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

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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; $P < 0.05$).

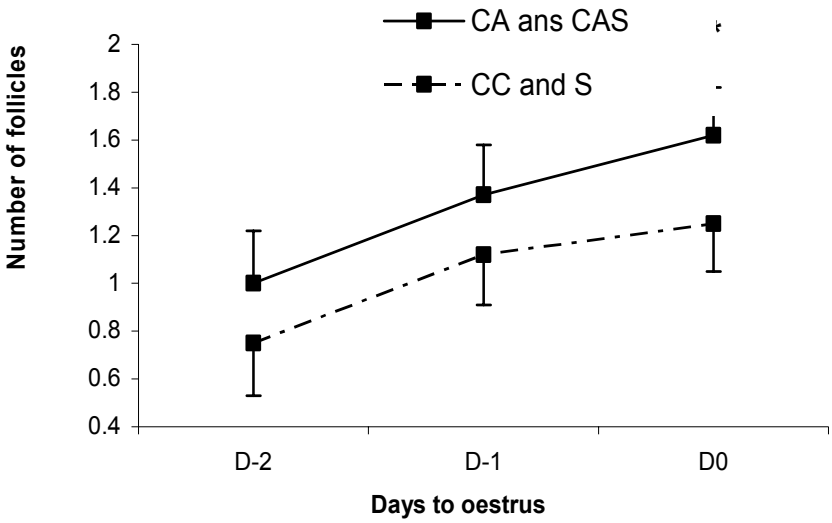

Fig. 1. Variation of the number of large follicle (≥6 mm) of ewes supplemented with cactus or cactus-soybean meal (CA & CAS) and concentrate or soybean meal (CC & S).

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.

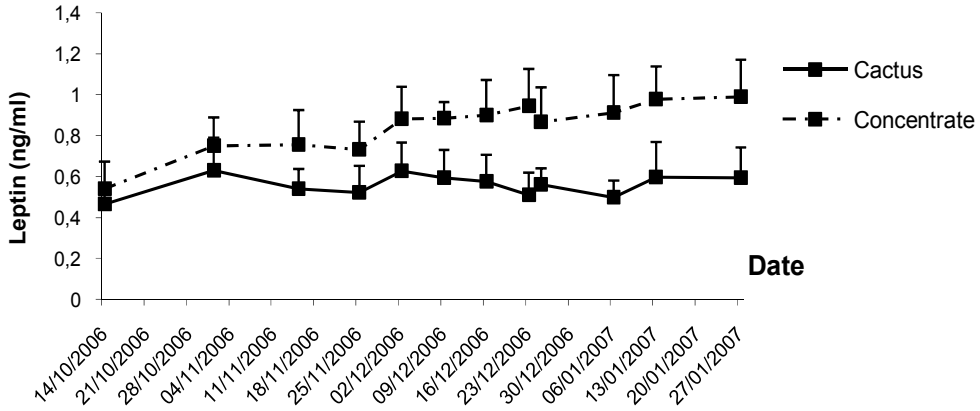

Fig. 2. Plasma leptin concentration for concentrate or cactus supplemented Barbarine rams.

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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References

Adamiak S.J., Mackie K., Watt R.G., Webb R. and Sinclair K.D., 2005. Impact of nutrition on oocyte quality: Cumulative effects of body composition and diet leading to hyperinsulinemia in cattle. In: *Biol. Reprod*., 73, p. 918-926.

AFRC, 1993. Requirements for metabolizable energy. In: G Alderman (ed.), *Energy and Protein Requirements of Ruminants*. An advisor manual prepared by the AFRC technical committee on responses to nutrients. CAB International, Wallingford, UK, p. 21-32.

Ayadi M.A., Abdelmaksoud W., Ennouri M. and Attia H., 2009. Cladodes from *Opuntia ficus indica* as a source of dietary fiber: effect on dough characteristics and cake making. In: *Industrial Crops and Products*, 30, p. 40-47.

Ben Salem, H., Nefzaoui A. and Ben Salem L., 2004. Spineless cactus (*Opuntia ficus indica f. inermis*) and oldman saltbush (Atriplex nummularia L.) as alternative supplements for growing Barbarine lambs given straw based diets. In: *Small Ruminant Research,* 51, p. 65-73.

Ben Salem H. and Smith T., 2008. Feeding strategies to increase small ruminant production in dry environments. In: *Small Ruminant Research,* 77, 174-194.

Blache D. and Martin G.B., 2009. Focus feeding to improve reproductive performance in male and female sheep and goats- How it works and strategies for using it. Nutritional and foraging ecology of sheep and goats. In: *Options méditerranéennes, A*, 85.

Ferguson E.M., Ashworth C.J., Edwards S.A., Hawkins N., Hepburn N. and Hunter M.G., 2003. Effect of different nutritional regimens before ovulation on plasma concentrations of metabolic and reproductive hormones and oocyte maturation in gilts. In: *Reproduction* 126, p. 61-71.

González-Bulnes A., Santiago-Moreno J., García-López M., Gómez-Brunet A. and López-Sebastian A., 1994. Observación del ovario en la oveja y eficacia en la deteccion de foliculos y cuerpos luteos mediante ecografia transrectal. In: *Investigacion Agraria*, 10, p. 319-329.

Martin G.B., Rodger J. and Blache D., 2004. Nutritional and environmental effects on reproduction in ruminants. In: *Reprod. Fertil. Develop*., 16, p. 491-501.

Rekik M., Ben Salem H., Lasoued N., Chalouati H. and Ben Salem I., 2010. Supplementation of Barbarine ewes with spineless cactus (*Opuntia ficus-indica f. inermis*) cladodes during late gestation-early suckling: effects on mammary secretions, blood metabolites, lamb growth and postpartum ovarian activity. In: *Small Ruminant Research*, 90, p. 53-57.

Rekik M., Gonzalez-Bulnes A., Lassoued N., Ben Salem H., Tounsi A. and Ben Salem I., 2012. The cactus effect: an alternative to the lupin effect for increasing ovulation rate in sheep reared in semi arid regions. In: *Journal of Animal Physiology and Animal Nutrition*, 96, p. 242-249.

Sakly C., Rekik M., Ben Salem I., Lassoued N., Mtaallah B., Kraiem K. and González-Bulnes A., 2012. Reproductive response of Barbarine ewes to supplementation with alternative feed prior to and during mating under semi-arid extensive conditions. In: *European Association of Animal Production Publication*, 131, p. 235-239.

Vinõles C., Meikle A. and Martin G.B., 2009. Short-term treatments grazing legumes or feeding concentrates increase prolificacy in Corriedale ewes. In: *Animal Reproduction Science,* 113, p. 82-92.