malunga *et al.* (2009). This trend can be explained by the rapid rumen degradability of the starch to contained in the seeds of barley and triticale.

After five hours of the morning meal, the concentration of VFA from the schemes CC and SC reached the peak (89.03±0.82 and 87.28±1.05 mmol/l respectively), this would be attributed to degradation of starch grains of white maize and sorghum. This corroborates the results of Russell and Gahr (2000), no statistical difference between the means of four concentrates ($p>0.05$). It is also noted that the high concentration of VFA for concentrate TC is correlated with the low gas production especially at the beginning of incubation. At the end of the day, the VFA concentration is stabilized ($p>0.05$) for the different regimes, this decrease in concentration can be explained by the rate of absorption and activity of micro organisms in the rumen (Rouissi, 1994) (Table 1).

The proportion of acetic acid changes similarly for concentrated feeds TC and BC, the minimum value being observed after two hours of the distribution (65.4 and 66.06% respectively). Then, it increases after 5 hours and is stabilized at the end of the day with no statistical difference between the regimes ($p>0.05$). This trend is similar to that demonstrated by Chikagwa-Malunga *et al.* (2009) and can be explained by the orientation of the fermentation of starch grains of barley and triticale with a strong and rapid degradation thereby reducing the synthesis of acetate and promoting that of propionate, which increases after the circulation of morning meal and the maximum value is displayed after two hours ($p>0.05$) (17.08 and 16.83% respectively for TC and BC) (Jouany *et al.,* 1995) and partly explains what is reported by Giger *et al*. (1988) who observed that the concentration of acetate and propionate in the rumen are reversed during the day. For diets of slowly degradable starch resources minimum value is reached after 5 hours (65.63 and 65.58%), while propionate is maximum at this time. Comparing regimes, the proportion of acetate is stable at the beginning and the end of the day ($p>0.05$), and this is mainly due to the rate of absorption through the rumen wall and use by bacteria in the presence of ammonia nitrogen for the synthesis of their protein, whereas it is statistically higher ($p<0.05$) for CC and SC, 2 hours post prandial. The concentration of butyric acid in the rumen operates in the same direction as that of acetate; the proportion is 11 to 13%

during the day regardless the diet (p>0.05). This is consistent with those of Rouissi (1994) shows lower values than those of Jouany *et al.* (1995), especially when the diet was based on beet.

Table 1. Effect of the nature of energy sources on the ruminal pH and Total VFA (mmol/l)

	Hours after the morning feeding			
	0	**2**	**5**	**8**
pH				
CC	6.67 ^{a} ± 0.34	648 ^{a} ± 0.38	6.22 ^{a} ± 0.42	6.25 ^{b} ± 0.34
SC	6.28 ^{b} ± 0.22	6.18 ^{b} ± 0.13	5.97 ^{a} ± 0.22	5.99 ^{b} ± 0.31
TC	6.60 ^{a} ± 0.27	6.16 ^{b} ± 0.21	6.06 ^{a} ± 0.29	6.40 ^{a} ± 0.35
BC	6.71 ^{a} ± 0.27	6.16 ^{b} ± 0.12	6.01 ^{a} ± 0.12	6.34 ^{a} ± 0.24
SME	0.084	0.069	0.083	0.091
Total VFA (mmol/l)				
CC	76.85^{a} ±1.1	81.4^{b} ± 1.46	89.03^{a} ± 0.82	86.3^{a} ± 0.83
SC	70.33^{b} ±1.58	78.36^{b} ± 1.3	87.28^{a} ± 1.05	83.9^{a} ± 1.22
TC	75.45^{a} ± 0.59	86.5^{a} ± 1.76.	88.55^{a} ± 07	83.3^{a} ± 1.24
BC	74.68^{a} ±1.49	85.45^{a} ± 0.69	87.58^{a} ± 0.74	84.66^{a} ± 1.03
SME	1.96	2.74	1.09	1.87

a, b and c: Means with different superscripts within a row differ significantly (p<0.05).

The total volume of gas after 36 hours incubation was statistically different (p<0.05) between diets. The concentrate (BC) displays the highest value (87.00±17.29 ml), which is similar to the results of Selmi *et al.* (2009). This could be explained by the rich grain of barley and faba bean starch, rapidly degradable in the rumen, thereby fostering an environment rich in VFA and NH3-N used by bacteria and protozoa to their development and proliferation (Michalet-Doreau and Sauvant, 1989). The lowest volume of gas is observed in the concentrate (TC) (56.58±13.06 ml). The proportion of methane (CH4) produced for the four concentrates is in the range of 25 to 35% from the total gas as shown in Table 2. CH4 production in the rumen is significant difference (p<0.05) with the concentrate, which is similar to the work of Sauvant (2000). The potential gas production represented in the model Orskov and Macdonald (1979) by the constant "b" is higher (p<0.05) for the regime CC (58.7) while the BC diet displays lowest value (34.5).

Table 2. Gas volume and methane (ml) in the rumen

	Type of Concentrate				SME
	CC	**SC**	**TC**	**BC**	
Gas_{24} (ml)	66.41 ±11.53^{a}	64.33 ±16.37^{a}	44.33 ±12.83^{b}	70.91±14.79^{a}	2.78
Total gas (ml)	77.66 ± 11.65^{a}	78.41 ±16.61^{a}	56.58± 13.06^{b}	87.00 ±17.29^{a}	1.07
CH_4 (ml)	22.08 ± 4.18^{ab}	21.16 ± 3.21^{ab}	19.75 ± 3.88^{b}	24.91 ± 5.69^{a}	1.25
b	58.7^{a}	50.3^{ab}	48.1^{b}	34.5^{c}	0.84
c	0.001^{a}	0.002^{a}	0.002^{a}	0.003^{a}	0.03

Gas24: gas to 24 h of incubation; CH4: methane; b: potential gas production, c: velocity of gas production. a, b and c: Means with different superscripts within a row differ significantly (p<0.05).

IV – Conclusions

Following this experiment, it appears that the effect of the incorporation of local raw materials

instead of imported raw materials in the formulation of feed concentrate feed can maintain or improve some parameters of rumen and *in situ* digestibility of the basic ration. Indeed, the rumen pH varies intensely with concentrates based on cereals as their starches are rapidly degradable. This trend explains well the general shape of the concentration of ammonia nitrogen during the day. Gas production is remarkable for the concentrates containing barley compared with the other schemes so that the production of methane may represent a loss of energy up to 10% of the digestible energy of the ration.

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In vitro ruminal fermentation of diets containing wheat straw and date pits as forage

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Abstract. The aim of this study was to analyze the effect of partial replacement of wheat straw (WS) by date pits (DP) in diets with different forage:concentrate (F:C) ratio. Three feeds [WS, DP and a commercial concentrate (CON)] were used to formulate six diets according to a 3 × 2 factorial arrangement of treatments (3 F:C ratios and 2 forage sources). The diets had F:C ratios of 100:0, 80:20 (80) or 60:40 (60) with either WS or a 80:20 WS:DP mixture (MIX) as forage, and were designated as WS (100% WS), 80WS, 60WS, MIX, 80MIX and 60MIX. Samples (500 mg) of each diet were incubated with 50 ml of buffered rumen fluid at 39ºC. Final pH, production of volatile fatty acids (VFA), total gas and methane, NH_3-N concentration and organic matter apparently fermented (OMAF) were determined after 24 h. The neutral detergent fibre (NDF) content of WS and DP was 71.1 and 86.5% (dry matter basis), but NDF lignification was greater for DP (26.7% of NDF) than for WS (15.3% of NDF). Compared with WS, the ruminal fermentation of the MIX diet resulted in greater (P<0.05) butyrate proportions (11.7 *vs.* 14.0%) and lower (P<0.05) gas production (2768 *vs.* 2588 µmol) but there were no differences (P>0.05) in the rest of the fermentation parameters. For both forages (WS and MIX), increasing the proportion of CON in the diet increased (P<0.05) the production of gas and total VFA, the molar proportion of butyrate and the OMAF. There were no differences (P>0.05) between 80WS and 80MIX diets in any fermentation parameter. Compared with 60WS diet, the fermentation of 60MIX diet resulted in greater production of total VFA (3061 *vs.* 3264 µmol), but no differences were detected in the rest of the parameters measured. The final pH, methane production and NH_3-N concentrations ranged between 6.91 and 7.00, 434 and 516 µmol, and 330 and 359 mg/L, respectively, with no differences (P=0.059 to 0.121) among diets. The results indicate that WS can be replaced by DP at 20% of the forage portion of the diet without any detrimental effect on ruminal fermentation. Furthermore, in diets with 60:40 F:C ratio, incorporating DP resulted in greater VFA production. If this result is confirmed in vivo, it would be of great interest in sheep practical feeding.

Keywords. Date pits – Wheat straw – Ruminal fermentation – Sheep.

Fermentation ruminale* in vitro *de rations contenant de la paille de blé et des noyaux de dattes

Résumé. *L'objectif de cette étude était d'analyser l'effet du remplacement partiel de la paille de blé (WS) par les noyaux de dattes (DP) dans des rations avec différents rapports fourrage:concentré (F:C). Trois aliments [WS, DP et du concentré commercial (CON)] ont été utilisés comme ingrédients pour formuler 6 rations selon un dispositif factoriel (3 rapports F:C et deux sources de fourrages). Les rations avaient des rapports de F:C de 100:0, 80:20 (80) ou 60:40 (60) avec soit WS ou 80:20 du mélange WS:DP (MIX) comme fourrage, et étaient désignées comme WS (100% PB), 80 WS, 60 WS, MIX, 80MIX et 60MIX. Des échantillons (500 mg) de chaque ration ont été incubés avec 50 ml de jus de rumen tamponné à 39ºC. Le pH final, la production d'acides gras volatils (AGV), de gaz et de méthane, la concentration de NH_3-N et la matière organique apparemment fermentée (MOAF) ont été déterminés après 24 h de fermentation. La teneur en NDF de WS et DP était de 71,1 et 86,5% (DM), mais la lignification de la NDF était plus importante pour les DP (26,7% de NDF) que pour WS (15,3% NDF). Par comparaison à WS, la fermentation ruminale du mélange a permis d'avoir des proportions plus élevées (P<0,05) en acide butyrique (11,7* vs. *14,0%) et plus faibles (P<0,05) en gaz (2768* vs. *2588 µmol) mais il n'y avait pas de différence (P>0,05) entre les deux rations pour les autres paramètres de fermentation. Pour les deux fourrages (WS et MIX), l'augmentation de la proportion du CON dans la ration a augmenté (P<0,05) la production de gaz et les acides gras volatils totaux, ainsi que la proportion molaire du butyrate et la matière organique apparemment fermentée. Aucune différence (P>0,05) entre les rations 80WS et 80MIX n'a été*

enregistrée au niveau des paramètres de fermentation. Par comparaison avec la ration 60WS, la fermentation de la ration 60MIX a généré une plus grande production d'AGV totaux (3061 vs. *3264 µmol), cependant, aucune différence n'a été détectée pour le reste des paramètres mesurés. Le pH final, la production de méthane et les concentrations de NH_3-N ont varié entre 6,91 et 7,00, 434 et 516 µmol, et 330 et 359 mg/L, respectivement, sans aucune différence (P=0,059 à 0,121) parmi les rations. Les résultats indiquent que WS peut être remplacé par DP à raison de 20% de la proportion du fourrage de la ration sans aucun effet négatif sur la fermentation ruminale. Par ailleurs, dans les rations 60:40 F:C, l'incorporation des DP a résulté en une plus grande production d'AGV. Si ces résultats sont confirmés* in vivo*, ils pourraient être d'un grand intérêt dans la pratique.*

Mots-clés. *Noyaux de dattes – Paille de blé – Remplacement – Fermentation ruminale.*

I – Introduction

Algeria, with more than 20 million sheep, 3.8 million goats, 1.65 million cattle and 0.30 million camels (FAOSTAT, 2010) has an insufficient animal feed production, and livestock production relies heavily on imported and subsidized feed. To cope with this problem, Algeria decided to adopt in the medium-long term a politic favoring the national production of feeds, and consequently reducing imports until reaching the self-sufficiency level (MADR, 2003). In this context, the use of crop residues and agro-industrial by-products would play an important role. These categories include, among other feeds produced in Algeria, cereals straw and date palm by-products, mainly date pits (DP). Date pits become available in high quantities when pitted dates are produced in packing plants or in industrial date processing plants based on juice extraction, as Algeria has over 12 million palm trees and ranked in the top 10 of the date producers worldwide (FAOSTAT, 2010). At the rural level one may find some accumulation of DP when immature dates are pitted before sun-drying (Barreveld, 1993). To achieve an efficient utilization of any by-product, it is necessary to know its chemical composition and nutritive value. The aim of this study was to analyze the effect of partial replacement of wheat straw (WS) by DP in diets with different forage:concentrate ratio.

II – Materials and methods

1. Feeds and diets

Three feeds (WS, DP and a commercial concentrate) were used as ingredients to formulate six diets according to a 2 × 3 factorial arrangement of treatments. The diets had forage:concentrate ratios (dry matter (DM) basis) of 100:0, 80:20 (80) or 60:40 (60) with either WS or a 80:20 WS:DP mixture (MIX) as forage, and were designated as WS (100% WS), 80WS, 60WS, MIX, 80MIX and 60MIX. Ingredient composition of concentrate was barley, maize, wheat bran, soybean meal, NaCl and mineral-vitamin premix in the proportions of 270, 270, 277.3, 180, 1.7 and 1 g/kg, respectively (DM basis). Chemical composition of feeds and experimental diets is given in Table 1.

2. *In vitro* incubations

Samples of each feed and diet were ground through a 1-mm screen, and accurately weighed (500 mg) into 120-ml serum bottles for *in vitro* incubations. Ruminal fluid was obtained from four rumen-cannulated Merino sheep fed medium-quality alfalfa hay *ad libitum*. Ruminal contents of each sheep were obtained before the morning feeding, mixed and strained through four layers of cheese-cloth into an Erlenmeyer flask with an O_2-free headspace. Particle-free fluid was mixed with the buffer solution of Goering and Van Soest (1970) in a proportion 1:4 (v:v) at 39ºC under continuous flushing with CO_2. Bottles were prewarmed (39ºC) prior to the addition of 50 ml of buffered ruminal fluid into each bottle under CO_2 flushing. Bottles were sealed with rubber

stoppers and aluminium caps and incubated at 39ºC for 24 h. Four incubation runs were performed on different days, so that each treatment was conducted in quadruplicate.

Table 1. Chemical composition (g/100 g dry matter) of feeds and experimental diets

	Organic matter	Crude protein	Neutral-detergent fibre	Acid-detergent fibre	Hemicellulose	Cellulose	Lignin
Feeds							
Wheat straw	91.8	4.44	71.1	41.2	29.9	30.3	10.9
Date pits	99.0	5.11	86.5	62.1	24.4	38.9	23.1
Concentrate	93.5	12.85	58.9	7.18	51.7	5.05	2.13
Diets†							
WS	91.8	4.44	71.1	41.2	29.9	30.3	10.9
80WS	92.2	6.13	68.7	34.4	34.3	25.3	9.12
60WS	92.5	7.81	66.2	27.6	38.6	20.2	7.37
MIX	93.3	4.58	74.2	45.3	28.8	32.0	13.3
80MIX	93.3	6.23	71.1	37.7	33.4	26.6	11.1
60MIX	93.3	7.89	68.0	30.1	38.0	21.2	8.84

†WS: wheat straw; MIX: 80:20 wheat straw:date pits; 80WS: 80:20 wheat straw:concentrate; 60WS: 60:40 wheat straw:concentrate; 80MIX, 80:20 MIX:concentrate; 60MIX, 60:40 MIX:concentrate.

Bottles were withdrawn from the incubator 24 h after inoculation, total gas production was measured in each bottle using a pressure transducer and a calibrated syringe, and a gas sample (about 15 ml) was stored in a vacuum tube before analysis for CH_4 concentration. Bottles were then uncapped, the pH was immediately measured and the fermentation was stopped by swirling the bottles in ice. One ml of the bottle content was added to 1 ml of deproteinizing solution (10% of metaphosphoric acid and 0.06% crotonic acid; w/v) for volatile fatty acids (VFA) analysis and another 1 ml was added to 1 ml of HCl for NH_3-N analysis.

3. Analytical procedures, calculations and statistical analyses

Dry matter, ash and N were determined according to the AOAC (1999). Neutral-detergent fibre (NDF), acid-detergent fibre (ADF) and acid-detergent lignin analyses were carried out according to Van Soest *et al.* (1991) and Goering and Van Soest (1970). NH_3-N concentration was determined by a colorimetric method and VFA concentrations by gas chromatography as described by Carro *et al.* (1999). Methane was measured as described by Martínez *et al.* (2010).

The amounts of VFA produced were obtained by subtracting the amounts present initially in the incubation medium from those determined at the end of the incubation period. The amount of organic matter apparently fermented (OMAF) in each bottle was estimated from net productions of acetate, propionate and butyrate as described by Demeyer (1991).

Data were analysed using the MIXED procedure (SAS Inst. Inc., Cary, NC). The effects of type of forage (WS and WSDP), concentrate level (0, 20 and 40%) and the interaction type of forage x concentrate level were considered fixed, and incubation day effect was considered random. Significance was declared at $P<0.05$, whereas $P<0.10$ values were considered as trends and discussed. When a significant effect of treatment ($P < 0.05$) was detected, differences among means were tested using the Tukey's multiple comparison test.

III – Results and discussion

Organic matter, CP, NDF and ADF contents of WS and DP were in the range of those previously reported by others (Sipos *et al.*, 2010; Boudechiche *et al.*, 2009; Habib and Ibrahim, 2009). These results confirm DP as a good source of cellulose (38.9% of DM), but with a higher lignification compared with WS (26.8 and 15.3% of NDF, respectively).

The results of the *in vitro* fermentation of WS, DP and concentrate with ruminal fluid from sheep are shown in Table 2. The VFA production for DP was 1.3 times lower (P<0.001) than that for WS, which is consistent with the higher NDF and lignin content of DP (Table 1). Compared with WS, *in vitro* fermentation of DP resulted in lower (P<0.05) proportions of acetate and propionate, but higher (P<0.05) proportions of butyrate and other minor VFA (isobutyrate, valerate and isovalerate) and acetate/propionate ratio. In contrast, there were no differences (P>0.05) in methane production between WS and DP. Final concentrations of NH_3-N were lower (P<0.05) for DP than for WS, despite of their similar CP content (5.11 and 4.44 g/100 g of DM for DP and WS, respectively). This difference may be due to a lower CP degradability of DP compared with that of WS. As expected, fermentation of concentrate resulted in higher (P<0.001) VFA production and lower (P<0.001) acetate/propionate ratio than fermentation of WS and DP, which agrees well with the lower NDF and ADF content of concentrate compared with that of WS and DP. The higher CP content of the concentrate resulted in higher (P<0.05) NH_3-N concentrations in the cultures with concentrate as substrate than those in the cultures with DP (361 and 316 mg/L, respectively), but in all cultures NH_3-N concentrations were above the minimum level necessary for maximal rate of fermentation (Mehrez *et al.*, 1978) due to the use of a nitrogen-enriched buffer for the in vitro incubations. The higher (P<0.05) NH_3-N concentrations observed in the cultures with WS compared with those with DP could not be explained by differences in CP content, as both feeds had similar CP content (4.44 and 5.11 g/100 g of DM for WS and DP, respectively). Digestibility of CP of DP has been reported to be very low (Al-Yousef *et al.*, 1993) and even negative values have been found in some studies (El-Shazly *et al.*, 1963). A low CP degradability of DP would explain the lower NH_3-N concentrations observed in the cultures with DP.

Table 2. Final pH, production of of volatile fatty acids, gas and methane, NH_3-N concentration and organic matter apparently fermented (OMAF) after *in vitro* fermentation of wheat straw, date pits and concentrate samples (500 mg) in batch cultures of mixed ruminal microorganisms for 24 h (n=4)

Item	Wheat straw	Date pits	Concentrate	SEM	P value
pH	6.97[b]	7.10[c]	6.76[a]	0.014	<0.001
VFA, volatile fatty acids (µmol)					
Acetate	1613[b]	1207[a]	2118[c]	28.5	<0.001
Propionate	565[b]	385[a]	888[c]	31.9	<0.001
Butyrate	310[a]	308[a]	743[b]	14.7	<0.001
Other†	147[a]	139[a]	188[b]	6.1	0.002
Total	2635[b]	2039[a]	3937[c]	54.7	<0.001
Molar proportions of VFA (mol/100 mol):					
Acetate	61.3[a]	59.5[b]	53.9[b]	0.75	0.001
Propionate	21.4[b]	18.8[a]	22.4[b]	0.51	0.006
Butyrate	11.7[a]	15.0[b]	18.9[c]	0.43	<0.001
Others†	5.58[a]	6.73[b]	4.78[a]	0.238	0.003
Acetate/Propionate (mol/mol)	2.89[b]	3.18[c]	2.45[a]	0.081	0.002
Gas (µmol)	2768[b]	2109[a]	4676[c]	45.1	<0.001
Methane (µmol)	437[a]	419[a]	581[b]	22.4	0.004
NH_3-N (mg/L)	359[b]	316[a]	361[b]	6.0	0,031
OMAF (mg)	226[b]	179[a]	364[c]	4.9	<0.001

SME: Standard error of the mean. †Calculated as the sum of isobutyrate, isovalerate and valerate.
[a, b, c]Mean values within a row with unlike superscript letters differ (P<0.05).

The results of the in vitro fermentations of the experimental diets are shown in Table 3. For both forages (WS and MIX), supplementation with concentrate resulted in increased (P<0.05) total VFA, acetate, propionate and butyrate productions without changes (P>0.05) in the production of other minor VFA. These results are in agreement with those from other studies (García-Martínez *et al.*, 2005; Martínez *et al.*, 2010) in which diets with different forage/concentrate ratios were fermented in vitro with ruminal fluid from sheep. Moreover, the differences observed between the fermentation pattern of WS and MIX as the only feed and that of the diets including concentrate closely resembled the in vivo fermentation patterns reported when sheep fed low-quality forages were supplemented with concentrates (Fondevila *et al.*, 1994; Castro *et al.*, 2002).

Table 3. Final pH, production of volatile fatty acids (VFA), gas and methane, NH_3-N concentration and organic matter apparently fermented (OMAF) after *in vitro* fermentation of diets (500 mg) containing wheat straw, date pits and concentrate samples in batch cultures of mixed ruminal microorganisms for 24 h (n=4)

Item	Diet†						SEM	P value
	WS	80WS	60WS	MIX	80MIX	60MIX		
pH	6.97	6.92	6.91	7.00	6.92	6.93	0.020	0.059
VFA (µmol)								
Acetate	1613ab	1781cd	1711bc	1486a	1774cd	1892d	49.3	0.001
Propionate	565a	611ab	697bc	538a	580ab	660bc	30.1	0.017
Butyrate	310a	397b	492c	355ab	406b	523c	22.2	<0.001
Others††	147	150	161	160	152	179	10.2	0.315
Total	2635a	2940bc	3061cd	2539a	2911b	3254e	48.8	<0.001
Molar proportions of VFA (mol/100 mol):								
Acetate	61.3	60.8	56.4	58.6	61.1	58.2	1.52	0.191
Propionate	21.4	20.7	22.4	21.1	19.8	20.2	0.84	0.346
Butyrate	11.7a	13.5ab	15.9c	14.0b	13.9b	16.1c	0.60	0.001
Others††	5.58ab	5.09a	5.23a	6.27b	5.20a	5.51a	0.249	0.045
Acetate/Propionate (mol/mol)	2.89	3.00	2.68	2.78	3.11	2.90	0.146	0.394
Gas (µmol)	2768b	3203c	3510d	2588a	3136c	3513d	41.8	<0.001
Methane (µmol)	437	516	474	462	508	434	23.4	0.108
NH_3-N (mg/L)	359	341	348	330	331	341	7.3	0.121
OMAF (mg)	226ab	258bc	275cd	221a	245bc	292d	6.5	<0.001

†WS: wheat straw; 80WS: 80:20 wheat straw:concentrate; 60WS: 60:40 wheat straw:concentrate MIX: 80:20 wheat straw:date pits; 80MIX: 80:20 MIX:concentrate; 60MIX: 60:40 MIX:concentrate. ††Calculated as the sum of isobutyrate, isovalerate and valerate.
[a, b, c, d, e]: mean values within a row with unlike superscript letters differ (P<0.05).

Final pH ranged between 6.91 and 7.00 for all diets, being in the range of values for optimal cellulolytic activity (Stewart, 1977). There were no differences (P>0.05) between 80WS and 80MIX in any of the measured parameters, indicating that replacing 20% of WS by DP in the forage did not affect the ruminal fermentation of the diet. However, the production of acetate and total VFA was 11 and 6% higher (P<0.05), respectively, compared with that with the 60WS diet. There were no differences (P>0.05) between 60WS and 60MIX diets in the rest of the determined fermentation parameters. These results indicate that replacing WS by DP at 20% of the forage portion of the diet would be an easy means of increasing VFA production.

IV – Conclusions

The results of this study indicate that wheat straw can be replaced by date pits at 20% of the forage portion of the diet without any detrimental effect on ruminal fermentation. Furthermore, in diets with 60:40 forage:concentrate ratio, incorporating date pits in the forage portion resulted in greater volatile fatty acids and acetate production. If these results are confirmed *in vivo*, it would be of great interest in sheep practical feeding in countries having high palm by-products production. Including date pits in the diet would be especially interesting for dairy ruminants, as acetate is the main precursor for de novo milk-fat synthesis in the mammary gland.

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n-Alkanes for grazing studies with ruminants: where can we go?

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Abstract. An attempt was made to estimate the amount and quality of pasture consumed by sheep, using simultaneously faecal microhistology procedures and n-alkanes markers as markers. Cuticles of field-surveyed plants were characterized and n-alkane profiles of those consumed by the animals (cuticles found in faeces) determined to estimate diet composition and then dry matter intake and digestibility. Since in grazing conditions faecal recoveries of the different n-alkanes cannot be calculated directly, they were estimated by dosing through that of dotriacontane (C_{32}), an artificial artificially dosed alkane and quantifying its recovery in . In the present work large differences were observed when diet composition was estimated using different methods of calculation. No method was able to detect all species identified by faecal microhistology, except in the case of six out of the 24 animals in which the composition of the diet was estimated. It can be concluded from the results that good estimates of diet composition and intake are obtained in grazing animals, using n-alkanes as markers, including only the discriminant hydrocarbons in the calculations. Also better results of diet composition (more compatible with microhistological findings in the faeces) and intake (more compatible with live weight changes of the grazing animals) are obtained applying faecal recovery to diet components instead to faeces. Besides, it seems necessary to estimate accurately the faecal recoveries of *n*-alkanes, even in grazing conditions, as they largely influence the digestibility results. Furthermore, it is important to use a large population of experimental animals as in our case only 20 % presented consistent results of diet composition and intake.

Keywords. *n*-Alkanes – Diet selection – Intake – Sheep.

Les n-alcanes pour l'étude des ruminants sur les pâturages : jusqu'à où peut-on aller ?

Résumé. *Une tentative a été faite pour estimer la quantité et la qualité des pâturages consommés par les ovins, en utilisant conjointement la microhistologie fécale et la technique des n-alcanes. Les cuticules des plantes du terrain étudié ont été caractérisées et les profils des n-alcanes des plantes consommées par les animaux (cuticules trouvées dans les fèces) déterminés afin d'estimer la composition de la diète et l'ingestion de la matière sèche et sa digestibilité. Puisque, dans les conditions de pâturage, les récupérations fécales des différents n-alcanes ne peuvent être calculées directement, ils ont été estimés à partir d'un alcane dosé (dotriacontane-C_{32}) qui a montré une haute concentration dans les fèces. Dans le présent travail, il a été démontré que de grandes différences apparaissent lorsque la composition du régime est estimée en utilisant différentes méthodes de calcul. Aucune méthode n'était capable de détecter toutes les espèces identifiées par la microhistologie fécale, sauf dans le cas de six des 24 animaux dans lesquels la composition du régime a été estimée. À partir des résultats obtenus, il peut être conclu que de bonnes estimations de la composition de la diète et d'ingestion en utilisant la technique des n-alcanes chez les animaux aux pâturages sont obtenues notamment lorsque les hydrocarbures discriminants sont inclus dans les calculs. Également, de meilleures estimations de la composition de la diète (plus compatibles avec les résultats de la microhistologie fécale) et d'ingestion (plus compatibles avec les changements de poids vif des animaux aux pâturages) sont obtenues lorsque la correction pour la récupération fécale est appliquée aux composants de la diète plutôt qu'aux échantillons de selles. Il est également nécessaire d'évaluer avec précision les récupérations fécales des n-alcanes, même en pâturage, car elles influencent largement sur les résultats de digestibilité. En outre, il est important d'utiliser une large population animale dans ce genre d'estimations vu que dans notre cas, seulement 20% ont présenté des résultats cohérents en ce qui concerne la composition du régime alimentaire et l'ingestion.*

Mots-clés. *n-Alcanes – Sélection du régime – Quantités ingérées – Ovins.*

I – Introduction

Small ruminants provide cheap animal protein in many areas of the Mediterranean basin, being their production mainly based on extensive pasture grazing. In these conditions, animal performance depends primarily on intake and diet quality, which are both extremely difficult to accurately estimate in sheep and goats grazing heterogeneous (multispecies) dryland pastures. The *n*-alkane methodology (Mayes *et al*., 1986) is currently one of the most used for this purpose, although it requires a correct sampling and identification of the plant species (and/or parts) actually consumed by the herd (Dove and Mayes, 2005). Microhistological examination of cuticles from oesophageal, ruminal or faecal samples has been used to this purpose for a long time (Holechek and Gross, 1982), although it presents the disadvantage that a huge number of reference images are needed. These techniques are also time consuming and give poor estimates of the proportion of an individual diet component (Dove and Mayes, 2006).

In the last twenty-five years more than fifty annual or biannual species have been identified in the grazed lucerne fields of the Ebro valley (North-East Spain), although no characterization of their cuticles has been performed. On the other hand, no information exists about their *n*-alkane profiles which knowledge is compulsory for intake and diet composition estimations.

In this work cuticles and *n*-alkanes profiles of the cited species were characterized, and then diet composition and dry matter intake and digestibility were estimated in sheep. To this purpose, different methods (Mayes *et al.*, 1994; Dove and Moore, 1995) were compared.

II – Materials and methods

The study was carried out in 2003, and a survey aimed at identifying as many plant species as possible was conducted in a 1-ha paddock of dryland lucerne. Specimens of each identified plant kind were taken, and two subsamples obtained: one for cuticle characterization (Stewart, 1967) and one for *n*-alkane analysis. Then a grazing trial was performed with 24 non-pregnant, non-lactating Rasa Aragonesa ewes (average live weigth 48.5 ± 1,36 kg) during 28 consecutive days. Barley straw was available *ad libitum* in the field, its cuticle characterized and its n-alkane concentrations analyzed. Animals' live weight was registered weekly.

During the whole grazing period a once-daily dose of 1.5 g of paper pellets containing equal amounts of tetracosane (C_{24}), dotriacontane (C_{32}) and hexatriacontane (C_{36}) was given to each animal with a dosing gun at 09:00 h. About 5% of the pellets were sampled and analysed for alkane concentration. Average concentration (± SEM) of C_{24}, C_{32} and C_{36} in the dosed pellets was 76.2±4.07, 76.8±4.25 and 74.4±4.29 mg/pellet. Spot faecal samples were collected daily, directly from the rectum, at the same time as alkane dosing, freeze-dried and pooled, on a DM basis, to a single sample per animal for analysis.

Estimates of diet selection were obtained using different mathematical approaches. First, concentrations of individual alkanes in the consumed plant species (those which cuticles were present in the faeces, including barley straw) were expressed as proportion of the total amount, and the ratios were arcsin transformed in order to satisfy normality. A discriminant analysis was then performed to obtain centroids for every group (species) using subsequent functions. Only discriminant functions increasing at least 10% of accumulated variability were analysed (Dove *et al.*, 1996). Results obtained were subjected to a one-way analysis of variance and then validated using the cross-validation method. The statistical package SPSS 19.0 was used.

Estimates of diet composition were obtained with either all alkanes (C_{21} to C_{36}, except dosed alkanes and C_{22} and C_{24} which were used as internal standards) or only discriminant alkanes (different depending on the animal considered) using the 'Solver' routine of the 'Microsoft Excel' programme with non-negative restrictions (Mayes *et al.*, 1994) or the 'EatWhat' programme (Dove and Moore, 1995). In the first case, diet composition was estimated by minimisation of the sum of the squared discrepancies between the measured faecal proportions of individual

alkanes (recovery-corrected and expressed relative to the total faecal alkane (R), and diet alkane proportions (of the total alkane) calculated from alkane profiles of dietary components (E), as follows:

$$\sum [R-E]^2_{alk:1...n} = \sum \left[\frac{H_i}{H_t} - \frac{xA_i + yB_i + zC_i}{xA_t + yB_t + zC_t} \right]^2_{alk:1...n} \quad \text{[Eq. 1]}$$

where x, y and z represent the proportions of components A, B and C in the diet; Hi, Ai, Bi and Ci the concentrations of alkane i in faeces (recovery-corrected) and components A, B and C, and Ht, At, Bt and Ct total alkane concentrations (recovery-corrected in the case of the faeces). Since in grazing conditions faecal recoveries of the different n-alkanes cannot be calculated directly, they were estimated relative to the dosed alkane C_{32} (which showed the highest faecal concentration/dose ratio) as suggested by Dove *et al.* (1999).

In the present work differences between the two methods of calculation appeared and for the sake of understanding reasons for that the following equation was used with the 'Solver' routine:

$$\sum [R-E]^2_{alk:1...n} = \sum \left[\frac{H_i}{H_t} - \frac{(xA_i + yB_i + zC_i) * FR_i}{x(A_i * FR_i + A_j * FR_j + ...) + y(B_i * FR_i + B_j * FR_j + ...) + z(C_i * FR_i + C_j * FR_j + ...)} \right]^2_{alk:1...n} \quad \text{[Eq. 2]}$$

In this equation *i, j,* … represent different n-alkanes, and FR_i, FR_j, … their faecal recoveries. This is the form data must be introduced in the 'EatWhat' programme. As a third option, the expression:

$$\sum [R-E]^2_{alk:1...n} = \sum \left[\frac{H_i}{H_t} - (x * \frac{A_i}{A_t} + y * \frac{B_i}{B_t} + z * \frac{C_i}{C_t}) \right]^2_{alk:1...n} \quad \text{[Eq. 3]}$$

was used, recovery-correcting either faecal (Equation 3.1) or diet components (Equation 3.2) *n*-alkane concentrations.

Intake estimates were performed in animals where diet composition was accurately obtained [$\Sigma (R-E)^2$ equal or very close to zero] using the n-alkane pair C_{31}/C_{32} (Mayes *et al*., 1986).

Dry matter digestibility was estimated, in the same animals as intake, using the 'EatWhat' programme or hentriacontane (C_{31}) as internal marker.

III – Results and discussion

Thirty-two plant species were identified in the field (including barley straw-*Hordeum vulgare*), although only eleven were found in the faeces of sheep (Table 1).

A great variability between animals was noted in diet selection, and the most appreciated species were *Medicago sativa* (selected by 92% of the animals), *Poa* ssp. (83%) and *Marrubium vulgare* (50%). Cuticle fragments of barley straw (*Hordeum vulgare*) were identified only in 67% of the animals' faeces. It might be argued that some cuticles could have not been identified because of digestion processes, but faecal samples were taken along 28 consecutive days, and hence the probability of identifying some relatively intact structures was supposed to be high.

Table 2 shows the sum of the squared discrepancies between the measured faecal proportions of individual alkanes (R), and diet alkane proportions (of the total alkane) calculated from alkane profiles of dietary components (E). Values were obtained using the 'Solver' routine of the 'Microsoft Excel' programme and Equation 1, Equation 2, Equation 3.1 and Equation 3.2.

Table 1. Plant species identified in the faeces of sheep grazing a dryland lucerne field

	Animal number																	
	4	3	2	1	6, 9, 14	7	5, 16, 20	8, 23	11	10	12	13, 24	15	18	19	17	21	22
Medicago sativa	x	x	x		x		x	x	x	x	x	x	x	x	x	x	x	x
Poa ssp.	x	x			x	x	x	x	x	x	x	x	x		x	x		x
Hordeum vulgare	x		x	x	x	x		x	x		x	x		x			x	x
Marrubium vulgare						x	x	x		x				x	x	x	x	x
Anacyclus clavatus		x		x		x				x	x	x						
Dactylis hispanica	x		x	x										x				x
Carduus tenuiflorus		x							x		x		x					
Erodium cicutarium	x	x																
Salvia verbinaca															x	x		
Descurainia sophia																x		
Plantago lanceolatus						x												

Table 2. Sum of the squared discrepancies (±SEM) between the measured faecal proportions of individual alkanes (expressed relative to the total faecal alkane), and diet alkane proportions (of the total alkane) calculated from alkane profiles of dietary components (n = 24). Values were obtained using the 'Solver' routine of the 'Microsoft Excel' programme, and Equations 1, 2, 3.1 and 3.2 are defined in the Materials and Methods section

	Equation 1	Equation 2	Equation 3.1	Equation 3.2
Including all alkanes	0.008±0.0132	0.007±0.0128	0.008±0.0132	0.007±0.0128
n	0	0	0	0
Including only discriminant alkanes	0.002±0.0235	0.001±0.0211	0.002±0.0235	0.001±0.0211
n	6	6	6	6

n: number of sheep in which all plant species detected in faeces by microhistology were included in the estimates of diet composition.

Including only discriminant alkanes in the different equations improved the accuracy of diet composition estimates compared to when using all alkanes, although even in that case only in 6 out of 24 sheep all species identified in faeces by microhistology appeared in the estimated diet composition. In addition, Equation 1 and Equation 2, and Equation 3.1 and Equation 3.2, provided identical results only when $\sum (R\text{-}E)^2 = 0$ or very close to zero (individual data not presented). On the other hand, equations Equation 2 and Equation 3.2 gave more accurate estimates of diet composition than equations Eqn.1 and Eqn.3.1. Values of $\sum (R\text{-}E)^2$ were identical regardless the mathematical models used were Equation 1 or Equation 3.1, or Equation 2 or Equation 3.2.

A comparison between diet composition estimates obtained using the 'Solver' routine of the 'Microsoft Excel' programme or the 'EatWhat' programme (only with Eqn.2) was carried out in order to try and understand the reasons for the different estimates of diet composition obtained using the different mathematical models. Results of this comparison are given in Table 3. The 'S' values, which are a measure of the goodness of fit of the solution, with units of alkane concentration (Dove and Moore, 1995), were much lower when only the discriminant alkanes were used. Hence, only these results were included in Table 3. Only when 'S' values were = 0 or very close to zero estimates of diet composition obtained with the two programs were

practically identical (animals 5, 6, 9, 10, 16 and 20). In these cases, also the $\sum (R-E)^2$ values obtained with the 'Solver' were = 0 or very close to zero (data not presented). In other cases, a lower value of 'S' did not mean more accuracy, and vice versa (e.g. O1 vs O15 in Table 3).

Table 4 shows different estimates of intake and dry matter digestibility (DMD) for the six animals in which diet composition was assumed to have been accurately obtained.

Table 3. Estimates of diet composition (as proportions) obtained using the 'Solver' routine of the 'Microsoft Excel' programme or the 'EatWhat' programme, and Equation 2. Only discriminant alkanes were employed, and data from selected animals (O) are given

		Solver	EatWhat	S			Solver	EatWhat	S
O1	*Hordeum*	0.6856	0.8640	9.52	O10	*Medicago*	0.0867	0.0860	0.00
	Anacyclus	0.0000	0.0000			*Poa*	0.2933	0.2920	
	Dactylis	0.3144	0.1360			*Marrubium*	0.0622	0.0630	
						Anacyclus	0.5578	0.5590	
O5	*Medicago*	0.7943	0.7940	0.00	O15	*Medicago*	0.4352	0.4050	77.67
	Poa	0.0249	0.0250			*Poa*	0.5648	0.5950	
	Marrubium	0.1809	0.1810			*Carduus*	0.0000	0.0000	
O6	*Medicago*	0.1087	0.1090	0.00	O16	*Medicago*	0.8965	08960	0.00
	Poa	0.1982	0.2010			*Poa*	0.0026	0.0030	
	Hordeum	0.6931	0.6890			*Marrubium*	0.1009	0.1010	
O9	*Medicago*	0.0875	0.0870	0.00	O20	*Medicago*	0.8972	0.8970	0.00
	Poa	0.1279	0.1230			*Poa*	0.0048	0.0050	
	Hordeum	0.7846	0.7910			*Marrubium*	0.0980	0.0980	

Table 4. Intake (g/day) and dry matter digestibility (DMD; %) in animals (O) where diet composition estimate was considered accurate

	O5	O6	O9	O10	O16	O20
Intake Equation 1- Equation 2	731	2161	1748	1588	846	457
Intake Equation 3.1	739	1282	1056	1073	846	449
Intake Equation 3.2	729	1276	959	1067	844	444
DMD EatWhat	58.68	79.76	84.94	76.8	60.63	60.16
DMD C_{31} Equation 1- Equation 2	35.74	79.78	83.56	76.77	34.96	38.49
DMD C_{31} Equation 3.2	33.32	65.35	72.17	62.77	32.00	35.94
DMD C_{31} Equation 3.1	30.86	65.19	69.51	62.54	30.72	34.46

Intake Equation 1- Equation 2: estimates obtained using diet composition values derived from Equation 1 or Equation 2. Intake Equation 3.1: estimates obtained using diet composition values derived from Equation 3.1. Intake Equation 3.2: estimates obtained using diet composition values derived from Ec.3.2. DMD EatWhat: estimates obtained using the 'EatWhat' programme. DMD C_{31} Equation 1- Equation 2: estimates obtained using the C_{31} alkane as internal marker and equations Equation 1 or Equation 2. DMD C_{31} Equation 1: estimates obtained using the C_{31} alkane as internal marker and equation Equation 1. DMd C_{31} Equation 2: estimates obtained using the C_{31} alkane as internal marker and equation Equation 2.

In the case of intake, values obtained from diet composition derived from equations Equation 3.1 or Equation 3.2 agreed much more with changes in body weight for all six animals included in Table 4. On the contrary, estimates of DMD were not considered satisfactory in any case. As an example, estimates of straw intake in animals O6 and O9 were high (34% and 45% of the

total dry matter intake), whereas DMD obtained was also abnormally high. It is speculated that an accurate estimation of faecal recovery of n-alkanes (relative to that of C_{32} in the present work) might play an important role.

IV – Conclusions

From the results obtained, it can be concluded that good estimates of diet composition and intake using the n-alkanes technique in grazing animals can be obtained including only the discriminant hydrocarbons in the calculations. Also better results of diet composition (more compatible with microhistological findings in the faeces) and intake (more compatible with live weight changes of the grazing animals) were obtained applying faecal recovery to diet components instead to faeces. For intake estimates, the use of equation Equation 3 gave the best results and hence is proposed as an alternative to the classical approaches of Mayes *et al.* (1994) or Dove and Moore (1995). It seems also necessary to estimate accurately the faecal recovery, as it largely influences the digestibility results. A large population of experimental animals should be used as in our case only 20% presented sound results.

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Session 3
Animal welfare / Product quality

The role of biotic and abiotic stress factors on sheep welfare: The example of parasites and climatic changes in European countries

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Abstract. Traditional Mediterranean small ruminants' production systems mostly involve local breeds, which are kept in outdoor conditions with natural ligneous vegetation and cereal stubble, as major dietary components. Biotic stress factors, in particular, gastrointestinal nematodes remain one of the main threats for the health and the welfare in such 'low input' production conditions. Gastrointestinal parasites can cause production losses, increased susceptibility to other diseases and/or pests, and even death. Abiotic stress factors (e.g. temperature stress and imbalanced diets) are known to result in significant reductions in yield and product quality and may increase the susceptibility of animals to parasites and other diseases. Predicted changes in climate are expected to increase heat stress incidence, especially in Southern Europe. Abiotic stress is also known to increase the susceptibility of sheep to gastrointestinal parasites. The predicted impacts of climate change include increased heat stress, changes in semi-natural vegetation cover used for grazing, damaged ecosystems, and rising sea levels. The actual effects are predicted to heterogeneous and to differ between regions. Yet, in most cases, the negative effects are expected to outweigh the benefits and disproportionately hurt traditional small ruminant farmers which in many regions are among the poorest population groups, and have the least capacity for adaptation. Climatic changes will reduce grain yields, will impair pasture composition, quality and quantity, will direct affect pathogen prevalence, incidence and severity and thereby influence animal health and productivity and resource (especially feed) use efficiency. Adaptation of livestock to the more variable environmental conditions predicted as a result of climate change should therefore be a primary focus of R&D focused on improving small ruminant management and breeding systems/strategies. A particular focus should be on improving the sustainability and robustness via the utilization of robust, indigenous breeds rational waste utilisation and management, development of more balanced diets and re-integration of (and nutrient cycling between) local small ruminant and crop production systems.

Keywords. Stress – Sheep – Gastrointestinal nematodes – Climatic changes.

Le rôle des facteurs de stress biotique et abiotique sur le bien-être des ovins: L'exemple des parasites et des changements climatiques dans les pays européens

Résumé. *Les systèmes de production traditionnels méditerranéens des petits ruminants concernent surtout les races locales, qui pâturent et qui utilisent la végétation ligneuse et les chaumes de céréales comme principaux composants alimentaires. Les facteurs de stress biotique, tels que l'infestation par des parasites gastro-intestinaux, restent l'une des principales menaces pour la santé et le bien-être des petits ruminants élevés en conditions extensives. Ces parasites gastro-intestinaux peuvent provoquer des pertes économiques dues aux baisses de production, aux coûts de la lutte, à une augmentation de la sensibilité aux maladies et parfois même la mort des animaux. Les facteurs de stress abiotique (ex : température, régimes alimentaires déséquilibrés) sont connus pour influencer la quantité et la qualité des productions* et ils peuvent augmenter la sensibilité des animaux aux parasites et autres maladies. *Selon les prévisions de changement climatique il faut s'attendre à une hausse du stress thermique, en particulier dans le sud de l'Europe. Ce stress thermique est aussi connu pour augmenter la sensibilité des petits ruminants aux parasites gastro-intestinaux. L'impact du changement climatique comprend une augmentation de la chaleur, des modifications de la végétation seminaturelle, le bouleversement des écosystèmes et l'augmentation du*

niveau de la mer. Les prédictions sur les effets réels sont hétérogènes et spécifiques à chaque région. Pourtant, dans la plupart des cas, il est apparent queles effets négatifs sont supérieurs aux effets bénéfiques et qu'ils toucheront spécialement les éleveurs de petits ruminants, un groupe de population peu favorisé et à faible capacité adaptative. Le changement climatique va réduire les rendements en grains, affaiblir la qualité et la quantité des pâturages, affecter la prévalence, l'incidence et la sévérité des pathogénies , influençant l'état de santé et la productivité des animaux. L'adaptation de l'élevage dans un environnement en mutation devrait être un élément clé dans tous les choix et stratégies de recherche et développement. Ces stratégies devraient soutenir le développement durable et une gestion moderne de la production animale qui inclura l'adaptation de systèmes d'élevage appropriés, la sélection de races indigènes robustes, la gestion rationnelle des déchets et des régimes alimentaires équilibrés, ainsi que la ré-intégration (à travers des cycles de nutriments) entre systèmes de production de petits ruminants et de cultures.

Mots-clés. *Stress – Moutons – Nématodes gastro-intestinaux – Changements climatiques.*

I – Introduction

Small ruminants (sheep and goats) are a major component of the dairy sector in the Mediterranean basin. Sheep and goat production often occupies marginal lands that are unsuitable for crop (including feed crop) production. As such, the major areas where sheep production is the dominant agricultural activity are often relatively mountainous and/or arid regions, for example the hills of the northern Europe and the Mediterranean basin (Dyrmundsson, 2006; de Rancourt *et al.*, 2006). However, sheep and goats are also widely distributed as part of diversified farming systems and have a role in the conversion of grain and other feed crops into meat, milk and wool, as well as forming an important part in nutrient cycling into crop rotations. Local tradition and demand for sheep products, not only basic economics, underpin the wide distribution of grazing-based systems of small ruminant production and in semi-natural environments they deliver important environmental benefits such as biodiversity conservation, wildfire risk reduction and landscape management (de Rancourt *et al.*, 2006).

Climate change is projected to increase the average temperature, affect rainfall pattern and increase the length of the growing season in Europe (e.g. IPCC, 2007). The projected influence on the timing and volume of crop biomass production is expected to affect a number of interlinked ecosystems. Changes in the timing and length of the growing season may not only have far reaching consequences for plant and animal ecosystems but persistent increases in growing season length may lead to long-term increases in carbon storage (White *et al.*, 1999) and changes in vegetation cover which may in turn affect the global climate system (Robeson, 2002; Linderholm, 2006). It is estimated that there has been an average global decadal increase of 0.13^{o}C between 1956 and 2005; a further increase of around 0.4^{o}C is expected within the next 2 decades (IPCC, 2007). Changes in the phenology, distribution and biomass of several species in response to climate change have been observed worldwide; a meta-analysis of published data for over 1700 species from a range of taxa, estimated that the range limits of these species had shifted on average 6.1 km (±2.4 km) per decade towards the poles. Other species have shifted their range to higher altitudes. In addition, the timing of spring events such as frog breeding and tree budburst has advanced, on average, 2.3 days per decade (Parmesan and Yohe, 2003).

Parasitic helminths are commonly found in all ruminant species and farming systems worldwide. The economically most important helminths are members of a group of gastrointestinal nematodes, lungworms or trematodes (liver and rumen flukes). Among the gastrointestinal nematodes, *Teladorsagia circumcincta, Haemonchus contortus, Trichostrongylus* spp. and *Nematodirus battus* have become the major production-limiting nematode parasite species affecting sheep in temperate climates, with their relative importance being influenced by

regional and temporal differences in climate and sheep management. Other genera, such as *Cooperia*, *Chabertia*, and *Oesophagostomum* can be important in some contexts but usually as part of a mixed parasitic infection. Parasites reduce the animals' productivity by feeding on the host or its blood (e.g. *H. contortus*), taking up nutrients from the hosts' gastrointestinal tract, damaging the absorptive lining of the gastrointestinal tract and stimulating the immune system (Greer, 2008). The outcome is inefficient feed utilisation, inducing a state of relative protein deficiency, fluid and electrolyte or macro-element imbalances and anaemia, leading to clinical signs, such as hyporexia, daily weight gain decrease, diarrhoea, and in some cases death. The uptake of parasites is ubiquitous in grazing sheep and control of parasitism is generally achieved by frequent administration of anthelmintic drugs to suppress egg output and consequently decrease infection pressure. The spread and increasing prevalence of resistance to anthelmintic drugs, however, threatens the efficacy of this control approach and the sustainability of nematode control in sheep (Coles, 2002). Overall, the greatest economic importance of nematode parasites is suboptimal productivity arising from continuous low-level exposure to infective larvae (Coop *et al.*, 1982).

In relatively recent times, the evolutionary balance between parasites and, for example, their sheep hosts has been upset by domestication and the subsequent development of intensive livestock management practices, which create environments that are suited to the development and survival of free-living stages of the parasites, enhance sheep exposure to infective larvae, inadvertently alter the host innate or adaptive immune responses to infection and thus enable exposure to previously unrecognised parasitic nematode species or strains. Furthermore, these conditions may have affected different parasitic nematode species to differing extents, upsetting the equilibrium that may exist within the sheep host between different parasites (Mapes and Coop, 1973; Dobson and Barnes, 1995), affording a competitive advantage to some and allowing potentially pathogenic species to predominate.

If the above mentioned changes in climate are sustained as predicted, various effects on sheep and goat production could be foreseen. Climate influences the abundance of infective larval stages of parasites via direct effects on the development and survival rate of the free-living stages in pastures leading to an increase of animals' infection rates (O'Connor *et al.*, 2006). Also, the spatial distribution of different species and the fauna is closely related to climatic conditions. Fig. 1 shows some ways in which climate change may affect different parasites species and therefore the risk of disease. The consequences of climate change are, therefore, particularly important in helminth parasites because of its potential impact on their free-living stages and/or their intermediate or paratenic hosts.

II – Climate and parasites

The rates of physiological processes in the majority of invertebrates are highly dependent on ambient temperature. The existing level of challenge and seasonal patterns of infection also show climate-driven spatial variation (Smith and Grenfell, 1994; Kao *et al.*, 2000; O'Connor *et al.*, 2006, Morgan, and van Dijk, 2012).

Changes in climate might be expected therefore to have a direct effect on parasite distribution, abundance, and population dynamics leading (depending on the species) to an increased or a decreased prevalence. For example, eggs of the several ovine trichostrongylids develop into the infective third larval stage (L3) above a threshold of around 4^{o}C, some species, such as *H. contortus*, have a higher threshold of around 8^{o}C (Kao *et al.*, 2000; O'Connor *et al.*, 2006). Above the threshold, development rate is proportional to temperature. The rate of this increase, which is likely to be an important determinant of the response of these nematodes to global warming, varies between species. Since mortality also increases within increasing temperature, optimal temperatures, at which the maximum number of larvae is produced from a given number of eggs, are difficult to predict. The optimum temperature for development also varies in a large range from 16.30^{o}C for *T. circumcincta* to 25.37^{o}C for *H. contortus* (O'Connor *et al.*,

2006). Early stage larvae (L1 and L2) are more vulnerable to extreme temperature and desiccation than L3, the last are able to withstand much harsher conditions. L3 can survive to for several months in water at 3°C; they are destroyed by freezing (Jasmer *et al.*, 1987; O'Connor *et al.*, 2006). At higher temperatures, L3 survival declines with increasing temperature. In part, this is due to the persistence of the second larval stage cuticle, while protecting them from adverse environments effects, it enables it from feeding, leading to a decrease of lifespan when temperature decreases. Thus, L3 *H. contortus*, for instance, has a population half-life of around 93 days at 12°C and only 9 days at 28°C (Barger *et al.*, 1972; O'Connor *et al.*, 2006).

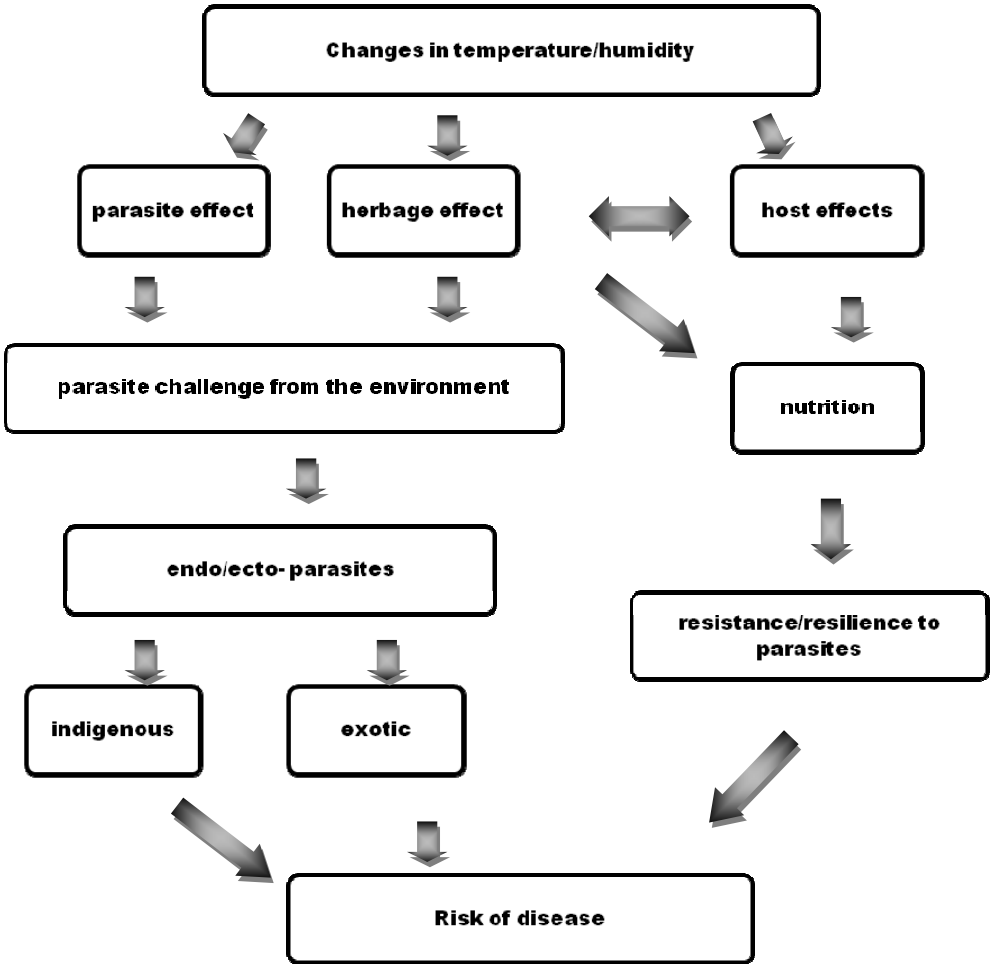

Fig. 1. Scheme on how climate change may affect different parasites and the risk of disease (Kenyon *et al.* 2009, modified).

Other climatic variables can also have high impact on L3 survival. For example, desiccation and ultraviolet irradiation increase mortality, but there are differences in susceptibility between parasite species (Kao *et al.*, 2000; Van Dijk *et al.*, 2009). At 25°C in water, continuous exposure to ultraviolet radiation at comparable levels to temperate areas resulted in the death of over half

of *H. contortus* L3 population within 5 days, *T. circumcincta* within 3 days and *N. battus* within 2.5 days (Van Dijk *et al.*, 2009). Under natural solar irradiation in the United Kingdom, the mortality rate of trichostrongyloid L3 was lower than under artificial ultraviolet irradiation, but was more than twice higher that of sheltered control L3 in sunny days (Van Dijk *et al.*, 2009). In the field in Europe, high temperatures often occur alongside dry and sunny conditions, so desiccation and ultraviolet irradiation should be considered in addition to temperature when estimating the persistence of L3 in pastures. In temperate regions, the seasonality of trichostrongylid infections in ruminants is driven by temperature variation (Roberts and Grenfell, 1992; Stromberg, 1997). Thus, the L3 of most species overwinter well on pastures and remain infective for especially newborn lambs. Increasing temperatures through spring accelerate development and result in rising L3 levels on pasture through summer, before immunity establishment, decreasing stocking rates and slower development lead to falls in L3 levels in autumn and through winter (Armour, 1986; Smith and Grenfell, 1994). Most clinical cases of nematode infection therefore occur in growing lambs in late summer (Van Dijk *et al.*, 2008). Where L3 survival over winter is low, especially for *H. contortus* in cooler climates, survival in the host becomes relatively more important and hypobiosed larvae in the mucosa play a large role in carrying infection from year to year (Waller *et al.*, 2004). In milder climates, temperature can be high enough during autumn and winter to allow development of some species, such as *Trichostrongylus* spp. and, possibly, *T. circumcincta* to the infective stage, so that extended end-of-season grazing carries an elevated infection risk (Van Dijk *et al.*, 2008).

III – Climate and husbandry

Parasites' infection patterns are impossible to understand and predict and control without considering sheep management/husbandry systems. Management systems vary between regions and are themselves strongly influenced by climate (Chiotti and Johnston, 1995). Understanding the interactions between climate, larval availability and sheep management practices therefore provides the key to designing effective and sustainable parasite control programmes (Barger, 1999).

Climate change is likely to affect many aspects of animal husbandry, both directly and indirectly. For example, it may influence the reproduction cycles, housing period and duration, sheep diet's quality and quantity, breed selection, and management interventions such as shearing. Changing growth patterns of grassland and semi-natural vegetation used for grazing, for instance, may change grazing pattern, with strongly affects the seasonality of parasite infection. Thus, in temperate grasslands, global change is predicted to increase grass growth early and late in the year and could extend the grazing season (Fitzgerald *et al.*, 2005; Keady *et al.*, 2007), while decreasing rainfall in more arid areas could, conversely, increase the need for grain/conserved forage supplementation during the dry season (Nääs *et al.*, 2010), and possibly increase housing periods. Some parasites, such as lice, are transmitted more efficiently in housed or constrained livestock, whereas for others such as gastro-intestinal nematodes longer grazing seasons into a warmer and wetter spring or autumn may result in a stronger challenge and/or longer period of exposure. As well as effects on overall parasite abundance, changes in the timing of transmission could affect disease risk in a non linear way, for example by exposing livestock of different age or nutritional status to infestation (Colditz *et al.*, 1996; Bianchi *et al.*, 2003; Faccini *et al.*, 2004). Climate change may indirectly affect host susceptibility, for example via increasing nutritional stress by reducing the digestibility of protein in grass grown at higher temperatures (Craine *et al.*, 2010). Poor nutrition from grass could in turn be offset by increased legume growth (and availability of feed crops) due to higher atmospheric CO_2 concentrations (Campbell and Stafford Smith, 2000). Interactions between crop growth, parasite transmission patterns and livestock susceptibility are, therefore, likely to be strongly modified by management.

There is a great diversity of small ruminant production systems. They may have contrasting

grazing practices, production cycles and economic expectations and, therefore, modify the climate-driven epidemiology of nematode infection outlined above. For example, management systems, and the sheep breeds varies depending on whether meat, milk or wool production is the primary aim. Meat is mainly produced from lambs in their first year of life, which in many regions are grazed following lambing at the beginning of the season during which forage is most abundant. In some locations, lambs are finished on grain or other feed indoors or kept over the winter for finishing in the second grazing season. To maximise use of grass, especially in dry summer or cold winter period, and/or to achieve more convenient utilisation of feed, seasonal movement of sheep (e.g. through transhumance) is still used in mountainous regions (Vatn, 2009). The extent and timing of grazing therefore varies widely depending on location and sheep production system and this has a major effect on the level and timing of nematodes' challenge. GI nematodes infection, for instance, is generally much attenuated in housed or very early lambing flocks. The selection and development of sheep farming systems takes place in a complex environment and is shaped by physical (including climatic) factors, as well as immediate and more general aspects of agricultural economics/timing of demand for lamb and dairy products (de Rancourt *et al.*, 2006). Although much academic research on the factors shaping livestock production systems has focused on economic and social factors (Pardos *et al.*, 2008; Sturaro *et al.*, 2009), climate is increasingly recognised as an important factor, since the majority of systems rely on grazing/pasture growth, which depends on temperature and rainfall (Chiotti and Johnston, 1995).

The production system will affect the age structure of the sheep population, stocking density, seasonality of grazing and many other factors that underpin the epidemiology of nematode infection. Precisely in the case of meat lamb production on pasture, the scenario involves high concentrations of susceptible hosts (i.e lambs just after weaning which have no fully developed immunity) and potentially rapid cycling up of infection, while co-grazing with lactating ewes which contaminate the pasture with nematode eggs especially during the periparturient rise (Armour, 1986). The impact of infection on growth rates of untreated lambs is likely to be high, and financially highly significant, because of small margins and a need to finish lambs early. By contrast, milk production system involves mainly ewes, having already, at least a proportion of them, acquired immunity to these parasites leading to more tolerable low infestation intensities. However, as milk production is concentrated in warmer parts of Europe, *H. contortus* is more likely to cause problems and immunity to this species is not as strong as to other trichostrongylids species. By virtue of its high biotic potential and rapid development in warm conditions, *H. contortus*, can cause sudden disease outbreaks. In meat lambs, nourished by milk produced by ewes affected by the emergence of hypobiosed larvae in the spring, growth rates could be adversely affected. The timing of lambing and weaning in relation to peak of challenge can therefore strongly influence the epidemiology of nematode infection in sheep. In parts of Europe, where autumn and winter temperatures determine the duration of the grazing season, and, thereby, the likelihood of finishing meat lambs on grass, it is likely that global warming will provide farmers with an opportunity to lamb earlier in the year. This can be achieved through the use of either reproductive control methods or by using breeds, such as the Dorset, with the capacity to achieve reproductive activity for long periods. The potential impact of using such breeds on the epidemiology of gastrointestinal nematodes may vary significantly between parasite species. For *H. contortus* (which predominantly over-winters in its host) the effect of early lambing would expected to be marginal, assuming that the time frame between the periparturient rise and the onset of egg development and grass growth, would remain constant. In the short term, any decoupling of the timing of lambing and outdoor temperatures consistent with parasite development could negatively affect *Haemonchus* populations. In contrast, for *T. circumcincta* (where a large proportion of the parasite population over-winters at pasture), the overall effects may vary strongly between years and are likely to depend on an interplay between autumn and winter temperatures. A warm autumn may result in high numbers of L3 at pasture; thus, bringing the lambing season forward could result in heavier infections of lambs during winter or early spring. The importance of the phenomenon will depend on winter temperatures. Warmer winters are likely to negatively influence survival of L3 of this originally

Arctic species in pasture (Van Dijk *et al.*, 2008), thereby potentially nullifying any increased autumn development. Then again, if either lambing is brought forward sharply or a warm autumn is followed by a cold winter, first generation teladorsagiosis may be observed in young lambs (Sargison *et al.*, 2002). *Nematodirus* spp., overwintering at pasture but inside the eggs, is unlikely to be influenced by warmer winters. An earlier lambing season would be predicted to desynchronise the traditional availability of young, susceptible lambs with a spring hatch of *N. battus* eggs, thereby negatively influencing populations of this parasite. However, as both temperatures and ultraviolet levels are low over winter, larvae hatched in autumn are likely to survive very much longer than those hatching in spring. This, combined with a climate-change driven pressure on the parasite to hatch from non-chilled eggs in autumn, could realign the availability of larvae with the presence of non-immune young lambs at pasture in autumn or winter and lead to severe, unexpected, disease establishment. While such modelling studies are valuable, they often assume either that production systems are fixed or that they can be changed easily in the interests of parasite control. In actual fact, dramatic changes in parasite challenge can be driven by small changes in farm management, even if they are motivated by other factors than parasite control (Morgan and Wall, 2009). General advances in understanding the effect of climate on parasite epidemiology will be made by integrating the climatic factors that shape production systems with those that drive nematode epidemiology, but such approaches have yet to gain traction in Europe.

Additionally, patent increase in disease incidence are likely to provoke changes in farmer behaviours that could include altered husbandry practices as well as changes in their approach to chemical prophylaxis and reactive treatment (Morgan and Wall, 2009). A change in the perception of disease risk may lead to changes in approach to intervention, with perhaps a greater willingness to prevent or treat earlier. Since, once triggered, intervention is often targeted at an entire group of animals, irrespective of individual susceptibility, the overall effect may be a reduced incidence of infestation. It must also be borne in mind when considering impact of any changes in climate that livestock face a range of parasites and other health problems, and interactions between them are common. Furthermore, farmers are also likely to respond in a way that maximises overall productivity within the context of the farm and market environment. Farm adaptation to climate change depends strongly on human as well as biophysical factors, and the interactions between them at farm and global levels are only beginning to be properly appreciated by the research community (Chiotti and Johnston, 1995; Olesen and Bindi, 2002; Kabubo-Maraira, 2008; Boomiraj *et al.*, 2010; Morgan, and van Dijk, 2012). These interactions are likely to become even more complex as policy seeks to mitigate greenhouse gas emissions from livestock production (Gill *et al.*, 2010).

Hence, the factors affecting management of livestock are often as complex as those affecting the biology of their parasites, and need to be taken into account when attempting to predict the effects of climate change.

IV – Future climate-driven challenges

Sustainability is even more important when it comes to animal production systems. This translates to the development of such production systems that will produce high quality products with the lowest possible negative effects for the environment. The latter refers not only to the climate but to the protection of environment (water, air, soil, landscape, biodiversity ...) in order to achieve sustainability. Especially as regards sheep and goats breeding parasites are among the most important animal health and welfare problems and a major cost factor in sheep production in Europe. Due to the intensification of production, the use of anthelmintics and antibiotics to control these diseases has increased rapidly over the last 20 years. With increasing anthelmintic resistance, there is a need to target treatment more effectively, to suppress nematode infection below economically damaging levels, while preserving susceptible genotypes in refugia (Van Wyk, 2001; Coles, 2002).

It is widely accepted that animals with large population sizes and high genetic diversity, as well as many endemic parasites, are in a strong position to be able to adapt to any stress factors, including those posed by climate changes. Renewed appreciation of the influence of climate on nematode epidemiology is therefore essential and, in the modern era, can be supplemented by improved quantitative understanding of the effects of climate and management, and interactions between them, on larval availability and infection of sheep (Smith and Grenfell, 1994).

Such understanding can be used, inter alia, in order to (i) predict main infection periods and intervene accordingly, (ii) track the fate of eggs produced by resistant nematodes and ensure adequate dilution in refugia and (iii) model effects of climate changes and suggest rational strategic and farm level responses accordingly.

Early evidence suggests that, on balance, global warming will increase nematode challenge to grazing sheep in temperate Europe, with faster development of L3 in summer and prolonged development into autumn outweighing effects of lower survival in milder winters for *Teladorsagia* and *Trichostrongylus* spp., while milder winters would facilitate overwintering survival of *Haemonchus* spp. L3 (Van Dijk *et al.*, 2008).

Drier summers could compensate to some extent, especially in southern Europe, by limiting development of larvae in summer. However, drought would also substantially reduce grass growth and the viability of summer grazing for sheep production, so the potential benefits of a reduced parasite challenge might not be realised.

Any response to increased nematode challenge that relies on increasing anthelmintic use is likely to be self-defeating through the development of drug resistance. This applies to targeted treatments, as well as generally increased treatment frequency in summer. For example, dose-and-move is likely to select for resistance most strongly if timed to coincide with clean pastures (Waghorn *et al.*, 2009) and if increasingly undertaken in dry summers, as they become more frequent with climate change, could select even more rapidly for resistance.

Similarly, treatment of sheep during winter in colder climates targeting *H. contortus*, on account of low overwinter survival on pasture (Waller *et al.*, 2006), would enable surviving resistant parasites to quickly dominate. Mathematical and simulation models of nematode population dynamics can improve predictive ability in the face of variable nematode challenge and focus judicious use of anthelmintics in the present and in the future. The available toolkit includes general models aiming to improve strategic understanding of nematode epidemiology (Smith and Grenfell, 1994) and farm-specific models for decision support (Learmount *et al.*, 2006). However, application of predictive models to practical nematode control in Europe has been limited to date. Models that require real-time detailed climatic data produce predictions too late to be of practical benefit to farmers in making decisions on treatment or management.

Future advances could incorporate stochastic variation of climatic parameters to generalise predictions and capture uncertainty in outcomes (Morgan *et al.*, 2007) and refine knowledge of the relationships between macroclimatic parameters such as average temperature and rainfall and the microclimates experienced by the free-living stages (O'Connor *et al.*, 2006). Future models should ideally also take explicit account of management factors, which can have a dominant effect on infection patterns (Morgan and Wall, 2009; Wall *et al.*, 2011). The factors that shape management practices should be included in the most resilient models dealing with prediction of future disease patterns, and this is likely to require deeper knowledge of social factors that influence on-farm decision making and the adoption of new technologies (Gibon *et al.*, 1999; Pardos *et al.*, 2008; Sturaro *et al.*, 2009; Marshall, 2010). Precise models will be able to not only focus anthelmintic treatment to maximum sustainable economic effect, but also to identify suitable management interventions to reduce the negative effects of climate change on parasite challenge (Morgan and Wall, 2009). Thus, decreased overwinter survival of *Teladorsagia* and *Trichostrongylus* might create opportunities for control by delayed grazing of contaminated pastures in spring.

All the above mentioned facts may lead us to some conclusions-suggestions in an effort to better face the impact of future climate changes to sheep and goats parasitism. More precisely we need: (i) to better understand the parasite epidemiology, the population genetics and the phenotypic and genotypic basis of adaptation to a continuous and gradual climate change; (ii) to accurately predict the changes in the distribution of vegetation in a precise region, especially in the Mediterranean area, where the combination of an increase of temperature and decrease of annual rainfall may strongly influence vegetation and have an impact on the feeding resources of small ruminants; (iii) to better study the behavioural changes of the animals under the new climatic conditions and their influence on their productivity and generally on their welfare; (iv) to develop/improve robust breeds of small ruminants fully adapted to arid conditions; and (v) to include animal welfare policies in the development research programmes at region level e.g. Mediterranean area and at different climate change scenarios such as Northern European climatic conditions.

All of this knowledge will help the development of sustainable, effective control regimes that can be used implemented farmers in order to maintain animal productivity and welfare.

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Comparison of meat and carcass sensory quality in organically and conventionally pasture-fed lambs at two levels of herbage availability

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Abstract. We compared the sensory qualities of meat and carcasses from pasture-fed lambs reared organically or conventionally (O vs. C) at 2 levels of herbage availability (High H *vs.* Low L). Mean lamb growth profile was kept similar between the two production systems. The experiment was conducted over 2 years from weaning until slaughter with 12 OH, OL, CH and CL Limousine castrated lambs each year. The O and C treatments differed in the level of on-pasture mineral N fertilisation. The experimental pastures were regrowths; the H and L pastures were rotationnally managed to lead to a mean lamb age at slaughter of 5 and 6 months in the H and L groups respectively. Sensory evaluation indicated that the overall liking of loin chops was lower in the O than in the C treatment. Redness of *longissimus thoracis et lumborum* muscle after 2h blooming was higher in the L than in the H treatment, sheding some light on the potential effect of the intensification of organic farming via an increase in stocking rate.

Keywords. Organic farming – Lamb – Meat – Herbage availability.

Comparaison des qualités sensorielles de la viande et de la carcasse d'agneaux élevés au pâturage en production biologique ou conventionnelle à deux niveaux de disponibilités en herbe

Résumé. *Nous avons comparé les qualités sensorielles des carcasses et des viandes d'agneaux engraissés au pâturage en élevage biologique ou conventionnel (O* vs. *C) à deux niveaux de disponibilités en herbe (Haut H vs. Bas L). Le profil de croissance a été maintenu similaire entre les deux systèmes de production. L'expérimentation a été conduite pendant deux années avec 12 agneaux mâles castrés de race Limousine dans chaque groupe OH, OL, CH et CL chaque année. Les traitements O et C différaient par le niveau de fertilisation azotée minérale épandu sur les parcelles. Les parcelles expérimentales étaient des repousses après fauche et elles étaient conduites en pâturage tournant pour conduire à un âge moyen des agneaux à l'abattage de 5 et 6 mois dans les lots H et L respectivement. Les côtelettes O ont été moins appréciées que les côtelettes C. L'indice de rouge du muscle* Longissimus thoracis *et* lumborum *après 2h d'exposition à l'air a été plus élevé chez les agneaux L que chez les agneaux H, indiquant les effets possibles d'une intensification de l'élevage biologique à travers une augmentation du chargement.*

Mots-clés. *Agriculture biologique – Agneau – Viande – Disponibilités en herbe.*

I – Introduction

Organic farming embodies extrinsic features that consumers value, but studies on the intrinsic properties of organic products remain scarce. The organic farming specifications make commitments on pasture-feeding, which is favourable from a nutritional point of view, since meat from pasture-fed lambs has been shown to have a nutritionally more desirable fatty acid composition than meat from lambs fed concentrate diets (Aurousseau *et al*., 2004). However, pasture-feeding may lead to a greater occurrence of off-odour and off-flavour of the meat (Rousset-Akrim *et al*., 1997) and to a less desired meat colour (Priolo *et al*., 2002). The pastoral

flavour is mainly due to the branched-chain fatty acids, 3-methylindole (skatole) and some products of oxidation of linolenic acid and its derivates (Schreurs *et al*., 2008). Lamb growth rate and age at slaughter, which may strongly vary in pasture-fed lambs, may also affect the sensory characteristics of lamb meat (Rousset-Akrim *et al*., 1997). Therefore, as organic farming promotes pasture-feeding and 'natural rhythm' of animals, and limits the incorporation of concentrate within animal diet, this production system may be prone to the occurrence of sensory defects and to high variability in sensory characteristics. Moreover, as the organic products supply in France is lower than the demand, there is also a need to investigate to what extent the intensification of organic farming (via an increase in stocking rate for example) may affect lamb meat and carcass quality.

The odour and flavour of the meat may be even stronger when the pasture is rich in legume species, such as white clover (*Trifolium repens*) or lucerne (*Medicago sativa*), due to their prominent role in the ruminal synthesis of skatole and indole (Schreurs *et al*. 2007a,b). Moreover, higher proportions of white clover in the lamb's diet may lead to softer subcutaneous fat due to a higher polyunsaturated-to-saturated FA ratio (Lourenço *et al*., 2007). These results were recently confirmed by Prache *et al*. (2011) with lambs reared either organically either conventionally. Although there are strong reasons for having high proportions of legume species in organic livestock systems to compensate for avoiding mineral fertilisers and to reduce reliance on bought-in concentrate feed, the outcome of these studies demonstrates that this may increase the occurrence of sensory defects in meat and carcass sensory quality.

This study was therefore conducted to compare meat and carcass sensory quality in organically and conventionally pasture-fed lambs at two levels of herbage availability. The two production systems mainly differed in the level of on-pasture mineral N fertilization (0 *vs.* 100 U per ha for the organic and the conventional pasture respectively). It was hypothesized that the level of mineral N fertilization used on swards may affect lamb meat and carcass quality by modulating the proportion of legumes in the diet, and that the level of herbage availability may affect lamb meat and carcass quality via an effect on: (i) diet composition, with animals being more or less able to express a degree of selectivity for legume species during grazing; and (ii) lamb growth rates and thus age at slaughter.

II – Materials and methods

This study was carried out over 2 years (2010 and 2011) at the Unité Expérimentale des Monts d'Auvergne experimental farm at Orcival, France, run by the INRA's Clermont-Ferrand Theix Center and located at 950 m high. The animals were handled by specialized personnel in accordance with the list of specifications of organic farming.

The experiment used 96 Limousine weaned castrated male lambs from weaning until slaughter in the following 2 x 2 experimental design: production system (organic O *vs.* conventional C) x herbage availability (high H *vs.* low L). The experimental pastures were grazed rotationally and herbage availability was managed using weekly lamb weighings to ensure a mean age at slaughter of 5 months and 6 months for H and L lambs respectively. Each O and C treatment comprised 24 lambs per year over 2 years using the same experimental pastures. The two production systems differed in terms of mineral N fertilization level (0 *vs.* 100 U per ha for O pasture and C pasture, respectively). The differential in mineral N fertilisation started from year 2000 onwards.

1. Animals and diets

The lambs were born between 9 March and 15 April in 2010 and between 17 March and 5 April in 2011. Each year, 24 Limousine lambs from a conventional flock and 24 Limousine lambs from an organic flock were classified into 12 blocks of four lambs according to birth weight, ADG between birth and weaning and birth date, and then assigned to 1 of the 4 treatments (OH, OL,

CH, CL). Weaning occurred at a mean lamb age of 90 d and a mean liveweight of 24.8 kg. All the lambs were finished at pasture. All lambs were treated against internal worms at weaning (i.e. the beginning of the experiment). The level of parasite infection was then individually surveyed every 15 days and controlled using faecal samples to enable manipulation of growth rate via herbage availability and avoid bias linked to parasitism level. Lambs with a number of parasites' eggs per gram faeces higher than 550 received an anthelminthic treatment.

C and O lambs grazed a non-fertilised natural pasture between turning out (10 May 2010, 2 May 2011) and weaning (21 June 2010 and 17 June 2011). After weaning, the lambs were fattened without any supplementation on the experimental plots (regrowths after herbage mowing and harvesting). We used the same experimental plots for both experimental years.

2. Methods

Lambs were weighed once weekly in order to manage herbage availability to ensure similar average growth patterns between O and C lambs. The botanical composition of the pastures was visually assessed before the beginning of the experiment using the method of Daget and Poissonet (1971).

Lambs were slaughtered when they attained 35 kg and 36 kg LW for H and L lambs respectively to take account for differences in carcass weight/liveweight ratio. The lambs had access to food and water until 1 h before slaughter, and were transported by truck to the abattoir 25 km from the experimental site. Immediately on arrival at the abattoir, the animals were electrically stunned and slaughtered by throat cut. The carcasses were placed in a chill at 4°C until 24 h post-mortem.

At 24 h *post mortem*, the carcass was weighed, graded for conformation and assessed for external fat using the methods described by Priolo *et al*. (2002). The perirenal fat and the kidneys were then removed from the cold carcass. Fat was separated from the kidneys and then weighed. The reflectance spectrum and colour variables (lightness, redness, yellowness, chromaticity and hue angle) of subcutaneous caudal fat were measured using a MINOLTA CM-2002 spectrophotometer equipped with a protective glass visor (2° viewing angle and D65 illuminant). Five reflectance measurements were taken. Firmness of subcutaneous dorsal fat was measured by a trained assessor on a 15-point scale from 3, 'very soft', to 15, 'very hard', using a finger test. The left *longissimus thoracis et lumborum* (Ll) muscle was excised 24 h post mortem and the ultimate PH was measured. A two centimetre-thick slice was placed on a polystyrene tray, wrapped in air-permeable film (10,000 cm^3 O_2/m^2/24 h, polyvinyl chloride film) and stored in darkness at 4°C. The reflectance spectrum and colour variables were measured at 2 h post-sampling using an Uvikon 933 (Kontron) spectrophotometer equipped with an integrating sphere of Teflon (2° viewing angle and A illuminant). Lipid oxidation was measured by the thiobarbituric acid reactive substances (TBARS) method described in Prache *et al.* (2011). The results were expressed as mg of malondialdehyde per kg of meat (TBA units).

Lamb sensory quality was evaluated on six chops per lamb, according to the method described by Prache *et al.* (2011). Briefly, at each sensory evaluation session, 12 experienced panellists evaluated the lambs from one block, with OH, OL, CH and CL lamb presented in randomized order. Full details of the method used and of the criteria evaluated are given in Prache *et al.* (2011); the chops overall liking was added as a synthetic evaluation criteria.

Data were subjected to ANOVA, the model including the production system, the level of herbage availability, the experimental year and the interaction between production system and level of herbage availability.

III – Results and discussion

The proportion of white clover was 14.0, 8.5, 26.7 and 16.9 in OH, OL, CH, CL pastures in 2010

and 8.5, 8.5, 10.8 and 4.6 in OH, OL, CH, CL pastures in 2011. Against our expectation, the differential in N mineral fertilisation therefore did not induced corresponding differences in the proportion of legume in the swards. Moreover, the contribution of white clover in these pastures, which was measured every year since more than 10 years, seemed to be somewhat cyclic.

As planned, lamb growth rate between birth and slaughter and age at slaughter were affected by herbage availability (P<0.001), but were not different between production systems or experimental years. There was therefore no confounding effect between production system and lamb age and weight at slaughter. Mean lamb growth rate between birth and slaughter was 201 and 163 g/d for H and L lambs respectively, and mean age at slaughter was 158 and 197 d for H and L lambs respectively. As it was expected, cold carcass weight was not different between the 2 levels of herbage availability, between the 2 production systems and between experimental years; it averaged 14.72 kg. As expected, perirenal fat weight and dorsal fat thickness were not affected by the experimental factors, but they were slightly higher in the second experimental year. We therefore avoided potential bias linked to carcass fatness in the loin chops sensory evaluation.

Carcass conformation and morphology were not affected by the experimental factors and was not different between experimental years (data not shown).

There was no effect of experimental factors on subcutaneous dorsal fat firmness and colour. Subcutaneous dorsal fat firmness averaged 8.73 (medium) and it was not different between years. Yellowness of subcutaneous dorsal fat was not different between years, but its ligthness and redness were higher in the second year.

There was no effect of experimental factors on *longissimus lumborum* (Ll) muscle PH, but redness of Ll muscle after 2h blooming was higher for lambs raised on the low level of herbage allowance than on those raised on the high level of herbage allowance (21.28 *vs*. 19.45, P<0.005). There was no effect of experimental factors on the other Ll muscle colour parameters, nor on Ll muscle lipid oxidation intensity (Table 1).

Table 1. *Longissimus thoracis et lumborum* muscle colour and lipid oxidation intensity after 2h blooming, and chops overall liking in organically-reared and conventionally-reared pasture-fed lambs at two levels of herbage availability

	Organic		Conventional			P-value			
	High	Low	High	Low	RMSE[1]	PS[2]	HE[3]	PS x HE	Y[4]
n	24	24	24	24					
L*	34.24	34.27	36.04	33.89	3.452	NS	NS	NS	*
a*	19.15	21.36	19.76	21.20	3.052	NS	***	NS	**
b*	7.38	7.97	8.27	7.75	2.094	NS	NS	NS	*
TBARS (mg mDA/kg meat	0.57	0.59	0.56	0.61	0.096	NS	NS	NS	***
Chops overall liking	3.92	3.95	4.12	4.54	1.44	***	NS	NS	-

[1]Root mean square error; [2]Production System; [3]Level of herbage availability; [4]Experimental year; *P<0.05; **P<0.01; ***P<0.005.

For lambs produced in year 1 experiment, the overall liking of the panellists was lower for O chops than for C chops (P<0.001). This result is in line with a previous study (Prache *et al*., 2011). In the latter paper, the explanation that was put forward for the higher level of abnormal fat odour in O chops was the higher proportion of white clover in the diet because of differences in the corresponding pastures (37.3% vs 20.7% in the O and C pastures respectively). The

reason for differences in the present study is less clear, as white clover represented a low proportion of total biomass in both pastures and was not different between the O and the C pasture. The lamb chop sensory evaluation is still being processed for lambs produced in year 2 experiment. The distribution of fatty acids in LI muscle is also being processed.

The mean number of anthelminthics treatments per lamb after weaning was higher in year 2 than in year 1 (1.3 *vs.* 0.2, P<0.001) and, as expected, it was higher for L lambs than for H lambs (1.0 *vs.* 0.5, P<0.001). However, it remained at a low level.

IV – Conclusions

The low level of herbage availability led to a higher redness of the meat and a higher use of chemical anthelminthics, which shed some light on the potential effects of the intensification of organic farming via an increase in stocking rate. The chops' overall liking was lower for organically-reared than for conventionally-reared pasture-fed lambs, confirming results obtained in a previous study. Further experiment will investigate: (i) the effect of concentrate supplementation on lamb meat and carcass quality in organically and conventionally-reared pasture-fed lambs; and (ii) the dose-response curve of lamb meat and carcass quality to the proportion of legume:grass species in the diet, using lambs individually penned indoors.

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Effects of feeding system and *Acacia cyanophylla*-condensed tannins on lamb growth and meat characteristics

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Abstract. This experiment was conducted to investigate the effects of two feeding systems (pasture and feedlot (FL)) and natural protection of protein from microbial degradation in the rumen by acacia tannins on lamb's growth, carcass and meat characteristics. For this trial, 28 lambs were divided into four groups, two groups were raised in FL and fed a hay concentrate ration; the two remaining groups were reared on vetch pasture (VP). Animals of all groups received 400 g/day of concentrate in stalls. In each feeding system, one group received 100 g of acacia as a tannin source (FL-T and VP-T); the two others were tannin-free (FL-TF and VP-TF). At the end of the growth trial (77 days), all lambs were slaughtered and meat quality was determined in the longissimus dorsi muscle. Average daily gain (ADG) was higher (P< 0.01) for pasture than FL lambs (184 vs. 130 g), but it was not affected by dietary condensed tannins. However, the interaction between feeding system and tannin incorporation was significant (p<0.05); ADG was higher for FL-T than FL-TF, while VP-T has lower ADG than VP-TF. Lambs from all groups had similar muscle and fat proportions. Meat lightness (L*) was higher for FL-T and VP-TF (p<0.05) than FL-TF and VP-T, respectively. The cooking loss was reduced by tannin introduction in both feeding systems. No differences were found in meat chemical composition and ultimate pH. Inclusion of tannins decreased intensity of flavour (P< 0.05) but had no effect on overall acceptability. It could be concluded that tannin inclusion did not affect meat and carcass characteristics; however, it improved lamb's growth on poor tannin basal diet but lead to a decrease in growth rate on rich tannin basal diets (vetch).

Keywords. Lambs' growth – Carcass – Meat – Condensed tannins – Feeding system.

Effets du mode de conduite et des tannins condensés de l'*Acacia cyanophylla *sur la croissance et les caractéristiques des carcasses et de la viande des agneaux

Résumé. *Ce travail a été entrepris pour étudier les effets de deux systèmes d'alimentation (bergerie (B) et pâturage) et de l'apport de feuillage d'*Acacia cyanophylla *(acacia) riche en tanins condensés comme protecteur naturel des protéines de la dégradation microbienne dans le rumen sur la croissance et les caractéristiques de la carcasse et de la viande de moutons. Vingt-huit agneaux de race Barbarine ont été répartis en 4 lots ; deux lots ont été maintenus en bergerie avec du foin d'avoine à volonté, les deux autres ont été conduits sur pâturage de vesce (PV). Les agneaux de tous les lots reçoivent en bergerie 400 g de concentré/j/tête. Dans chaque système d'alimentation, un lot reçoit 100 g d'acacia (B-T et PV-T) et les deux autres n'en reçoivent pas (B-0T et PV-0T). À la fin de l'essai (77 jours), tous les agneaux ont été abattus et la qualité de la viande a été déterminée sur le muscle* Longissimus dorsi*. Le gain moyen quotidien (GMQ) a été plus élevé (p<0,01) sur pâturage qu'en bergerie (184 vs. 130 g), mais il n'a pas été affecté par l'apport d'acacia (P>0,05). Cependant, l'interaction entre le système d'alimentation et l'incorporation d'acacia dans la ration était significative (p<0,05); le GMQ a été plus élevé pour B-T que pour B-0T, alors que PV-0T avait un GMQ supérieur à celui de PV-T. Les carcasses des agneaux de tous les lots avaient les mêmes teneurs en muscle et gras. La luminosité de la viande (L*) était plus élevée (p<0,05) pour B-T et PV-0T que pour B-0T et PV-T. Le pH final et la composition chimique de la viande n'étaient pas affectés par les deux traitements alimentaires. La perte à la cuisson a été réduite par l'apport d'acacia dans les deux systèmes d'alimentation. Cet apport a induit une réduction de l'intensité de saveur mais n'a pas affecté l'acceptabilité globale de la viande. On peut conclure que l'apport d'une source de tanins en l'occurrence l'acacia n'affecte pas les caractéristiques des carcasses et de la viande, cependant il améliore la croissance des agneaux ayant des régimes pauvres en tanins et réduit celle des agneaux sur régimes riches en tanins.*

Mots-clés. *Agneaux – Croissance – Carcasses – Viande – Tanins – Système alimentaire.*

I – Introduction

The lamb meat is perceived by consumers as a natural product with a distinctive flavour and utile nutrients for human health. The feeding system and supply nature have considerable effects on meat quality (Atti *et al*., 2005; Priolo *et al.* 2001). The intake of rumen undegradable protein (RUP) may favour the production of less fatty carcasses (Fattet *et al.*, 1984; Vipond *et al.*, 1989) than that product by rumen degradable protein (RDP). The manipulation of dietary protein in order to protect them from microbial degradation has been generally achieved by using chemical formalin. This method can have undesirable effects on the quality of product. Tannins, naturally occurring plant metabolites, can protect proteins against ruminal degradation. The use of small amounts of Acacia cyanophylla Lindl foliage, rich in tannin, can naturally play this role (Ben Salem *et al.*, 2005; Maamouri *et al.*, 2011) and allows a better flow of amino acids. On the other hand, the meat of grazing animals is generally preferred by consumers who consider it natural, healthy and respectful of animal welfare. Therefore, it is important to differentiate between meat from lambs reared on pasture or kept in feedlot (FL) throughout the carcass characteristics. The carcasses and meat of ruminant reared on grazing conditions has different characteristics compared to stall-based production systems (Atti and Abdouli, 2001; Priolo *et al.*, 2001). The aim of this work is to study the growth performance and carcass and meat quality of Barbarine lambs grazing a cultivated vetch pasture or kept in FL condition and receiving a small quantity of acacia as a source of tannin.

II – Material and methods

The experiment was conducted at Lafareg, experimental farm of the National Institute of Agricultural Research of Tunisia (INRAT). Twenty eight lambs (22.5 ± 3.6 kg and 150 days old) were divided into four groups. Two groups were raised in FL and fed a hay concentrate ration; the two remaining groups were reared on vetch pasture (VP) on rotational grazing with a stocking rate of 90 lambs per ha. Animals of all groups received 400 g/day of concentrate in stall. Within each feeding system, one group received 100 g of acacia as a tannin source (FL-T and VP-T); the two others were tannin-free (FL-TF and VP-TF). Concentrate was administered to lambs after complete consumption of acacia. At the end of the growth trial (77 days), all lambs were slaughtered.

Lambs BW before slaughter and then carcass weights were recorded. The left half-carcasses were cut into six joints; all joints were dissected. Samples of *longissimus dorsi* (LD) muscle were taken for meat quality determination, chemical and sensorial analysis.

The initial and ultimate pH was measured with an Orion 9106 penetrating probe after calibrating with two buffers (7.00 and 4.00) 1 and 24 h post mortem, respectively. Color variables (L* (lightness), a* (redness), b* (yellowness)) were measured, 24 h post mortem, using a Minolta CR 400 color meter calibrated to a standard white tile. Cooking loss in muscle samples aged in plastic bags at 4°C until 72 h post mortem was determined. Meat samples were weighed (Wi), held in plastic bags and then immersed in a water-bath at 75°C and heated for 30 min until the internal temperature reached 75°C. The bags were then cooled under running tap water for 30 min and blotted dry with paper towels. The cooked meat was weighed again (final weight, Wf) and cooking loss was calculated as 100 (Wi - Wf)/Wi. For sensory evaluation, meat samples were roasted in aluminum paper in a pre-heated oven at 180ºC. Each sample had been cut into six coded pieces and served to panelists in random order for testing. Panelists were trained during two sessions on sensory properties of meat from different animal species before the assessment of meat samples.

Data statistical analysis was performed by analysis of variance using the GLM procedure of SAS (SAS, 1987). A 2x2 factorial design was adopted to test the effect of feeding system and tannins supplementation

III – Results and discussion

1. Forage production, diet intake, and lamb growth

The contents of condensed tannins (CT) were 0, 31.6 and 15.9 (g tannic acid equivalent per kg DM) in hay, acacia and vetch foliage, respectively; the vetch CT is very close to mean value reported by Makkar *et al.* (1996).

The grass yield in the whole plot during the grazing period was 880 kg DM. The mean daily herbage availability was 1.084 kg DM per lamb of grass, while average hay DM intake was 450 g/d. The mean CP content of grass was 220 vs. 67 g/kg DM for hay. The Average daily gain (ADG) was higher ($p<0.01$) for pasture than FL lambs (184 *vs.* 130 g). This difference could be related to the higher forage consumption and quality in VP. Growth of animals receiving tannin was 160 vs. 150 g/d for none tannin without significant difference. However, the interaction between feeding system and tannin incorporation was significant ($p<0.05$). The ADG was higher for FL-T than FL-TF (152 *vs.* 110 g), while VP-T had lower ADG than VP-TF (167 *vs.* 192 g). As consequence, slaughter BW was significantly affected by feeding system ($p<0.001$). The tannin supply had no significant effect on this parameter but the interaction was significant (Table 1). Dressing percentage and testicular weight were improved by condensed tannins supplementation particularly for FL animals (83 for FL-T *vs.* 58 g for FL-FT).

For FL feeding system the tannin content was weak; so the supply with small amount of acacia, rich in tannin, lead to an improvement of amino acids flow and absorption which resulted in a higher growth rate. While grazing regimen, based on vetch, containing tannins; the acacia supply lead to an excess of tannin supply and resulted in a negative reaction and lower growth rate (Ben Salem *et al.*, 2005).

2. Carcass composition, meat characteristics and sensory evaluation

Lambs from all groups had similar muscle and fat proportions (Table 1). This result was in contradiction with previous studies showing that grazing animals had significantly less fat than FL ones (McClure *et al.*, 1994; Atti and Abdouli, 2001; Nuerberg *et al.*, 2005).

Table 1. Average daily gain (ADG), slaughter body weight (SBW) and carcass tissue

	VP-FT	VP-T	FL-FT	FL-T	FS effect	TS effect	Interaction FS x TS
ADG (g)	192	167	110	152	0.01	0.11	0.02
SBW (kg)	33.2	32.2	28.0	29.6	0,05	0,731	0.05
Muscle (%)	61.1	61.8	63.0	62.8	0,541	0,159	0.25
Fat (%)	15.9	16.5	12.9	14.2	0,047	0,138	0.36

VP: reared on vetch pasture; FL: reared in feed-lot; T: supplemented with acacia (tannin source); FT: tannin-free; FS: feeding system; TS: tannins supplementation.

Both initial and ultimate pH values were similar for all groups, while cooking loss was affected by tannin supply which decreased this parameter (Table 2). Meat of all treatments presented lightness in the range of 41 - 45, L* values remained in the range of acceptability. Meat with a lightness value equal to or exceed 34 was acceptable on average and above 44 was acceptable by 95% of consumers (Khliji *et al.*, 2010). The lightness and redness parameters were not significantly affected by both dietary treatments (Table 2); However the interaction between feeding system and tannin incorporation was significant ($p<0.05$). The yellowness was significantly higher for grazing lambs than FL ones.

No significant differences were found for moisture (25%), crude protein, ash and fat contents

between groups. However, the interaction between feeding system and tannin incorporation tended to be significant ($p<0.08$ for CP and 0.05 for crude fat). The tannin supplementation resulted in an increase of CP and decrease of fat for FL lambs and in an opposite situation for VP lambs (Table 2). This result converged with that of lamb's growth concerning the positive effect of tannin supply on poor basal regimen tannin content (fat reduction for FL animals).

The results of sensory analyses were summarized in Table 2. No significant effect ($P>0.05$) of dietary treatment was recorded on the sensory attributes except intensity of flavour, which was decreased ($P<0.05$) by tannin incorporation. Meat for all groups was judged moderately tender (5.9) and juicy (5.5). Inclusion of tannins had no effect on overall acceptability.

Table 2. Physical, chemical and sensorial characteristics of meat

	VP-FT	VP-T	FL-FT	FL-T	FS effect	TS effect	Interaction FS x TS
pH1	6.8	6.9	6.8	6.7	0.149	0.640	0.149
pH 2	5.9	5.8	5.8	5.7	0.083	0.384	0. 831
Cooking loss	16.6	14.3	16.6	13.4	0.758	0.062	0.758
L*	43.5	40.9	43.4	45.4	0.096	0.637	0.05
a*	13.7	16	15.1	13.6	0.533	0.208	0.133
b*	9.1	8.6	7	7.3	0.005	0.916	0.225
Ash (%)	6.0	4.7	5.5	4.5	0.579	0.102	0.795
Crude protein	86.5	83.8	84.1	85.7	0.880	0.578	0.080
Fat (%DM)	8.5	10.5	11.4	8.8	0.720	0.872	0.052
Tenderness	6	5.7	5.9	6	0.662	0.884	0.356
Juiciness	5.7	5.1	5.8	5.6	0.370	0.113	0.865
Flavour	6.7	6.3	6.8	6.2	0.924	0.051	0.253
Overall acceptability	5.9	5.5	6	5.6	0.761	0.165	0.542

VP: reared on vetch pasture; FL: reared in feed-lot; T: supplemented with acacia (tannin source); FT: tannin-free; FS: feeding system; TS: tannins supplementation.

IV – Conclusion

Grazing animals had higher growth rate than FL lambs. Tannin inclusion improved lamb's growth on poor tannin basal diet but lead to a decrease in growth rate on basal diets containing tannins (vetch). It tends to improve the meat composition (reducing meat fat and increasing meat protein for FL) in the same way and did not affect meat overall acceptability. Hence, the supply of tannins should take into account the basal diet composition to avoid negative effects on animal performance.

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In vitro anthelmintic activity of some Mediterranean plants against *Haemonchus contortus* infective stage

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Abstract. The use of bioactive tanniniferous plants seems a promising alternative to control infections parasitic nematodes of the gastro intestinal tract in small ruminants. Both *in vitro* and *in vivo* studies, have confirmed the anthelmintic properties of several forage legumes, or tropical and browse plants. The aim of the present study was to evaluate the *in vitro* anthelmintic (AH) effect of 3 Tunisian plants (*Trigonella foenum graecum, Periploca angustifolia* Labill*., Ceratonia siliqua*) on *Haemonchus contortus* infective larvae. The larval exsheathment inhibition assay (LEI) was used to determine the potential inhibitory effects of 5 plant extracts at different concentrations (1200, 600, 300, 150µg/ml). The inhibition effect at the highest concentration ranged between 82.97-100% for *C. siliqua* (leaves), *P. angustifolia* (pods and leaves)*, T. foenum graecum* (whole plant) and at 53.5% for *C. siliqua* (fruits). The possible implication of polyphenols and/or tannins in the anthelmintic activity was showed after measuring the total phenols, total tannins and the biological activity of the extracts tested.

Keywords. Tannins – Larval exsheathment assay – Third stage larvae (L3) – Tunisia.

***Activité anthelminthique* in vitro *de quelques plantes de Tunisie contre les larves infestantes d'*Haemonchus contortus**

Résumé. *L'utilisation de plantes bioactives riches en métabolites secondaires, comme les tannins, semble une alternative pour la lutte contre les nématodes parasites gastro-intestinaux chez les petits ruminants. Des études,* in vitro *et* in vivo*, ont confirmé les propriétés anthelminthiques de plusieurs plantes légumineuses fourragères, tropicales ou de plantes de parcours. L'objectif de notre étude était l'évaluation* in vitro *des effets anthelminthiques (AH) de 3 plantes tunisiennes (*Trigonella foenum graecum*,* Periploca angustifolia *Labill.,* Ceratonia siliqua*) sur les larves infestantes d'*Haemonchus contortus*. Le test d'inhibition de dégainement larvaire a été utilisé pour déterminer les effets anthelminthiques de 5 extraits de plantes aux concentrations suivantes (1200, 600, 300, 150 µg/ml). L'effet d'inhibition de dégainement, à la plus forte concentration utilisée, a varié de 82,97-100% pour* C. siliqua *(feuilles),* P. angustifolia *(gousses et feuilles), et* T. foenum graecum *à 53,5% pour* C. siliqua *(fruits). L'implication possible de polyphénols ou/et de tannins dans l'activité anthelminthique a été soupçonnée après avoir mesuré les phénols totaux, tannins totaux et l'activité biologique des extraits testés.*

Mots-clés. *Tannins – Dégainement des larves L3 – Larve infestante (L3) – Tunisie*.

I – Introduction

The parasitic nematodes of the gastrointestinal tract remain a major worldwide concern for the health and welfare of grazing ruminants because of the pathological problems and the

production looses that they cause. For more than 50 years, the control of these parasitic diseases has relied on the repeated use of chemical anthelmintics (AH). However this extensive use of synthetic molecules, is nowadays facing some limits. The increasing concern of consumers on the use of chemical drugs in farm animals, the possible impact of chemical residues in the environment (Mc Kellar, 1997) but mainly the development of resistance to AH in worm populations (Jackson and Coop, 2000; Kaplan, 2004) explain the need to seek alternative solutions. Among those solutions, the possible exploitation of bioactive plants, rich in secondary metabolites such as tannins, seem one of the option for the sustainable control of these parasites. Many *in vitro* and *in vivo* studies, have shown that the use of bioactive plants seems to modulate the biology of nematode parasites, either by reducing the parasitic infection in the host or the pasture contamination (Hoste *et al.,* 2006). Most of these studies have focused on the anthelmintic properties of legume forages such as sulla (*Hedysarium coronarium*), (Niezen *et al.* 1995, 1998, 2002), or sainfoin (*Onobrychis viciifolia)* (Paolini *et al.*, 2005; Heckendorn *et al*., 2007; Manolaraki *et al.,* 2010). Moreover, some other studies have explored the potential AH properties of several browses exploited in Mediteranean area such as *Ceratonia siliqua*, *Pistacia lentiscus*, *Castanea sativa* (Manolaraki *et al.*, 2010) or heather (*Calluna vulgaris*) (Osoro *et al.*, 2007).

The main objective of the current study was to verify the possible AH activity of three Tunisian browse plants by evaluating in vitro 5 plant acetonic extracts on *Haemonchus contortus* third stage larvae.

II – Materials and methods

1. Plant samples and preparation of extracts

The plant samples were collected from the field in spring-summer 2011. In total 5 samples from 3 different plants were collected: *Trigonella foenum graecum* (whole plant), *Periploca angustifolia* (leaves and pods), *Ceratonia siliqua* (leaves and fruits). After freeze drying, 10g of each plant samples were extracted by shaking (1 hour, T≤35°C) in 100 ml of acetone:water (70:30). The filtrate was concentrated under low pressure (T≤35°C) and washed 3 times with dichloromethane in order to eliminate lipids and chlorophylls. The final extract tested was obtained after freezing and lyophilisation.

2. Bioassay: Larval exsheathment inhibition

The larval exsheathment inhibition assay (LEI) (Bahuaud *et al*., 2006) was used to evaluate the AH activity of the samples on ensheathed third-stage larvae (L3) of *H. contortus.* The L3s were obtained from donor goats monospecifically infected with *H. contortus* (MAFF,1986). The larvae were then stored at 4°C before use. One thousand 3-month-old L3 were incubated for 3 h at 20°C either with four different concentrations (1200, 600, 300, 150 of extract µg/ml) of each plant extract diluted in phosphate buffer saline solution (PBS: 0.1M phosphate, 0.05M NaCl, pH 7.2) or with PBS (used as a negative control). After washing with PBS, the larvae were submitted to the artificial process of exsheathment by contact with a solution of sodium hypochloride (2% w/v) and sodium chloride (16.5% w/v) diluted in 1 to 400 in PBS. The kinetics of L3 exsheathment, according to the different treatments, was identified under microscopic observation at a magnification of x 200 at 0, 20, 40 and 60 min after contact with the solution to induce the artificial exsheathment. These measurements of the exsheathment rates at regular time (20 minutes) interval were performed because it is important to assess the linearity of the response for the control (PBS) and to verify that nearly 100% of the infective larvae were exsheathed after 60 minutes. This is a prerequest to make possible the calculation of IC50 and their comparison/interpretation.

3. Evaluation of tannin content

A. Biological activity

The biological activity of the plant samples, related to the tannin content was measured using the radial diffusion method (Hagerman, 1978) which is based on the property of tannins to form complexes with proteins. We used Bovine Serum Albumin (BSA) (Sigma Aldrich Ltd) as protein source and tannic acid (Sigma Ltd) as a standard. The results were expressed in g-equivalents of tannic acid/100 g of dry plant (DP)

B. Folin-Ciocalteu assay

The Folin Ciocalteu method (Makkar, 2003) was used to determine the total polyphenols (TP) and total tannins (TT) in the extracts. After the initial measurements of TP, an inhibitor of tannins, the polyvinylpolypyrrolidone (PVPP) (Sigma Aldrich Ltd) was added to the extract and the measurement was repeated. Then TT was calculated as the difference between TP measured with or without addition of PVPP in the same extract. The TP and TT were determined by recording the absorbance at 725 nm using of a spectrophotometer (UV-Visible Spectronic Unican, Genesys 8). A tannic acid standard curve was performed and the results were expressed as tannic acid equivalents/100 g of dry plant (DP).

III – Results and discussion

Under Mediterranean conditions, the breeding of animals relies on the exploitation of rangelands covered by several plants from various families. In Tunisia, and especially in arid zones, which cover more than 70% of the total area (Floret and Pontanier,1982), shrub species such as *P. angustifolia, C. siliqua* and *T. foenum graecum* constitute feeding resource for livestock especially during the summer, when the alternative herbaceous species have wilted (Papanastasis *et al*., 1998). The AH properties of *C. siliqua* have previously been shown *in vitro* and *in vivo* (Manolaraki et al., 2010). In contrast, according to our knowledge this is the first time that the AH activity of *P. angustifolia and T. foenum graecum* was examined. Most of the browse plants found in rangelands are rich in plant secondary metabolites (PSMs) including tannins. This observation was confirmed in our study, since 3 out of 5 plant extracts [*C. siliqua* (leaves), *P. angustifolia* (pods and leaves)] have shown high TP and TT values reaching 11.63 and 6.11 g equivalent Tannic acid/100 g of dry plant for TP and TT, respectively (Table1).As far as the two other plant, *C. siliqua* (fruits) and *T. foenum graecum,* is concerned the lower TP, TT values that have shown corresponds to lower AH (Table 2).

Table 1. Polyphenolic compounds and biological activity of the tannin content for the five plant samples

	TP	TT	BA
C. siliqua (leaves)	11.63 (±0.14)	6.11 (±0.28)	8.97 (±0.99)
P. angustifolia (pods)	9.01 (±0.17))	2.19 (±0.19)	2.00 (±0.17)
P. angustifolia (leaves)	6.09 (±0.25)	0.72 (±0.06)	2.42 (± 0.23)
C. siliqua (fruits)	3.58 (±0.09)	2.07 (±0.08)	0.70 (± 0.05)
T. foenum graecum	1.37 (±0.39)	0.59 (±0.15)	0.16 (±0.04)

TP, TT, BA values are expressed as g of tannic acid equivalent/100g of dry plant.

During the last decade, repeated evidence tend to confirm the hypothesis that AH properties are associated with some PSMs and especially tannins (Hoste *et al.*, 2006; Rochfort *et al.*, 2008). Our results tend to confirm this hypothesis since the plant sample with the highest BA and TP

and TT content [*C.siliqua* (leaves)] has shown the highest AH activity. Manolaraki *et al.* (2010) have shown the implication of tannins in the AH activity of *C.siliqua* (fruits) by using PVPP, since the results were restored to control values after addition. The overall results in the current study show the AH activity of the 5 plant samples, as measured by the LEI assay, which ranged from 100 to 53.5 % at the highest concentration (Table 2). These results complete previous data obtained from studies that show the AH properties of various plants browsed by small ruminants in the Mediterranean basin (Manolaraki *et al.*, 2010; Hoste *et al.,* 2009; Bahuaud *et al.*, 2006).

Table 2. Mean values (±SD) of proportion exhseathed larvae and inhibition values (%) compared to the PBS control at different concentration and at 60 minutes after the start of the LEI assay

	Kinetics of L3 exsheathment			
	150µg/ml	**300µg/ml**	**600µg/ml**	**1200µg/ml**
C. siliqua (leaves)	24.2 (±24.4)	8.3 (±7.2)	6.0 (±13.0)	0.0 (±0.0)
Inhibition (%)	74.14	91.12	93.63	100.0
P. angustifolia (pods)	15.6 (±12.5)	5.9 (±4.9)	3.9 (±4.4)	2.6 (±2.4)
Inhibition (%)	84.16	94.02	96.09	97.82
P. angustifolia (leaves)	24.4 (±32.4)	6.7 (±4.4)	4.0 (±3.2)	2.6 (±3.7)
Inhibition (%)	72.05	93.2	95.92	97.35
C. siliqua (fruits)	91.1 (±6.5)	73.7 (±13.2)	77.2 (±32.6)	40.3 (±25.7)
Inhibition (%)	0	14.99	10.94	53.5
T. foenum graecum	89.0 (±7.4)	76.5 (±21.9)	59.1 (±42.1)	15.5 (±8.8)
Inhibition (%)	1.47	15.88	35	82.97

IV – Conclusions

Over all the present study confirmed the potential AH properties of the 3 browse Tunisian plants tested, although further studies are needed to better understand the nature of active compounds and the possible mode of action on parasites. This knowledge is essential to acquire for the correct use of plants rich in PSM and/or tannins in small ruminant livestock.

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Changes in the rumen *Butyrivibrio* group in lactating ewes fed a diet supplemented with sunflower oil with or without marine algae

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Abstract. In ruminants, microbial biohydrogenation (BH) of unsaturated fatty acids (FA) can be modulated by diet supplementation with lipids (e.g., sunflower oil -SO- or marine algae -MA-) to improve the fatty acid profile of the milk. The *Butyrivibrio* group contains the most active biohydrogenating bacteria isolated from the rumen and, for this reason, it has been extensively considered the main responsible for the BH process. With the aim of examining the effect of lipid addition on the *Butyrivibrio* population, as well as time-dependent variations, thirty-six lactating ewes were divided in 6 lots (3 lots/treatment) and offered a diet supplemented with either 2.5% SO or 2.5% SO plus 0.8% MA. After 0, 26 and 52 days on treatments, individual samples of rumen fluid were collected through a stomach tube, composited for each lot, and analysed using the terminal restriction fragment length polymorphism (T-RFLP) molecular technique. Results showed no significant variations, due to either diet or time, in the *Butyrivibrio* T-RFLP profiles or in the relative abundances of the major terminal restriction fragments (T-RF) detected. However, some less abundant fragments (i.e., representing less than 4.3% of the total) varied significantly. For example, the frequency of a T-RF compatible with 18:0-producing bacteria increased on day 52 in the diet with only SO (from 0.4 to 4.3%), whereas MA addition precluded this effect. The few changes caused in the *Butyrivibrio* group by a lipid supplementation that is known to alter rumen BH would indicate a low relevance of these bacteria in the ruminal FA metabolism in dairy sheep. Nevertheless, the effect on some small subpopulations would not allow to rule out their involvement in the process.

Keywords. Lipid supplementation – Ruminal bacteria – Biohydrogenation – T-RFLP.

Modifications du groupe* Butyrivibrio *dans le rumen de brebis laitières alimentées avec un régime supplémenté en huile de tournesol en présence ou en absence d'algues marines

Résumé. *Chez le ruminant, la biohydrogénation (BH) microbienne des acides gras (AG) insaturés peut être modulée par des régimes supplémentés en lipides (par exemple en huile de tournesol -HT- ou en algues marines -AM-) pour améliorer le profil en AG du lait. Le groupe* Butyrivibrio*, auquel appartiennent les bactéries les plus actives impliquées dans la BH ruminale, a été considéré comme le principal responsable de ce processus. Afin d'étudier les effets de régimes supplémentés en lipides sur l'évolution au cours du temps de la population* Butyrivibrio*, 36 brebis laitières ont été réparties en 6 lots et ont reçu un régime expérimental supplémenté avec 2,5% de HT ou avec 2,5% de HT et 0,8% de AM (3 lots par traitement). Après 0, 26 et 52 jours de traitement, des échantillons individuels de liquide ruminal ont été prélevés par voie œsophagienne, puis mélangés pour réaliser un échantillon représentatif par lot, et analysés en utilisant la technique moléculaire d'étude du polymorphisme de longueur des fragments terminaux de restriction (T-RFLP). Les résultats montrent qu'il n'y a pas de variations significatives des profils T-RFLP de* Butyrivibrio *ou dans l'abondance relative des principaux fragments terminaux de restriction (T-RF) en fonction de la nature de la ration ou du temps de prélèvement. Cependant, quelques fragments de faible abondance (moins de 4% du total) varient de manière significative. Par exemple, la fréquence d'un T-RF liée aux bactéries produisant le 18:0 a augmenté avec le régime HT après 52 jours de traitement, alors que le régime AM n'a produit aucun effet. Ces premiers résultats semblent donc montrer que les bactéries du groupe* Butyrivibrio *sont peu impliquées dans la BH ruminale chez des brebis laitières nourries avec des régimes supplémentés en lipides. Cependant, les quelques modifications observées sur de petites sous-populations bactériennes ne peuvent pas exclure complètement leurs implications dans le processus de BH.*

Mots-clés. *Supplémentation lipidique – Bactéries du rumen – Biohydrogénation – T-RFLP.*

I – Introduction

In ruminants, dietary lipid supplementation with sunflower oil (SO) in combination with marine lipids, such as marine algae (MA), has been reported to increase the milk concentration of potentially beneficial bioactive lipids (Shingfield *et al*., 2006; Toral *et al*., 2010). This effect has been related to diet-induced alterations in ruminal biohydrogenation (BH) of unsaturated fatty acids (FA), which are mediated by rumen microorganisms. The *Butyrivibrio* group, which includes the genera *Butyrivibrio* and *Pseudobutyrivibrio* as well as phylogenetically related microbes, contains the most active biohydrogenating bacteria isolated from the rumen. For this reason, it has been extensively considered to be the main responsible for the BH process (Lourenço *et al*., 2010). However, studies using culture-independent molecular methods have suggested that identified biohydrogenating bacteria, such as the 18:0-producing *B. proteoclasticus*, may not play a major role in this process, whereas other yet-uncultured bacteria, within the *Butyrivibrio* group, might be more relevant (Boeckaert *et al*., 2008; Belenguer *et al*., 2010; Toral *et al*., 2012).

Results on the milk FA profile in lactating ewes supplemented with SO alone or in combination with marine lipids (Toral *et al*., 2010) suggest long-term variations in the rumen biohydrogenating bacteria. Despite that, information on the *Butyrivibrio* group is limited to a relatively short period (up to 28 days on supplemented diets; Belenguer *et al*., 2010; Toral *et al*., 2012). Therefore, the aim of this study was to investigate the effect of the addition of SO and MA to the diet of dairy ewes on the ruminal *Butyrivibrio* group, as well as time-dependent variations over an extended period, using a culture-independent molecular technique.

II – Materials and methods

Thirty-six Assaf ewes (82.4 ± 5.30 kg body weight; mean ± SD) in mid lactation were randomly distributed in 6 lots (6 ewes/lot) and assigned to one of 2 dietary treatments (3 lots/treatment). Diets consisted of a total mixed ration based on alfalfa hay and a concentrate (forage: concentrate ratio 40:60) and supplemented with either 2.5% SO (Control diet) or 2.5% SO plus 0.8% MA (SOMA diet; DHA Gold Animal Feed Ingredient, Martek Biosciences Corp., Columbia, MD, USA). All ewes were fed the control diet for a 20-day adaptation period before the start of the study. Fresh diets were offered daily *ad libitum* at 09:00 and 19:00 h and clean water was always available. After 0, 26 and 52 days on treatments, samples of ruminal fluid were individually collected 3 h after the morning feeding using a stomach tube. Samples were strained through 2 layers of muslin, composited for each lot, and immediately frozen at –80°C for DNA extraction.

After thorough mixing, DNA was extracted from freeze-dried samples of rumen fluid as described in Belenguer *et al*. (2010). Duplicate DNA samples were used as templates for terminal restriction fragment length polymorphism (T-RFLP) analysis, which was based on *Butyrivibrio* group-specific primers (Boeckaert *et al*., 2008) and one restriction enzyme (*HhaI*; Belenguer *et al*., 2010). The lengths of the fluorescently labelled terminal restriction fragments (T-RF) were determined using the size standard ET-550-R (GE Healthcare Life Sciences, Buckinghamshire, UK) with the GeneMarker Analysis software (SoftGenetics, State College, PA, USA).

To infer the potential phylogenetic bacterial assignment of the fragments, *in silico* restriction for the *Butyrivibrio* group bacteria with the primers and enzyme used in the analysis were obtained from the Ribosomal Database Project II (http://rdp.cme.msu.edu/; Cole *et al*., 2009).

Data from T-RFLP (size in base pairs -bp- and peak area for each T-RF) were analyzed as outlined by Abdo *et al*. (2006), and the number of T-RF and the relative abundances over the total peak area of each T-RF were calculated. Hierarchical clustering analysis with the Ward´s method based on Jaccard distances was performed, using R-project software (R Development Core Team, 2011), to build a dendrogram.

For each T-RF, the relative abundance was analysed by repeated measures, using the MIXED procedure of the SAS software package, version 9.2 (SAS Inst. Inc., Cary, NC, USA). The statistical model included the fixed effects of treatment, day of sampling and their interaction, and the lot as a random effect. Means were separated using the "pdiff" option of the "lsmeans" statement. Significant differences were declared at $P < 0.05$ and tendencies at $P<0.10$.

III – Results and discussion

As plotted in the dendrogram (Fig. 1), results showed no significant variations, due to either diet or time, in the bacterial profiles, which seems to indicate that the addition of MA to a SO-containing diet did not alter noticeably the structure of the rumen *Butyrivibrio* group after 26 or 52 days on treatments. Although similar results were reported for lactating ewes fed fish oil (Belenguer *et al*., 2010), a certain separation of the *Butyrivibrio* group profiles was observed in sheep receiving the same diets as in this study, even though the effect was less clear on this group than on total bacteria (Toral *et al*., 2012).

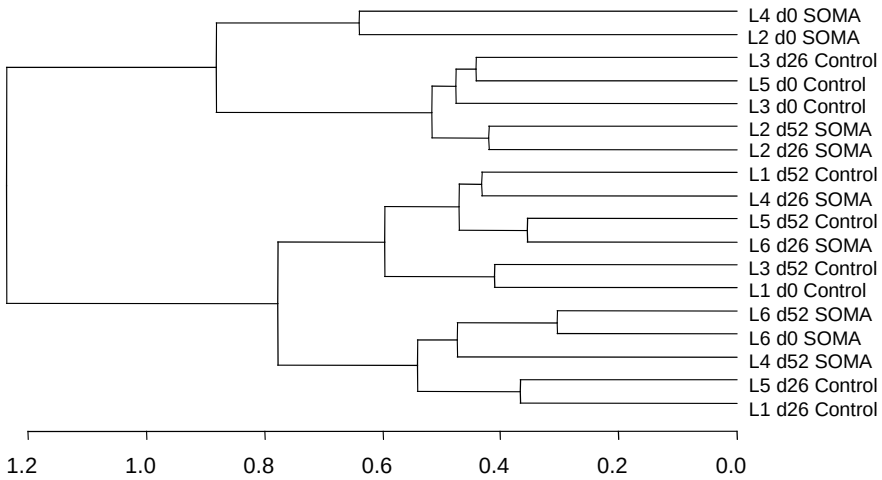

Fig. 1. Terminal restriction fragment length polymorphism (T-RFLP) derived dendrogram showing the relationships among the profiles of the *Butyrivibrio* group. The DNA was extracted from rumen fluid of lactating ewes after 0 (d0), 26 (d26) and 52 (d52) days on a diet supplemented with 2.5% SO (Control) or 2.5% SO plus 0.8% MA (SOMA) (L = lot).

The number of T-RF averaged 29.6 ± 5.75, which is in line with previous studies in sheep fed marine lipids (Belenguer *et al*., 2010; Toral *et al*., 2012), and was only reduced significantly after 52 days of MA supplementation ($P<0.05$). This is consistent with the lack of effects of fish oil (Belenguer *et al*., 2010) or MA (Toral *et al*., 2012) supplementation on this parameter observed for shorter periods of time (up to 28 days).

Samples were composited per lot to reduce the high inter-animal variability described in the rumen bacterial composition (Li *et al*., 2009) and favour the detection of significant effects. Nonetheless, the between-lot variation was still considerable, and there were no modifications, due to diet or time, in the relative abundance of the major T-RF detected (namely, those of 149, 162 and 405 bp). The 162-bp fragment may correspond to bacteria phylogenetically related to 18:0-producers (Boeckaert *et al*., 2009) and the 405-bp T-RF is compatible with uncultured *Lachnospiraceae* bacteria potentially involved in BH (Boeckaert *et al*., 2009). The same lack of

changes in the relative abundance was found for other fragments, such as the 164 and 300 bp T-RF, which are compatible with potentially biohydrogenating *Lachnospiraceae* bacteria (Boeckaert *et a*l., 2009). Although this seems to contrast with previous studies showing irregular variations in these fragments (Belenguer *et al*., 2010; Toral *et al*., 2012), it is probably noteworthy that apparently inconsistent changes may be accounted for by different bacteria, with different sensitivity to polyunsaturated FA (Maia *et al*., 2007), resulting in the same fragment size.

Table 1. Number of fragments (T-RF) and relative abundance over the total peak area (%) of several T-RF obtained by T-RFLP in rumen fluid samples of lactating ewes fed a diet supplemented with 2.5% SO (Control) or 2.5% SO plus 0.8% MA (SOMA), on days 0, 26 and 52.

	Control			SOMA			SED[2]	P[1]		
	0	26	52	0	26	52		T	D	T×D
Number of T-RF	28.3	29.0	32.3	33.3[a]	28.0[ab]	26.3[b]	6.49	0.85	0.63	0.02
Length (bp)										
149	31.16	30.16	32.31	30.66	24.88	29.45	7.430	0.32	0.54	0.79
159	0.00[b]	0.15[b]	0.66[a]	0.10	0.28	0.11	0.160	0.45	0.01	<0.01
161	0.44[b]	0.32[b]	4.26[a]	1.40	1.88	2.02	1.024	0.92	<0.01	0.01
162	16.86	10.65	9.53	15.39	11.05	8.54	4.217	0.65	0.13	0.95
164	3.17	3.06	3.90	4.41	1.90	2.43	1.369	0.57	0.43	0.34
300	5.82	5.41	5.42	3.62	5.11	4.45	1.866	0.49	0.79	0.55
405	19.57	22.09	20.16	20.62	16.39	27.51	5.185	0.77	0.44	0.25

[a,b] For each treatment, means within a raw with different superscripts differ significantly ($P < 0.05$). [1] Probability of significant effects due to experimental treatment (T) or day (D). [2] SED = standard error of the difference.

On the other hand, less abundant fragments (representing less than 4.3% of the total) varied significantly over time. For example, the frequency of the T-RF of 161 bp increased on day 52 in the diet with only SO (from 0.4 to 4.3%), whereas MA addition precluded this effect. This T-RF might be compatible with a strain of the 18:0-producing *B. proteoclasticus*, although in previous experiments with sheep (Belenguer *et al*., 2010; Toral *et al*., 2012) and cattle (Huws *et al*., 2011) no changes in this group, due to lipid supplementation, were observed using real time PCR. Nevertheless, as mentioned above, other unclassified bacteria of *Lachnospiraceae* might also match the same T-RF.

IV – Conclusion

Overall, the few changes caused in the *Butyrivibrio* group by a lipid supplementation (sunflower oil + marine algae) that is known to alter rumen BH would indicate a low relevance of these bacteria in the ruminal FA metabolism in dairy sheep. However, some small subpopulations were affected by the addition of MA, which would not allow to rule out their involvement in the process.

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Effect of carob pulp on growing performances, nutritional, and technological quality of meat and perirenal fat from goat

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Abstract. The objective of this study is to evaluate the effect of condensed tannins (CTs) distribution using carob pulp in diet on meat and carcass quality and growth performance of kids. Three diets with 0%, 25%, and 45% of carob pulp (C0, C5 and C10, respectively) were distributed to 3 groups of kids from weaning to slaughter at 6 months of age. C5 kids showed highest $ADG_{90\text{-}180}$ and final weight at 180 days (73.33 g/day; 18.50 kg respectively). Meat moisture, fat, pH and water retention showed no variation. However, C5 contains more protein (P<0.05).The linoleic acid composition in meat increase with C10 (P<0.05). The low intakes of CTs seem to be insufficient to fully protect certain unsaturated fatty acids against bio-hydrogenation of fatty acids (DFA 67.03% *vs* 64.91% and 66.54% respectively for C0, C5 and C10, P>0.05). The same result was observed for perirenal fat. Omega 6 and C18:1n9 experienced an increase in meat from goats fed with the C10 diet (P>0.05). Carob use in kids diet is accompanied by an improvement of protein and desirable fatty acids, especially linoleic acid, in meat.

Keywords. Carob – Meat quality – Growth – Kids.

Effet de la pulpe de caroube sur la qualité nutritionnelle et technologique de la viande et du gras périrénal des chevreaux

Résumé. *L'objectif de ce travail est d'évaluer l'effet de la distribution des tanins condensés (TCs) en utilisant la pulpe de caroube dans l'alimentation, sur la qualité (carcasse; viande) et sur la croissance des chevreaux. Trois rations avec* 0%, 25%, 45% *de pulpe de caroube (C0, C5 et C10 respectivement) ont été distribué à 3 groupes de chevreaux du sevrage jusqu'à l'abattage à 6 mois. Les chevreaux C5 ont enregistré un* $GMQ_{90\text{-}180}$ *et un poids final à 180 jours les plus élevés (73,33 g/jour; 18,50 kg). L'humidité de la viande, la matière grasse, le pH 0 et 24h post mortem et la rétention d'eau n'ont révélé aucune variation. Toutefois, la viande C5 contient plus de protéines (P<0,05). La composition de la viande en acide linoléique a augmenté avec C10 (P<0,05). Les faibles apports de tanins semblent insuffisants pour protéger complètement certains acides gras insaturés contre la biohydrogénation des acides gras (DFA 67,03%* vs *64,91% et 66,54% respectivement pour C0, C5 et C10, P>0,05). Le même résultat a été observé pour la graisse périrénale. Oméga 6 et C18:1n9 ont connu une augmentation dans la viande de chevreaux recevant la ration C10 (P>0,05). L'utilisation de la pulpe de caroube est accompagnée d'une amélioration des protéines et des acides gras désirables, en particulier linoléique, de la viande.*

Mots-clés. *Caroube – Qualité de la viande – Croissance – Chevreaux.*

I – Introduction

In northern Morocco, goat meat is produced on pastures that are known by their forage resources rich in phenolic compounds mainly the condensed tannins (CTs) (Chebli *et al.*, 2012). The latter are anti-nutrients that weaken productivity in meat from these farms (Makkar, 2003). However, CTs can protect certain beneficial nutrients, such as desirable fatty acids, against ruminal biodegradation and thereafter these nutrients can be found in animal products, and

consequently improve their quality (Min *et al.*, 2003; Ramírez-Restrepo and Barry, 2005). However, studies showing the impact of CTs on productivity and quality of goat meat are not locally available in Morocco. Thus, optimal use of feed resources rich in CTs is to be undertaken, in order to preserve the effectiveness of food digestibility and at the same time the quality of animal products. This work aims to identify the effect of CTs distribution using carob pulp in supplement diet, focusing on growth performance and fattening and also in the carcass and the meat quality of kids of the local goat population in northern Morocco.

II – Materials and methods

Three concentrate supplementations (C0, C5 and C10) with respective intake levels of CTs (0, 2.7, 5.6 g/day/kid) were distributed respectively to 3 groups of kids (7 per group) from weaning (at 90 days) until the age of 180 days. The control group (C0) received a concentrate supplement containing grain of barley, maize, faba bean and sunflower cake. In the group tests, the carob pulp (*Ceratonia siliqua*) was used as a source of CTs by incorporating 25% and 45%DM of the carob pulp, respectively, in the C5 in C10 concentrate diet. The local carob pulp of northern Morocco contains an average of 20% of TCs.

Oat hay was distributed in all groups equitably during the period of the experiment. In the three groups, concentrate diet distributed had an equal level of energy and protein (0.8 UFV, 70 g PDI). The growth control is performed every 15 days in the morning on fasting animals.

24 hours after slaughter, meat samples were taken from the *Longissimus dorsi* muscle (LD), the *Semimembranosus* muscle (SM) of thigh and from perirenal fat in order to perform analysis on dietary, technological and organoleptic meat quality. Measurements were taken on carcass weight just after slaughter and cold carcass weight (24 hours after slaughter at a room temperature of an average of 20°C), the weighing of perirenal fat, the carcass length, thickness and length of the thigh. Also measurements were taken on: Consumption index [feed Intake (kg)/Average daily gain ADG (kg)]; Carcass yield (%) [(HW/LW)*100; where HW: carcass weight after removal of the head, skin, offal and fours; LW: live weight before slaughter]; Compactness index [carcass weight/carcasse length]; Muscle index [thickness of the thigh/leg length]; and Conformation index [which is the sum of the two indexes].

The colour of the cover fatty tissue was measured on a thickness of 1 to 1.5 cm of the LD 12 h post-mortem using the Minolta Chromameter CR410. The values of Lightness (L*), Redness (a*) and Yellowness (b*) were given by Chromameter once it is placed on a specific location of the carcass. Color score of fat cover was calculated as cited in Normand and Brouard-Jabet (2002). Meat texture was assessed 24 hours post-mortem using a Texturometer (Texture Analyzer -PRO-TMS) on a piece of LD 1 cm thick and 3 cm long. The pH was determined at 0 and 24 hours post-mortem with a portable pH meter HANNAHI 99163. Retention capacity of water is measured according to Grau and Hamm (1953; cited in Ait Bella, 2006) by exerting a force of 2.250 kg weight for 5 minutes.

To perform the analysis of meat fatty acids, the samples were taken from the LD (between 12th and 13th ribs). Content of meat protein, fat, ash and moisture is carried out on *Semimenbranosus* muscle of the thigh according to the AOAC (1997). Fatty acids were extracted by the method of Folch *et al.* (1957) and esterified according to Christie (1993) and Shehata *et al.* (1970 respectively) for meat and fat samples. The esters of fatty acids were determined using gas chromatography (Varian CP-3800) equipped with a flame ionization detector and a capillary column of 100 m.

For the different parameters studied, the analysis of variance, multiple comparison of means and the calculation of standard error of means were performed using the statistical package SAS (2002).

III – Results and discussion

Moderate rate (C5) of carob pulp incorporated in the concentrate diet improved significantly the growth performance of kids (Table 1). Indeed, C5 group have registered a highest weight gain between 90-180 days compared to C0 and C10 (73.33 *vs* 42.90 and 38 g/day, P<0.05) and a highest weight at 180 days (18.50 *vs* 15.46 and 15.22 kg, P<0.05) respectively. With C5 group, consumption index is lower compared to C0 and C10 group (7.72 *vs* 12.18 and 19.56 respectively, P<0.05). CTs in carob used in high quantities (C10) inhibit the activity of digestive enzymes, which reduce the growth of animals. The decrease in growth in C10 kids is due to the inhibition of digestive enzymes caused by CTs contained in carob used at large quantities (Vasta *et al.*, 1999, El Otmani *et al.,* 2011).

Table 1. Effect of carob pulp on growth performance of kids in control (C0) and test (C5 and C10) group (n = 7/group)

	C0	C5	C10	SEM	Probability
Initial live weight (kg)	11.6	11.90	11.80	0.7979	0.9641
Final live weight (kg)	15.46^{ab}	18.50^{a}	15.22^{b}	0.9880	0.0466
$ADG_{90\text{-}180}$ (g/day)	42.90^{b}	73.33^{a}	38.00^{b}	7.3981	0.0111
Feed intake (g DM/kid/day)	500^{c}	535^{b}	620^{a}	0.000	0.0001
Consumption index	12.18^{ab}	7.72^{b}	19.56^{a}	2.6713	0.0262
Cold carcass weight (kg)	5.87	6.44	5.87	0.5570	0.7123
Carcass yield (%)	40.48	36.80	39.88	1.6856	0.2905

Within the same row, means with different superscript are significantly different (P<0.05).
SEM: Standard error of the mean.

Carob incorporation shows no significant effect of compactness index but there is a difference between treatment C5 and C10 concerning muscle and conformation index (P<0.05). Slightly lower indices are obtained with C5 (0.46 and 0.57 respectively, P <0.05). This difference is due to the carcasses and thigh length of C5 kids which are longer than TC10 (Table 2).

Table 2. Effect of carob pulp on carcass characterization of kids in control (C0) and tests (C5 and C10) group (n = 7/group)

	C0	C5	C10	SEM	Probability
Carcass length (cm)	56.20	57.50	54.70	0.7810	0.0760
Thigh length (cm)	26.20	27.50	24.70	0.7810	0.0760
Thigh thickness (cm)	12.42	12.54	12.48	0.1706	0.8849
Compactness index	0.10	0.11	0.11	0.0090	0.8427
Muscle index	0.47^{ab}	0.46^{b}	0.51^{a}	0.0108	0.0213
Conformation index	0.58^{ab}	0.57^{b}	0.61^{a}	0.0120	0.0435
Color score of fat cover	5.52^{a}	4.49^{ab}	4.15^{b}	0.4104	0.0472
Lightness (L*)	40.07^{b}	43.23^{a}	42.70^{a}	0.7964	0.0346
Redness (a*)	21.52	21.29	21.57	0.5115	0.9161
Yellowness (b*)	6.81^{a}	4.99^{b}	5.78^{ab}	0.3833	0.0179

Within the same row, means with different superscript are significantly different (P<0.05). SEM: Standard error of the mean.

C5 fat cover shows a higher color score than C10 (4.49 *vs.* 4.15 respectively, P<0.01). About the C5 meat of LD, lightness (L*) is higher and yellowness is low (43.23 and 4.99 respectively, P<0.05). These indices indicate a satisfactory color of the C5 carcass and meat.

About the dietary quality, meat protein content obtained with C5 diet is higher than in C10 (17.24 vs. 15.35 respectively, P<0.05; Table 3). This result shows that the protein content of goat meat is improved with moderate level of carob pulp not exceeding 25% DM (equal to 2.7 g/day/kid of CTs). This result can be explained by the minimization of ammonia losses of nitrogen in the urine which result from the reduction of amino acids degradation with the presence of moderate amounts of CTs contained in carob. Protein contents slightly higher ranging between 19.5% and 22.2% are reported by Ding *et al.* (2010), Werdi Pratiwi *et al.* (2007), Sen *et al.* (2004) and El Otmani *et al.* (2011).

No significant effect of carob has been recorded on meat moisture, content fat and pH (0 and 24 hours) post mortem. Also, there was no significant variation in the water holding capacity of *Semimenbranosus* or *Longissimus dorsi* meat between the different tested diets (Table 3). These results are consistent with those of Ding *et al.* (2010), Sen *et al.* (2004) and Werdi Pratiwi *et al.* (2007). However, we notice a tendency to a decrease in meat fat and an increase in acidity (0 and 24 hours) post mortem with C10 diet (P>0.05).

Table 3. Effect of carob pulp on dietetical and technological parameters of kids meat in the control (C0) and tests (C5 and C10) group (n= 7/group)

	C0	C5	C10	SEM	Probability
Protein (%)	16.32^{ab}	17.24^{a}	15.35^{b}	0.4823	0.0292
Ash (%)	2.88	2.84	2.7	0.0589	0.0834
Thigh moisture (%)	74.05	75.2	76.29	0.6505	0.091
Longissimus moisture(%)	72.96	75.69	76.02	1.019	0.1065
Fat (%)	4.61	3.24	3.86	0.732	0.418
pH (0 hours)	6.52	6.62	6.42	0.1767	0.1391
pH (24 hours)	5.68	5.75	5.60	0.0125	0.3221
Water-holding capacity (thigh)	45.28	47.12	44.12	1.7715	0.5015
Water-holding capacity (*Longissimus dorsi*)	25.36	25.94	25.95	2.7187	0.9849

SEM: Standard error of the mean.
Within the same row, means with different superscript are significantly different (P<0.05).

Regarding the fatty acids composition (Table 4), goat meat contains predominantly oleic, palmitic, stearic, arachidic acid and desirable fatty acids. This result is consistent with Mahgoub *et al*. (2002), Santos *et al.* (2007), Beserra *et al.* (2004), Werdi Pratiwi *et al.* (2007), Ding *et al.* (2010), and Zerrouk *et al.* (2010). However, there is an increase in conjugated linolenic acid (CLA, 18:2n6c) when CTs are distributed at a high amount (0.14% vs. 0.09%, P<0.05, respectively for C10 and C5). Moderate intake of CTs (C5) seems to be insufficient to fully protect certain unsaturated fatty acids against the bio-hydrogenation of carbon chains of fatty acids. The proportion of CLA obtained with C5 diet is, indeed, below that of the control group (0.09% vs. 0.12%, P<0.05). While, the voluntary intake of concentrate containing 45% of carob pulp explains the obtaining of high CLA content in meat (0.48%, Vasta *et al.* 1999). El Otmani *et al.* (2011) also reported a significant increase in CLA content by incorporating *Lupinus angustifolius* containing 0.075% of alkaloids which are also phenolic compounds such as CTs. Linolenic acid which is an essential fatty acid of Omega 3 group and eicosatrienoic acid of omega 6 group, both showed an increase in the LD with high intake of CTs in diet (0.69% *vs*. 0.32%, and 1.03% *vs*. 0.32% respectively for C10 and C0, P>0.05).

Globally, the use of CT in the diet of growing and fattening kids is accompanied by a significant improvement in meat content of protein and fatty acid particularly the desirable linoleic acid, especially with use of high CT use in diet (5.6 g/day/kid of CTs).

Table 4. Effect of carob pulp on fatty acids composition (% of total fatty acids) of *Longissimus dorsi* of the control (C0) and tests (C5 and C10) group of kids (n= 7/group)

Fatty acids	C0	C5	C10	SEM	Probability
Myristic acid (C14)	3.11	2.91	2.87	0.21	0.6985
Palmitic acid (C16)	22.02	20.63	21.54	0.97	0.6089
Stearic acid (C18)	13.08^{b}	15.11^{ab}	16.99^{a}	0.98	0.0494
Oleic acid (C18:1n9)	53.24	48.72	48.15	2.28	0.2654
Linoleic acid (C18:2n6c)	0.12^{ab}	0.09^{b}	0.14^{a}	0.01	0.0132
Linolenic acid (C18:3n3)	0.32	0.59	0.69	0.22	0.4905
Arachidic acid (C20)	7.53	10.95	8.35	1.94	0.4538
Eicosatrienoic acid(C20:3n6)	0.32	0.46	1.03	0.38	0.4122
Behenic (C22)	0.26	0.53	0.23	0.16	0.4202
Desirable fatty acids	67.03	64.91	66.54	2.03	0.7465
Mono-unsaturated fatty acids	53.24	48.72	48.15	2.28	0.2654
Poly-unsaturated fatty acids	0.71	1.08	1.40	0.47	0.5905
Unsaturated fatty acids	53.95	49.80	49.55	2.75	0.4042
Saturated fatty acids	46.06	50.20	50.44	2.49	0.4046

Means within the same row with different superscript are significantly different (P<0.05).
SEM: Standard error of the mean.

IV – Conclusions

Moderate intake of CTs provides leaner and drier meat with more protein content. But, with no changes in technological quality. However, the quantity of desirable fatty acids improves when a high rate of CTs is distributed in the concentrate diet. CTs exist in most plants and in agricultural by-products, particularly in fodder shrubs most eaten by goats (Ayadi *et al.*, 2010). Therefore, high intake of CTs on these pastures could occur. This confirms the good quality of fatty acids in kids meat produced on pasture. However, this quality is obtained despite the dry matter digestibility, resulting in a decrease in weight productivity of kids (Chentouf *et al.*, 2006). Developing of a technique for improving the digestibility of ingested matter in goats and preserving the quality of fatty acids composition of meat can increase the goat farming income in mountain areas where animal diets are based on grazing.

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Dietary effects on meat chemical traits and fatty acids composition in intramuscular lipids of Sarda x Ile de France heavy lambs

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Abstract. The effect of diet on meat chemical traits and fatty acids content were evaluated in Sarda x Ile de France lambs. Eighteen lambs that are off-springs from Ile de France rams and Sarda ewes, after a suckling period of 50 days, were divided into three groups homogeneous for sex and twin birth: (i) group HL with lambs raised in stall and fed alfa-alfa hay and commercial concentrate; (ii) group PL in which lambs were raised at pasture (*Lolium italicum* L) for 24 hours a day; and (iii) group ML with lambs suckled by their mothers. The mothers grazed for 7 hours per day and received as supplements alfalfa hay and commercial concentrate. Lambs were slaughtered at 20-26kg live weight. Chemical composition of meat samples (*Longissimus dorsi* muscle) was similar for all dietary treatments. Fatty acid composition was strongly affected by feeding system. Meat from grazing lambs and lambs suckled by their mothers showed higher proportions of ω3 PUFA, conjugated linoleic acid (CLA 9c 11t, P<0.05) and lower ω6/ω3 ratio than that of meat from stall-fed lambs (P<0.001). In conclusions, meat fatty acid composition was improved in the PL and in the ML groups than in the HL groups.

Keywords. Feeding mode – Heavy lambs – Meat quality – Fatty acids.

Effet de l'alimentation sur les caractéristiques chimiques et sur la composition en acides gras des lipides intramusculaires de la viande d'agneaux lourds Sarda x Ile de France

Résumé. *L'effet de l'alimentation sur les caractéristiques chimiques et sur la composition en acides gras a été évalué sur la viande des agneaux Sarda x Ile de France. Dix-huit agneaux, descendance de béliers Ile de France et de brebis Sarda, après une période d'allaitement de 50 jours, ont été répartis dans les groupes suivants, homogènes pour le sexe et la naissance de jumeaux : (i) groupe sevré en stabulation et soumis à un régime composé de foin de luzerne et de concentré commercial ; (ii) groupe sevré en pâturage* (Lolium italicum*) pendant 24 heures par jour ; et (iii) un groupe d'agneaux allaités par leurs mères. Les mères pâturaient pendant 7 heures par jour et ont reçu, en tant que compléments, du foin de luzerne et du concentré commercial. Les agneaux ont été abattus à 20-26 kg de poids vif. La composition chimique des échantillons de viande* (Longissimus dorsi*) était similaire pour tous les traitements alimentaires. La viande des agneaux au pâturage (groupe Herbe) et des agneaux allaités par leurs mères (groupe Lait) a montré une plus grande proportion de ω3-PUFA, d'acide linoléique conjugué (CLA 9c 11t, P<0,05) et une valeur moins élevée pour ce qui concerne le ratio ω6/ω3 (P <0,001) que celle de la viande des agneaux nourris à l'étable (groupe en stabulation). En conclusion, la composition en acides gras de la viande des agneaux des groupes en pâturage et allaités par leurs mères est meilleure que celle de la viande du groupe en stabulation.*

Mots-clés. *Mode d'alimentation – Agneaux lourds – Qualité de la viande – Acides gras.*

I – Introduction

In Sardinia the light suckling lamb is a traditional and typical product, which in 2001 received the PGI identification, named "Agnello di Sardegna" (Reg. 138/01). Similarly to what occurs in other countries of the Mediterranean basin, Sarda suckling lambs are raised following their mothers at pasture and are slaughtered at a cold carcass weight < 7 kg. The PGI product specification

provides also the “heavy lamb” typology (cold carcass weight 10-13 kg), but in this case lambs come either from Sarda sheep in purity or from cross-breeding with other highly specialized meat breeds like Ile de France and Berrichon du Cher. The product specification requires that heavy lambs are raised at pasture and fed with fresh forages.

It is known that the ruminant diet can play a significant role in improving meat quality in terms of nutraceutical compounds that are beneficial to consumer health (Wood *et al.*, 2004). Changes in intramuscular fatty acid composition are linked to the respective fatty acid content in the feed offered, though rumen biohydrogenation has a considerable impact in reducing the concentration of polyunsaturated fatty acids (PUFA) in ruminants. When compared to grain feeding, pasture increases the ω3-PUFA content and decreased the C18:2-ω6/C18:3-ω3 ratio in lamb meat (Popova, 2007; Santos-Silva *et al*., 2002). Likewise, French *et al.* (2000) demonstrated that, when grown at the same rate, muscle from cattle fed a high grass intake had a higher PUFA/SFA ratio and a lower ω6/ω3 PUFA ratio than that of muscle from cattle fed concentrates rich in barley and maize grain.

The aim of the present study was to compare the effects of three different dietary treatments on meat quality in terms of macronutrients and fatty acid content in Sarda x Ile de France heavy lambs.

II – Materials and methods

Eighteen Sarda x Ile de France lambs were raised at pasture together with their mother until a live weight of 10-14 kg. After weaning, they were randomly assigned to one of three dietary treatments: PL (pasture lambs, 6 animals), fed at pasture on a ryegrass sward for 24h/day; HL (housed lambs, 6 animals) fed with alfalfa hay (765 g DM/head day) and commercial concentrate (516 g DM/head day); ML (suckled lambs, 6 animals) suckled by their mothers . The animals were weighed weekly and they were slaughtered at 20-26 Kg live weight (Sitzia et al., 2011). The carcass weight was recorded after 24 hours at 4°C and M. *longissimus dorsi* was taken for chemical analysis and intramuscular fatty acid (FA) composition. Meat samples were analysed for dry matter, fat, ash, protein in accordance with ASPA indications (1996). Muscle lipids were extracted by means of a hexane/2-propanol solution (3:2 v/v), according to Hara – Radin method (Hara *et al.*, 1978). Fatty acids were converted to methyl esters (Chin *et al.*, 1992), separated and quantified using a Varian 3900 gas chromatograph, with a SP2560 capillary column (100m x 0.25mm x 0.2µm). Statistical treatment of the data was performed using the STATGRAPHIC software (STATGRAPHICS Centurion XV, version 15.1.02. StatPoint, INC.). The effect of feeding treatments on chemical parameters and fatty acids content was assessed using the general linear model (α=0.05) procedure. A multiple comparison test (Tukey) was used to separate means.

III – Results and discussion

The proximate composition of *Longissimus dorsi* muscle is shown in Table 1. Dietary treatment had no significant effect (P>0.05) on moisture, fat, protein and ash content. Feeding system significantly affected the fatty acid composition of lambs’ intramuscular fat (Table 2). Meat from lambs suckled by their mothers (ML group), was characterized by a higher content of medium chain fatty acids, particularly C12:0 (P<0.05), C14:0 (P<0.01) and C16:0 (P<0.05), when compared to meat of grazing lambs (PL group). Suckled lamb, from a metabolic point of view, can be considered as a non ruminant, and consequently qualitative and quantitative fatty acid profile of suckling lamb meat may reflect the composition of the ingested milk (Velasco *et al.*, 2004,) that is commonly characterized by a high content of medium chain fatty acids. Meat from lambs fed at stall showed an intermediate concentration of medium and long chain fatty acids, but a significantly lower content of monounsaturated fatty acids (C18:1 9 cis, P<0.05 and C18:1 11 trans, P<0.01) when compared to meat from lambs reared at pasture.

Table 1 Chemical parameters (mean ± sd) in M. *Longissimus dorsi* of Sarda x Ile de France lambs

	Treatment†			Effect of feeding
	ML	HL	PL	
Moisture (%)	75.08 ± 1.12	75.78 ± 0.91	75.72 ± 0.67	ns
Fat (%)	2.33 ± 0.75	1.98 ± 0.48	2.09 ± 0.27	ns
Protein (%)	20.91 ± 0.40	20.79 ± .0.26	21.06 ± 0.39	ns
Ash (%)	1.22 ± 0.07	1.18 ± 0.02	1.21 ± 0.02	ns

†ML, suckled lambs; HL, housed lambs; PL, pasture lambs.
ns: not significant.

Table 2. Fatty acid composition (mean ± sd)in intramuscular fat of M *Longissimus dorsi* of Sarda x Ile de France lambs

FAME (%)†	Treatments††			Effect of
	ML	HL	PL	feeding
C10:0	0.38 ± 0.12	0.28 ± 0.05	0.40 ± 0.20	ns
C12:0	0.75^{a} ± 0.17	0.59^{ab} ± 0.17	0.41^{b} ± 0.05	*
C14:0	6.76^{a} ± 0.83	4.94^{b} ± 0.73	4.52^{b} ± 0.58	**
C16:0	26.15^{a} ± 2.17	25.07^{ab} ± 0.92	23.47^{b} ± 1.40	*
C18:0	13.79^{b} ± 1.05	16.76^{a} ± 0.86	17.83^{a} ± 1.53	***
C18:1 11t	2.23^{b} ± 0.31	2.08^{b} ± 0.29	2.97^{a} ± 0.49	**
C18:1 9c	31.92^{ab} ± 3.86	30.26^{b} ± 2.11	34.96^{a} ± 1.73	*
C18:2 9c,12c	8.73^{b} ± 1.38	11.67^{a} ± 1.24	7.12^{b} ± 0.26	***
C18:3 9c,12c,15c	2.89^{a} ± 0.62	2.12^{b} ± 0.31	2.98^{a} ± 0.32	*
CLA 9c,11t	1.10^{a} ± 0.11	0.76^{b} ± 0.16	0.98^{ab} ± 0.19	*
CLA 11t,13c	0.04 ± 0.01	0.04 ± 0.01	0.06 ± 0.05	ns
C20:4 5c,8c,11c,14c	2.64^{ab} ± 0.34	3.10^{a} ± 0.73	2.21^{b} ± 0.39	*
C20:5 5c,8c,11c,14c,17c	0.55 ± 0.18	0.42 ± 0.09	0.46 ± 0.15	ns
C22:5 7c,10c,13c,16c,19c	1.41 ± 0.35	1.41 ± 0.26	1.19 ± 0.37	ns
C22:6 4c,7c,10c,13c,16c,19c	0.66^{a} ± 0.12	0.48^{b} ± 0.14	0.44^{b} ± 0.07	*
SFA	47.83 ± 2.94	47.65 ± 1.25	46.63 ± 0.37	ns
MUFA	34.15^{ab} ± 3.72	32.34^{b} ± 2.05	37.92^{a} ± 1.28	*
PUFA	18.02^{ab} ± 2.89	20.01^{a} ± 2.16	15.44^{b} ± 1.26	*
UFA	52.17 ± 2.94	52.35 ± 1.25	53.37 ± 0.37	ns
ω3	5.51 ± 1.20	4.43 ± 0.58	5.07 ± 0.74	ns
ω6	11.37^{b} ± 1.70	14.78^{a} ± 1.91	9.33^{b} ± 0.56	***
ω6/ω3	2.09^{b} ± 0.19	3.36^{a} ± 0.43	1.86^{b} ± 0.22	***
AI	1.04^{a} ± 0.16	0.87^{ab} ± 0.09	0.79^{b} ± 0.06	**
TI	1.20 ± 0.16	1.27 ± 0.07	1.18 ± 0.06	ns
P/S	0.38^{ab} ± 0.07	0.42^{a} ± 0.05	0.33^{b} ± 0.03	*

†SFA: Saturated fatty acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; UFA: Unsaturated Fatty Acids. ω3: omega 3 fatty acids; ω6: omega 6 fatty acids;. AI = (C12:0 + (C14:0*4) + C16:0)/UFA;TI = (C14:0+C16:0+C18:0)/((0.5*ω6)+(0.5*MUFA)+(3* ω3)+(ω3/∑ω6); P/S = PUFA/SFA. ††ML, suckled lambs; HL, housed lambs; PL, pasture lambs; ns: not significant. *p <0.05; **p<0.01; ***p<0.001; a-b Means within row with different superscript letters differed significantly.

The content of polyunsaturated fatty acids (PUFA), determined in intramuscular fat of

Longissimus dorsi muscle, closely reflects the fatty acid composition of ingested fed. Pasture and suckled lambs (PL and ML) showed a very similar qualitative and quantitative profile of polyunsaturated fatty acids. The intramuscular fat of both PL and ML groups showed significant higher values of linolenic (C18: 3 9cis, 12cis, 15cis, P<0.05) and rumenic acids (CLA 9cis, 11trans, P<0.05) compared to that of HL lambs. ML lambs, following their mothers out to pasture, may have ingested small amounts of grass in addition to milk, that is characterized, by a high proportion of linolenic acid (C18:3 9cis, 12cis, 15cis, 78.57%) . It is reknown that grass-based diets increase linolenic acid contents in lamb tissues (Bas et al., 2000) and that the level of CLA 9cis, 11trans increases with the incidence of pasture in the diet since it is related with the level of the CLA precursor, linolenic acid, in the ingested grass (Schimd et al., 2005). Conversely, meat from lambs reared at stall is characterized by a significantly higher content of linoleic acid (C18: 2 9cis, 12cis) and of omega-6 fatty acids (ω6) (P <0.001) than that of other groups; indeed, these animals were fed a diet based on commercial concentrate that is rich in linoleic acid (C18:2 9cis 12cis 46.51%).

In order to assess the effects of feeding treatments on nutritional value of fat some indices have been determined (ω6/ω3 ratio, P/S ratio, AI and TI indices). Due to the concentration of ω6 and ω3 fatty acids, meat from PL and ML lambs showed a significantly lower ω6/ω3 ratio (P<0.001) than that of HL group (1.86, 2.09 and 3.36 respectively). It is highly recommended that in the human diet this ratio is less than 4 (Department of Health, 1994); in this experiment the ω6/ω3 ratio was below the recommended value in the meat from lambs in all the groups. The P/S ratio in all groups was found to be less than the recommended limit of 0.45, although the lowest value was observed in meat of lambs kept on pasture. The atherogenic index (AI) significantly varied (P<0.01) from 0.79 for grazing lambs to 1.04 for suckled lambs. These values were lower than those reported by Vacca *et al.* (2008) for Sarda lambs fed with maternal milk.

IV – Conclusions

In conclusion, according to the assessment of the nutritional quality indexes ω6/ω3, P/S and AI, meat from grazing lambs was found to be qualitatively higher than that of other groups and particularly with respect to that of lambs held in stall and supplied with hay and concentrate.

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Effect of lupin on growth performance, carcass characteristics and meat quality of growing and fattening kids

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Abstract. To determine lupin grain (*Lupinus angustifolius)* effect in kids diet, we analyzed its incorporation effect on growth performances, carcass characteristics and meat quality. Four rations of concentrate (Rm, R2, R3 and R4) respectively with 0%, 12%, 21% and 35% DM of lupin physically treated and completed with barley and faba beans, were distributed to 4 groups of 7 local kids in each one from 90 days until 180 days of age. Results showed no effect of lupin on final weight, ADG (41.30, 47.50, 42.06, 49.76 g/day respectively for Rm, R2, R3 and R4), carcass yield, gastric pouch weight, adipose tissue and bone tissue importance, length of carcass and thigh and compactness index, while its incorporation improved significantly muscle and conformation index (P<0.05). For carcass color, rates higher than 20% increased significantly redness of tail and saddle (P<0.01), and decreased significantly saddle lightness (P<0.05). For the nutritional quality of meat, lupin induced significant reduction of humidity (P<0.01) with 75.76, 77.59, 73.85 and 74.11% respectively for Rm, R2, R3 and R4, while minerals, protein and fat were not affected. Lupin incorporation can reach 35% DM of the concentrate of kids without negatively affecting growth performance, carcass characteristics and meat quality.

Keywords. Lupin – Kids – Growth performances – Carcass characteristics – Meat quality.

Effet du lupin sur les performances de croissance, les caractéristiques de la carcasse et la qualité de la viande des chevreaux en croissance-engraissement.

Résumé. *L'objectif de ce travail est d'analyser l'effet de l'incorporation du lupin dans la ration des chevreaux sur les performances de croissance, les caractéristiques de la carcasse et la qualité de la viande. Quatre rations de concentré (Rm, R2, R3 et R4) iso-énergétiques et iso-azotées composées respectivement de 0%, 12%, 21% et 35% MS de lupin traité physiquement et complétées avec de l'orge et de la féverole, ont été distribuées à quatre lots de 7 chevreaux de 90 à 180 jours d'âge. Les résultats obtenus montrent qu'il n'y a pas d'effet du lupin sur le poids final, le GMQ (41,30, 47,50, 42,06, 49,76 g/jour respectivement pour Rm, R2, R3 et R4), le rendement de la carcasse, le poids du réservoir gastrique, l'importance du tissu adipeux et du tissu osseux*, *la longueur de la carcasse et de la cuisse, et l'indice de compacité. Cependant, le lupin améliore significativement l'indice de muscle et de conformation (P<0,05). Les taux d'incorporation de lupin supérieurs à 20% augmentent significativement l'indice de rouge de la queue et la selle (P<0,01), et diminuent significativement l'indice de luminance de la selle (P<0,05). Concernant la qualité nutritionnelle de la viande, le lupin induit une réduction significative de l'humidité (P<0,01) avec 75,76, 77,59, 73,85 et 74,11% respectivement pour Rm, R2, R3 et R4. Cependant, les cendres, les protéines et la matière grasse ne sont pas affectées. L'incorporation du lupin peut atteindre 35% de MS de la ration de concentré des chevreaux sans affecter négativement les performances de croissance, les caractéristiques de la carcasse et la qualité de la viande.*

Mots-clés. *Lupin – Chevreaux – Performances de croissance – Caractéristiques de la carcasse – Qualité de la viande.*

I – Introduction

In the north west of Morocco, the goat is the dominant livestock species. It represents 41% of ruminant livestock, about 690,000 heads (MAPM, 2008). Herds are concentrated in the

mountainous areas where the diet is based on the use of forest range with forage supply characterized by strong seasonal variability and a deficiency on protein resource (Chentouf *et al.,* 2004). Thus, diversification and improvement of feeding practices are necessary, and lupin can, undoubtedly, improve protein supply in the feeding calendar and can find its place in. However, studies on the introduction of lupin grain in the diet of goats are rare and mainly concern dairy goats (Broqua 2002, Masson 1981; Morales *et al*., 2008). In this context, this study aims to analyze the effect of incorporating physically treated lupin grain in the diet of kids on the performance of growth and fattening, the carcass characteristics and the quality of meat.

II – Materials and methods

Twenty eight kids from the local goat population of Northern Morocco, aged 3 months, were assigned to 4 groups (Rm, R2, R3 and R4) of 7 kids each and were followed from weaning to three months until the age of 6 months. These animals were fed a diet containing oat hay supplemented with 4 iso-energetic and iso-nitrogenous concentrate supplements. These supplements were made of barley, lupin grain, faba bean and a mineral-vitamin supplement. The lupin used was yellow lupin (*Lupinus angustifolius*) due to its availability in the region. Grains naturally exhibit relatively high levels of alkaloids; these are responsible for bitter taste and at high levels of intake can cause poisoning. To reduce their alkaloid content, lupin was subjected to physical treatment (Schoeneberger *et al*., 1982 cited by Mukisira *et al*., 2001) which consists of cooking the lupin in water at a temperature of 100°C followed by soaking it in cold flowing water for 24 hours and then drying for 24 hours at a temperature of 65°C in a ventilated oven.

The incorporation of lupin in the concentrate supplement was in the levels of 0, 12, 21 and 35% of DM respectively for lots Rm, R2, R3 and R4. During the test, the weighing of animals was conducted weekly to calculate average daily gain between 90 and 180 days of age (ADG90-180). After slaughter, more weightings and measurement were conducted to evaluate the carcass, yield hot and cold (24 hours *post mortem*), color, importance of adipose tissue, bone and muscle tissue. Compactness index represents ratio of weight on carcass length. While muscle index is thickness relative to length of thigh. The sum of these two indices is Conformation index. The color of the carcass was measured using a portable colorimeter (Chromameter Minolta CR410). Samples were taken on the *Semimembranosus* muscle to determine ash, fat, protein and moisture content. These analyses were carried out according to the AOAC (1979). Statistical analysis was performed by Excel 2007 and software (SAS, 2001).

III – Results and discussion

The final live weight and ADG90-180 are not affected by diet (Table 1). This result is similar to Vicenti *et al.* (2009) who reported that the lupin substitution of soybean had no significant effect on growth performance of bull calves for fattening.

Table 1. Effect of lupin on growth performance of goats

	Initial live weight (kg)	final live weight (kg)	ADG 90-180 (g/day)
Rm	14.1	16.39	41.3
R2	13.93	17.06	47.5
R3	14.04	17.29	42.06
R4	14.06	17.63	49.76
probability	0.99	0.89	0.79
significance	NS	NS	NS

NS: not significant.

Diets had no significant effect on carcass characteristics including yield and hot and cold (24 h *post mortem*) carcass weight (Table 2). Similar results were reported by El Maadoudi (1997) in Timahdite lambs and Vicenti *et al.* (2009) with Podolian bulls.

Table 2. Effect of lupin on carcass weight, yield, pluck and gastric pouch

	Carcass weight (kg)	Cold Carcass yield (%)	Pluck† (kg)	gastric pouch full (kg)	gastric pouch empty (kg)
Rm	7.24	44.12	0.9a	4.21	2.37
R2	7.72	45.08	0.76b	4.24	2.03
R3	8.33	48.77	0.94a	4.73	2.33
R4	7.31	41.34	0.87ab	4.13	1.91
P	0.56	0.09	0.03	0.34	0.06
	NS	NS	*	NS	NS

†Consists of all liver, lung, heart, spleen and trachea.
a, b, ab: in a same column, followed by the letters distinguished values are statistically different than 5%.
P : probability ; NS: not significant; *: P<0.05.

Also, the diet did not affect the weight of perirenal and mesenteric fat, and gastric pouch while the differences observed for the weight of the pluck seem to be more related to the characteristics of animals than to the diet (Table 3). For carcass length, there is no significant difference between lots and the same is also noted for the length of the thigh (Table 3).

Table 3. Effect of lupin on adipose and bone tissue

	Mesenteric fat (kg)	Perirenal fat (kg)	Carcass length (cm)	thigh length(cm)
Rm	0.32	0.08	51.8	21.2
R2	0.3	0.1	53.3	20.0
R3	0.49	0.13	55.3	20.4
R4	0.38	0.09	53.4	20.1
P	0.09	0.23	0.27	0.6
	NS	NS	NS	NS

P : probability ; NS: not significant.

According to the appreciation of the importance of muscle tissue, no differences were found between lots as regards the thigh thickness and the compactness index , while we note that the incorporation of lupin in the diet promoted positively muscle index (P<0.05) and the conformation index (P<0.05) (Table 4).

Concerning carcass color, lupin improved color index. Indeed, it induced a significant reduction of the brightness value of the saddle (P<0.05), an increase of red index of the saddle (P<0.01) and tail (P<0.01). No significant difference was observed for the color indices in the back (Table 5).

Moisture of *Semimembranosus* muscle decreased with increasing rate of lupin in the diet (P<0.05). This result contrasts with that reported by Vicenti *et al.* (2009) who observed no effect of substitution of soybean by lupin in the diet of Podolian bulls on the moisture of this muscle. While fat, protein and ash were not affected by the incorporation of lupin (Table 6).

Table 4. Effect of diet on muscle tissue.

	Thighs thickness (cm)	Compactness Index	Muscle index	Conformation index
Rm	10.62	0.13	0.5b	0.64b
R2	11.13	0.14	0.56a	0.7a
R3	11.19	0.14	0.55a	0.7a
R4	10.53	0.13	0.53ab	0.66ab
P	0.37	0.69	0.02	0.02
	NS	NS	*	*

a, b, ab: in a same column, followed by the letters distinguished values are statistically different than 5%. P : probability ; NS: not significant; *: P<0.05.

Table 5. Effect of diet on carcass color

	L* saddle	a* saddle	b* saddle	L* tail	a* tail	b* tail	L* back	a* back	b* back
Rm	61.09ab	4.38b	9.37	53.91	8.06b	8.31	68	3.53	10.79
R2	63.47 a	3.07b	9.84	54.91	8.20b	8.04	66.37	2.4	8.15
R3	57.40b	6.55 a	9.34	48.62	12.76a	8.83	62.19	3.72	9.94
R4	58.83b	6.31a	9.17	52.89	10.89a	9.41	64.82	4.12	10.51
P	0.013	0.002	0.93	0.13	0.002	0.24	0.06	0.25	0.1
	*	**	NS	NS	**	NS	NS	NS	NS

* Lightness, a * redness; b *yellowness. a, b, ab: in a same column, followed by the letters distinguished values are statistically different than 5%. P : probability ; NS: not significant; *: P<0.05; **:P<0.01.

Table 6. Effect of lupin on moisture, fat, ash and protein of *Semimembranosus*

	Moisture (%)	Fat (%)	Protein (%)	Ash(%)
Rm	75.76a	3.15	20.46	2.17
R2	77.59 a	2.33	20.52	2.15
R3	73.85b	3.38	20.98	2.18
R4	74.11b	3.43	22.15	2.17
P	0.0011	0.054	0.8	0.64
	**	NS	NS	NS

a, b : in a same column, followed by the letters distinguished values are statistically different than 5%. P : probability ; NS: not significant; **:P<0.01.

IV – Conclusions

Lupin can be incorporated in the diet of kids at rates that reach 35% of the concentrate ration and thus improve the carcass quality without adversely affecting fattening performance and meat quality.

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Effects of replacing corn and soya beans with white sorghum and faba beans on milk quality of Sicilo Sarde dairy ewes in Tunisia

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Abstract. The effects of using white sorghum seeds and faba beans as feed resources were studied on milk performances in dairy Sicilo-Sarde ewes during the milking phase. Twenty ewes were divided into two homogeneous batches according to age, litter size, rank of lactation and live body weight. Ewes received 1.5 kg DM of oat hay /ewe/day supplemented with 500 g/ewe/day of a standard concentrate diet (group C) or a concentrate composed of local white sorghum, faba beans and a sheep vitamin and mineral supplement (group S). Milk quality was determined once a week on samples of bulk milk batch to measure the fat content by Lactoscan. The concentration of urea in milk was determined by the DMAB method. The extraction of lipids was determined following Folch *et al.* (1957) method; the fatty acid composition was determined by gas chromatography. The use of the experimental concentrate (white sorghum and faba beans) affected the protein, non fat solids and lactose contents ($p<0.05$). The average level of urea was 53.5±8.76 and 35.5 ±3.4 mg/dl for the C and S ($p<0.05$), respectively. The main fatty acids were palmitic acid (27.75±1.29% *vs.* 27.77±1.21% of total fatty acids for the S and C diets, respectively; $p>0.05$) and miristic acid (12.06±0.82% and 12.47±1.21% for CC and CS, respectively; $p>0.05$). The average of capric acid was comparable in both concentrates (7.17±1.17%) in the control group (C) and 7.38 ± 1.39% in the experimental group (S). The concentration of conjugated linoleic acid (CLA) was 0.48 ± 0.03 and 0.36 ± 0.09% for the for the C and S ewe groups, respectively ($p>0.05$).

Keywords. Dairy sheep – Conjugated linoleic acid – Fatty acids – Milk quality – Urea.

Effet du remplacement du maïs et du soja par le sorgho blanc et la féverole sur la qualité du lait, la concentration d'urée, la composition des acides gras et l'acide linoléique conjugué (CLA) dans le lait des brebis Sicilo-Sardes en Tunisie

Résumé. *L'effet de l'utilisation du sorgho blanc et de la féverole comme ressources alimentaires locales a été étudié sur les performances laitières de la brebis Sicilo-Sarde durant la phase de traite. Vingt brebis ont été divisées en deux groupes homogènes selon l'âge, la taille de la portée, le numéro de lactation et le poids vif. Les brebis ont reçu 1,5 kg MS/brebis/jour de foin d'avoine complémenté par 500 g/brebis/jour d'aliment concentré commercial (lot C), et 500 g/brebis/jour d'aliment concentré local à base de sorgho blanc, féverole et CMV ovins (lot S). La qualité du lait durant les semaines de contrôle a été déterminée par un Lactoscan Milk analysis. La concentration de l'urée du lait a été déterminée par la méthode DMAB. L'extraction des lipides du lait a été déterminée selon la méthode de Folch* et al. *(1957). Le remplacement du maïs et tourteau de soja par le sorgho blanc et la féverole a affecté l'extrait sec dégraissé et le lactose du lait ($p<0,05$). La moyenne de l'urée du lait était de 53,5 ± 8,76 pour le lot C et 35, 5 ± 3,4 mg/dl pour le lot S ($p<0,05$). La moyenne des acides gras était pour l'acide palmitique C16 (27,75 ± 1,29% vs. 27,77 ± 1,21% des acides gras totaux respectivement pour les lots S et C) et pour l'acide myristique C14 (12,06 ± 0,82 % et 12,47 ± 1,21% respectivement pour C et S). La moyenne de l'acide caprique C10 était comparable ($p>0,05$) pour les deux aliments concentrés: 7,17 ± 1,17 pour le lot témoin (C) et 7,38 ± 1,39 % pour le lot expérimental (S). La concentration de l'acide linoléique conjugué (CLA) était 0,48 ± 0,03 et 0,36 ± 0,09% respectivement pour les lots C et S. Les acides gras totaux étaient comparables ($p>0,05$) pour le lait des brebis des deux groupes.*

Mots-clés. *Acide gras – Acide linoléique conjugué – Brebis laitière – Urée – Qualité du lait.*

I – Introduction

Currently, the Tunisian ovine population reached 4 million females (DGPDIA, 2005), which is represented mainly by the Barbarine, Noire of Thibar and fine Queue races. These three breeds have been used for meat production. The Sicilo-Sarde, localised in the Northern areas of Tunisia (e.g. Mateur and Beja), is the only breed used for milk production (DGPDIA, 2005). Although intensification of livestock in Tunisia is increasing, its integration with crops is limited. Integration of forage into rain-fed farming has not really succeeded.

The performances of dairy production of the Sicilo-Sarde ewes depend largely on food complementation especially during the gestation and suckling (Bocquier and Caja, 2001) periods. In the last few years, the economic world conjuncture involved rising prices of corn and soybean meals which constitute the basic raw materials in concentrated food formulations for Tunisian livestock.

However, with the new policy guidelines in agricultural, the research for alternatives feeds is imperative (Rouissi *et al*., 2008). The aim of our study was to evaluate the effect of the supplementation with local feed resources (white sorghum and faba bean) on production and quality of Sicilo- Sarde ewe's milk.

II – Materials and methods

1. Animals and diets

Twenty (20) Sicilo-Sarde ewes were divided into two homogeneous batches according to age (5.3±1.25 years as against 5.7±1.15 years), the litter size (1.1±0,31), the rank of lactation (4.3±1.25 *vs.* 4.6±0.96) and live body weight (33.83±5.63 *vs.* 33.95±5.58 kg) received a common base ration (oat hay) at 1.5 kg DM/ewe/day supplemented by a 500 g/ewe/day of a standard concentrate [C: 10% barley, 43.3% corn, 25% wheat bran, 17.7% soybean meal, 4% sheep Vitamin and Mineral Mixture (VMM)], or an experimental concentrate (S: 66% white sorghum, 30% faba, 4% sheep VMM). Two weeks of adaptation take place before starting the measurements for 10 weeks control. The chemical composition (organic matter (OM), total nitrogenous matter (CP), crude fiber (CF) and lipids (FAT) (AOAC, 1990) and the nutritive values (Sauvant, 1981) of concentrates and hay are given in the Table 1.

Table 1. Chemical composition and nutritive value of feeds (% DM)

	Concentrate feed		Oat hay
	C	S	
DM %	94.7	94.7	92
Organic matter	91.0	88.3	92.1
Crude protein	16.3	14.65	4.9
Crude fiber	12.7	3.7	35.6
FAT	2.8	7.1	1.8
UFL/Kg DM	0.98	0.99	0.47
PDIN(g/kg DM)	99	96	33.6
PDIE (g/kg DM)	104.9	95	63.9

2. Milk sampling and analysis

Throughout the period of the experiment, milk production was recorded daily with 2 manual milking per day. The study of milk quality was performed once a week on a sample of bulk milk of each batch. During the whole trial milk samples have been collected from each group. The following milk quality parameters (pH, CP, FAT, urea) were determined by Lactoscan (Milkotronic LTD, serial No. 4696, Hungary) and urea by the DMAB method (JOCE, 2009).

Fatty acids in milk were determined by gas chromatography. A sample of 30 g, 200 ml of chloroform and 100 ml of methanol are added and homogenized vigorously for 2 minutes. The mixture was filtered with a filter bucher paper. The residue was dispersed in chloroform-methanol (2/l, v/v, 300 ml), mixed for 3 minutes and filtered again . The remaining solids were washed with chloroform-methanol (2/l, v/v, 60 ml). All filtrates were collected in a funnel. The filtrates were added to 0.2 volume of a solution of NaCl 0.7%. After the formation of two phases. A supernatant containing the non- lipid compounds and an organic phase contain substantially all of the lipids. The organic phase was collected in a calibrated flask and the solvent was removed at 50°C in a rotary vacuum evaporator. The fat content was determined gravimetrically.

In a 20 ml tube, 10 mg of lipid extract, 1 ml hexane and 2 ml 0.5 M sodium methoxide (1.35 g in 50 ml methanol). The mixture was vortexed and placed 15 minutes at 50°C in a heating block and allow to cool and then added 1 ml of methanol/HCl 5% v/v (to be prepared extemporaneously) and was vortexed vigorously. The solution was centrifuged at a rate of 3000 rpm for 5 minutes and recovered hexanoic stage transfer in chromatography vials .The spectrophotometer was adjusted to zero through the reagent blank and read the absorbance at 420 nm. Before the analysis of milk samples by chromatography for fatty acids determination, extraction of lipids took place following the method of Folch *et al.* (1957). The device used was a gas chromatograph FID type of fatty acid methyl esters, with a column OMEGAWAX 250. The temperature at the detector and the injector was 220°C while the level of the column was programmed from 45°C to 190°C.

3. Statistical analysis

The results of the effect of diets on the milk quality parameters were subjected to a one way analysis of variance using the GLM procedure in SAS (1989).

III – Results and discussion

The fat content of milk was 7.58±0.6% and 7.21±0.41% for the experimental (S) and the control groups (C), respectively. Statistical analysis revealed that there was no difference between the two groups (p> 0.05). This result is consistent with that reported by Rouissi *et al*. (2008) and Selmi *et al*. (2010). This result can be explained by the high energy content in white sorghum seeds and their fat content compared to corn and by the nature of faba bean seeds which are more energetic because of the large amount of starch easily degradable in the rumen (Sauvant, 2004). The average fat content obtained in this trial is higher compared to that reported by Hammami *et al.* (2009) who found an average fat value ranging from 5.34 to 5.83% by supplying a concentrate based on barley during the lactation. This explains the results of Barrillet *et al.* (2002) who reported that the fat content is influenced by the amount of milk produced (decrease causes an increase in these rates) and length of use (the end of the milking phase is accompanied by an increase in the fat content).

The protein content of milk was statistically comparable between diets (p>0.05), (6.04±0.57% and 5.86±0.54% for S and C, respectively). This result converges with those of

Santos *et al.* (1998). This is attributed to the fact that the milk protein content is positively correlated with the energy balance of the diet since energy intake stimulates the synthesis of microbial protein in the rumen (Bocquier and Caja, 2001). The comparable effect of soybean meal and faba bean as protein sources in feed concentrates can be explained by the wealth of faba bean seeds in essential amino acids such as lysine and methionine (Bocquier and Caja, 2001).

Statistical analysis showed that the lactose content is not significantly different ($p>0.05$) according to the dietary regime. However, the average content of lactose in milk of ewes of experimental group is slightly higher compared to that of the control sheep (4.27±0.43% for S and 4.15±0.5% for C, respectively). This agrees with the result found by Rouissi et al (2008). It is about 4.27±0.43% for S and 4.15 ± 0.5% for C, this The slight difference in milk lactose content between S and C groups could be due to the richness of faba bean in easily degradable carbohydrate (starch) present especially at the seed coat of faba bean by comparing it with that of soybean meal (Pottier, 2002).

The content of urea in milk is considered as an indicator of protein utilization (Cannas *et al*., 1998). The average values were 53.5±8.76 and 35.5±3.4 mg/dl respectively for C and S groups ($P<0.01$). This result is consistent with that found by Lagriffoul *et al.* (1999) and different from that reported by Maâmouri *et al.* (2009) who supplied diets rich in tannins. The ratio of protein/energy in the diet that appears to be the factor having the greatest nutritional impact on the rate of urea (Cannas, 2002). This trend could be attributed to soya bean meal rich in protein degradable in the rumen compared to faba beans that contain a large amount of starch but also influences negatively urea concentration. Indeed, the energy level affects the amount of protein and non protein nitrogen to be used by micro-organisms (Benazzouz *et al*., 2007). Thus, an increase in energy intake in the diet will cause a decrease of urea on the one hand and secondly the tannin content in the integument of faba beans may reduce protein degradation and therefore reduce the amount of urea.

The influence of the inclusion of white sorghum in the ewe's concentrate as an energy source to replace corn was assessed through the analysis of fatty acids in The statistical analysis revealed that there were no differences among fatty acids in milk of both ewe groups regardless of the regime (Table 2). The main saturated fatty acids were palmitic acid C16 (27.75±1.29% *vs.* 27.77±1.21% of total FA for diets S and C, respectively) and miristic acid C14 (12.06±0.82% for C and 12.47±1.21% for S). White sorghum as an energy source rich in fat (Pottier, 2002) maintained the concentrations of key fatty acids compared with corn energy source commonly used as a main ingredient in concentrates made for sheep The stearic acid (C18: 0) represented respectively 6.9±0.74 and 6.82±0.82% of TFA in milk of the C and S ewe groups. The concentration of the oleic acid C18: 1 n-9 was not affected by the diets (20.7±3.2 and 22.5±4.6% for C and S groups respectively; $p>0.05$). The capric acid content was 7.17±1.17 and 7.38±1.39% ($p>0.05$) in the group C and group S respectively. These contents are higher compared to those reported by Luna *et al.* (2005) and Collomb *et al.* (2006) who worked on at different levels of altitude.

The CLA content of sheep milk is dependent on feeding conditions and the nature of the complementation. The concentration of CLA from this study was similar to that reported by Atti *et al.* (2006) who fed ewes with a diet based on concentrate feed but was lower compared with milk from sheep grazing on ryegrass or barley reported by the same authors in the same paper. CLA concentration was not different between the groups (0.48±0.03 and 0.36±0.09%, $p>0.05$); this shows that the white sorghum is a good alternative to corn because of the important amount of fat in its seeds.

The percentage of saturated fatty acids was higher compared to the polyunsaturated fatty acids, regardless of the diets. This can be explained by the biohydrogenation of PUFA in the diet. The percentage of monounsaturated fatty acids was 23.9±6.1 and 25.33±4.76, for C and S, respectively, with no statistical difference ($p>0.05$). All the observed results on milk

fatty acids composition could be explained by the comparable fat composition of maize and white sorghum that are rich in linoleic acid (1305 mg), oleic acid (964 mg) and palmitic acid (407 mg). The ratio PUFA/SFA is similar for both groups (p>0.05) and compared to that observed by Atti *et al.* (2006) who worked on sheep fed on pasture. This can be explained by the significant contribution of polyunsaturated fatty acids in feed concentrates compared to forage that are rich in short chain fatty acids (Table 2).

Table 2. Concentration of Fatty acids profile and CLA in Milk (% of total FA)

	C	S	Pr >F	SEM
Capric Acid (C10)	7.17±1.17	7.38±1.39	Ns	0.4
Miristic Acid (C14)	12.06±0.82	12.47±1.21	Ns	1.03
Palmitic Acid (C16)	27.75±1.29	27.77±1.21	Ns	1.06
Stearic Acid (C18)	6.9±0.74	6.82±0.98	Ns	0.87
Conjugated Linoleic Acid (CLA)	0.48±0.11	0.36±0.1	Ns	0.11
SFA	65. 76± 4.04	66.84±4.92	Ns	0.96
MUFA	23.9 ±6.1	25.33±4.76	Ns	0.84
PUFA	3.88±1.1	3.7±0.8	Ns	0.56
SCFA	14.62±4.21	14.54±5.35	Ns	1.1
LCFA	53.17±2.42	53.67±3.63	Ns	0.57
PUFA/SFA	0.59	0.55	Ns	0.73
FA omega-3	0.369	0.364	Ns	0.44
FA omega-6	0.09	0.15	Ns	0.82
Ratio n-6/n-3	0.32	0.46	Ns	0.67

SFA: Saturated fatty acids; MUFA: mono-unsaturated FA, PUFA: poly-unsaturated FA; SCFA: Short chain FA; LCFA: Long chain FA. SEM: standard error of the mean; Ns: not significant (p>0.05).

IV – Conclusions

The results from this experiment suggest that the effect of the incorporation of local raw materials instead of imported raw materials in the formulation of concentrate feed can maintain or improve some physico-chemical characteristics of Sicilo-Sarde ewes' milk, including its fatty acid concentrations. The higher content of urea in the control group put into evidence the waste of protein used in that diet and the coverage rate of nitrogen needs of this sheep The elevated urea level in the milk receiving the concentrate feed with a soy protein source reflects the quality level of soybeans protein in one hand, and in the other hand the presence of anti nutritional substances in faba beans.

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Effects of rosemary extracts incorporation on Barbarine lamb's growth and carcass characteristics

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Abstract. The aim of this experiment was to study the effect of rosemary extracts (RE) incorporation on diet intake, lambs growth, carcass characteristics and composition. Thirty two fat tail Barbarine lambs (19.9±2 kg body weight (BW)) were divided into 2 homogeneous groups according to BW. All sheep were fed 50% alfalfa caps and 50 % concentrate. Two types of concentrate were used, the Control (C) and the experimental (RE) in which 0.06% of RE (measured on fresh weight of the concentrate) was added. At the end of the growth trial (60 days), all animals were slaughtered. The BW at slaughter was similar for both groups (25 kg). Dressing percentage was not affected by RE incorporation (54.4 *vs.* 51.7 for C and RE, respectively). The gut was more developed for C group (1614 *vs.* 1495 g). The carcass weight was similar for both groups (10.9 *vs.* 10.7 kg for C and RE, respectively). Carcasses of RE group showed comparable fatness (22%), less muscle (50.6 *vs*. 52.5%) and a higher bone proportion than C one. In conclusion, small quantity of RE did not show significant effects on lambs' growth and carcass composition.

Keywords. Rosemary extracts – Lambs - Growth – Carcass composition.

Effets de l'incorporation des extraits de romarin sur la croissance et les caractéristiques de la carcasse des agneaux de race Barbarine

Résumé. *Le but de cette expérience était d'étudier l'effet de l'incorporation des extraits de romarin (RE) sur la croissance et la composition de la carcasse des agneaux. Trente-deux agneaux de race Barbarine (19,9 kg de poids vif (PV)) ont été divisés en 2 lots homogènes en fonction de PV. Tous les agneaux ont reçu une ration composée de 50% de bouchons de luzerne et 50% d'aliment concentré. Deux types d'aliment concentré ont été utilisés, le témoin (T) et l'expérimental, qui correspond à T avec 0,06% de RE. À la fin de l'expérience (60 jours), tous les animaux ont été abattus. Le PV à l'abattage a été similaire pour les deux groupes (25 kg). Le rendement en carcasse n'a pas été affecté par l'incorporation de RE (54,4* vs *51,7 % respectivement pour T et RE). Le tube digestif a été plus développé pour le lot T (1614* vs *1495 g). Le poids de la carcasse froide était similaire pour les deux lots (10,9* vs *10,7 kg respectivement pour T et RE). Les carcasses du lot RE ont montré la même composition en gras (22%), moins de muscle (50,6* vs *52,5%) et plus d'os que le lot T. En conclusion, la faible dose de RE n'a pas montré d'effet significatif sur la croissance des agneaux et la composition de la carcasse.*

Mots-clés. *Extraits de Romarin – Agneaux – Croissance – Composition de la carcasse.*

I – Introduction

The livestock extensive system has become ineffective in spite of the use of different diets rich in energy. These plans can be improved by a number of additives and growth promoters (antibiotics, hormones, etc.). In recent years, the prohibition of use of antibiotics and growth promoters (OJEU, 2003), which had shown adverse effects on human and environment, required the use of new alternatives. At this level, plant extracts such as essential oils have

received increased attention as potential alternatives to growth promoters for animal production (Chaves *et al.,* 2008; Nieto *et al.*, 2010). The objective of this experiment was to study the effect of rosemary (*Romarinus officinalis*) extracts (RE) incorporation on lambs growth, carcass characteristics and composition.

II – Material and methods

1. Animals and diets

The experiment was carried out at INRAT experimental farm of Bourebiaa. Thirty-two Barbarine lambs with an average body weight (BW) of 19.9±2 kg were used. They were divided into 2 homogeneous groups according to BW and concentrate type. All lambs were fed 50 % alfalfa caps + 50% concentrate (two meals a day) and had free access to water. Two types of concentrate were used: the control (C) was composed of barley (80%), soybean meal (18%) and CMV (2%), while the experimental concentrate (RE) represents the C concentrate mixed with of 0.06% (measured on fresh weight of the concentrate) of rosemary extract.

2. Measurements

Feed intake was recorded daily and sheep BW weekly. At the end of the growth trial (60 days), all animals were slaughtered. Body weight at slaughter (BWS) was recorded. Red cut-down (liver, kidneys, spleen, and heart); omental and mesenteric fat (OMF) and all fraction of the digestive tract were weighed. Cold carcass weight (CCW) was recorded after 24 h of storage of the carcasses at 4°C. After removing the tail, each carcass was divided longitudinally into two halves; the left sides were dissected into fat, muscles and bones.

3. Statistical analysis

A one-way analysis of variance for diet effect on growth, slaughter parameter and carcass composition using GLM procedure in SAS (1989) was applied. Then, the test Duncan was used to compare diet mean effects (α=0.05).

III – Results and discussion

1. Growth performance and slaughter parameters

Feed intake was similar for all lambs. Consequently, lambs had similar daily weight gain without significant difference between groups (Table 1). The growth rates recorded in this study (81 *vs.* 76 g for C and RE, respectively) are generally lower than the average daily gains reported for the same breed in other studies (Atti and Abdouli, 2001; Mahouachi and Atti, 2005). RE incorporation did not affect CCW and, consequently, DP which was slightly higher for C group. The lack of significant effects can be explained by the similarity between the energetic level of diets and considering the fact that these parameters are strongly correlated to the SBW (Sents *et al.*, 1982; Atti and Khaldi, 1988; Atti *et al.*, 2003), which, in the present study, was unaffected by RE incorporation.

2. Non-carcass components

No significant difference was recorded for the weight of the different red cut-down (Table 2) between groups. RE incorporation slightly ameliorated testicles weight (30 *vs.* 27 g for RE and C group, respectively) which is correlated to spermatozoa production (Mahouachi, 1985). Thus, RE may have an important interest on reproduction parameters. Conversely, RE did not show positive effect on digestive tract which had higher weight for control group. This result was not in

agreement with those of Noirot *et al.* (2007) reporting positive effects of dietary essential oils effect on digestion. Since the lambs were slaughtered at similar body weights, there were no significant differences for all parameters mentioned above which confirms the results of Atti and Khaldi (1988).

Table 1. Body weight (BW) parameters, carcass weight and dressing percent (DP)

Group	C	RE	SEM	P- values
Daily gain (g)	81	76	1.01	0.64
SBW (kg)	25.1	24.9	0.97	0.90
EBW (kg)	20.1	20.7	0.85	0.52
CCW (kg)	10.9	10.7	0.54	0.71
RDP %	54.4	51.7	0.15	0.41

C, control group; RE, rosemary essential oils group; SEM: standard error.

Table 2. Rosemary extracts incorporation effect on non-carcass component

Group	C	RE	SEM	P-values
Red cut-down (g)	1046	1051	3.5	0.90
Testicles (g)	27.4	30.3	0.5	0.57
Rumen (g)	618.9	583.6	2.5	0.21
Intestines (g)	868.3	793.3	10.3	0.52

C, control group; RE, rosemary essential oils group; SEM: standard error.

3. Carcass composition

The results of weight (g) and proportion (%) of different tissues in whole carcasses are presented in Table 3. There were no significant differences between groups. Muscle weight and proportion were slightly higher for C group (5413 *vs.* 5346 and 52.5 *vs*. 50.6% for C and RE group, respectively). On the contrary, RE incorporation increased bone weight (2667 *vs.* 2404 g for RE and C group, respectively) and proportion (25.5 *vs.* 23.8% for RE and C group, respectively). Lambs of both groups presented the same carcass fat proportion (22%). This result was in agreement with those of Atti *et al*. (2011) who did not find significant differences in carcass composition (muscle and fat) between Barbarine lambs in feedlot receiving control concentrate and 200 g of aromatic plants (artemisia and rosemary) and others receiving only control concentrate. The similar intake and the comparable energetic level of the diets, together with the short experimental period of the experience could explain the absence of significant differences between groups.

IV – Conclusion

Small quantity of RE incorporation did not show significant effects on lamb's growth and carcass composition.

Table 2. Rosemary extracts incorporation effect on proportion of different carcass tissues

Group	C	RE	SEM	P values
Muscle (g)	5413	5346	22.1	0.79
Muscle (%)	52.5	50.6	0.1	0.23
Fat (g)	2402	2376	23.0	0.92
Fat (%)	22.6	22.4	0.1	0.90
Bone (g)	2404	2667	12.8	0.07
Bone (%)	23.8	25.5	0.1	0.11

C, control group; RE, rosemary essential oils group; SEM: standard error.

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Growth performances and carcass composition of Barbarine lambs: Effect of the substitution rate of soya bean cake by faba beans (*Vicia faba*)

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Abstract. The objective of this study was to examine the effect of faba beans level in the diet on lamb growth performance and carcass composition. Thirty male lambs of the fat tail Barbary breed were divided into three groups of ten on the basis of live weight (33.4±2.56 kg). Three different iso-nitrogen and iso-energetic concentrates, where soya beans cake was partially substituted by faba beans were compared (concentrates comprising 0, 100 g/kg and 200 g/kg faba beans). Lambs underwent an adaptation period of 15 days. Five lambs from each group were slaughtered after a 30 days-finishing period. The other lambs were slaughtered after a 60 days-finishing period. Lambs carcasses were dissected. Average daily gain during the first 30 days-finishing period (125 g/day) and during all the trial (75 days) (137 g/day) was similar for all dietary treatments. Faba bean levels in diets had no significant effect on the carcass quality. However, lambs fed by concentrates comprising 100 g/kg faba bean had the lowest proportion of fat in the carcass. It be concluded that carcass lambs which received faba beans were less fatty.

Keywords. Barbary lamb – Growth – Carcass quality – Faba bean – Soya bean cake.

Performances de croissance et composition de la carcasse des agneaux de race Barbarine : Effet du taux de substitution du tourteau de soja par la féverole (*Vicia faba*)

Résumé. *L'objectif de cette expérience est d'étudier l'impact de la substitution partielle du tourteau de soja par de la féverole durant différentes périodes de finition sur les performances zootechniques, le rendement à la découpe et la composition de carcasse d'agneaux. Trois groupes de 10 agneaux ont reçu différents types de concentré iso-énergétiques et iso-azotés. Un groupe (SBM) a reçu un concentré à base de tourteau de soja. Les groupes FB1 et FB2 ont reçu un concentré contenant 100 g/kg et 200g/kg de féverole respectivement. La durée d'adaptation au régime a été de 15 jours. La moitié des agneaux de chaque groupe ont été abattus après une période de finition de 30 jours, qui suivait la période d'adaptation à l'alimentation expérimentale. Les autres agneaux ont été abattus après une durée de finition de 60 jours. L'incorporation de la féverole dans la ration n'a pas affecté la vitesse de croissance des agneaux (137g/ jour en moyenne). Les agneaux qui ont reçu le concentré contenant 200g/kg de féverole durant toute la période de finition ont le rendement vrai le plus élevé (59,17%), tandis que les agneaux du FB1 ayant été abattus après 30 jours seulement ont la carcasse la plus maigre et le rendement à la découpe au niveau du gigot le plus élevé.*

Mots-clés. *Barbarine – Croissance – Qualité de la carcasse – Féverole – Tourteau de soja.*

I – Introduction

The feeding of sheep in Tunisia is often provided by natural vegetation and fallow resources. These resources vary considerably depending on climatic conditions particularly rainfall. Moreover, nearly two thirds of lambings takes place in autumn so that the lambs and their mothers can take advantage of the surge of grass. In good years, lambs are marketed in

spring at a live weight of 20 to 25 kg, without being fattened indoors. However, in dry years, the growth rate of lambs may be very low and their live weight at weaning (5 months) may not exceed 16 to 17 kg (Abderrabba, 1989). In recent years, in arid and semi-arid areas, the energy and protein requirements of fast growing, intensively fattened lambs have been met using soyabean and maize, which are the major ingredients in concentrates and largely imported at high costs (Lanza *et al.*, 2003). Therefore, the use of legume grains such as faba beans (*Vicia faba*), in animal nutrition, is expected to increase further in the near future. In lambs' feeding, the substitution of soya bean meal by faba beans does not affect growth performance (Surra *et al.*, 1992; Purroy *et al.*, 1993; Atti and Mahouchi, 2009). The objective of this study was to evaluate the effects of partial substitution of soya bean by faba beans and finishing period duration on lamb growth rate and the carcass quality.

II – Materials and methods

1. Measurements

The experiment was conducted on a farm in North West of Tunisia. The feeding trial was carried out with a total of 30 fat-tailed Barbarine male lambs. Before the experiment period, lambs received wheat straw and concentrate where soybean meal was the main protein source. At 180 days of age, the lambs were divided into 3 groups balanced according to live weights (33.4±2.56 kg), with 10 animals per treatment. Treatments included: Lambs fed with concentrate where soybean meal was the main protein source (SBM group); lambs fed with concentrate where comprising 100 g/kg faba bean, faba beans being the main protein source (FB1 group) and lambs fed with concentrate comprising 200 g/kg faba bean, faba beans being the main protein source (FB2 group). The duration of the experiment was 75 days (with 15 days-adaptation period the experimental diets). The lambs were raised in stalls and fed a diet based on the experimental concentrates and wheat straw. The wheat straw was offered *ad libitum*. In detail, SBM lambs received a concentrate that comprised mainly barley and soybean meal, FB1 and FB2 lambs received a concentrate in which part of soybean meal and maize were replaced by faba beans (the concentrate comprising 100 g/kg and 200 g/kg faba beans respectively) (Table 1). Moreover, the concentrate fed to the SBM group contained small quantities of bran, to obtain iso-proteic and iso-energetic diets. Animals of all groups had free access to water. They were weighed weekly just prior to feed distribution. Five lambs per group were slaughtered 30 days after the beginning of the experimental treatments at an average body weight of 36 ±1.03 kg. At the end of the experiment (75 days), all remaining lambs were slaughtered at an average body weight of 40 ± 1.6 kg. The left half-carcasses were cut according to Colomer Rocher *et al.*, (1972) into six joints (leg, lumbar region, flank, thoracic region, neck and shoulder). All regions were dissected into fat, muscle and bones.

The Net dressing percentage was calculated within this equation= (Hot carcass weight / (liveweight – Digestive contents))*100.

The yield of different carcass pieces was calculated by the ratio of piece weight to cold carcass weight.

The chemical composition of the experimental diets is reported in Table 1. Mineral content was determined by ashing at 550°C for 8h. Nitrogen was determined by the Kjeldahl method (CP=N x 6.25). The NDF (Neutral detergent fiber), ADF (Acid detergent fiber), ADL (Acid detergent lignin) were determined using the Van Soest method.

2. Statistical analysis

All statistical analyses were performed using SAS 9.1. Data were analysed using the GLM procedure to determine the effects of the experimental factors (faba bean level and duration of the finishing period) and their interaction on final live weight, daily gain, hot carcass, net

dressing, cutting yields and carcass. Duncan's test was used for pairwise comparison. Differences were considered significant at the p≤ 0.05 level.

Table1. Chemical composition and constituents of experimental foods

	Wheat straw	Concentrate		
		SBM	FB1	FB2
Composition				
Dry matter (%)	93.62	88.29	88.55	88.84
Crude protein (%DM)	2.74	15.77	15.71	16.33
NDF (%DM)	81.22	0.16	0.16	0.21
ADF (%DM)	47.21	0.38	3.4	4.6
ADL (%DM)	7.22	1.1	1.4	2.1
Energy (UF/kg DM)	0.31	0.96	0.97	0.96
MM (%DM)	7.56	6.61	6.32	6.53
Ingredients (%)				
Barley		29	42.5	48.5
Maize		42	32	18
Bran		10	-	-
Soya bean meal		14	10.5	8.5
Faba bean		-	10	20
MVS[b]		5	5	5

NDF: Neutral detergent fiber; ADF: Acid detergent fiber; ADL: Acid detergent lignin; UF: Feed unit = French Feed Unit System for ruminants; MM: Mineral matter; MVS: Mineral- vitamin-supplement.

III – Results and discussion

Lambs performances are presented in Table 2. Except for the net dressing percentage, the level of faba bean incorporation within concentrates did not affect lamb performances (p>0.05). Consequently, there was not a significant effect of the level of incorporation of faba beans within concentrates on final liveweights. This result is line with those previously reported by Atti et Mahouchi (2009), Lanza *et al.* (1999) and Caballero *et al*. (1992). This legume seed leads to a similar daily gain than soya bean (Surra *et al.*, 1992) and a higher daily gain than that observed with other legume seeds like lupins and lentils (Purroy *et al.,* 1993). These results can be explained by the amino acids profile of faba beans, which generally completes that of cereal grains (Popova, 2007). Moreover, the introduction of barley in the SBM concentrate should optimize the use of the faba beans' rapidly degradable protein fraction (about 90%, Jarrige, 1988) and improve the microbial protein synthesis in the rumen. The FB2 and SBM lambs had the highest net dressing percentage compared to FB1 lambs (57.75, 56.14 and 55.29 for FB2, SBM and FB1 respectively). This result can be explained by the weight of digestive tube of different groups.

Daily gain and net dressing percentage were affected by the duration of the finishing period. The lambs that had the longer finishing period had a higher liveweight and hot carcass weight than the lambs that had the shorter finishing period. The daily gain during the last four weeks increased comparatively to the first four weeks of the finishing period. Consequently, feed conversion ratio was more favourable for FB1 and FB2 lambs during the long than for the short finishing period. Therefore, the FB2 lambs having the long finishing period had the highest net dressing percentage than SBM lambs and FB1 lambs. The interaction between faba beans level and duration of the finishing period affected final weight lambs, hat carcass weight and net

dressing percentage. The incorporation of faba bean in concentrates improved net dressing percentage with 3% to 4% if the duration of the finishing period is extended to 60 days. The duration of the finishing period has the global effect on hot carcass weight and net dressing percentage also.

Table 2. Lamb performances

Duration of finishing period	30 days			60 days			P		
	SBM	FB1	FB2	SBM	FB1	FB2	FBL	DFP	I
Initial weight (kg)	33.33	33.35	33.55	33.33	33.35	33.55	ns	ns	ns
Final weight (kg)	37.32^{b}	36.28^{b}	36.36^{b}	39.86^{a}	41.03^{a}	40.91^{a}	ns	****	**
Daily gain (g/day)	123	113	119	140	135	135	ns	ns	ns
Hot carcass weight (kg)	17.44^{b}	16.86^{b}	17.66^{b}	19.72^{a}	20.52^{a}	21.12^{a}	ns	****	***
Net dressing (%)	55.91^{b}	53.98^{b}	56.33^{ab}	56.38^{ab}	56.61^{ab}	59.17^{a}	ns	*	*

SBM: Lambs fed with concentrate where soybean meal was the main protein source; FB1: Lambs fed with concentrate where comprising 100g/kg faba bean; FB2: Lambs fed with concentrate comprising 200 g/kg faba bean; FBL: faba bean level; DFP: duration of the finishing period ; I: Interaction (Faba beans level* duration of the finishing period).
ns = Not significant ; (*): p<0.05 ; (**): p< 0.01 ; (***): p< 0.001; (****): p<0.0001; a, b: within a column, means without a common superscript letter differ (p<0.05)

The yield of different carcass pieces is presented in the Table 3. The ratio of hind leg weight to cold carcass weight was only affected by faba beans level. The ratio of hind leg weight to cold carcass for the FB1 was the highest compared to those for SBM and FB2 groups (31.93, 33.66 and 32.88 respectively to SBM, FB1 and FB2). Indeed, the FB1 lambs slaughtered after 30 days had the highest leg percentage (34.59%).

Table 3. Lambs cutting yields

Duration of the finishing period	30 days			60 days			P		
	SBM	FB1	FB2	SBM	FB1	FB2	FBL	DFP	I
Leg (%)	31.96^{b}	34.59^{a}	32.96^{b}	32.26^{b}	32.74^{b}	32.61^{b}	*	ns	ns
Lumbar region (%)	10.22^{a}	10.03^{a}	10.16^{a}	9.27^{ab}	8.70^{b}	9.20^{ab}	ns	***	**
Flank (%)	4.39^{b}	4.27^{b}	4.42^{b}	6.06^{a}	5.78^{a}	5.53^{a}	ns	****	***
Thoracic region (%)	25.24	24.76	23.27	23.42	24.08	23.97	ns	ns	ns
Neck (%)	8.91^{ab}	9.43^{ab}	9.32^{ab}	8.87^{ab}	8.31^{b}	10.43^{a}	ns	ns	ns
Shoulder (%)	18.9	18.32	18.7	18.6	18.89	18.31	ns	ns	ns

SBM: Lambs fed with concentrate where soybean meal was the main protein source; FB1: Lambs fed with concentrate where comprising 100g/kg faba bean; FB2: Lambs fed with concentrate comprising 200 g/kg faba bean; FBL: faba bean level; DFP: duration of the finishing period; I: Interaction (Faba beans level* duration of the finishing period).
a, b: within a column, means without a common superscript letter differ(p<0.05)

The duration of the finishing period affected the lumbar region percentage (p<0.001) and the flank percentage (p<0.0001). The lumbar region percentage decreased while the flank percentage increased with the duration of the finishing period. The interaction between faba beans level and duration of the finishing period affected lumbar region yield and flank yield. The extension of duration of the finishing period to 60days promotes the abdominal region development (flank). The duration of the finishing period has the global effect.

Carcass composition is presented in Table 4. Faba beans level did not affect the muscle

percentage neither fat or bone percentage (p>0.05). Fat and bone carcass percentage were affected by finishing period and interaction between faba beans level and duration of the finishing period. The FB1 lambs having the shorter duration of finishing period had the less fat percentage and the highest muscle percentage (Table 4).

Table 4. Carcass composition

Duration of finishing period	30 days			60 days			P		
	SBM	FB1	FB2	SBM	FB1	FB2	FBL	DFP	I
Muscle (%)	55.13^{b}	60.04^{a}	55.94^{b}	55.34^{b}	56.10^{b}	57.37^{b}	ns	ns	ns
Fat (%)	22.35^{a}	16.94^{b}	21.23^{a}	24.06^{a}	22.98^{a}	22.64^{a}	ns	*	*
Bone (%)	20.24^{a}	20.6^{a}	20.61^{a}	18.41^{ab}	18.35^{ab}	17.78^{b}	ns	****	**

SBM: Lambs fed with concentrate where soybean meal was the main protein source; FB1: Lambs fed with concentrate where comprising 100 g/kg faba bean; FB2: Lambs fed with concentrate comprising 200 g/kg faba bean; FBL: faba bean level; DFP: duration of the finishing period ; I: Interaction (Faba beans level* duration of the finishing period).
a, b: within a column, means without a common superscript letter differ(p<0.05).

Most studies have reported no effect of different nitrogen sources on carcass composition. Thus, Purroy *et al.* (1992) and Lanza *et al.* (1999) were reported that lambs fed faba beans had the lowest proportion of fat in the carcass than those fed soybean meal or lentils.

IV– Conclusion

This study was designed to examine the effects of using faba beans as an alternative protein source in the diet growth performances and carcass composition of Barbarine lambs. Partially replacing concentrates based on soya bean meal as protein source by concentrates comprising 100 g/kg or 200 g/kg faba beans did not affect lamb daily gain. Lambs fed by concentrates comprising 200 g/kg faba beans slaughtered on the end of experiment (75 days) had the highest net dressing percentage. However, the FB1 lambs had the highest ratio of hind leg weight to cold carcass weight. Lambs from the FB1 group had the lowest proportion of fat in the carcass. In conclusion, the use of concentrates comprising 100 g/kg faba beans in the diet of Barbarine lambs for 30 days is feasible and has a little positive effect on growth performances and on carcass quality.

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Effect of substitution rate of soya bean cake by faba beans (*Vicia faba*) on meat quality of black of Thibar and fat tail Barbary breed lambs

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Abstract. This study aims to compare the effect that different level of faba bean distributed to two local Tunisian lamb breeds on the quality of meat during 5 days *postmortem*. Thirty male black of Thibar and fat tail Barbary lamb breeds were divided into three groups. After weaning the lambs were offered access to one of three different iso-nitrogen and iso-energetic concentrates, where soya beans were substituted at 0%, 10% or 20% by faba beans cake in diets. At the end of the trial (75 days), all lambs were slaughtered. Colour (L,a*,b*), pH and shear force of *longissimus dorsi* and *semitendinous* muscle were significantly affect by breed and nitrogen source ($p<0.001$). This study showed that, lambs receiving faba bean had tender meat and better quality scores than meat from lambs receiving only soya bean.

Keywords. Meat quality – Barbary – Black of Thibar breed – Faba bean – pH – Colour – Shear force.

Effef de la substitution du tourteau de soja par la féverole (*Vicia faba*) sur la qualité de la viande des agneaux de races Noire de Thibar et Barbarine

Résumé. *Cette étude vise à comparer l'effet de l'incorporation de différents niveaux de féverole distribués à des agneaux de deux races locales tunisiennes sur la qualité de la viande pendant 5 jours post-mortem. Trente mâles simples de race Noire de Thibar et de race Barbarine ont été divisés en trois groupes de 10 agneaux chacun. Après le sevrage, les agneaux avaient accès à l'une des trois rations iso-azotées et iso-énergétiques, où le tourteau de soja a été remplacé par 0%, 10% ou 20% de féverole. À la fin de l'essai (75 jours), tous les agneaux ont été abattus. La couleur (L, a *, b *), le pH et la force de cisaillement du* Longissimus dorsi *et du* Semitendinosus *ont été affectés de manière significative par la race et par la source d'azote (p<0,001). Cette étude a montré que les agneaux recevant la féverole avaient une viande tendre et de meilleurs scores de qualité que la viande d'agneaux ne recevant que du soja.*

Mots-clés. *Qualité de la viande – Barbarine – Noire de Thibar – Féverole – pH – Couleur – Force de cisaillement.*

I – Introduction

Soya bean is the most common nitrogen source included in the concentrate for ruminants. However, in recent years an important objective of Mediterranean farmers has been to promote the use of alternative protein sources, preferably from local feedstuff, in animal feeding to try to reduce production costs. Legumes seeds, such as faba beans, lupins and peas, have attracted attention as supplements for ruminants in recent years. Indeed, faba bean has a high protein (30%) and starch (42%) content (Yu et *al*., 2002) and is a potentially valuable protein and energy supplements for animals. Several studies showed that their use did not negatively affect growth, slaughter performances or meat quality (Hadjipanayiotou, 2002; Lanza *et al*., 2003b; Lanza et Priolo, 1999; Purroy *et al*., 1992; Surra *et al*., 1992).

Faba bean (*Vicia faba*) is a legume seed available in the Mediterranean area and is comparatively cheap despite its relatively high nutritional value. Its crude protein content is high

(30-32% of dry matter) and the aminoacidic profile has a high lysine content (Palander *et al*., 2006). The use of diets based largely on faba bean for lamb fattening gave similar growth performance and meat characteristics as traditional diets based on soybean meal as the main protein source (Caballero *et al*., 1992; Lanza *et al*., 1999).Thus, the objective of the present trial was to evaluate the effect of replacing dietary soybean meal by faba bean in the concentrate fed to lambs breeds on the quality of meat during 5 days *postmortem* (pH, colour, shear force).

II – Materials and methods

1. Experimental design, animals and diets

The experiment was conducted on a farm in North West of Tunisia. The feeding trial was carried out with a total of 15 fat-tailed Barbarine and 15 Black of Thibar male lambs. The thirty lambs were born on the same farm. At 180 days of age, the lambs were divided into 3 groups balanced according to their weights (34.13 kg±2.75 kg), with 10 animals per treatment. Treatments included: Lambs fed with concentrate where soybean meal was the main protein source (SBM group); lambs fed with concentrate where faba bean was the 10% of protein source (FB1 group) and lambs fed with concentrate where faba beans were the 20% of protein source (FB2 group). The lambs, after 15 days of adaptation to the experimental diet, were fed for 60 days. The lambs were raised in stalls and fed a ration based on wheat straw and concentrate. The wheat straw was offered of ad libitum intake. Although, the concentrate was amount 800 g per head.

The animals were slaughtered 240 days of age in a licensed abattoir. After slaughter, the carcasses were cooled to 4°C for 24 h according to the normal working practice in the abattoir. They were then halved carefully, and the left side of each carcass was divided into standardised commercial cuts: leg, shoulder, rib and loin, breast, and neck, and transported to the laboratory in an appropriate refrigerated system. Slices of *Longissimus dorsi* (LD) and *Semitendinous* (ST) muscles were taken to measure pH, colour and shear force during meat maturation. Legs were frozen at -20°C to carry out the sensory analysis.

2. pH

The initial pH (pH_i) was measured one hour after slaughter on the carcass at the long dorsal and at the leg. The meat pH was measured at 24 h post-mortem, at 4 ± 2°C, in the LD (9th-13th rib level) and in ST with a penetrating electrode connected to a portable pH-meter type Hi 8424 microcomputer (HANNA instruments). Besides, the pH was measured during five days post mortem in the aim to study the evolution of pH.

3. Meat colour

Meat colour was measured at 24 h post-mortem, at 6 ± 2°C on the surface of the section of the LD (9th-13th rib level). Meat colour was also measured at 5^{th} day of maturation meat. Colour measurements of the muscle surfaces obtained using a Minolta CR-410 colorimeter (Minolta). Before use, the colorimeter was standardized using a white tile with Illuminant D65. The CIELAB colour space was used in colour measurement. The L (lightness), a* (redness), and b*(yellowness) colour co-ordinates were measured three times on the muscle surfaces and averaged for statistical analysis.

4. Texture

Slices of LD and ST were cut rectangular parallelepipeds, around 1*1 cm-thick and 2-3 cm long, were cut parallel to the muscle fibres. Shear force was measured on meat aged 24 h and 5

days post-mortem. Raw meat texture was measured in the longitudinal configuration using a TA-XT plus (Texture analyser, Stable Microsystem, UK).

5. Sensory analysis

In order to evaluate the influence of faba beans level substitution and breed on the sensory characteristics, 30 cooked lamb legs were assessed by a trained panel of 14 members, using classical sensory analysis by attribution of note to each presented sample (from 1 to 10). The day before the event tasting meat samples were thawed in a refrigerator at 4°C. Each lamb leg muscles were cut into slices according to the number of guests tasters. The slices should be of uniform size and thickness. These were placed in aluminum trays covered with aluminum foil identified and put in an oven pre-heated to 240°C for 50 to 55 minutes. They were cooked with the fat cover may be present on the surface. The panel was held at 12 a.m. the attributes were immediately served on glass plates. A glass of about 100 ml of water was provided for each assessor between samples. All sessions were done in eight booth sensory panel room. Panelists have given a score the meat samples. Each attribute was scored on a scale of 10 cm for each characteristic: color, tenderness, flavor, juiciness, and odor. The attributes were ranged from the lowest intensity of each trait to the highest.

6. Statistical analysis

All statistical analyses were performed using SAS 9.1. Data were analysed using the GLM procedure to determine the significance of effects (breed, faba bean level) on pH, colour and instrumental texture and their interactions. In addition, the muscle effect difference was analysed. Duncan's test was used to measure differences among means. Differences were considered significant at the $p \leq 0.05$ level.

III – Results and discussion

1. pH

The effects of breed and faba beans level in concentrate on pH are summarized in Table 1. The pH values 24 h after slaughter ranged from 5.6 to 5.7 in *Longissimus dorsi* muscle, indicating that the animals were not stressed at the time of slaughter. Furthermore, the pH at 24 h ranged from 5.7 to 5.9 in *Semitendinosus* muscle, is higher than the pH 24h in LD muscle. The results are in accordance with those previously reported by Velasco *et al.*, 2004.This result is probably due to the nature of the muscle (skeletal muscle) and its glycolytic, oxidative and oxido-glycolytic fibers content (Hocquette *et al*., 1998) and/or its glycogen content (Bendall, 1962). In general, there were no differences in pH measurements among breeds or feed, although there were individual differences according to Duncan's test.

Breed did not significantly influence pH values in either LD or ST muscles. This effect has been widely studied. Compared with the Barbarine lambs, Black of Thibar lambs presented lower pH values at 24 h ($P<0.001$) in the m. ST. Most studies have reported no significant difference between breeds. Thus, Dransfield *et al.* (1979), using English breeds (Texel, Dorset Down, Suffolk, Oxford, Cotswold, and Southdown); found no difference in pH 24h. Sañudo *et al.* (1996) obtained the same results with Spanish breeds. However, Beriain *et al*. (2000) found differences in ultimate pH of about 0.2 at LD muscle between Aragonesa lambs and those of Lacha breed.

Faba beans level in concentrate did not significantly influence pH values in either LD or ST muscles. The ultimate pH in LD and ST muscle were similar. The pH in LD or ST muscle aged 5 days of Barbarine lambs are significantly different by feed group ($p<0.05$). The pH (LD) of FB1 Barbarine lambs was higher than those of SBM and FB2 groups. Besides, the pH (ST) of SBM Barbarine lambs was lower than FB1 and FB2 groups. Furthermore, other workers did not find any effect of concentrate in rate on pH in lambs (Carson *et al.*, 2001; Lanza *et al*., 2003), and

are thus consistent with the results of the present study. However, Solomon and Lynch (1988) showed that male lambs of 45 kg fed by different diets have pH values at 3 and 5 h post-mortem were higher but they became weaker after 48 h post-mortem in the case of lambs fed diets richer compared to those fed the diet poorest. After 1 h post-mortem, glycogen content was higher and lactic acid was the lowest for lambs richer fed diets compared to other. Furthermore, Díaz *et al.* (2002) reported non-significant differences in ultimate meat pH among feeding systems were also available.

Table1. The pH of *Longissimus dorsi* (LD) and *Semitendinous* (ST) muscles from lambs of different feed groups and different breeds

	Fat-tailed Barbarine			Black of Thibar			P		
	SBM	**FB1**	**FB2**	**SBM**	**FB1**	**FB2**	**Feed**	**Breed**	**Interaction**
Longissimus dorsi									
pH_i	6.43	6.35	6.35	6.23	6.30	6.30	ns	ns	ns
pH_{24h}	5.69^{d}	5.74^{d}	5.74^{d}	5.72	5.71	5.67^{d}	ns	ns	ns
pH(5 day aged)	5.53^{ac}	5.71^{bd}	5.52^{ad}	5.7	5.58	5.63	ns	ns	**
Semitendinous									
pH_i	6.59	6.49	6.55	6.44	6.45	6.52	ns	ns	ns
pH_{24h}	5.96^{c}	5.93^{c}	5.98^{c}	5.78	5.8	5.9^{c}	ns	***	*
pH(5 day aged)	5.65^{bc}	5.9^{ac}	5.91^{ac}	5.77	5.7	5.7	ns	ns	*

ns = Not significant differences; (*) p<0.05 ; (**) p< 0.01 ;(***) p< 0.001; a,b: Different superscripts represent significant differences among feeds (within breed) (p<0.05); c,d: Different superscripts represent significant differences among muscles (p<0.05).

2. Meat colour

Meat colour was influenced by both effects breed and faba beans level in concentrate, but the effect of breed was greater than that of feed group (Table 2). Only the lightness L_{24h} and yellowness b* (5 day aged) at LD muscle, also, L_{24h} and redness index (a* 24h) at ST muscle, didn't affected by breed. The redness index (a*24h) measured at LD muscle for Barbarine lambs was higher than Black of Thibar (16.58 *vs* 14.42). The LD muscle at 24h was more yellow in Barbarine lambs. After 5 days post-mortem, the LD for Black of Thibar was more lighter (54.80 *vs* 53.56). However, the ST muscle for Black of Thibar was more yellow than Barbarine lambs (8.39 *vs* 7.75) at 24 h after slaughter. In addition, after 5 days post-mortem, the ST muscle for Black of Thibar was lighter, redder and more yellow than those for Barbarine lambs (58.82, 22.97,12.65 and 52.04, 20.45, 9.22 for L, a* and b* parameters for Black of Thibar and Barbarine lambs, respectively). Martínez-Cerezo *et al*. (2005) did find an effect of breed on colour meat and are thus consistent with the results of the present study.

Feed groups affected significantly the L_{24h}, a^*_{24h} ($p<0.01$) and L (5 day aged) ($p<0.05$) at LD muscle. The LD for FB1 and FB2 groups had the highest redness index (a*) comparatively at those for SBM group. The L_{24h} for SBM group at LD muscle was lighter (55.24) than FB2 (53.48). After 5 days post-mortem, redness index at LD muscle decreased.

The ST muscle for SBM group was less light (54.17) and less red (18.62) than lambs received faba beans in concentrate. The yellow index b* was higher at ST muscle 5 day aged for SBM group (11.85). The incorporation of faba beans increased the redness and yellowness index of LD and ST muscle for Barbarine lambs. Lanza *et al.* (2003b) were reported that lightness (*L*), redness (*a**) and yellowness (*b**) values of LD muscle were identical between treatments, lambs received peas and those did not.

Table 2. The colour of *Longissimus dorsi* (LD) and *Semitendinous* (ST) muscles form lambs of different groups and different breeds

	Fat-tailed Barbarine			Black of Thibar			P		
	SBM	**FB1**	**FB2**	**SBM**	**FB1**	**FB2**	**Feed**	**Breed**	**Interaction**
Longissimus dorsi									
L 24h	54.95	54.54	52.82	55.53	54.44	54.13	*	ns	ns
a*24h	15.98	16.40	17.32	13.40	14.8	15.06	*	****	****
b*24h	9.20	9.83	9.42	5.96	6.80	6.92	ns	****	****
L (5 day aged)	54.58	53.87	52.28	54.97	53.55	53.88	**	**	***
a* (5 day aged)	13.81	14.14	14.93	14.99	14.13	15.05	ns	ns	ns
b* (5 day aged)	9.34	8.93	8.71	7.37	7.41	8.25	ns	****	****
Semitendinous									
L 24h	54.51	51.15	52.69	53.83	53.61	53.34	**	ns	****
a*24h	17.73	20.07	19.59	19.50	19.03	18.74	*	ns	****
b*24h	7.96	7.67	7.60	8.44	8.47	8.27	ns	**	*
L (5 day aged)	52.91	51.30	51.77	60.50	58.70	58.10	ns	****	****
a* (5 day aged)	20.15	20.90	20.40	23.69	22.25	22.96	ns	****	****
b* (5 day aged)	10.73	8.44	8.34	12.96	12.51	12.47	*	****	****

ns = Not significant differences; (*) p<0.05 ; (**) p< 0.01 ;(***) p< 0.001; (****)p<0.0001.

3. Meat tenderness

Mean values of meat shear force are presented in Table 3. The faba beans level did not affect the mean shear force levels at 24h and 5th day post mortem measured in *Longissimus dorsi* and *Semitendinous* muscles (p>0.05), only the shear force of ST at day 5 post-mortem(p<0.05). The lambs received 20% of faba beans in concentrate had the tenderest ST at day 5 post-mortem (37.47N) than those of SBM and FB1 groups (52.18, 41.50 for SBM and FB1group, respectively). Lanza *et al.* (2003b) reporting non-significant differences in shear force among feeding systems.

Table 3. Shear force of *Longissimus dorsi* (LD) and *Semitendinous* (ST) muscles form lambs of different groups and different breeds

	Fat-tailed Barbarine			Black of Thibar			P		
	SBM	**FB1**	**FB2**	**SBM**	**FB1**	**FB2**	**Feed**	**Breed**	**Interaction**
Longissimus dorsi									
Shear force 24h (N)	40.62	47.83	43.76	46.55	48.59	48.56	ns	ns	ns
Shear force 5 day aged (N)	37.92^{b}	38.04^{b}	40.44^{a}	53.33^{a}	54.86^{a}	43.72^{b}	ns	****	**
Semitendinous									
Shear force 24h (N)	59.06	41.08	43.43	51.80	51.03	63.01	ns	ns	**
Shear force 5 day aged (N)	51.46^{a}	29.73^{b}	29.48^{b}	52.95^{a}	42.64^{b}	56.53^{a}	*	**	***

ns = Not significant differences; (*) p<0.05 ; (**) p< 0.01 ;(***) p< 0.001; (****)p<0.0001. a, b: Within a column, means without a common superscript letter differ (p<0.05).

The breed affect the tenderness of LD muscle aged 5 day (p<0.0001) and shear force of ST muscle measured at day 5 post-mortem (p<0.001). Barbarine lambs had the lower shear force for LD muscle aged 5 day comparatively to Black of Thibar lambs (38.90 *vs* 49.55). Indeed, the

ST muscle from Black of Thibar had a higher shear force measured at day 5 post-mortem (49.55 vs 36.70). Martínez-Cerezo *et al.,* 2005, showed that breed had significant effects on instrumental texture. The effect of breed was greater than that of feed group. Therefore, shear force LD at 5th day post-mortem from Barbarine of SBM had the lower shear force than those of FB2 group (P<0.05). Furthermore, the LD from Black of Thibar of FB2 group was more tender than those of SBM and FB1 groups. Significant differences in shear force among production systems/feeding systems have been attributed to the variation of enzymes activities in muscle during maturation meat. At ST muscle aged 5 days, Barbarine lambs from FB1 and FB2 groups had the lower shear force. Indeed, Black of Thibar lambs whose received 10% of faba beans (FB1) had the tenderest ST muscle. All the results obtained by this present study demonstrating that the using of faba beans in concentrate tends to decrease the myofibrillar toughness.

4. Sensory characteristics

Results of sensory characteristics of meat in different feed groups and breeds are presented in Table 4. Except odour intensity score, feed group didn't have significant influence on sensory characteristics. Odour scores given to meat of FB2 lambs were significantly lower (p<0.05) than that of lambs of other feed groups.

Table 4. Meat sensory characteristics of leg form lambs of different groups and different breeds

	Fat-tailed Barbarine			Black of Thibar			p		
	SBM	FB1	FB2	SBM	FB1	FB2	Feed	Breed	Interaction
Colour intensity	5.59^{a}	6.70^{a}	6.40^{a}	5.58^{a}	4.36^{b}	6.25^{a}	ns	*	*
Tenderness	5.06^{b}	5.55^{b}	4.76^{b}	5.29^{b}	6.22^{a}	6.02^{b}	ns	*	ns
Juiciness	4.01^{b}	4.12^{b}	3.94^{b}	5.40^{a}	5.71^{a}	5.02^{b}	ns	**	*
Odour intensity	6.04^{b}	5.46^{b}	5.83^{b}	6.68^{a}	4.96^{b}	5.87^{b}	*	ns	ns
Flavour intensity	5.04	5.27	5.20	5.94	5.35	5.57	ns	ns	ns

ns = Not significant differences; (*) p<0.05 ; (**) p< 0.01 ;(***) p< 0.001; (****)p<0.0001. a, b:Within a column, means without a common superscript letter differ(p<0.05).

Breed affected significantly the meat colour, tenderness and juiciness scores (p<0.05). The black of Thibar had the higher tenderness and juiciness scores but the lower colour score. This result might be related to the carcass fatness or/and intramuscular fat content in meat of Black of Thibar lambs. Priolo *et al.* (2002) noted that influence of fatness level on meat tenderness could be related to a direct effect of the fat which is softer than lean and/or to an indirect effect of reduced muscle fibre shortening. In the current study, tenderness values were positively correlated with carcass fatness score (r=0.366; P<0.05). There were significant differences among breeds within feed groups. Except the Black of Thibar form FB1 group, the others had similar scores. Acceptance of meat by consumers is influenced by numerous criteria such as meat flavour, tenderness and juiciness (Sañudo *et al.*, 1998). Therefore, Panellists gave had a lower meat colour and odour scores to Black of Thibar from FB1 group. In addition, they had a higher tenderness and juiciness scores. These results were in accordance with reports of Yu *et al.* (2001), whose found that the scores of the overall acceptability of meat from lambs fed the narbon beans diets was comparable to that of the lambs fed the Lucerne diets.

IV – Conclusion

Breed had a significant effect on the physic-chemical characteristics and sensory quality of lamb meat and should be considered in programs to improve meat quality. Using faba beans in

concentrate also affected meat lamb quality. Using faba beans in concentrate increased the different parameters of meat colour. After the maturation meat, the meat from black of Thibar was the lighter, redder and more yellow. The Barbarine lambs from FB1 or FB2 groups had the lower shear force. However, the planelists preferred meat from black of Thibar received 10% of faba beans in concentrate.

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The effect of the incorporation of sorghum in the diet of fattening kids on performances, carcass characteristics and meat quality

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Abstract. The effect of including sorghum in the diet for fattening kids on growth performance, carcass characteristics and meat quality was studied. Three concentrate rations (Rm, R2 and R3) containing respectively 0%, 25% and 50% DM of sorghum grain completed with barley and faba beans, were distributed to 3 groups of 5 local kids for 3 months. The results showed that sorghum had no effect on ADG (47.9, 48.9, 44.5 g/day respectively for Rm, R2 and R3), while the intake and feed conversion showed a significant difference (12.35, 11.65, 13.20 kg DM/kg weight gain (P<0.001).This difference was positive with 25%DM of sorghum. As for carcass characteristics, sorghum had no effect on performance and weight of carcass, pluck, gastric pouch, perirenal and mesenteric fat. Bone tissue was not affected, as the length of the carcass and thigh did not differ significantly. No differences were observed with regards to the muscle tissue because thigh thickness, compactness, conformation and muscle index were similar. Sorghum did not affect carcass color indices. In terms of technological (pH and water retention) and organoleptic (color and tenderness) meat quality traits, no effect of sorghum inclusion was observed. Sorghum incorporation can reach 50% DM of kids concentrate without negatively affecting fattening performance, carcass characteristics and meat quality.

Keywords. Sorghum – Kids – Fattening performances – Carcass characteristics – Meat quality.

Effet de l'incorporation du sorgho dans la ration des chevreaux en engraissement sur les performances, les caractéristiques de la caracasse et la qualité de la viande

Résumé. *Pour déterminer l'effet de l'incorporation du sorgho grain dans la ration des chevreaux, les performances de croissance, les caractéristiques de la carcasse et la qualité de la viande ont été évaluées. Trois formules de concentré (Rm, R2 et R3) contenant 0%, 25% et 50% MS de sorgho grain associé à l'orge et la féverole, ont été distribuées à 3 lots de 5 chevreaux durant 3 mois. Les résultats montrent que le sorgho n'a pas d'effet sur le gain moyen quotidien (GMQ = 47,9, 48,9 et 44,5 g/jour respectivement pour Rm, R2 et R3. Cependant, la quantité ingérée et l'indice de conversion ont montré une différence significative (12,35, 11,65, 13,20 kg MS/kg gain de poids) (P<0,001). Quant aux caractéristiques de la carcasse, le sorgho n'affecte pas les performances de la carcasse. Les qualités technologique (acidité et taux de rétention d'eau) et organoleptique (couleur et tendreté) de la viande ne sont pas affectées par l'incorporation du sorgho. Le sorgho peut atteindre 50% MS du concentré des chevreaux sans affecter négativement les performances d'engraissement, les caractéristiques de la carcasse et la qualité de la viande.*

Mots-clés. *Sorgho – Chevreaux – Performances d'engraissement – Caractéristiques de la carcasse – Qualité de la viande.*

I – Introduction

In the north west of Morocco, goat is the dominant livestock species and its diet is based on the forest path which provides forage but it is subject to strong seasonality, conditions in which an adequate productivity of flocks is not guaranteed (Chentouf *et al.,* 2004). Thus, the improvement and diversification of food calendar appears to be necessary. Sorghum is adapted to the

northern region of Morocco where it expresses high levels of production and can be exploited in green or grain (Noutfia and Baya, 1997). High yields can contribute to satisfying the concentrates requirements of the goat herd. In this context, this work aims to analyze the effect of the incorporation of sorghum grain in the diet of kids on fattening performance and carcass characteristics and meat quality.

II – Material and methods

Fifteen kids from the local goat population aged 4 months were followed from weaning to 3 months until 7 months of age. The animals were assigned to 3 groups (Rm, R2 and R3) and received a food ration consisting of oat hay and 3 types of iso-energetic and iso-nitrogenous concentrate supplements. These supplements were composed of barley grain, faba bean, vitamin-mineral supplement and sorghum grain. The incorporation rate of sorghum was in the range of 0, 25 and 50% dry matter of concentrate respectively for groups Rm, R2 and R3. During the test, animals were weighed weekly to calculate average daily gain. After slaughter, measurement were conducted to evaluate the carcass, yield, color, importance of adipose, bone and muscle tissue. The color of the carcass was measured using a portable colorimeter (Chromameter Minolta CR410). To perform the analysis of meat quality, the samples were taken on the *Longissimus dorsi* (between 12th and 13th rib) and *Semimembranosus* muscle of the leg (on the back of the leg). On the *Longissimus dorsi*, the pH was measured using a pH meter HANNA HI99163 portable for this purpose. Tenderness was measured using a texture analyzer (Texture Analyzer – PRO-TMS) and color using the portable colorimeter. The *Semimembranosus* served to determine the water-holding capacity. The statistical analysis was performed by the Excel 2007 and SAS (2001).

III – Results and discussion

The incorporation of sorghum had no effect on final live weight and average daily gain. The ADG obtained was between 40.0 and 47.9 g/d. This gain is higher than that recorded by Sen *et al.* (2004) with goats in semi arid conditions. Contrary to the previous parameters, feed intake and feed conversion showed a significant difference. The best results were recorded in animals in the control group (Table 1).

Table 1. Effect of sorghum on growth performance of kids

	Final live weight (kg)	ADG (g/d)	feed intake (kg DM/day)	Feed conversion (kg DM/kg weight gain)
Rm	15.6	47.9	0.592[a]	12.3[c]
R2	15.3	43.5	0.570[c]	13.1[b]
R3	14.2	40.0	0.587[b]	14.7[a]

a, b, c: in a same line, followed by the letters distinguished values are statistically different than 5%.

The dietary treatment had no effect on carcass characteristics including yield and carcass weight. The carcass yield obtained ranged from 39.7 to 42.3%. Keli *et al. (*2008) obtained similar yields of about 40.8% and 41.4% respectively with animals fed flax and control. The absence of diet effect was also observed for the weight of pluck and gastric pouch full and empty (Table 2).

Table 2. Effect of sorghum on carcass weight, yield, pluck and gastric pouch

	Carcass weight (kg)	Carcass yield (%)	Pluck† (g)	Gastric pouch full (kg)	Gastric pouch empty (kg)
Rm	6.4	41.4	636.3	3.8	1.5
R2	6.2	42.3	614.3	3.7	1.3
R3	5.5	39.7	565.6	3.7	1.2

†Includes all liver, lung, heart, spleen and trachea.

After evaluation of adipose tissue, the diet did not affect significantly the weight of the mesenteric and perirenal fat (Table 3). Regarding the importance of bone tissue, there was no significant difference between batches because the carcass length and thigh were not affected by the sorghum incorporation (Table 3).

Table 3. Effect of sorghum on adipose and bone tissue

	Mesenteric fat (kg)	Perirenal fat (kg)	carcass length (cm)	thigh length (cm)
Rm	421.35	165.08	57.50	26.5
R2	289.28	174.14	55.20	25.8
R3	282.2	143.65	55.17	25

Regarding the importance of muscle tissue, no differences were found between treatments with regards to the thickness of the thigh, the index of compactness of muscle and conformation (Table 4).

Table 4. Effect of diet on muscle tissue

	Thickness thigh (cm)	Compactness Index	Index muscle	Index conformation
Rm	10.55	0.11	0.40	0.51
R2	11.28	0.11	0.44	0.55
R3	10.37	0.10	0.42	0.52

Also the incorporation of sorghum did not affect the color indices of carcass at saddle, tail and back (Table 5).

Table 5. Effect of diet on carcass color

	L* saddle	a* saddle	b* saddle	L* tail	a* tail	b* tail	L* back	a* back	b* back
Rm	52.62	14.76	9.40	51.82	12.38	4.60	60.30	7.54	4.67
R2	52.20	14.37	9.15	49.6	14.99	5.86	59.70	8.22	4.83
R3	50.64	14.64	10.13	49.57	15.28	5.75	55.80	9.92	5.90

L* Lightness, a * Redness , b *yellowness

In terms of parameters of technological meat quality, the incorporation of sorghum had no effect

on the pH measured at 0 and 24 hours post mortem and on the water-holding capacity. The values of pH at 24 hours were higher than those found by Werdi Pratiwi *et al.* (2007) reporting pH values of 5.7 and 5.6 respectively for goats from Australia of 10 kg and 70 kg live weight, and those reported by Sen *et al.* (2004) who obtained an average pH of 5.48. Our results are similar to those found by Ding *et al.* (2010) with pH ranging from 6.05 to 6.38 for goats and Boer cross Guanzhong Dairy. The water-holding capacity varied between 17.7% for the group receiving 50% of sorghum and 19.8% for those who received 20% (Table 6).

Table 6. Effect of diet on parameters of meat quality

	pH 0h	pH 24	Water holding capacity (%)	Tenderness (N)	*Longissimus dorsi* color		
					L*	a*	b*
Rm	6.95	6.05	19.7	102.05	52.62	14.76	9.40
R2	6.97	6.13	19.8	78.33	52.20	14.37	9.15
R3	6.80	6.12	17.7	87.6	50.64	14.64	10.13

Regarding the organoleptic quality, these parameters were not affected by diet. The tenderness ranged between 78 and 102 N (Newton). The values obtained for the groups receiving sorghum were similar to those found by El Otmani *et al*. (2011) reporting values ranging from 75.77 and 89.36 N for kids receiving lupin or a control diet. Color indices of *Longissimus dorsi* were not affected by diet. The yellow index obtained is similar to that obtained by El Otmani et al. (2011), while the red index is less and the luminance is higher than that found by the same authors (Table 6).

IV – Conclusions

Sorghum grain can be incorporated in the diet of kids at rates that can reach 50% of the concentrate ration without adversely affecting fattening performance, carcass characteristics and meat quality.

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Session 4
Options for mitigating / coping with climate change

Green house gas emissions from organic and conventional systems of food production, with and without bio-energy options

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Abstract. This study uses input and output data from 8 year rotations in the Nafferton Factorial Systems Comparison experiments to model Life Cycle Assessment (LCA) to the farm gate, estimating green house gas emissions (GHG) from a range of farming systems. Six scenarios were assessed that allowed the comparison of: (i) organic *vs* conventional production; (ii) stockless arable *vs* dairy (cow) production; and/or (iii) composting of straw based manure *vs* biogass production from slurry manure in a dairy (cow) unit. Systems were compared on the basis of; gross GHG, the potential off-set from energy generation using biogas (both as CO_2 equivalents/ha; tCO_2e/ha) and the yield of human food energy, throughout the 8 year rotation. Including a dairy enterprise under both organic and conventional management substantially increased gross GHG (3.2-3.3 and 4.3-4.4 t CO_2e/ha respectively) compared with stockless scenarios (0.6 and 2.0 t CO_2e/ha respectively) due to enteric methane output, yet biogas production contributed a relatively low counter to off-set these high emissions (0.4 -0.5 t CO_2e/ha for organic and conventional systems). In all comparison conventional systems generated more GHG than organic production (differences ranged from 0.9 to 1.4 t CO_2e/ha) largely due to greater reliance on manufactured inputs including feed and fertiliser along with higher N_2O emissions resulting from fertiliser use. Food energy output was also higher under conventional management with little difference between the 3 scenarios (44-47 GJ per Ha). On the other hand, the organic stockless system yielded substantially less food energy (22 GJ/ha) with the introduction of a dairy enterprise raising this to 31-32 GJ/ha, largely due to direct utilisation of forage crops grown in 3 out of 8 years during the rotation.

Keywords. GHG – Organic – Livestock – Bio-energy.

Emissions de gaz à effet de serre à partir des systèmes organique et conventionnel de production des aliments avec ou sans options bioénergétiques

Résumé. *Cette étude utilise des données d'entrée et de sortie issues de 8 années de rotations dans le cadre des essais de comparaison des systèmes factoriels Nafferton pour l'évaluation du modèle de cycle de vie (LCA) de la ferme en estimant les émissions de gaz à effet de serre (GES) à partir d'une gamme de systèmes d'élevage. Six scénarios ont été évalués et ont permis la comparaison de : (i) la production conventionnelle* vs *la production organique ; (ii) système de production sans animaux* vs *système de production laitière ; et/ou (iii) compostage du fumier à base de paille* vs *production de biogaz à partir du lisier dans une unité de vaches laitières. Les systèmes ont été comparés sur la base de : GES, potentiel de compensation à partir de la génération d'énergie utilisant le biogaz (sous forme d'équivalent CO2/ha : tCO2e/ha) et rendement de l'énergie des aliments consommés par l'homme pendant 8 années de rotation. L'intégration d'une entreprise laitière soumise à une gestion conventionnelle et organique a considérablement augmenté les GES (3,2-3,3 et 4,3-4,4 t CO_2e/ha respectivement) par comparaison aux scénarios sans animaux (0,6 et 2,0 t CO_2e/ha respectivement) à cause de l'élimination du méthane entérique, donc la production de biogaz a faiblement contribué à compenser ces importantes émissions (0,4 -0,5 t CO_2e/ha); pour les systèmes organique et conventionnel). Pour toutes les comparaisons, les systèmes conventionnels ont généré plus de GES que la production organique (différence variant de 0,09 à 1,4 t CO_2e/ha, cela est dû en grande partie à la grande dépendance vis-à-vis des intrants fabriqués tels que les aliments et les fertilisants parallèlement aux émissions plus élevées de N_2O résultant de l'utilisation de fertilisants. L'énergie alimentaire en output a été aussi plus élevée avec une gestion conventionnelle avec une légère différence entre les 3 scénarios (44-47 GJ per ha). D'autre part, le système organique sans animaux a produit sensiblement moins d'énergie alimentaire (22 GJ/ha) qui est passée à 31-32 GJ/ha avec l'introduction d'une entreprise laitière, résultant de l'utilisation directe des fourrages cultivés lors de 3 années sur 8 pendant la rotation.*

Mots-clés. *GES – Organique – Bétail – Bioénergie.*

I – Introduction

The initial step in reducing the environmental cost of food production is to quantify agriculture's contribution to green house gas (GHG) emissions and climate change. The next stage might be to compare farms or production systems to identify strengths and scope to reduce their impact without penalising output or quality. (Krammer *et al.*, 1999 and Dalgaard *et al.*, 2001). To this end, it is generally considered that producing bio-energy on farm (e.g. biogas digestion, biomass burning) can partially offset GHG emissions from food production (Boodoo *et al.*, 1977 and Fredrickson *et al.*, 2006).

This paper is an extract from a wider Life Cycle Assessment (LCA) comparing GHG emissions from organic and conventional production systems (Cooper *et al.*, 2011); it considers the role of livestock and how alternative manure management might affect emissions. Two baseline systems from the Nafferton Factorial Systems Comparison trial are compared with alternative models varying end-uses of agricultural by-products, considering on-farm and upstream emissions only.

II – Materials and methods

The Nafferton Factorial Systems Comparison (NFSC) trial was established in 2003 and compares organic and conventional systems of crop rotation (see Table 1), crop protection, and fertility management in a factorial design. GHG balances were compared for 2 of the scenarios along with 4 other simulated options (all listed in Table 2). Calculations are based on recorded input and output data from the trial (with a 10% reduction in yield for crops grown after straw incorporation in stockless systems) along with published default emission factors (IPCC, 2006).

Table 1. Crop rotations in the Nafferton Factorial Systems Comparison experiments

Crop rotation	Rotation year							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Conventional	Winter wheat	Winter wheat	Winter barley	Potatoes	Winter wheat	Winter barley	Grass/ clover	Grass/ clover
Organic	Winter wheat	Potatoes	Spring beans	Cabbages	Spring barley	Grass/ clover	Grass/ clover	Grass/ clover

Livestock numbers are based on feed energy produced relative to requirements for dairy production, assuming a 25% replacement rate for milking cows calving at 24 months in both systems. Milk yield was assumed at 8250 litres for conventional cows and 6750 litres under organic management (Anon 2008) with 65% and 80% respectively of feed energy supplied from forage (50,903 *vs* 56,205 MJ ME) (Butler *et al.*, 2008). Enteric methane production was estimate at 155 kg per cow per year in the conventional systems compared with 176 kg per cow per year for organic production with greater fermentation losses on the higher forage diet (Butler *et al.*, 2007). Additional nutrients, necessary for target milk production were supplied initially from homegrown feeds then balanced with purchased cereal and protein (based on feed composition and nutritional requirements in McDonald *et al.*, 2002), while excess cereals or beans were assumed to be sold off the farm.

Table 2. Agricultural systems used for baseline scenarios and alternative scenarios (baseline scenarios in bold)

Code	System
O+LS	**Organic crop rotation with organic management; forage crops fed to dairy cattle; straw for bedding; composted manure returned to the field; potatoes sold off the farm (stocked)**
O-LS	Organic crop rotation with organic management; straw and forage crops incorporated into the soil; cereals beans, potatoes and cabbages sold off the farm (stockless)
O+BG	Organic crop rotation with organic management; cereals, beans and forage crops fed to dairy cattle; straw returned to the soil; manure slurry used for biogas, then returned to the field; potatoes and cabbages sold off the farm (stocked)
C-LS	**Conventional crop rotation with conventional management; all crops (and straw) sold off the farm (stockless)**
C+LS	Conventional crop rotation with conventional management; forage crops fed to dairy cattle; straw for bedding; composted manure returned to the field; potatoes sold off the farm (stocked)
C+BG	Conventional crop rotation with conventional management; straw returned to the soil; manure slurry used for biogas, then returned to the field; potatoes sold off the farm (stocked)

On site calculations accounted for direct and indirect GHG arising from: burning of fossil fuel in farm activities (listed in Table 3), fuel extraction and transport, the energy costs of producing farm machinery and other inputs as well as IPCC default values for N_2O (from soil, fertiliser and manure) and CH_4 (from manure and enteric fermentation) (IPCC, 2006). As standard in LCA modelling, all emissions were converted to CO_2 equivalents (CO_2e) using factors of 23 for CH_4 and 310 for N_2O (Baggott *et al.*, 2007).

Calculations for GHG off-set by energy generation used published estimates of energy yield from anaerobic digestion of slurry as suggested by Balsam *et al.* (2006). The total human food energy produced over the 8 year rotation in each scenario was calculated using recorded crop yields, dairy outputs as stated earlier and food nutrition composition as published by USDA (2010).

III – Results and discussion

1. Gross emissions per hectare

Estimated gross GHG emissions per hectare for the 6 scenarios are shown in Fig. 1. Generally conventional production generated higher emission per hectare than the organic systems. Livestock systems generated considerably higher emissions than cropping only options largely due to the relatively high contribution of enteric methane from dairy cows and replacement heifers. There was little difference between both organic dairying scenarios (O+LS and O+BG); both are dominated by enteric fermentation contributing 75 to 76% of gross emissions, which in magnitude are 3 times the entire emissions from the stockless system (O-LS). Gross emission on an area basis are higher for conventional production largely explained by a combination of (a) greater dependence on manufactured inputs (including fertiliser and feed), accounting for 26-27% of total gross emissions on the livestock systems (C+LS, C+BG) and 36% on the stockless system (C-LS), in addition to (b) higher N_2O originating from mineral fertiliser application; representing 18-20% on the livestock systems and 34% of gross emission on the stockless conventional system. As with organic production, there are only slight differences in gross emissions between the 2 conventional dairy scenarios although in these cases, enteric methane contributes a lower proportion of total emissions (38%) because of higher use of manufactured inputs including fertiliser.

Table 3. Default figures used for calculating greenhouse gas emissions from field activities (direct emissions) for the Nafferton Factorial Systems Comparison experiments

Activity		Source
Emissions calculated on an area basis	kg CO_2e/ha	
Ploughing	131.6	Kramer *et al.* (1999)
Seeding	23.7	Kramer *et al.* (1999)
Rolling	23.7	Kramer *et al.* (1999)
Pesticide spray	41.2	Kramer *et al.* (1999)]
Weeding	23.7	Kramer *et al.* (1999)
Fertilizer spreading	36.2	Kramer *et al.* (1999)
Combining	91.2	Kramer *et al.* (1999)
Secondary tillage	43.1	Kramer *et al.* (1999)
Mowing	16	Dalgaard *et al.* (2001)
Ridging	19.2	
Potato harvest	54.4	
Potato planting	19.2	
Flailing	16	
Emissions calculated on a weight basis	Total CO_2e/tonne	
Baling	6.4	Dalgaard *et al.* (2001)
Compost application	1.92	Dalgaard *et al.* (2001)

Emissions for baling straw and silage were based on a figure of 2 litre diesel fuel per tonne of biomass; diesel fuel use was converted to CO_2 emissions using a factor of 3.2 kg CO_2 emitted per litre of diesel fuel burned; ridging and potato planting were assumed to be equivalent in energy use to heavy seedbed harrowing; emissions for rolling were assumed to be equivalent to sowing; potato harvesting was assumed to be equivalent to sugar beet harvest; flailing was assumed to be equivalent to mowing; compost application figures were based on the values for loading and spreading manure from Dalgaard *et al.* (2001)

Although nitrogen fertiliser manufacture is considered energy intensive and its application to crops results in significant emissions of nitrous oxide, these calculations show it does not contribute a high proportion of emissions in the wider picture, especially in options with livestock where emissions are dominated by methane. For example, in the stockless conventional scenario (C-LS), emissions from the manufacture of off-farm inputs include N fertiliser only accounted for 14% of the total. Hillier *et al.* (2009) found N application rates, whether from inorganic or farmyard manure (FYM) sources, explained 95% of the variation in the carbon footprints of different farm types in Scotland. If we exclude enteric methane emissions from our calculations, the average annual on-site emissions for our O-LS baseline scenario are 841 kg CO_2e ha^{-1}, which is similar to the carbon footprints of the farms in the Hillier *et al.* study (728 kg CO_2e ha^{-1}). Estimates of on-farm emissions for a comparable stockless conventional system in our analysis were 2019 kg CO_2e ha^{-1} (for C-LS) compared with 1541 kg CO_2e ha^{-1} for the conventional farm types in the Scottish study.

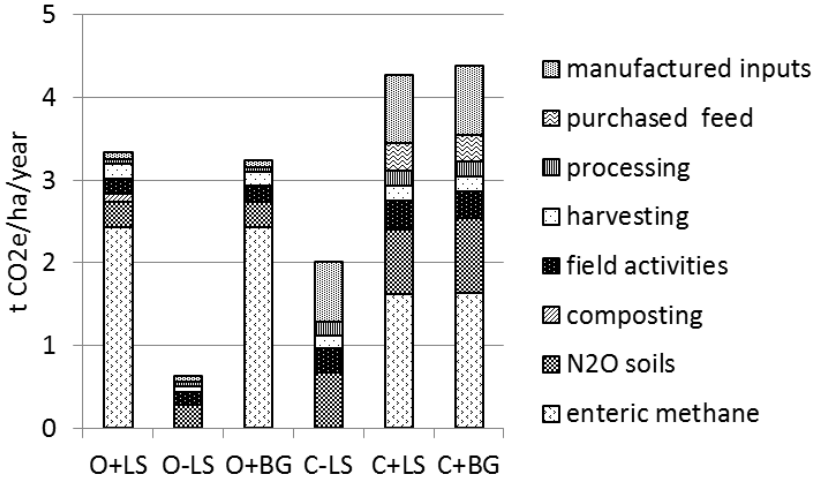

Fig. 1. Breakdown of gross Greenhouse Gas emissions from 6 scenarios detailed in Table 2.

2. Net greenhouse gas emissions including on-farm bio-energy production

In the two bio-energy scenarios (O+BG and C+BG) emissions of GHG can be offset if the energy produced on-farm displaces electricity from coal; assumed to be 410 kg CO_2e ha^{-1} in the O+BG scenario and 451 kg CO_2e ha^{-1} in the C+BG scenario in this study. The inclusion of biogas digesters on farms with ruminants is one strategy to compensate for the high emissions of methane from the rumen. However, the modest off-set of only 12% of total emissions in the O+BG scenario and 6% in the C+BG scenario are swamped by the relatively high contribution of enteric methane; 75-76% of the total emissions from the stocked organic systems and 38% from the stocked conventional systems (Fig. 2). Therefore, it is apparent that a multi-faceted approach to addressing methane emissions from ruminant systems needs to be implemented and we cannot rely solely on energy generation to reduce the overall impact. This could include a reduction in the fibre content of dairy diets, the inclusion of vegetable oil to suppress rumen protozoan activity or longer term benefits from selective breeding (of livestock, forage plants and/or rumen microbes). Effective methane output per unit of food (be it milk or meat) can also be reduced if progress is made to improve longevity and/or productivity, spreading the methane generated during the rearing phase over greater output. This whole area is complex and in its infancy; it is interesting to note that current modelling (IPPC, tier 2) lacks the sensitivity to acknowledge any progress due to such action (IPCC, 2006 and IPCC, 2007).

The offsetting of GHG emissions by soil carbon (C) sequestration is not included in this analysis but on average, organic land has higher soil C levels than conventionally farmed land. Reganold *et al.* (1987) reported that a side-by-side comparison of an organically and conventionally managed wheat field showed higher soil C levels in the organic field. This has been followed by more comprehensive paired comparisons of organic and conventional farms. In 1992 Armstrong Brown *et al.* surveyed 30 pairs of organic and conventional farms in the UK and reported a trend towards higher soil organic matter (SOM) for organic horticultural and arable farms compared with their conventional equivalents. They attributed the differences to the greater use of farm year manure, reduced tillage intensity, and more periods under temporary ley or permanent pasture.

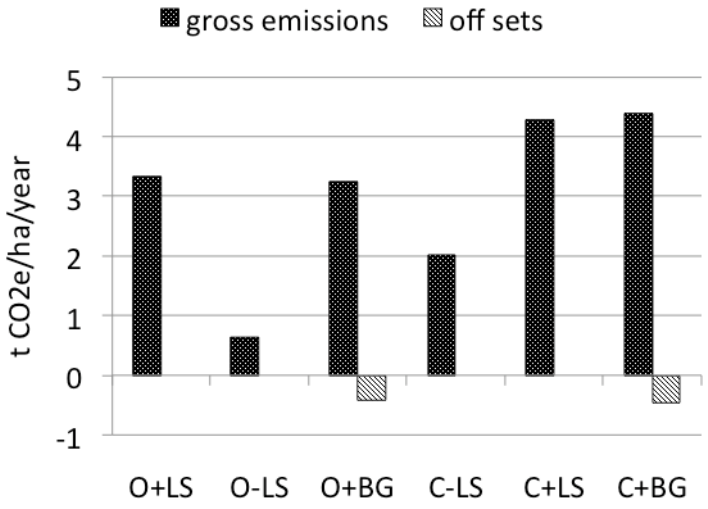

Fig. 2. Gross emissions and off set GHG from anaerobic digestions of slurry for 6 senarios detailed in Table 2.

Including soil C in GHG inventories remains a contentious issue. Changes in soil C occur gradually, and eventually reach an equilibrium value related to annual inputs of soil C balanced against losses. For some scenarios in this study, composted manure was returned to the soil (e.g. O+LS and C+LS), while in the biogas cases, C is lost from the system through conversion to CH_4 and burning of the gas for energy. The remaining C, however, may be relatively resistant to decomposition since it is stabilised by the digestion process (Moller *et al.*, 2009). Therefore it is likely that different quantities and qualities of carbon would be returned to the land in each scenario, and that this would result in variations in equilibrium soil C among the scenarios. To include changes in soil C in our GHG balance we would have needed to estimate differences in equilibrium soil C between the baseline systems and the alternative scenarios and then calculated annual losses or gains of soil C for each scenario relative to the baseline (e.g. O+LS and C-LS). The rates of gain or loss of soil C would be expected to diminish each year, so we would also have needed to arbitrarily choose a specific year since conversion from the baseline to the alternative scenario. The estimation of these values was beyond the scope of this study, and would ideally involve the use of a recognised soil C model such as CENTURY (Paustian *et al.*, 1992) or ROTH-C (Jenkinson 1990). This type of analysis is planned for future LCAs of the NFSC trial data.

3. Gross emissions per MJ human energy

Figure 3 shows average calculated yields for human food energy from each of the farming systems over the 8 years for each rotation. Actual crop yields from the Nafferton Factorial Systems Comparison experiments were used as the baseline for calculations and, typically yields were lower under organic management. However, since records used were taken in the early years after organic conversion, these represent a system in transition to organic status when it is common to experience reduced yield as the soil adjusts to a fully biological system of production (Huxham *et al.*, 2005). Entz *et al.*, (2005) reported grain yields 23-27% lower on a survey of organic farms compared to conventional; however, they also reported maximum yields on organic farms that were greater than the long-term averages for conventional farms, indicating that there is potential in organic systems to improve yields. In the Nafferton

experiments, N is supplied either from the legumes in the rotation (grass/clover or beans) or applied compost. Since 95-100% of the N in the compost was in an organic form (for composts used between 2004 and 2007), it may not be readily available to the growing crop during the year of application and plant growth could have been N-limited. As reserves of soil organic N build, subsequent mineralisation will increasingly contribute to crop growth and long-term inputs of organic matter can alter the composition and activity of the soil microbial community relative to conventional management (Widmer *et al.*, 2006 and Fließbach *et al.*, 2007). This can lead to a soil microbial community that is more adapted to cycling of N in organic systems i.e. with enhanced biological N fixing capacity and more efficient pathways for mineralization of nutrients from organic matter.

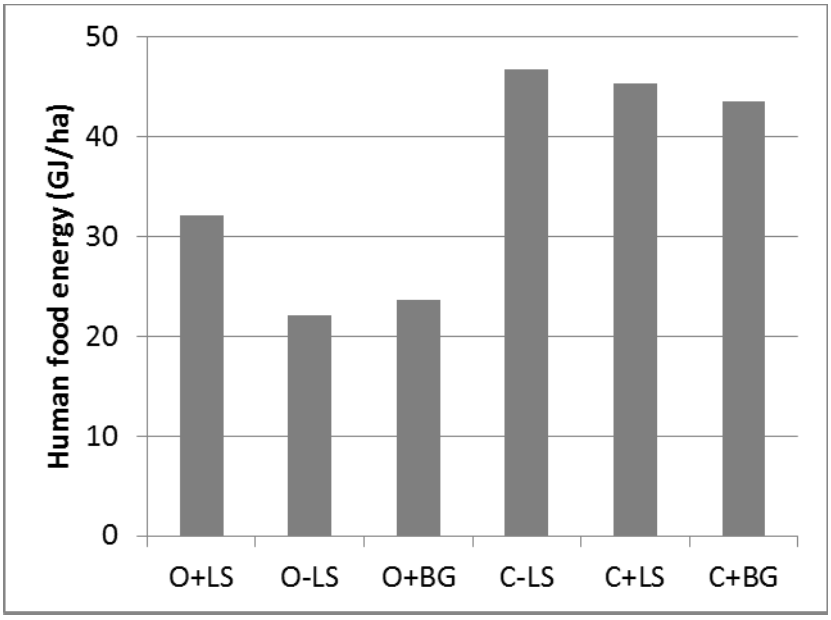

Fig. 3. Human food energy produced (GJ) per hectare of land over 8 years in each of the scenarios detailed in Table 2.

Food energy output ranged from an average of approximately 47 GJ ha^{-1} over the 8-year rotation for the conventional dairy system (C+LS) to 22.4 GJ ha^{-1} for the stockless organic scenario (O-LS) (Fig. 3). In the organic scenarios, the best way to maximise food production was to include livestock (in this case dairy cattle), which utilise the productive grass/clover swards that dominate the crop rotation, and convert forage plants into human food. Food yields for the stockless scenario under organic management (O-LS) are low since food crops/energy are only produced in five out of the eight years of the rotation, with the grass/clover grown in the remaining years left *in situ* as mulch. In the stockless system under conventional management (C-LS) this practice is unnecessary as mineral fertiliser can replenish nutrient balances and forage crops can be exported from the system, hence the higher output of food energy relative to the organic system.

4. Optimising food production and minimising GHG emissions

The ideal food production system will maximise food production, while minimising environmental damage. If we plot the net GHG emissions versus the food energy produced in each of the

scenarios (Fig. 4) we can see that both stocked conventional systems (C+LS and C+BG) and the stockless conventional (C-LS) system, in which all crops are exported off the farm for livestock feed, produce the largest amounts of energy per hectare, however this food production comes at the cost of high GHG emissions. All of the organic systems produce less food, but they also are all relatively low in emissions.

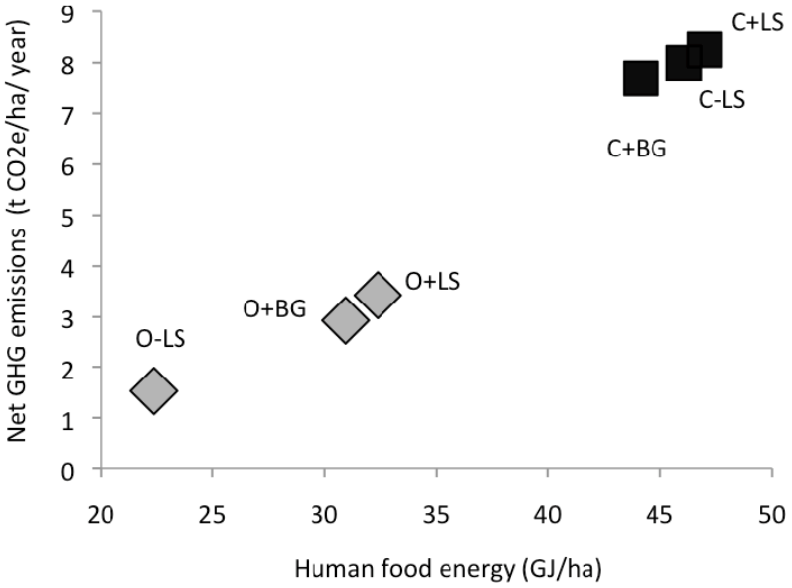

Fig. 4. Relationship between net GHG emissions and food energy production for 6 production scenarios detailed in Table 2.

This simple analysis highlights some key aspects of life cycle analysis that need to be considered if the results are to be meaningful. In this analysis a full 8 year crop production cycle has been studied. In the real world most farms follow a crop rotation and while some phases may have relatively low emissions of GHG (the ley phase of the O+LS scenario in this analysis emitted less than 100 kg CO_2e ha^{-1} y^{-1}), other phases may emit considerably more (e.g. the cabbage phase of the O+LS scenario emitted ~1400 kg CO_2e ha^{-1} y^{-1} on-site). Therefore it is necessary to consider a full cycle of the crop rotation when comparing different farming systems using LCA.

Likewise, it is important to look at the full impact of GHG emissions beyond the farm gate, to effectively compare systems. Although not fully explored in this small study, the conventional systems exported most of their emissions beyond the farm gate, although it will depend on the ultimate use of the forage and food crops sold from the farm. If subsequently fed in pig or poultry production (or forages for ruminant systems), down-stream emissions would be considerably higher than direct human consumption. These externalised environmental costs are not accounted for in this LCA that stops at the farm gate. This was particularly evident when the emissions associated with pig farming (the ultimate consumers of crops produced on many arable farms in the UK) were also included in the balance (Cooper *et al.*, 2011). It is also inconsistent to use a system boundary of the farm gate for downstream emissions, when most LCAs account for emissions from inputs well before they reach the farm (e.g. the upstream emissions associated with manufacture of farm inputs).

IV – Conclusions

Food production remains the primary goal of farming in Europe and we are facing increasing challenges globally to feed the expanding world population. Therefore any LCA of farming systems needs to place this analysis in the context of food production. The scenarios in this study demonstrate trade-offs that often exist between food production and environmental sustainability. The systems with the lowest emissions (O-LS) also produced the lowest food energy per hectare; while highest emissions were associated with the most productive systems. But the fact remains that the highly productive, conventional systems in these scenarios are dependent on imported nutrients for production. Nitrogen fertiliser is produced using energy from a non-renewable resource (fossil fuels) and P fertilizer is mined from soil reserves whose supply is finite. It has been estimated that known P-deposits may be depleted within 50 years (Fantel *et al.*, 1985). These high levels of production may therefore not be sustainable in the long-term.

The bio-energy scenarios show innovations can be adopted on farms to at least partially offset emissions from farming practices. In addition, on-farm bio-energy systems may generate additional farm income, making their implementation a “win-win” situation although the relatively low off-set suggests other measures are also necessary to reduce the environmental impact of livestock production.

Further studies are needed to clarify the uncertainties associated with LCAs of farming systems especially to monitor and reduce methane emissions from ruminants. Overall, improvements in N use efficiency at the farm level will reduce emissions from food production systems, and at the same time minimise environmental damage.

Acknowledgements

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Local feed resources for poultry

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Abstract. In poultry 'no input', 'low input' and commercial production can be distinguished. 'No input' implies scavenging poultry with some kitchen waste or crop residues as supplemental feed. Input is negligible and economic efficiency is high, provided there is any output. Commercial production is capital intensive and completely based on supplied feed. Birds might be given outside access for foraging, but this is for behavioural and welfare reasons, not for nutrition. Chickens are real omnivores. The feed industry utilizes all kinds of ingredients and by-products for least cost rations. Literature provides a tremendous amount of information on feeding value of a wide variety of feed ingredients. Low input systems are a difficult category for economic evaluation. Birds often have to get part of their diet from scavenging, but also receive on a regular basis (compound) feed. This can be home-made from local resources or industrial and thus out-of-pocket costs. Purchase of feed is only possible if sufficient income can be generated from sales of eggs or birds. Lack of market access (buying resources and selling products) and competition from industrially produced eggs and meat are more a barrier than knowledge on feed resources. With regard to management no input and low input systems have a tendency to 'over-graze' the resources for scavengers, with high mortality and low productivity as a consequence. Reducing numbers of birds might increase productivity.

Keywords. Poultry – Commercial production – Feed resources – Low input systems.

Ressources locales destinées aux volailles

Résumé. *Dans le secteur avicole on peut distinguer différents types de production : production sans intrants (no input), production à faible niveau d'intrants (low input) et production commerciale. La production sans intrants 'No input' est basée sur l'utilisation exclusive des déchets ménagers ou des résidus de récolte comme compléments. Les coûts sont négligeables et l'efficacité économique peut être élevée s'il y existe une production finale. La production commerciale est intensive et complètement basée sur l'apport des aliments de l'extérieur. Les volailles pourraient bénéficier d'accès à l'extérieur, mais c'est pour des raisons de bien-être, pas pour la nutrition. Les poulets sont des omnivores. Les fabricants d'aliments pour bétail utilisent différents ingrédients pour formuler des aliments au moindre coût. La littérature fournit beaucoup de données relatives à la valeur alimentaire d'une large gamme d'aliments. L'évaluation économique des systèmes à faibles intrants est considérée difficile. Les oiseaux sont souvent astreints à se nourrir sur les résidus mais reçoivent aussi et de façon régulière des aliments composés qui pourraient être fabriqués au niveau de la ferme à partir de ressources alimentaires locales ou à partir de ressources industrielles achetées. L'achat des aliments n'est possible que lorsqu'un revenu est généré à partir de la vente des œufs et des oiseaux. Le manque d'accès au marché (achat des ressources et vente des produits) et la concurrence avec la production d'œufs et de viande à l'échelle industrielle représentent une barrière plus importante que la maîtrise des ressources alimentaires. Quant à la gestion, les systèmes « sans intrants » et « à faibles intrants » ont tendance à surexploiter les ressources alimentaires issues de résidus, ce qui se traduit par une mortalité élevée et une faible productivité. La réduction du nombre de volailles pourrait améliorer la productivité de ces animaux.*

Mots-clés. *Volaille – Production commerciale – Ressources – Systèmes à faibles intrants.*

I – Introduction

Poultry is present in almost all systems in all parts of the world (Pym and Alders, 2012). The demand for animal products is increasing faster than the overall demand for food and among animal products the demand for poultry meat and eggs is expanding most prominent. (FAO, 2009). Among the poor, many families are for their livelihood dependent on livestock and

especially on poultry. FAO (2012) distinguishes industrial and integrated poultry production, large scale and small scale commercial production and village or backyard production. Non-industrial poultry production can be categorized as (modified after FAO, 2012):

(i) 'no input', the birds forage themselves, while at best some shelter for the night and some kitchen waste or crop residues are provided: free range extensive and backyard extensive systems.

(ii) 'low input', foraging is important, but the birds receive supplemental feed: semi-scavenging system.

(iii) 'high input', the birds receive a complete diet; access to a foraging area might be provided, but rather for welfare reasons than for nutrition: intensive systems (Singh *et al.*, 2011; Rushton, 2010).

McLeod *et al.* (2008) characterised no and low input systems as 'safety net' and the small scale high input systems as 'asset builder'. The so-called family poultry production systems are always small scale with up to a few hundred birds. Often they are multipurpose: providing both eggs and meat on top of a next generation, with a tendency to specialize more if input increases. Industrial poultry production is always specialized for either meat of eggs (or reproduction) and is based on high input systems. It involves much larger numbers of birds, from several thousands to up to several millions on a location. No and low-input poultry production systems are often based on indigenous, traditional breeds of chickens and on local feed resources. High input family systems still might rely on indigenous breeds and local resources, but develop into systems with commercial hybrid stock (Thieme and Besbes, 2010; Rushton, 2010) and compound feed based on ingredients, that might originate from larger distances. The small scale family farms operate in a local and informal supply chain, while large scale, industrial enterprises tend to be organised in formal, well regulated supply chains (McLeod and Sutherland (2012).

II – Local resources

Scavenging is the ultimate form of production based on local resources. This type of production requires no input in the form of capital and/or imported genotypes or feed ingredients. Goromela *et al.* (2006) reviewed the information on the diet scavenging birds collect. Scavenging diets contain about 10% protein, 1.17% Ca and 0.5% P and have an energy content of 11.2 MJ/kg. The amount of waste per day per household that can be fed to poultry is estimated at 300-600 g on a dry matter basis, with protein and energy contents comparable to the crop content. This is below recommendations for commercially producing poultry (NRC 1994). Goromela *et al*. (2007) indicated that it is also below the genetic potential of indigenous genotypes. For production according to the genetic potential supplementation is required (Goromela *et al.*, 2007). Starvation is a major cause of mortality (Olukosi and Adeola, 2010). Productivity of local poultry could be improved by supplying extra feed (Chowdhury and Luseba, 2012). The question is how this can be done in a profitable way.

During an e-conference on family poultry production, organised by FAO and the International Network on Family Poultry Development (INFDP) (Chowdhury and Luseba, 2012) local feed resources and interaction with other production systems were explored. Some general conclusions from this e-conference are that it is expected that the amount of scavengeable feed will reduce due to urbanisation and climate change and that there is a lack of knowledge on how to use commercial feed in a scavenging system. Many contributors indicate that commercial feed might be too concentrated for indigenous poultry and that it is in general too expensive for profitable production with indigenous breeds. A more or less successful strategy is to feed young birds for a limited period on commercial feed and later on dilute commercial compound feed with local available grains or grain products (Chowdhury and Luseba, 2012; Sonaya and Swan, 2004). The overall tendency among the comments is that for higher productivity

commercial feed is required. However, commercial poultry has a much higher inherent production potential than indigenous birds and consequently 'standard' commercial feeds contain too much protein, resulting in poor performance and waste of protein, especially in laying stock. Poultry has an inherent capacity to select adequate diets themselves. Faruk (2010) found good results with choice feeding or roughly mixing a premix of essential amino acids, vitamins and minerals with a locally available energy source (fi millet).

In poultry production feed costs are about 70% of the total production costs. Goromela (2009) did a cost benefit analysis for supplying extra feed and for management strategies of laying hens. He calculated that supplemental feed gave a net profit, but management of laying hens in such a way that they are not involved in hatching and rearing chicks was much more profitable. Mutayoba *et al.* (2010) estimated in Tanzania the revenues of supplementation of scavenging birds. With a homemade complete diet the performance of a commercial diet was approached and both gave about 25% more output than scavenging only, but the extra costs for the feed supplied were not outweighed by the revenues from extra production, Sonaya and Swan (2004) indicate that if balanced feed, good health-care and day-old chicks of hybrid varieties are locally available, intensive poultry management is an option. If these are not available, then local breeds under scavenger free-range systems is still the best choice.

A limited number of participants in the e-conference emphasised the role of poultry in integrated production systems to control weeds and insects. Then not only meat and eggs are produced, but also 'labour'. Knowledge on how to handle such integrated systems is limited. The same is true for the value of poultry manure, which can be considered as an important local resource for crop production.

From the discussions it became clear that there is a lack of knowledge on how much can be produced on local available resources. If only local resources are available, managing the number of chicks being hatched in order to increase survival rate and the number of eggs available for consumption might be a more profitable strategy than increasing input. The general tendency is however to find ways to shift to commercial compound feed and hence a shift from no or low input to high input systems.

There is a wide body of knowledge on feeding value of possible ingredients available at the compound feed industry. These data become publicly available through internet and internet. Among others the Working Group Nutrition of the WPSA (1989) published feeding values for a large number of ingredients on-line. Less is known on nutrient requirements of indigenous strains. For growing birds the NRC tables for growing White Leghorns are a reasonable approach (NRC, 1994). The NRC also gives indications for laying hens in relation to adult body weight and egg production that can be used to estimate the energy requirements of indigenous birds. Sonaiya and Swan (2004) summarize information on available feed ingredients. Combined with the calculation programmes it is possible to compose a diet from available ingredients (Thomson and Nolan, 2001). Based on this information and programmes and with some training in farmer field schools it should be possible to produce locally compound feed.

III – Transition from no or low input to high input systems is critical

Due to an increasing demand for animal products and to increasing urbanisation there is a pronounced tendency to shift from poultry keeping for subsistence to commercial poultry keeping. A gradual growth to commercial production is a difficult transition (Rushton, 2010). Commercial poultry production is going into a value chain with many different interests (Thieme and Besbes, 2010). Besides technical performance and economics, also ethics, animal health, and environmental concerns enter the overall picture.

Commercial production can still be based on indigenous (traditional) breeds. However, with indigenous breeds the production potential is too low to afford complete commercial feed (Olukosi and Adeola, 2010). With free range/scavenging and some supplemental feed it is possible to have a positive margin between costs and value of eggs and meat, provided labour is not taken into account. If a choice is made to go for higher production levels decisions have to be made on how much capital is available and how logistics can be organised. Thieme and Besbes (2010) and Hamon (2010) indicated that for higher production levels besides commercial feed also a shift from indigenous to commercial genotypes might be required. A shift to high input, intensive systems implies investments in purchased feed and purchased genotypes. Because of the value of these investments, also investments in housing and veterinary care (vaccination and medication) become necessary, which make the production system capital intensive. For commercial production many different types of resources (birds, feed, veterinary care, capital, labour, logistics and market access) have to be available locally. Feed might be the easiest one. Simulation studies might help to get indications which conditions are required to make a shift from low or no input to high input systems profitable.

Rushton (2010), however, indicates that economic models for no and low input family poultry production are extremely complex, because of the variety of inputs and often lack of data on output, even more so as most no and low input poultry production is multipurpose: eggs and meat plus delivering the next generation. Systems with commercial genotypes are in general single purpose, either eggs or meat and do not breed the next generation on the production farm. Due to the complex logistics (buying feed, laying hens or broiler chicks, veterinary care, get products to the market and find a market for the products) and introduction of formal quality systems it is difficult for small scale family farms to compete with the industrial farms (Pym, 2010; McLeod and Sutherland, 2012). Industrial poultry production is often highly integrated and controlled by one party, while family farms are only one link in the production chain.

Labour is an important factor in poultry production. With family poultry often women, children, or older generations take care of the chickens. If the time they spend with the chickens does not compete with other productive tasks, the income from poultry is an extra. If it does compete the value of the time spend in other tasks determines the costs of labour in poultry production. Industrial poultry farms by definition work with hired labour.

IV – Options for production based on local resources

Transition from no or low input poultry production to commercial production should be considered as a complete change of system; a change that is in fact a giant leap. For family farmers shifting to commercial production market access both for inputs and for outputs is essential. If the logistics are lacking to get birds, feed and products at the right time at the right place a transition to commercial production does not make sense. Not only the feed has to be available locally, but also all the other types of resources, capital and labour included. No and low input poultry production is in such a situation still a profitable enterprise, which can be optimised by managing the number of chicks hatched and by supplying extra feed. Selecting indigenous stock for higher productivity is theoretically possible, but approaching the genetic potential of current commercial stock is virtually impossible. Gradually shifting to commercial production is therefore not an option.

Opting for a niche market might be interesting. In several countries eggs and meat from indigenous birds can be marketed for a premium price compared to eggs and meat from commercial production. In a mixed crop livestock production system, where the chickens feed on insects, weeds, crop residues and kitchen waste and produce manure for crop production, poultry is very valuable.

In conclusion no and low input poultry systems can be optimised to generate more profit by optimising management of the birds, in principle manage reproduction: use more eggs for

consumption and less for reproduction, raise less chicks with some supplemental feed and thus much lower mortality (Sonaya and Swan, 2004; Singh *et al.,* 2011; Chowdury and Luseba, 2012). Due to the inherent capacity of chickens to select their own diet, also egg production can be increased with local resources without very detailed knowledge on those resources as feed ingredient. Low input production systems are essentially very little affected by changes in other production sectors and thus food and feed prices. With no and low input systems niche markets with premium prices might be accessible. Such a strategy might be much more profitable than trying to compete with industrial productions systems.

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Genetic aspects of heat stress in pigs expressed in fertility traits

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Abstract. The number of pig breeding programs has reduced in recent years. This leads to a small number of pig breeding companies producing pigs for many different environments. Therefore, pig breeding programs have to breed pigs capable of facing heat stress challenges during their productive life. Management practices such as cooling offer one option to reduce heat stress and warrant performance during hot seasons. A more sustainable alternative is to breed sows for improved heat tolerance. When evaluating breeding goals for dam lines we were confronted with different appreciation of two dam lines by pig producers in the Netherlands (temperate climate) and in Spain (warmer climate).These two dam lines, differed in the relationship between ambient temperature and reproductive performance. One of the dam lines showed no influence of temperature on performance, the other showed a decrease of 0.1 piglet per 1°C increase in ambient temperature. In a subsequent study, estimates of heritability reinforced the idea that genetic selection for sow heat stress tolerance is possible. Genetic correlations between reproductive performance in a temperate climate and reproductive performance in a hot climate tend to be unfavourable. In other words, improving reproduction traits without taking heat tolerance into account will lead to animals which have higher performance under temperate conditions, but which are also more sensitive to heat stress.

Keywords. Heat stress – Fertility – Pigs – Genetics.

Aspects génétiques du stress thermique chez les porcs exprimés par des paramètres de fertilité

Résumé. *Ces dernières années, le nombre de programmes de sélection porcine a baissé. Ainsi, le nombre de grandes organisations de Sélection Porcine produisant des animaux pour de nombreux environnements a chuté. En conséquence, les programmes de sélection doivent produire des porcs capables de faire face aux problèmes liés au stress thermique au cours de leur carrière de production. Les pratiques de gestion d'élevage telles que le refroidissement sont un moyen de réduire le stress thermique et de garantir des performances de production pendant la saison chaude. L'alternative la plus durable consiste à sélectionner des truies ayant une meilleure tolérance à la chaleur. Lors de l'évaluation des objectifs de sélection pour les lignées femelles, nous étions confrontés à différentes appréciations de deux lignées femelles par des producteurs de porcs aux Pays-Bas (climat tempéré) et en Espagne (climat plus chaud). Ces deux lignées femelles présentaient des différences au niveau de la relation entre la température ambiante et les performances reproductives. Chez l'une des lignées, la température n'avait aucune influence sur les performances alors que l'autre présentait une baisse de 0,1 porcelet par augmentation de 1°C de la température ambiante. Dans une étude suivante, les estimations d'héritabilité ont renforcé l'idée que la sélection génétique pour la tolérance au stress thermique des truies est possible. Les corrélations génétiques entre les performances reproductives dans un climat tempéré et les performances reproductives dans un climat chaud ont tendance à être défavorables. En d'autres termes, l'amélioration des caractéristiques reproductives sans tenir compte de la tolérance à la chaleur entraînera la production d'animaux ayant de meilleures performances dans des conditions tempérées mais qui seront plus sensibles au stress thermique.*

Mots-clés. *Stress thermique – Fertilité – Porcs – Génétique.*

I – Introduction

Genetic potential of pigs has increased during the past 50 years as a result of well-organized pig breeding programs. This improved genetic potential has led to improvement of economically important traits such as daily gain, back fat thickness, feed efficiency, and litter size (Merks, 2000; Brown-Brandl *et al.*, 2004). Implementation of crossbreeding, specialised sire and dam line breeding, BLUP genetic evaluation programs and well-defined selection indices have been responsible for a remarkable reduction of backfat (-75%), improvement in growth rate (+100%), and since the 1990s larger litter sizes (Merks *et al.*, 2012). Since the 21st century, breeding goals within modern pig breeding programs have shifted more and more towards improving product quality as well as to traits related to animal welfare such as piglet survival, sow longevity, and disease resistance (Kanis *et al.*, 2005). During recent years, the number of pig breeding programs has been dramatically reduced and it is expected that in 2020 only a couple of them will be left over. This leads to a small number of pig breeding companies producing pigs for many different environments (Gibbs et al, 2009;Dekkers *et al.*, 2011; Merks *et al.*, 2012).

In major pig breeding organisations, selection takes place in nucleus farms in mainly temperate climates and under high management standards (Knap, 2005). In general, selection on production under improved environmental conditions leads to increased environmental sensitivity (Van der Waaij, 2004). It is anticipated that differences between environmental conditions at nucleus farms and commercial farms will become even larger in the coming years. Meat production is expected to double from 229 million tones in 1999 to 465 million tones in 2050, the growth predicted to be the fastest in regions with hotter climates such as Latin America and South and East Asia (FAO, 2006). Furthermore, the temperature is expected to increase worldwide as a result of climate change (Hofmann, 2010). Therefore, pig breeding programs have to breed pigs capable of facing heat stress challenges during their productive life.

The economic losses from heat stress in the United States in the swine industry in 2003 were estimated to be $299 million and was mainly a result from an increased number of days open for sows and reduced growth for finishers (St-Pierre *et al.*, 2003). Pig management is to some extend a way to deal with high temperatures/ heat stress and can be done via implementation of cooling systems in pig farms and to adapt nutrition (Quiniou *et al.*, 2001). Another way to deal with heat stress would be to breed animals which are tolerant to high temperatures. Breeding for heat tolerance leads to a permanent change in the genetic composition of the swine population. To be able to genetically improve heat tolerance within a population, there is a need to find genetic variation and to choose specialized sire and dam lines and select them for improved production under the given conditions. Genetic variation in heat tolerance has been studied in dairy cattle, sheep, and finishing pigs (Ravagnolo and Misztal, 2000; Finocchiaro *et al.*, 2005; Zumbach *et al.*, 2008). However, there are hardly any studies focusing on genetic variation in sows with respect to heat tolerance.

II – Heat stress and fertility

1. Physiological background of heat stress

Pigs are highly sensitive to elevated ambient temperatures because they cannot sweat and even have problems coping with heat stress through increased respiration or panting. Pigs suffering from cold stress increase their internal heat production by shivering, or by increasing their feed intake. This increased feed intake is mainly used to raise the body temperature rather than to increase production (Quiniou *et al.*, 2001). Pigs also have various physiological processes to cope up with heat stress. Through radiation, conduction, convection, and evaporation pigs can transfer heat from their body to their environment. Wild pigs suffering from

heat stress in nature, regulate their body temperature by wallowing in mud or water and changing their activities from day to night. In modern pig industry mud or water is not available and pigs have to cope with the given environment. In fact, the internal heat production of pigs has increased during the past 50 years due to increase in leanness (Brown-Brandl *et al.*, 2004). Higher internal heat production lowers heat tolerance capacity, resulting in increased susceptibility to heat stress (Brown-Brandl *et al.*, 2001).

Heat stress can be measured typically via rectal temperature, respiration rate, skin temperature, and heat production (Omtvedt *et al.*,1971). The rectal temperature increases with initial exposure to heat stress, but gradually decreases with length of exposure to heat stress. However, there is hardly any relationship between rectal temperature and fertility traits in pigs (Omtvedt *et al.*,1971). Next to these physiological measures of heat stress, the detrimental effect of high temperatures can be also measured in reduced performance. In literature, several authors describe that high temperatures reduce feed intake in pigs. This negative effect on feed intake has consequences for reproductive performance of sows as well (Black, 1993). In general, heat stress affects fitness traits, i.e. an animal's ability to produce and reproduce. In pig production, this relates mainly to reproduction.

2. Reproductive performance

Reproductive performance in sow lines is of great importance in current commercial pig breeding programs (Hanenberg *et al.*, 2001). Several factors affect reproductive performance such as breed, parity, lactation length, nutrition, season, day light length and temperature. Litter size has been included in almost all pig breeding programmes and it is one of the most important reproduction traits based on the economic importance (Rhydmer, 2000). Next to litter size, pig breeding programmes should include piglet survival, number of teats, number of stillborn, and longevity of sows as well (Merks *et al.*, 2012). Negative influences of heat stress on reproductive performance, such as litter size and farrowing rate, have been described by several authors (Wetteman *et al.*, 1988; Peltoniemi *et al.*, 1999; Tummaruk *et al.*, 2004). Heat stress during the first two weeks after breeding reduces conception rate and number of viable embryos (Omtvedt *et al.*,1971). The effect during the second week of gestation is more severe. Heat stress during last week of gestation increases the number of still born piglets and reduces the number of live born piglets (Omtvedt *et al.*,1971).

3. Concept of upper critical temperature

Pigs suffer from heat stress when temperature exceeds the upper critical temperature of the thermo-neutral zone. The thermo-neutral zone is the range of ambient temperatures between the lower critical temperature and the upper critical temperature (Bianca, 1976). Below the lower critical temperature the animal needs to increase heat production via shivering and other processes to maintain body temperature. Above the upper critical temperature, the animal starts to be stressed by heat and energy is used to maintain body temperature particularly through the lungs by increased respiration (Black, 1993). The thermo-neutral zone is based on the body temperature of the pig. One could argue that there is as well a lower critical temperature and an upper critical temperature for pig production (e.g. finisher growth, litter size, farrowing rate). Below the lower critical temperature the pig has to reduce performance to use the energy for maintaining body temperature and above the upper critical temperature the pig has to reduce performance to avoid extra heat production (Bloemhof *et al.,* 2008). This concept is visualized in Fig. 1.

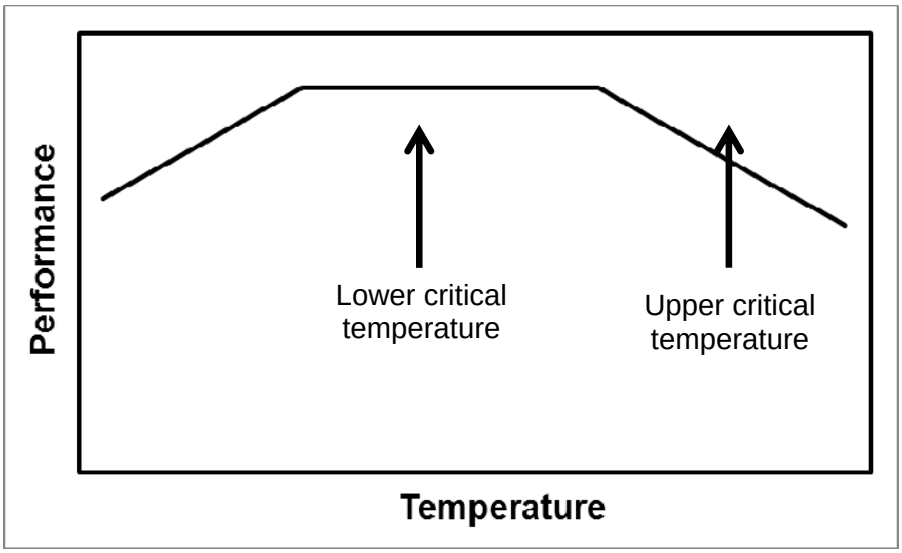

Fig. 1. Concept of thermo-neutral zone for pig performance.

III – Genetic differences

1. Genetic differences between lines

When evaluating breeding goals for dam lines we were confronted with different appreciation of 2 dam lines by pig producers in the Netherlands (temperate climate) and in Spain (warmer climate). The dam line preferred by the Spanish producers was not preferred by the Dutch and vice versa. Reproduction differences between Spanish and Dutch farms supported this opinion. Therefore an analysis was performed. Data included 32,361 records on reproductive performance from 11,935 sows on 20 farms in Spain, collected from 2003 to 2005. Sows belonged to two purebred dam lines named Dutch (a Yorkshire dam line, originally selected in the Netherlands) and International (a Large White dam line, selected based on data from all around the world). For each parity first insemination records were used and combined with maximum outside temperature at day of insemination (from a weather station close to the farm). First of all a descriptive analysis was performed and means for farrowing rate, litter size and the combination of farrowing rate and litter size (total number piglets born per first insemination) were calculated for each maximum outside temperature class (range from 10ºC to 36ºC). The results from this descriptive analysis are shown in Fig. 3. For temperatures below 23ºC farrowing rate, litter size, and total number piglets born per first insemination were higher for sows from the Dutch line than for sows from the International line. Above 24ºC litter size was almost the same in both dam lines, but farrowing rate and total number piglets born per first insemination were higher for the International line sows when temperature exceeded 25ºC (Bloemhof *et al.*, 2008). In a study by Bergsma and Hermesch (2012) the interaction between temperature and lactation feed intake of 4 dam line crosses was studied, the results of this study are shown in Fig. 2. For three of the four dam line crosses highest feed intake was recorded during the medium temperature range from 14ºC to 24ºC. The reaction norms for lactation feed intake on temperature were similar for cross A and cross B, even though cross A had a higher lactation feed intake. Cross D had a lower lactation feed intake than cross A and B and had in the extreme temperatures a larger decline in feed intake than cross A and B. Cross C showed a different reaction norm curve than the other 3 crosses. At temperatures below 5ºC these sows responded by increasing lactation feed intake. Cross C could be called the least

temperature sensitive dam line and intriguingly this dam line cross descends from the International dam line which was investigated in the study by Bloemhof *et al.* (2008).

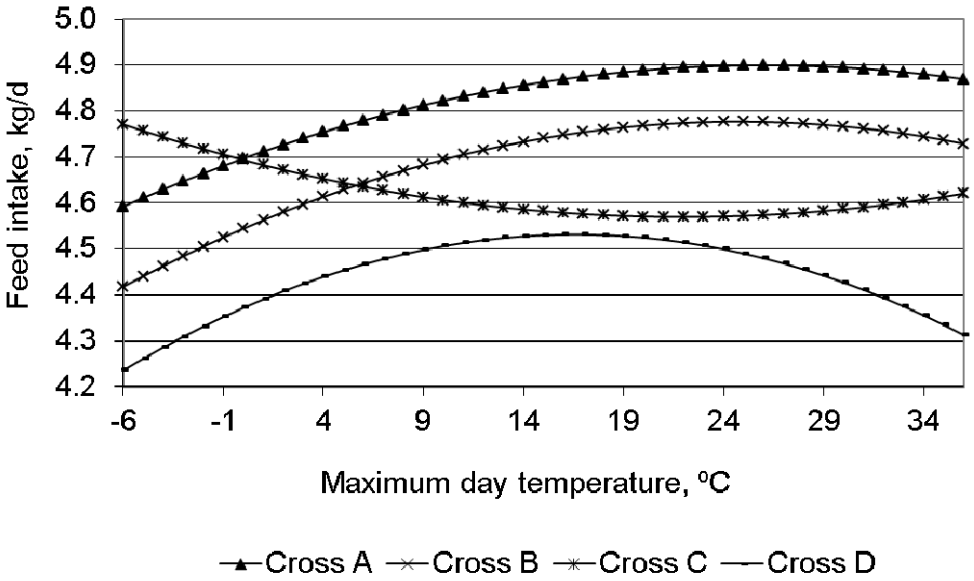

Fig. 2. Reaction norms for 4 dam line crosses of daily lactation feed intake records on maximum outside temperature measured at the nearest weather station (Adapted from Bergsma and Hermesch, 2012).

2. Genotype-environment interactions

Pig breeding programmes are more and more operating on a worldwide basis. This results in pigs that have to perform in a variety of environments. Therefore, genotype-environment interactions are expected to occur. However, their magnitude may vary depending upon the degree of differences between the genotypes or lines and the level of differences between the environments (Mathur and Horst, 1994). Due to genotype-environment interaction, pigs selected for best performance in one environment might not be the best in a different environment (Falconer, 1960).

In the study by Bloemhof *et al.* (2008), the thermo-neutral zone concept was adapted for the two dam lines to the relation between temperature and reproductive performance (Fig. 1). Subsequently, upper critical temperatures were estimated for reproductive performance of these two dam lines. Two models were compared for goodness of fit: a linear model, in which it was assumed that temperature above 10°C affects reproductive performance with a linear decrease, and a plateau-linear model with the plateau representing the thermo-neutral zone and a linear decrease above the upper critical temperature of this zone. Results of this analysis are shown in Figure 3. The goodness-of-fit tests showed that for the Dutch dam-line sows the plateau-linear model fitted best for all 3 reproductive performance traits (farrowing rate, litter size and total number piglets born per first insemination). This means that there is an upper critical temperature for the reproductive performance of the Dutch line sows. The upper critical temperature of this zone varied from 19.2°C for farrowing rate, to 21.7°C for litter size, to 19.6°C for total number piglets born per first insemination. Above these temperatures the decrease in

farrowing rate was 1% per °C, 0.05 piglet in litter size per °C and 0.13 piglet per °C per first insemination (Fig. 3). For the sows from the International dam line no upper critical temperature could be estimated for reproductive performance. However, a linear significant adverse effect of temperature on litter size and total number piglets born per first insemination was found. The decrease in performance of International line sows with increasing temperature was much less than in the Dutch line sows (Bloemhof *et al.*, 2008). These results suggest a significant genotype-environment interaction. This is a typical example of a genotype-environment interaction in livestock breeding programs (Mathur and Horst, 1994) where there is a larger reduction in performance of one genotype (Dutch line) compared to the other (International line) due to heat stress.

Dutch line sows were selected on reproductive performance based on data collected in the Netherlands, which is a temperate climate, and these sows showed quite a large reduction in farrowing rate and litter size with increasing temperatures in Spain. The International line sows showed less problems with high temperatures and were originally selected based on international data from mainly tropical countries (Brazil, Spain, Italy, Philippines). So even though Dutch line sows had higher levels of farrowing rate and litter size under relatively stress-free (e.g. lower temperature) conditions, sows of the International line were superior than sows of the Dutch line when temperatures where higher than 25°C. This is a clear indication for a genotype- environment interaction. We think that for the International line families were selected which did well for farrowing rate and litter size in these tropical conditions, adapting therefore to the local environments. These differences suggest that genetic selection on sow heat stress tolerance might be possible (Bloemhof *et al.*, 2008).

In view of the genotype-environment interactions one could consider using specific genotypes for specific environments, e.g. the International line for hot environments and the Dutch line for temperate environments. Maximum selection response can then be obtained by recording data under the production environment and using them for genetic selection (Mulder and Bijma, 2005).

3. Environmental sensitivity

The presence of genotype-environment interactions for pig breeding in hot climates could be considered as an opportunity rather than a problem. This provides opportunity for breeding of more robust pigs for the desired environments. To our opinion a robust pig is a pig tolerant against environmental perturbations without compromising production. These environmental perturbations could be high temperatures, disease pressure, low-quality feed or other challenges in pig management. In pig breeding it is generally accepted that selection for increased performance in one environment will lead to increased production in that particular environment. However, it does not necessarily mean that these pigs with increased performance will perform as well in a second environment (van der Waaij, 2004). It might even occur that selection on production under improved environmental conditions leads to increased environmental sensitivity (van der Waaij, 2004). In breeding programs where a specific line is used for a variety of temperature conditions, sensitivity to temperature stress should be considered as part of the genetic evaluation and selection. Another option for genetic evaluation procedures for environmental sensitivity is to use genetic reaction-norm models. Reaction-norm of a genotype is the phenotype expressed over a range of environments and is a measure of environmental sensitivity or robustness (Kolmodin and Bijma, 2004). The advantage of using reaction-norm models is that they will produce BLUP breeding values for general performance potential and for the environmental sensitivity of animals. This allows implementing environmental sensitivity as a trait into the selection index (Kolmodin *et al.*, 2003; Knap, 2005; Knap and Su, 2008). Reaction-norm models provide measures especially for improving robustness across environments.

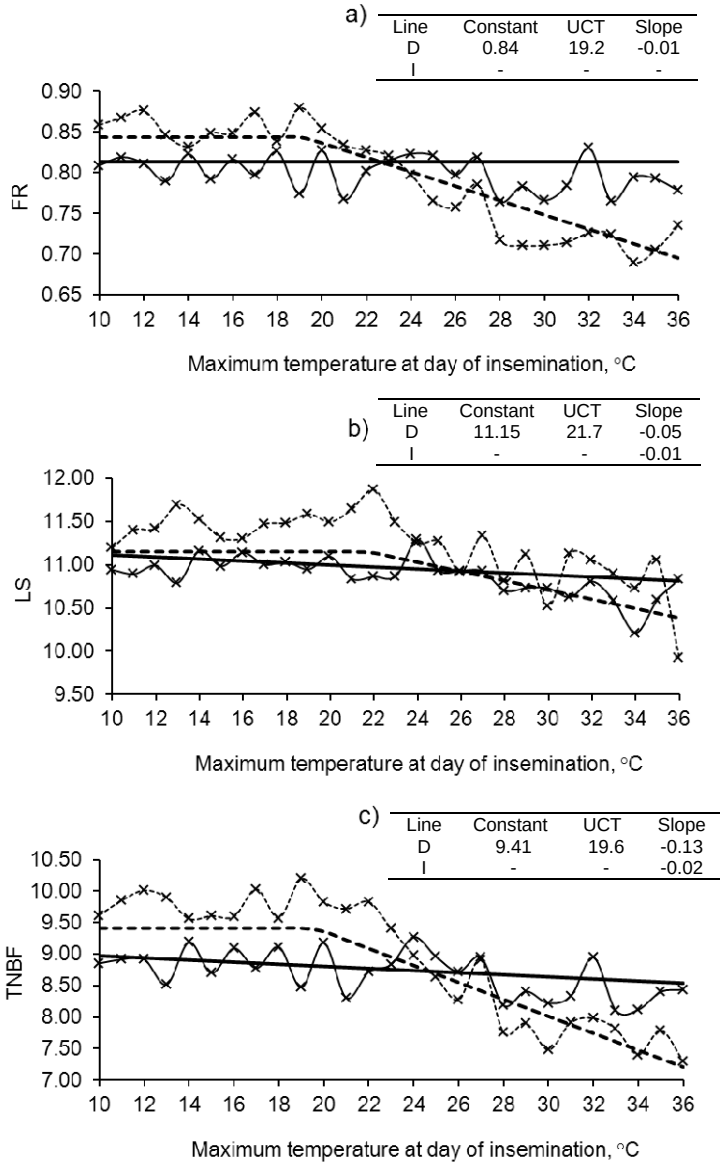

Fig. 3. Observed means per ºC maximum outside temperature at day of insemination for three reproductive performance traits (a= farrowing rate (FR), b= litter size (LS), c= total number piglets born per first insemination (TNBF)) calculated for two sow lines, a Dutch purebred Yorkshire line (- x - - - x - - -) and an International purebred Large White line (—x—) and the estimated effect of maximum outside temperature at day of insemination for these three reproductive performance traits for both sow lines, a Dutch purebred Yorkshire line (- - -) and an International purebred Large White line (—). For the Dutch line and the International line the estimates are given of the constant level of the reproductive performance trait where reproduction is not influenced by temperature (constant), of the upper critical temperature in ºC (UCT), and of the slope of the decrease above the UCT (slope), all estimates were significant different from 0 (adapted from Bloemhof *et al.,* 2008).

IV – Opportunities for genetic selection

1. Genetic parameters

An additional way to improve environmental robustness (e.g. breeding less sensitive animals) is to explicitly define robustness traits that can be included into profit equations and that can be used in breeding goals. An example of this might be breeding for improved heat tolerance.

To be able to genetically improve heat tolerance within a population, there is a need to find genetic variation. Therefore, heritability for the random regression slope of farrowing rate against increasing temperature at day of insemination (=heat tolerance) and the genetic correlation between farrowing rate and heat tolerance were estimated (Bloemhof *et al.*, 2012). The estimates were based on 93,969 first insemination records per cycle, from 24,456 sows inseminated between January 2003 and July 2008. Sows originated, again, from a Dutch purebred Yorkshire dam line and an International purebred Large White dam line and were raised in Spain and Portugal. Heritability estimates for farrowing rate were 0.05 for Dutch line sows and 0.08 for International line sows. Heritability estimates for heat tolerance at 29.3°C were 0.04 for Dutch line sows and 0.02 for International line sows. Genetic correlations between farrowing rate and heat tolerance were around 0 (Table 1).

Table 1. Estimated heritabilities and genetic correlations, with corresponding standard errors, for farrowing rate and heat tolerance for two damlines (h^2 = heritability, r_g genetic correlation)

Trait		Line sow	
		Dutch	International
Farrowing rate	h^2 production	$0.05_{0.02}$	$0.08_{0.01}$
	h^2 response to heat stress†	$0.04_{0.01}$	$0.02_{0.01}$
	$r_g$production, response	$0.16_{0.37}$	$-0.36_{0.17}$

†at heat load index 10 (=equal to temperature of 29.3°C).

2. Possibilities for selection

In the study by Bloemhof *et al.* (2012) it could be concluded that there were possibilities for genetic improvement of heat tolerance as expressed in farrowing rate. These results are in line with other studies in which additive variances for heat tolerance have been found to be important for non-return rate, milk production in dairy cattle, milk yield in sheep, and growth in finisher pigs. When heat stress was present the additive variances for heat tolerance were as large as the additive variances for non-return rate, milk yield and growth under non-stressed conditions (Ravagnolo and Misztal, 2000; Ravagnolo and Misztal, 2002; Finocchiaro *et al.*, 2005; Zumbach *et al.*, 2008). Selection for production traits often increases environmental sensitivity (van der Waaij, 2004). This was not fully supported by the results in Table 1. However, in finishing pigs a genetic correlation of -0.5 between carcass weight and heat tolerance has been reported (Zumbach *et al.*, 2008). In dairy cattle as well, genetic correlations between milk production and heat tolerance were negative, ranging from -0.30 to -0.95 (Aguilar *et al.*, 2009; Ravagnolo and Misztal, 2000). These correlations imply that selection strategies which focus on improving reproduction and production traits using data from only moderate climates will reduce heat tolerance and might lead to animals which are even more sensitive to environmental stress from high temperatures.

V – Pig breeding for hot environments

It is expected that heat tolerance may become more and more a limiting factor for worldwide pig production. First of all, as genetic progress in pigs has mainly focused in the past 50 years in reducing back fat thickness, animals have become more lean which results in an increased susceptibility to heat stress (Brown-Brandl *et al.*, 2001). Second, as a result of climate change temperature is expected to increase worldwide (Hofmann, 2010). A third reason for the increasing importance of heat tolerance is that meat production is expected to double in the next 50 years, with increase is predicted to be the fastest in warm climates (FAO, 2006). These 3 reasons show that breeding animals for improved heat tolerance is relevant. The differences described previously between the Dutch dam line and the International dam line can be used to select as a specific dam line for environments with high temperatures. However, one needs to realize that a line specifically bred for high temperatures will have a lower performance level under temperate climate (non-heat stressed conditions) than a line specifically bred for temperate climate. To ensure firm breeding value estimation for heat tolerance or robustness for all breeding candidates in a practical breeding programme, it should be ensured that selection candidates have relatives tested under both temperate and hot conditions in the field. Testing animals in an experimental heat chamber might also be a powerful alternative, however for testing large numbers of pigs this might be unpractical.

VI – Future perspectives

Due to globalization of pig breeding programs and concentration of genetic selection in fewer breeding organisations, pigs should be expected to perform in a different environment than the selection environment.These environmental differences could include differences in housing, feeding, climate and pig management. Selection is typically performed in nucleus populations under most optimal conditions while commercial pigs often have to perform under sub-optimal conditions and face therefore a variety of stressors. Heat stress is one of these stressors and could have significant adverse effects on sow reproduction. The results of our investigations show that there are differences between offspring from different lines in their responses to heat stress and in the relationship between heat stress and fertility traits. This provides an opportunity to select specific lines for specific environmental conditions e.g. one line for Dutch environment and another one for international use. Further, there are opportunities to select lines destined for international use to be genetically more robust to heat stress. Pig breeding programs especially for hot environments need to consider sensitivity of sows to heat stress in their selection decisions.

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Effect of a heat stress episode on feed and water intake in dairy goats bred under temperate climate

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Abstract. The effect of a heat stress episode was studied in eight dairy goats bred under temperate climate with *ad libitum* access to water. The increase in temperature from 19 to 28°C (recorded at 5 pm) modified neither feed intake nor the global shapes of feed and water intake patterns. However, there was a 40% increase in water intake and the latency from the beginning of the afternoon meal to the first water intake decreased from 35 to 26 min. Blood PCO_2 decreased because the animals hyperventilated to reduce their body temperature, whereas rectal temperature increased by around 0.6°C. Milk production was not modified, but milk fat content decreased. A significant goat effect was observed for almost all the results. Goats from the Alpine breed drunk more water when expressed on a dry matter intake basis than those from the Saanen breed. This could be explained either by their higher level of feed intake which enhanced the post-prandial heat production or by the difference in latent heat dissipated through sweating linked to coat colour.

Keywords. Heat stress – Intake behavior – Lactating goats – Temperate climate – Water intake.

Effet d'un stress de chaleur sur l'ingestion d'aliment et d'eau chez des chèvres laitières élevées en conditions tempérées

Résumé. *L'effet d'un stress de chaleur a été étudié chez huit chèvres laitières élevées en climat tempéré et disposant d'eau à volonté. L'augmentation de la température de 19 à 28°C (mesurée à 17 h) n'a modifié ni la quantité de ration ingérée, ni l'allure globale de la cinétique d'ingestion des aliments et d'eau. Par contre, la quantité d'eau bue a été accrue de 40 % et le temps de latence entre le début du repas et la première buvée, réduit de 35 à 26 min. La teneur en CO_2 du sang a diminué, car les animaux ont hyperventilé pour réduire leur température corporelle, alors que leur température rectale a augmenté de 0,6°C. La production laitière n'a pas été modifiée, mais le taux butyreux du lait a diminué. Un effet chèvre significatif a été observé sur la majorité des paramètres étudiés. En moyenne, les chèvres de race Alpine ont bu plus d'eau par rapport à la quantité de matière sèche ingérée que les chèvres de race Saanen. Ceci pourrait être dû à leur niveau d'ingestion plus élevé qui accroîtrait le dégagement de chaleur post-prandial, ou à une différence de chaleur latente dégagée par sudation liée à la couleur de leur pelage.*

Mots-clés. Stress de chaleur – Comportement d'ingestion – Chèvres en lactation – Climat tempéré – Ingestion d'eau.

I – Introduction

In recent years, temperate European regions suffered from unusually hot periods, as for example during the summer of 2003. The aim of this work was to study the effect of a hot period on feed and water intake of high-producing dairy goats bred in the Paris area, because most previous studies concerned ruminants under hot climates in areas where water is scarce. Moreover, drinking behavior is seldom studied in ruminants (Cardot *et al.*, 2007).

II – Material and methods

This study was conducted at the Experimental Farm of the research Unit INRA-AgroParisTech MoSAR (Thiverval-Grignon, France (48°51' N; 1°55' E); 70 m above sea level). Four Alpine and four Saanen goats (160 DIM at the start of the experiment) were housed in individual pens with free access to feed and water. Goats were fed a total mixed ration (TMR) twice daily after milking, in the proportion of two thirds at 4 p.m. and one third at 8 a.m., according to the milking intervals. The ingredients of the diet, on a dry matter basis, were dehydrated lucerne (30%), meadow hay (20%), pressed sugar beet pulp (30%) and compound concentrate feed (20%).

Animals were weighed weekly. Individual amounts of feed offered and refusals were weighed daily. Intakes of feed and water were recorded separately using, for each animal, two weighing-scales (Balea, Saint-Mathieu de Tréviers, France): one was fitted under the feeding trough and the other one under a water container linked to the drinking trough. This system continuously recorded every 2 min the weight of the feed in the feeding trough and of the water in the container with a precision of 5 g. Cumulative dry matter intake (DMI) or water drunk (WD) per kilogram of body weight (BW) was calculated for the 22 hours following the afternoon feeding, using the body weight and the dry matter percentage of the diet (ISO, 1983) measured weekly. Animals had no access to food during the two remaining hours of the 24 h period (around afternoon milking). The evolution of the percentages of DMI and water drunk was studied during the nycthemere by 20-min intervals. The percentage of DMI or water drunk during the first three hours after the afternoon feed allowance was also calculated and called P180DMI and P180WD respectively, because it was the period during which the maximum of variability between goats was observed for food and water intakes (Giger-Reverdin *et al.*, 2011). The latency from the beginning of the afternoon meal to the first water intake was also calculated.

Two periods of five consecutive days were compared: the first one was between the 17th and the 22th of June 2010, and the second one between the 26th of June and the 1st of July 2010. Milk production was measured at each milking and averaged for 5 days. Milk samples were analysed for fat and protein contents at the beginning and end of each period. Blood samples were also analysed twice at each period for gas composition with a blood gas analyzer ABL77 (Radiometer®). Temperature (T in °C) and humidity (H %) were measured in the experimental facility at three times each day: 9 am, 1.30 pm and 5 pm (GMT + 2 h). The temperature-humidity index (THI) was calculated according to the formula of West (1994):

$THI = 0.81\ T + (H/100) * (T - 14.3) + 46.3.$

Data were analysed using the MIXED procedure of SAS® for repeated measurements with the following model: $Y_{ij} = \mu + P_i + G_j + P_i*G_j + \varepsilon_{ij}$

where μ represents the overall mean, P_i the period effect, G_j the goat effect. P_i*G_j the interaction between the period and the goat effects.

The model included the repetition of the day within the period with the use of a mixed procedure First autoregressive AR(1). For the temperature and humidity data, the only effect tested was that of the period.

III – Results

1. Temperature and humidity in the goat unit

During both periods, the temperature increased during the day while the humidity decreased. However, the modifications were very small in the first period and more marked in the second one. This last period can be considered as a heat episode for the country with a mean temperature of 27.5°C at 5 pm (26.7 to 28.4°C) and a mean humidity of 41% (35 to 50%). The

period effect was significant except for the humidity in the morning (Table 1).

Table 1. Temperature and humidity in the goat unit during Periods 1 (control) and 2 (heat stress)

	Time	Period 1	Period 2	SEM	Period effect
Temperature (°C)	9 am	17.0	21.3	0.52	0.001
	1.30 pm	18.0	25.4	0.56	<0.0001
	5 pm	19.0	27.5	0.41	<0.0001
Humidity (%)	9 am	66.4	64.8	1.32	NS
	1.30 pm	60.0	45.4	2.95	0.01
	5 pm	57.4	40.6	2.83	0.01

NS: not significant.

2. Feed and water intake

Dry matter intake (DMI) was not modified by temperature, but water intake increased by 40% when the temperature increased (Table 2). The percentage of dry matter intake during the first three hours after the afternoon feeding did not vary, but there was a decrease in the percentage of water drunk. All the goats started to eat before drinking. Moreover, the latency to the first water intake after feeding allowance decreased with the increase in temperature. Feeding behavior expressed as % DMI per 20min intervals was not modified by heat stress (Fig. 1).

Table 2. Feed and water intakes during Periods 1 (control) and 2 (heat stress)

	Period 1	Period 2	SEM	Effect		Interaction Period*Goat
				Period	Goat	
DMI[1]	47.1	47.2	0.36	NS	<0.0001	0.02
Water drunk[2]	107	143	2.3	<0.0001	<0.0001	0.004
P180DMI[3]	0.38	0.37	0.009	NS	NS	NS
P180WD[3]	0.41	0.35	0.012	0.01	0.001	NS
Latency before the first water intake[4]	35	26	1.5	0.001	<0.0001	NS

[1]in g per kg of body weight; [2]in ml per kg of body weight; [3]Proportion of the daily intake eaten (DMI) or drunk (WD) during the first 180 min after the afternoon feeding; [4]in min after the afternoon feeding; NS: not significant.

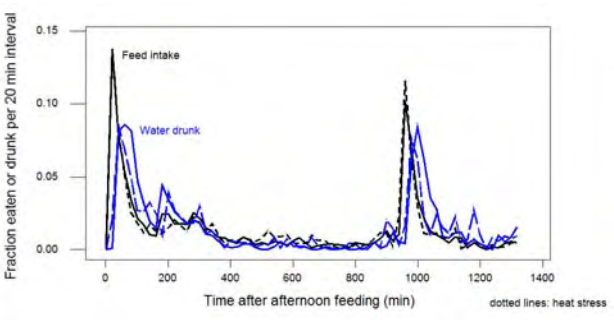

Fig. 1. Evolution of the quantities of water drunk and feed intake during the nycthemere.

3. Blood parameters

Blood PCO_2 decreased with the increase in temperature, whereas rectal temperature increased:

Table 3. Blood parameters and rectal temperature during Periods 1 (control) and 2 (heat stress)

	Period 1	Period 2	SEM	Effect		Interaction Period*Goat
				Period	Goat	
pH	7.39	7.40	0.004	0.04	0.001	NS
PCO_2 (mm Hg)	44.4	40.6	0.69	0.01	NS	NS
PO_2 (mm Hg)	35.6	36.6	1.12	NS	0.05	NS
Bicarbonates (mmol/L)	25.6	24.7	0.37	NS	NS	NS
Rectal temperature °C	38.5	39.1	0.07	<0.0001	NS	NS

NS: not significant

4. Milk production

Raw milk yield was not modified by heat stress, but the fat content and the fat yield decreased, (Table 4). Protein content and protein yield were not affected by the increase in temperature.

Table 4. Milk yield and composition during Periods 1 (control) and 2 (heat stress)

	Period 1	Period 2	SEM	Effect		Interaction Period*Goat
				Period	Goat	
Raw milk yield[1]	3.55	3.61	0.031	NS	<0.0001	0.03
Fat content[2]	33.3	30.2	0.31	<0.0001	<0.0001	0.01
Protein content[2]	31.9	31.3	0.09	0.01	<0.0001	NS
Fat yield[3]	116	108	1.7	0.01	0.001	NS
Protein yield[3]	113	113	1.0	NS	<0.0001	0.02

[1]in kg/day (mean of 5 days); [2]in g/kg of milk; [3]in g/day; NS: not significant.

5. Breed and goat effects

There was a breed effect on live-weight (Alpine: 58.3 kg *vs* Saanen: 64.2 kg; SEM = 0.52; $P < 0.001$) and intake: Alpine goats ate more dry matter per kg of body weight than Saanen (49.3 *vs* 45.9 g DMI/kg BW; SEM = 1.15; P = 0.02), and drank more water when expressed on a dry matter intake basis: 2.82 *vs* 2.46 L/kg DMI; SEM = 0.073; P = 0.01). The increase in this latter ratio with the temperature was more pronounced for the Alpine goats (Period 1: 2.38; Period 2: 3.26) than for the Saanen goats (Period 1: 2.15; Period 2: 2.76).

IV – Discussion

In this experiment, heat stress can be considered as a very moderate one, because the THI index was slightly above 72 during the afternoon in Period 2 which is the threshold level above which there is heat stress (West, 1994). It did not modify eating behavior on the contrary to other situations where goats were bred in harsh conditions (Silanikove, 2000). Nevertheless, total water intake was increased and the latency between the afternoon feeding and the first water intake was decreased, meaning that the animals felt thirsty earlier after TMR allowance

(Olsson *et al.*, 1997). The respiratory alkalosis (decrease in PCO_2) observed during Period 2 could be explained by the hyperventilation of the animals during the heat stress as already observed in cows (Kadzere *et al.*, 2002) or in goats (Augustinsson *et al.*, 1986). When the animals are panting, which is a way to decrease their inner temperature, they release CO_2. Although moderately heat-stressed, the experimental goats showed increased rectal temperature as previously observed in other animals exposed to hot periods (Augustinsson *et al.*, 1986; Kadzere *et al.*, 2002). The decrease in milk fat content has already been observed in dairy cows under a mild heat stress (Bandaranayaka and Holmes, 1976). The difference between breeds in terms of the water/DMI ratio might be explained by the higher level of intake of Alpine compared to Saanen goats and probably by a need to dissipate more heat from rumen fermentation (West, 1994; Kadzere *et al.*, 2002). It could also be due to the difference in coat colours: in Holstein cows, the temperature of the skin is higher in black areas than in white ones, leading to an increase in sweating rate in the black areas compared to the white ones (Da Silva and Maia, 2011). This could explain why Alpine goats with their dark-brown coat drank more water/kg DMI than the white coated Saanen goats.

V – Conclusion

High-producing European dairy goats seemed to cope well with short periods of moderate heat stress when water was available *ad libitum*. This study, which included feeding and drinking patterns, needs to be completed with a larger scale experiment and with measurements of the water fluxes and water losses *via* urine, faeces and milk. Long term adaptation and effects on production warrant future research in view of rampant global warming.

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Effect of antimethanogenic garlic-derived compounds on amylolytic and xylanolytic activities in the rumen

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Abstract. This work assessed the effect of adding two antimethanogenic garlic-derived compounds on *in vitro* rumen amylolytic and xylanolytic activities. A 12-day trial was performed using a continuous-culture system inoculated with rumen fluid from goats. At the end of the trial, a subsequent experiment was carried out by incubating the fermenters' content in batches culture to measure methane production over 24 h. The additives and doses tested were control (without additive), propyl propane thiosulfinate (PTS, 200 µl/L), diallyl-disulfide (DDS, 80 µl/L) and bromochloromethane (BCM, 160 mg/L) as positive control. The incubated substrate consisted of 50:50 alfalfa hay:concentrate mix. After 10 days of adaptation samples of the fermenters' contents were collected at 0, 2 and 4 hours after the morning feeding and the activity of amylase and xylanase enzymes quantified. In batch cultures, the addition of PTS and BCM resulted, in a reduction ($P<0.001$) of methane production of 40 and 96% respectively, while DDS did not have an effect ($P=0.575$), compared to control. In continuous-culture a reduction ($P<0.05$) of xylanase activity in samples treated with PTS at all sampling times was observed compared to the control, while amylase activity was not affected ($P\geq0.243$) by any garlic compound. On the contrary, and despite its potent anti-methanogenic effect, BCM did not affect ($P\geq0.647$) xylanase activity but increased ($P<0.05$) amylase activity at 2 hours compared to the control. Our results suggest that the reduction of methane synthesis in the rumen by some garlic-derived compounds may be accompanied by a detrimental decrease in xylanase activity and might compromise fermentation and intake.

Keywords. Xylanolytic and amylolytic activity – Methane – Rumen – Garlic-derived compounds.

Effet des composés dérivés de l'ail sur la production de méthane et l'activité amylolytique et xylanolytique dans le rumen

Résumé. *Ce travail a pour objectif d'évaluer* in vitro *l'effet de deux composés dérivés de l'ail sur l'activité des enzymes amylolytiques et xylanolytiques dans le rumen. Un essai de 12 jours a été réalisé en utilisant des fermenteurs à flux continu inoculés avec du liquide ruminal des chèvres. À la fin du processus, une expérience a aussi été réalisée en incubant du contenu des fermenteurs dans des bouteilles afin de mesurer la production de méthane pendant 24h. Les additifs et les doses testés étaient le contrôle (sans additif), propane propyle thiosulfinate (PTS, 200 µl/l), diallyle-disulfure (DDS, 80 µl/l) et bromochlorométhane (BCM, 160 mg/l) comme témoin positif. Le substrat incubé était un mélange 50:50 de foin de luzerne et de concentré. Après 10 jours d'adaptation aux différents régimes, des échantillons du contenu des fermenteurs ont été recueillis à 0, 2 et 4 h après le repas du matin et les activités amylanase et xylanase ont été quantifiées. Dans les bouteilles le PTS et le BCM réduisaient ($P<0,001$) la production de méthane de 40-96%, tandis que le DDS n'avait pas d'effet ($P=0,575$) en comparaison avec le témoin. Dans les fermenteurs a été observée une réduction ($P<0,05$) de l'activité xylanase avec du PTS par rapport au contrôle indépendamment du moment de l'échantillonnage tandis que l'activité amylase n'a été affectée par aucun des composés provenant de l'ail. Au contraire, et malgré sa puissante activité antiméthanogénique le BCM n'a pas d'incidence sur l'activité xylanase, mais il augmentait ($P<0,05$) l'activité amylase des échantillons obtenus après 2 et 4 heures d'incubation par rapport au contrôle et à des composés dérivés de l'ail. Les résultats obtenus suggèrent que la réduction de la synthèse du méthane dans le rumen par certains composés dérivés de l'ail peut être accompagnée par une diminution préjudiciable de l'activité xylanase.*

Mots-clés. *Activités xylanolytique et amylolytique – Méthane – Rumen – Composés dérivés de l'ail.*

I – Introduction

Research in animal production is currently focusing on developing cost effective production by improving productivity and product quality but also minimizing the impact on the environment, mainly by reducing methane (CH_4) emissions and N excretion. Methane production in the rumen represents a loss of energy for the host and an important source of greenhouse gases emissions (Benchaar and Greathead, 2011). In the last decade a wide range of plant derived compounds have been tested in order to reduce methane production in the rumen and some have exhibited significant effectiveness with potential practical use (Anassori *et al.*, 2011; Benchaar and Greathead, 2011). However, the mechanisms of action involved in the antimethanogenic effect are not fully understood, especially with regards to potential negative side effects, such as the reduction of fibrolytic activity resulting from less H_2 uptake (Soliva *et al.*, 2011). Among the plant extracts studied, garlic derived metabolites such as allicin, diallyl sulphide, diallyl disulphide and allyl mercaptan have shown *in vitro* capacity for mitigating ruminal CH_4 formation (Anassori *et al.*, 2011). In our group, some of these metabolites (diallyl disulfide and propyl propane thiosulfinate) have exhibited a substantial reduction of methane emissions in short term incubations in batch cultures (Martínez *et al.,* 2011). However, less information is available from longer period of treatments. Therefore, the aim of this work was to evaluate the effects of two garlic compounds on rumen methane production and the activity of some key enzymes using a medium-term *in vitro* approach such as single-flow continuous-culture fermenters (SFCCF).

II – Materials and methods

Eight single-flow continuous-culture fermenters (SFCCF) following the model of Muetzel *et al.* (2009) were used. Eight adult Murciano-Granadina goats fitted with ruminal canula were used in total as donors of ruminal contents. For each incubation run, two groups of three fermenters were inoculated with a different pool (700 mL) each one obtained from three different animals selected randomly. This resulted in four different pools as follows: pool 1 (goats 1, 2 and 3), pool 2 (goats 4, 5 and 6), pool 3 (goats 2, 5 and 7) and pool 4 (goats 3, 6 and 8). In each incubation run two fermenters received one of the 4 experimental treatments: diallyl disulfide (DDS, 3-prop-2-enyldisulfanyl prop-1-ene purity of 80%), propyl propane thiosulfinate (PTS, purity of 75%) and bromochloromethane (BCM, halogenated aliphatic hydrocarbon, purity of 12%) that was included as antimethanogenic positive control. Donor animals were adapted for 12 days to the diet used as substrate in the fermenters, which was composed of alfalfa hay and concentrate in a 50:50 ratio. Concentrate was made of barley, fava beans, corn, sunflower meal, gluten meal and by-pass fat. Rumen contents were collected before feeding and pooled under anaerobic conditions and 700 mL were inoculated into each fermenter, with an effective volume of 1000 mL. Each fermenter received daily 16 g of fresh matter of the experimental diet ground at 1 mm, in two equal portions at 09:00 and 14:00 h. Flow through fermenters was maintained by continuous infusion of artificial saliva (Muetzel *et al.*, 2009) at a rate of 40 mL/h and CO_2 was continuously infused to keep anaerobic conditions. Fermenters were maintained in a water bath at 39ºC and the effluent from each fermenter was collected into a flask maintained in a bath at 3ºC to prevent microbial growth. The doses tested were chosen from previous experiments (Martínez *et al.* 2011): 80 µl/L/day for DDS, 200 µl/L/day for PTS and 160 mg/L/day for BCM. After 10 days of incubation 1 mL of the fermenters content was collected at 0, 2 and 4 h after the morning feeding and frozen at -80ºC for determination of amylase and xylanase activities.

A 24 hours batch culture trial, based on Theodorou *et al.* (1994) protocol, was used by incubating fermenters content collected on day 12 to measure methane production. The content of each fermenter was filtered through two layers of cheesecloth while bubbling with CO_2. Basal diet (0.5 g) was incubated in 120 ml serum bottles with 60 ml of the fermenter content. Three replicates of each treatment previously described and a blank were used. Bottles were sealed

with rubber stoppers and aluminum caps and incubated at 39ºC in a water bath. Headspace pressure and volumes of gas and were measured with a Wide Range Pressure Meter (Sper Scientific LTD, Scottsdale, AZ) and a glass calibrated syringe, respectively, at 2, 4, 6, 8, 12, 24 h after inoculation. At 24 h a sample of the gas in each bottle was collected in a graduated syringe and transferred to a 5 ml vacuum tube and then kept at room temperature before methane content was measured by gas chromatography. An aliquot of the bottles' content was collected to measure total volatile fatty acids (VFA) concentration.

To measure the enzymatic activities in fermenters content, cells were lysed using a Mini-Beadbeater (BioSpec Products, Inc., Bartlesville, OK, USA) to release intracellular enzymes. Cell material was removed by centrifugation (10,000×g, 10 min, 4ºC) and the supernatant was used for analyses. Xylanase (EC 3.2.1.8.) and amylase (EC 3.2.1.1.) activities were determined as described by Giraldo *et al.* (2008) using oat beachwood xylan and soluble starch, respectively, as substrates. Enzymatic activities were expressed as mol of glucose or xylose released from the corresponding substrates per mL of sample in 1 min at 39ºC and pH 6.5. Data were analyzed comparing the additive effect as one-way ANOVA using SPSS (IBM SPSS Statistics v.19, IBM Corp., Somers, NY). Effects were considered significant at P≤0.05. When significant differences were detected, differences among means were studied using the LSD comparison test.

Table 1. Chemical composition (g/kg DM) of diets ingredients (n=2)

Item	Alfalfa hay	Concentrate
DM, g/kg fresh matter	907	915
OM	875	884
CP	203	168
NDF	513	245
ADF	330	118
ADL	99.2	36.3
Ether extract	8.1	15.3
GE, MJ/kg DM	18.4	19.5

III – Results and discussion

Total gas produced over 24 hours in batch culture and total VFA concentration were only significantly decreased by PTS and DDS treatments, respectively, as compared with the control (Table 2). Methane produced in batch cultures after 24 h of incubation was reduced (P< 0.001) with PTS (40%, P=0.008) and BCM (90%) as compared to the control, while DDS had no effect (P=0.575). Soliva *et al.* (2011) showed 90% inhibition of methane production with garlic oil (300 mg/L) in Rusitec fermenters and Busquet *et al.* (2005a) observed a decreased methane emission of about 70% after 17 h of incubation in batch cultures with a dose similar to the one used in the present study (300 mg/L) of garlic oil and DDS. However, Kamel *et al.* (2008) did not observe effect of DDS on methane emission after 24 h of incubation in batch cultures, although doses were lower than those used in other experiments. In agreement with the results observed with DDS in our experiment, Klevenhusen *et al.* (2011) reported no effect on methane production of DDS in sheep suggesting an adaptation of the rumen microbiota. The antimicrobial effect of garlic derived compounds has been suggested to be due to the inhibition of HMG-CoA reductase (Busquet *et al.*, 2005a; Busquet *et al.*, 2005b). The strong methane inhibition effect of BCM (up to 90%) is in agreement with observations made by Goel *et al.* (2009) *in vitro*. Tomkins *et al.* (2009) observed *in vivo* a reduction in methane emissions close to

90% after 14 days of treatment in beef and Abecia *et al.* (2012) reported a reduction of methane production of around 30% in lactating dairy goats after 60 d of BCM treatment.

Table 2. Effect of additives on total gas production, VFA concentration and methane produced (CH_4, mL) between 12 and 24 h of incubation in batch cultures inoculated with single-flow continuous-culture fermenters content collected after 12 days of incubation

	Treatments†				SEM	*P*-value
	Control	DDS	PTS	BCM		
Total gas, ml	59.1	59.0	43.8	59.6	3.11	<0.001
Total VFA, mmol/l	77.4[a]	65.7[b]	78.1[a]	78.9[a]	11.14	<0.001
Methane production	2.02[a]	1.87[a]	1.21[b]	0.08[c]	0.22	<0.001

†Treatments= PTS (propyl propane thiosulfinate), DDS (diallyl disulfide) and BCM (Bromochloromethane). SEM: standard error of the mean. [a-c] Within a row doses means without a common superscript letter differ, P < 0.05.

In SFCCF a reduction (P≤0.049) of xylanase activity by PTS at all sampling times was observed compared to the control (Table 3), while the other treatments did not have any effect (P≥0.157). The effect of PTS might be due to its antimicrobial activity (Ruiz *et al.*, 2010), that could specifically affect fibrolytic microorganisms, such as protozoa since it has been reported that about 38% of cellulase activity is associated to protozoa (Agarwal *et al.*, 1991). In this sense, we have recently observed that the addition of PTS in batch cultures reduced the abundance of protozoa (Martinez *et al.,* 2011). This is in agreement with Patra *et al.* (2006), who observed decreased xylanolytic activity with plant extracts in *in vitro* experiments using buffalo rumen fluid as inoculum. Also, the decreased xylanase activity with PTS agrees with the detrimental effect on total gas production. The amylolytic activity was only modified (P≤0.031) by BCM, increasing after 2 and 4 hour of incubation compared to the control and other treatments, respectively. Other authors (Hristov *et al.*, 2003) reported decreased amylolytic activity by several bioactive agents using bovine rumen fluid, in contrast to our results. These differences could be due to the different activity and chemical structure of compounds used in both studies.

Table 3. Effect of additives on xylanase and amylase activities in single-flow continuous-culture fermenters content sampled on day 10 after inoculation

	Hour	Treatments†				SEM	*P*-value
		Control	DDS	PTS	BCM		
Xylanase	0	6.91[a]	6.08[ab]	5.22[b]	7.10[a]	0.25	0.026
	2	5.53[a]	5.32[ab]	4.67[b]	5.33[ab]	0.13	0.123
	4	5.77[a]	5.69[ab]	5.11[b]	5.78[a]	0.12	0.133
Amylase	0	1.10	1.06	1.08	1.23	0.04	0.586
	2	0.59[b]	0.41[b]	0.57[b]	1.03[a]	0.08	0.014
	4	0.55[ab]	0.45[b]	0.46[b]	0.97[a]	0.09	0.085

†Treatments= PTS (propyl propane thiosulfinate), DDS (diallyl disulfide) and BCM (Bromochloromethane). SEM: standard error of the mean. [a-b] Within a row doses means without a common superscript letter differ, P<0.05. Amylase activity is expressed as nanomoles of glucose released from soluble starch by 1 mL of ruminal fluid in 1 min at 39ºC and pH=6.5. Xylanase activity is expressed as nanomoles of xylose liberated from oat beachwood xylan by 1 mL of ruminal fluid in 1 min at 39ºC and pH=6.5.

Janssen (2010) suggested that cellulolytic microbes produce more acetate and H_2, while amylolytic microbes produce less H_2 and more propionate, so more CH_4 is formed from forage

based diets. That could explain the different mechanisms of action of PTS and BCM, as while PTS could affect cellulolytic microorganisms, and reduce H_2 formation, BCM could affect competitors of amylolytic microorganisms, thus increases amylolytic activity and produces less H_2. This is important as often the anti-methanogenic effect of some compounds is confounded with a decrease in OM (mainly fibre) digestibility, which in turn will reduce animal intake and therefore productivity (Martin *et al.*, 2010).

IV – Conclusions

The results obtained in this work show that some garlic-derived compounds, such as PTS, have antimethanogenic effect over 12 days of *in vitro* incubation. Such reduction may be accompanied by a decrease in xylanase activity and gas production *in vitro*, which would need to be further investigated *in vivo* as could imply a detrimental effect on overall rumen fermentation and ultimately animal intake.

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Effect of polyethylene glycol addition on methane production from some Algerian browse plant species in an *in vitro* gas system

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Abstract. Biological activity of tannins of different browse plants was measured as the change in methane production when plant material was incubated with and without polyethylene glycol (PEG) using the *in vitro* gas production technique. Four dicotyledon browse plants (*Atriplex halimus*, *Artemisia campestris*, *Artemisia herba-alba*, *Calobota saharae*) and three monocotyledon browse plants (*Stipagrostis pungens*, *Lygeum spartum* and *Stipa tenacissima*), collected from an arid zone in Bousâada were evaluated. The increase in gas production upon the addition of PEG, compared with that without PEG, for the browse species varied widely (P<0.05), being particularly high in *S. tenacissima* (+35.0%) and low in *L. spartum* (+1.5%). The methane concentration in fermentation gas ranged from 7.9% with *A. halimus* to 18.6% with *L. spartum*. The higher increase in methane percentage was noted for *S. tenacissima* (+47.4%) and the lower percent value was observed for *L. spartum* (+1.5%). In presence of PEG, the methane production had positive correlation with crude protein (r = +0.78) while in absence of PEG, the methane production was correlated negatively with total condensed tannins (r = -0.88). The strongest correlation (r = 0.89; P<0.01) was between total condensed tannins and methane increase response to the addition of polyethylene glycol, suggesting that tannin compounds appeared to be useful to identify plants possessing antimethanogenic activity.

Keywords. Chemical composition – Browse – Methane – Rumen – Tannin.

Effet de l'addition de polyéthylène glycol sur la production* in vitro *de gaz et de méthane pour des plantes d'Algérie

Résumé. *L'activité biologique des tanins de diverses plantes par le microbiote ruminal d'ovins est mesurée* in vitro*, en présence et en absence de polyéthylène glycol (PEG). La fermentescibilité des substrats est évaluée par la production de gaz, retenus comme marqueurs métaboliques. L'étude est menée sur 4 plantes dicotylédones (*Atriplex halimus, Artemisia campestris, Artemisia herba-alba, Calobota saharae*) et 3 plantes monocotylédones (*Stipagrostis pungens, Lygeum spartum *et* Stipa tenacissima*), collectées de zones arides d'Algérie (Bousâada). Une augmentation nette de la production de gaz est constatée en présence de PEG (P<0,05). Elle est particulièrement élevée pour* S. tenacissima *(+35,0%) et faible pour* L. spartum *(+1,5%). Dans le pool gazeux fermentaire, la production de méthane varie de 7,9 % pour* A. halimus *à 18,6% pour* L. spartum*. Son augmentation la plus élevée est notée pour* S. tenacissima *(+47,4%) et la plus faible pour* L. spartum *(+1,5%). En présence de PEG, la production de méthane est corrélée positivement avec la matière azotée totale (r = +0,78), en son absence la production est corrélée négativement avec les tanins condensés totaux (r = -0,88). La forte corrélation, (r = 0,89 ; P<0,01), observée entre les tanins condensés totaux et l'augmentation de la production de méthane, en réponse à l'addition du PEG, suggère que le test des tanins couplé à la production de gaz peut être un moyen efficace pour identifier les plantes possédant des activités anti-méthanogènes.*

Mots-clés. *Composition chimique – Plantes – Méthane – Rumen –Tanins.*

I – Introduction

Tannins are water-soluble polyphenolic substances that bind and precipitate proteins and can have both negative and positive effects to livestock consuming fodder and shrubs containing a

certain concentration of condensed tannins (Nelson *et al.*, 1995). At high levels, tannin cause over protection of protein resulting in a low utilization of nitrogen (Silanikove *et al.*, 1994), while at concentrations below 50 g kg^{-1} DM, condensed tannins may increase supply of protein to the small intestine (Miller *et al.*, 1995). Due to its property to bind with condensed tannins (CT), polyethylene glycol (PEG) has been used to measure and reduce the adverse effects of CT in ruminant diets (Silanikove *et al.*, 1994; Barry and McNabb, 1999; Jones and Palmer, 2000; McSweeney *et al.*, 2001).

Methane production from ruminants contributes to total global methane emissions, which is an important contributor to global warming (Lassey, 2007). In the recent years, scientists have intensified efforts to exploit plants, plant extracts and natural plant compounds as potential natural alternatives to antibiotic growth promoters for enhancement of livestock productivity while minimizing its environmental impact (Anuraga, 2009; Makkar *et al.*, 2007), including decreased methane production (Soliva *et al.*, 2008). There is a need to develop or identify *in vitro* methods to screen large numbers of plants in a short time and with limited resources.

The objective of the present work was to study the biological activity of tannins of different browse plants collected from an arid zone in Algeria on methane production when plant material was incubated with and without polyethylene glycol (PEG) using the *in vitro* gas production technique.

II – Materials and methods

Plant material was collected in Bousâada district, north central Algeria (N 35° 15.768', E 04° 13.885', 496–981 m altitude), in the Saharan Atlas region, at the northern edge of the Sahara Desert between the Atlas Mountains and the el-Hodna depression and salt lake. The area has a dry desert climate characterized by high temperatures (24 to 41°C) and scarce and erratic annual precipitations (350-700 mm). Selection of the species was based on the available information on their consumption by grazing small ruminants, and on their relative abundance in the area of study. Seven browse plant species were used in this study: four dicotyledon plants, namely *Atriplex halimus* L., *Artemisia campestris* L., *Artemisia herba-alba* Asso and *Calobota saharae* (Coss. & Durieu) Boatwr. & B.-E. van Wyk (formerly *Genista saharae* or *Spartidium saharae*), and three monocotyledon plants, namely *Stipagrostis pungens* (Desf.) De Winter (formerly *Aristida pungens*), *Lygeum spartum* Loefl. ex L. and *Stipa tenacissima* L. Samples were collected in June 2009 (dry season), when plants were at a flowering (*A. halimus* and *L. spartum*) or at a mature stage (the rest of species) and they may be more important for grazing animals. Between six and ten specimens of each plant species were sampled to obtain a representative aliquot of the edible biomass, taken to the laboratory, pooled, oven-dried at 50 ºC (Makkar, 2003), and ground to pass a 1 mm screen.

Chemical composition of the plant material and the corresponding chemical analysis, especially those regarding tannins content (Makkar *et al.*, 1993; Makkar, 2003) are thoroughly described in Boufennara *et al.* (2012).

Six mature Merino sheep (body weight 49.4 ± 4.23 kg) fitted with a permanent ruminal cannula were used as donors of rumen fluid. Animals were fed lucerne hay ad libitum (167 g CP, 502 g NDF, 355 g ADF and 71 g ADL /kg DM) and had free access to water and mineral/vitamin block. Samples of rumen contents were withdrawn prior to morning feeding, transferred into thermos flasks and taken immediately to the laboratory, where rumen fluid was strained through four layers of cheesecloth and kept at 39 ºC under a constant flow of CO_2.

Gas production was measured using an adaptation of the technique described by Theodorou *et al.* (1994). Ground samples (500 mg) with and without the addition of polyethylene glycol (500 mg) were incubated in 50 ml of diluted rumen fluid (10 mL mixed rumen fluid + 40 mL medium (Theodorou *et al.*, 1994) prepared under a CO_2 atmosphere) in 120 mL serum bottles. Incubations were performed using two different inocula (rumen fluid from three sheep) giving

two replicates per treatment. Blanks (bottles containing only diluted rumen fluid) were used to compensate for gas production in the absence of substrate. All the bottles were closed with rubber stoppers, crimped with aluminium seals, shaken and placed in an incubator at 39ºC. The volume of gas produced in each bottle was recorded after 24 h of incubation, using a pressure transducer (Delta Ohm DTP704-2BGI, Herter Instruments SL, Barcelona) and a gas sample (10 ml) was taken into vacuum tubes and stored until analyzed for methane (CH_4) concentration. Methane was determined by gas chromatography (García-González *et al.*, 2008), each gas sample (0.5 ml) being manually injected (Pressure-Lok® syringes A-2 Series, Supelco, USA).

One way analysis of variance (Steel and Torrie, 1980) was performed on methane and gas production data, with browse species as the only source of variation (fixed effect) and source of inoculum (random effect) as a blocking factor. Multiple comparisons were done using LSD test. Pearson linear correlation coefficients were determined pair-wise between the variables studied. Analysis of variance and correlation were performed using the GLM and CORR procedures of the SAS software package (SAS Institute, 2008), respectively.

III – Results and discussion

Chemical composition and tannin composition of the plant material collected from the different species is shown in Table 1. Protein content in dicotyledon species ranged widely from 110 to 154 g/kg DM and was always greater than in monocotyledon grasses. The highest contents of total extractable tannins were observed in the Asteraceae *Artemisia* spp., whereas grasses, *A. halimus* and leguminous plants showed lower concentrations. Total condensed tannins varied widely among species, being highest in *S. tenacissima* and lowest for *A. halimus*.

Table 1. Chemical composition (g/kg DM) and phenolic compounds (g/kg DM, standard equivalent) of Algerian forages

Plant family	Plant species	NDF	ADF	CP	TET	TFC	TCT
Dicotyledons							
Chenopodiaceae	*A. halimus*	360	181	153.6	8.4	42.1	69.1
Asteraceae	*A. campestris*	330	212	115.0	57.1	62.7	114.3
	A. herba-alba	378	258	123.9	36.4	80.6	118.8
Fabaceae - Leguminosae	*C. saharae*	574	427	109.8	9.6	76.4	109.7
Monocotyledons							
Poaceae - Gramineae	*S. pungens*	771	425	95.2	4.8	46.5	78.7
	L. spartum	801	535	72.7	11.1	77.2	102.4
	S. tenacissima	793	476	74.6	3.8	165.5	213.9

NDF, neutral detergent fibre expressed with residual ash; ADF, acid detergent fibre expressed with residual ash; CP, crude protein; TET, total extractable tannins; FCT, free condensed tannins; TCT, total condensed tannins.

Data of gas and methane production of the studied plants without and with the addition of PEG, as well as the percentage of increment in response to PEG addition are shown in Table 2. The increase in gas production upon the addition of PEG, compared with that without PEG, for the browse species varied widely ($P<0.05$), being particularly high in *S. tenacissima* (+35.0%) and low in *L. spartum* (+1.5%). The percentage increase in gas production as result of the blocking effect of PEG on tannins would be an indicator of the biological activity of tannins on rumen microbial fermentation. Improved gas production due to addition of PEG to in vitro fermentation systems indicates a depressing effect of tannins on degradation of feed nutrients in the rumen

and, probably, their poor ruminal digestibility. In fact, PEG binds tannins, inactivates tannin anti-nutritive activity, and recovers feed nutrients bound by tannins (Canbolat *et al.*, 2005; Rubanza *et al.*, 2005). Based on this analysis, the species with a highest tannin anti-nutritional activity would be *S. tenacissima*.

Table 2. Gas and methane production *in vitro* (mmol/g DM) of Algerian forages with (+PEG) and without (-PEG) addition of PEG and percentage of increase (% incr., +PEG/-PEG)

Family	Susbtrate	Gas production			Methane production		
		-PEG	+PEG	% incr.	-PEG	+PEG	% incr.
Dicotyledons							
Chenopodiaceae	*A. halimus*	4.23^{b}	4.65^{c}	10.13^{bc}	0.86^{a}	0.89^{a}	3.96^{c}
Asteraceae	*A. campestris*	5.39^{a}	5.73^{a}	6.25^{cd}	0.76^{b}	0.80^{b}	5.43^{c}
	A. herba-alba	3.64^{c}	4.41^{c}	21.52^{ab}	0.62^{d}	0.75^{c}	20.23^{b}
Fabaceae- Leguminosae	*C. saharae*	5.18^{a}	5.43^{b}	4.74^{cd}	0.80^{b}	0.83^{ab}	4.42^{c}
Monocotyledons							
Poaceae - Gramineae	*S. pungens*	2.76^{d}	3.26^{d}	18.18^{bc}	0.62^{d}	0.69^{d}	12.30^{c}
	L. spartum	4.37^{b}	4.47^{c}	1.49^{d}	0.68^{c}	0.69^{d}	1.49^{c}
	S. tenacissima	2.32^{e}	3.13^{d}	35.00^{a}	0.30^{e}	0.44^{e}	47.41^{a}
R.S.D.†		0.140	0.117	6.071	0.019	0.017	4.652

†Residual standard deviation.
$^{a, b, c, d, e}$ Different superscripts in the same column indicate significant differences (P<0.05).

The higher increase in methane production in response to PEG addition was noted for *S. tenacissima* (+47.4%) and the lower increase value was observed for *L. spartum* (+1.5%) (P<0.05). The variations in methane concentration support the view that tannins decrease methane production. Other dietary components such as NDF also contributed to explaining total variation in methane production. Higher NDF increases methane production by shifting short chain fatty acid proportion towards acetate which produces more hydrogen.

Contrary to the work of Anuraga *et al*. (2009), in the present study we observed a strong positive and significant correlation between total CT and methane increase in response to the addition of PEG (r=0.88; P<0.05). This result suggests a role of total CT in methane reduction and that they seem to be useful to identify plants possessing antimethanogenic activity However, it must be taken into account that although amongst the tannin assays, determination of total CT is relatively simple and can be done using routine laboratory equipment, all chemical tannin assays measure tannins under conditions (i.e., temperature, pH, ionic strength) that differ from those of the rumen. Therefore, the results have limited applicability for predicting the activity of tannins. Nevertheless, PEG is considered to specifically bind tannins, and its use in the *in vitro* rumen assay is a better representation of tannin activity under rumen conditions.

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Blood parameters and feed intake in pregnant and lactating Barbarine ewes subjected to water deprivation

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Abstract. The effect of water restriction was assessed in Barbarine ewes: 24 adult ewes in the last 10 days of pregnancy were divided into two groups. Control ewes (C) had free access to water while deprived ewes (D) were *ad libitum* watered every 3 days during the last 10 days of pregnancy and the first 60 days of suckling. Body weight and score were measured every week, and feed and water intake were recorded daily for each animal. Venous blood was sampled during pregnancy, 2, 4 and 6 weeks of lactation and analyzed for electrolytes, glucose, urea, creatinine, total protein, triglycerides and cholesterol. A decrease in body weight was recorded at the end of the experiment in all ewes. However, the weight loss was significantly ($P<0.01$) greater in water-deprived animals as compared to the control. Feed intake was not affected by the treatment. Three days water deprivation induce changes in metabolism reactions which affected significantly ($P<0.01$) the level of the majority of electrolytes and metabolites measurers..

Keywords. Dehydration – Pregnancy – Lactation – Electrolytes – Metabolic – Sheep.

Métabolites sanguins et ingestion chez les brebis de race Barbarine en gestation ou allaitantes soumises à une privation d'eau

Résumé. *L'effet de la privation est évalué chez des brebis de race Barbarine: 24 brebis adultes gestantes (dans les derniers 10 jours de gestation) sont divisées en 2 lots. Un groupe témoin (C) qui a reçu de l'eau à volonté, et un groupe expérimental (D) qui n'a accès à l'eau qu'une fois tous les 3 jours. Le poids vif moyen a été mesuré chaque semaine et la prise alimentaire et de l'eau ont été enregistrées quotidiennement pour chaque animal. Les prélèvements sanguins sont effectués à partir de la veine jugulaire pendant la fin de la gestation, à 2, 4 et 6 semaines de lactation, pour analyse biochimique des électrolytes, glucose, urée, créatinine, protéines totales, triglycérides, cholestérol. Une diminution du poids corporel a été enregistrée à la fin de l'expérience. Cependant, la perte de poids était significativement (P <0,01) plus élevée chez les animaux du lot privé d'eau par rapport au lot témoin. La consommation d'aliments n'a pas été affectée par le traitement. La privation de 3 jours d'eau affecte significativement (P<0,01) la majorité des métabolites et des électrolytes mesurés*

Mots-clés. *Déshydratation – Fin de gestation – Lactation – Electrolytes – Métabolites – Brebis Barbarine.*

I – Introduction

Breeds of ruminants native to arid lands are able to withstand prolonged periods of water deprivation and graze far away from watering sites, sometimes 50 km or more far apart (Bayer and Feldmann, 2003). Within this context the largest population of the Barbarine sheep is raised in the Center and the South of Tunisia, a region that is characterized by arid conditions. Therefore, animals have to tolerate a stress environment due to high temperatures, and low food and water availability, especially during the dry season. Water deprivation was found to induce weight loss, hemoconcentration, increased serum protein, urea, creatinine, cholesterol and electrolytes concentration in sheep (Hamadeh *et al.*, 2006). On the other hand, the ewe's physiological status affects its biochemical responses to water deprivation (Aganga *et al.*, 1989). The process of milk synthesis imposes changes on almost all aspects of metabolism

(Annison *et al.*, 1984). The objective of this study is to determine whether water deprivation would change metabolic rate during late pregnancy and lactation in Barbarine ewes.

II – Materials and methods

The experiment was conducted in the experimental station of Bourbia of INRAT, Tunisia, from 10 December to 1 March. Twenty four adult ewes were divided into two groups balanced for age and weight. They were subjected to one of two treatments, Treatment C receiving water daily and Treatment D receiving water once every three days. Water restriction in Group D was imposed gradually first for one day, then for 2 days and thereafter for 3 days. Experimental ewes were kept in a single barn. The animals were fed a mixture of straw (1 kg/ewe/day) and barley (400 g/ewe/day) divided into two daily meals. Weight was recorded every 10 days. Throughout the experiment we recorded a daily feed and water intake for each animal. Blood samples were taken, every week, in the 3rd day of deprivation before watering and feeding, to determine plasma concentrations of glucose, urea, creatinine, total protein, cholesterol, triglycerides, sodium, potassium, calcium, magnesium, and phosphorus. Blood samples were collected in 10-ml vacuum tubes and centrifuged for 15 min at 3500 rpm. Blood metabolites and electrolytes were determined using electrolyte analyzer Genuis (Model GE200B; N.C: 300401101712). Data were subjected to statistical analysis using the ANOVA procedure of SAS program (SAS, 2005) with the water regime and time of measure as a source of variation.

III – Results and discussion

All the experimental animals lost weight by the end of the experiment. However, the weight loss was significantly greater in water-deprived animals as compared to the control, and in lactation stage as compared to the pregnancy (Table 1). this is thought to be due to deficient body liquids because water is requested for a correct rumination and digestion and the strong mobilization of fat for milk synthesis process. Li *et al*. (2000) found a decrease in body weight in winter (7%) and summer (11%) in sheep watered only in the evening (20:00 p.m.) as observed also by Cole (1995) in lactating sheep. Concerning food consumption, our results are in agreement with Mengistu *et al*. (2004) who did not find significant effects of water restriction on feed intake and that the loss of body weight is greatly influenced by the environmental temperature.

Table 1. Body weight and feed intake change (means±SD) under water deprivation on pregnant and lactating Barbarine ewes

Parameters	Treatment		Significance
	Once per 3 days watering	Daily watering	
Body weight (kg)			
Late pregnancy	60.17±7.59	58.46±5,37	ns
From birth up to 60 d in milk?	45.83±5.53	38.63±3.02	**
Feed intake (g)			
Last pregnancy	726.93±121.89	736.51±100.50	ns
From birth up to 60 d in milk?	1221.24±67.77	1203.06±76.61	ns

(ns) No Significant; **P<0.01.

Water deprivation had no significant effects on the blood levels of glucose. Hamadeh *et al.*, (2006) reported similar findings with respect to water restriction. The change in total protein, urea, creatinine, cholesterol, triglycerides, sodium and potassium was due to water deprivation because it causes hemoconcentration phenomena as result of a lower blood water level

(Casamassima *et al.*, 2008).Total protein concentration that often used to assess the level of hydration of the animal (Cork and Halliwell, 2002) was significantly affected by the treatment (P<0.001). In the study with Awassi sheep, Hamadeh *et al*., (2006) obtained the same results. Creatinine level, that was considered an indicator of reduced glomerular filtration rat (Keenan and Allardyce 1986), increased significantly under water deprivation hanging above the lactation. Similarly that Igbokwe, (1993) and Casamassima *et al.,* (2008) founded in different breeds. Contrarily results were previously reported in Aganga *et al.* (1989) obtained with Yankasa sheep. El-Shrif and Assad (2001) observed a sharp decrease in urea and creatinine in lactating ewes after parturition to approach those of the dry animals by the end of the first month. Water deprivation and lactation provoked a significant increase in cholesterol and triglycerides concentration as result of fat mobilization (Igbokwe, 1993). Similar results were detected by Hamadeh *et al.* (2006) in Awassi sheep.

Table 2. Metabolic changes provoked by water deprivation on pregnant and lactating Barbarine ewes

Parameters	Glucose (mmol/L)		Urea (mmol/L)		Creatinine (mmol/L)		Protein (g/L)		Triglyceride (mmol/L)		Cholesterol (mmol/L)	
Treatment	C	D	C	D	C	D	C	D	C	D	C	D
Pregnancy	2.80	2.90	9.24	9.08	87.80	82.00	74.00	67.80	0.16	0.16	1.62	1.60
Lactation	3.21	3.17	7.97	7.85	95.30	90.70	70.50	66.50	0.19	0.17	1.84	1.65
SE (p)	0.023	ns	0.079	ns	1.298	**	0.796	***	0.001	***	0.021	**

(ns) No Significant; (*)P < 0.05; (**) P < 0.01; (***) P < 0.001; SE (p): Standard error and significance.

Table 3. Electrolytes changes provoked by water deprivation on pregnant and lactating Barbarin ewes

Parameters	NA^+ (mmol/L)		K^+ (mmol/L)		Ca^{++} (mmol/L)		Mg^{++} (mmol/L)		P (mmol/L)	
Treatment	C	D	C	D	C	D	C	D	C	D
Pregnancy	138.20	136.60	4.00	3.99	1.74	2.04	0.62	0.69	1.45	1.22
Lactation	144.10	140.80	4.20	4.10	1.69	1.82	0.66	0.67	1.85	1.62
SE (p)	0.808	*	0.062	ns	0.043	**	0.009	**	0.018	***

(ns) No Significant; (*)P < 0.05; (**) P < 0.01; (***) P < 0.001; SE (p): Standard error and significance.

The effects of lactation on electrolytes reflect the mineral needs for milk production. Milk contains large amounts of Ca^{++} and K^+ (Collier, 1985) that would explain their decrease in plasma concentrations under lactation. Although Na^+ concentration is low in milk, the Na^+ requirements increase during lactation due to increased nutrient transport (Collier, 1985). On the other hand, concentration of plasma sodium was significantly higher under water deprivation according to results of Igbokwe (1993), while Ghosh *et al.* (1976) observed no significant effect of two-day water deprivation in Marwari sheep. This increase is probably the result of increased renal retention under the greater aldosterone activity. Water deprivation had no significant effects on the blood levels of K^+. Similar results are found By Igbokwe (1993) Hamadeh *et al*., (2006). However Aganga *et al.* (1989) indicated that plasma K^+ usually increases under dehydration in Yankasa sheep. Our result shown a significantly decrease in serum magnesium in the treated animals according Aganga *et al*. (1989). However, Parker *et al*. (2003) showed no effect of water deprivation on plasma magnesium.

IV – Conclusions

This study showed that during late autumn – winter, watering once every three days during late

pregnancy and lactation causes significant live weight loss especially in lactation and caused significant changes in many blood physiological indicators in lactating and pregnant Barbarine ewes. Despite these changes, we recorded a spectacular adaptive behavior which is developed by these ewes just after rehydration. Barbarine pregnant and lactating ewes can tolerate three successively days of water deprivation during 75 days between late pregnancy and the beginning of lactation without dramatic effect.

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