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Whole citrus fruits in total mixed rations for Mediterranean milking ewes. Milk production and composition

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Abstract. A total of 48 lactating ewes, belonging to 2 different Mediterranean breeds –Guirra (rustic native breed) and Manchega (mixed aptitude breed)– were used to evaluate the effect of the inclusion of whole citrus fruits (WCF) in their rations. Four iso-energetic and iso-protein total mixed rations were formulated containing fresh WCF at 0, 10, 20 and 30% replacing dry-rolled barley and pelleted beet pulp on a DM basis. The milk yield and composition were obtained once a week at morning and afternoon milking. Total milk yield values observed for 30% WCF group were 12% higher than for the others. Also, a reduction of the milk fat content with the inclusion of WCF was observed, being 8.2, 7.95, 7.69 and 7.1% for 0, 10, 20 and 30% WCF groups (P<0.05). Milk protein content was maximum for ewes on 10% WFC ration (6.4%) and minimum for 30% WCF group (6.0%; P<0.05), due to the lower milk casein content of 30% WCF group (-0.3% respect to 0 and 10% WCF groups; P<0.05), while soluble protein content was similar for all the groups (mean: 1.17%). Throughout the experimental period, a reduction of 6% fat corrected milk (6%FCM) production was observed with all the rations (-35 ml per week), but it was steeper for 20 and 30% WCF groups than for the other 2 groups. In conclusion, WCF could contribute to diminishing dependence of high-milking ewes on grains without affecting the milk output, however further studies related to WCF effect on body condition and on the capacity of lactating animals to mobilize body reserves should be made.

Keywords. Citrus – By-products – Lactating ewes – Milk production – Milk fat – Milk protein – Cereals – Beet pulp.

Agrumes entiers dans les rations totales mélangeés pour brebis laitières méditerranéennes. Production et composition du lait

Résumé. *Dans cette étude, 48 brebis en lactation, appartenant à 2 races méditerranéennes, la race Guirra (race autochtone rustique) ou la race Manchega (race mixte) ont été utilisées pour évaluer l'effet de l'inclusion de fruits d'agrumes entiers (WCF) dans leurs rations. Quatre rations complètes ont été formulées pour être iso-énergétiques et iso-azotées. Elles se différenciaient par le taux d'incorporation de WCF (0, 10, 20 et 30%) qui était substitué, sur une base matière sèche, à de l'orge concassée ou à de la pulpe de betterave en pellets. La production et la composition du lait ont été mesurées une fois par semaine sur deux traites successives (matin et soir). La production de lait obtenue avec le group recevant 30% WCF a été supérieure de 12% à celle des autres groupes. De plus, on a observé une réduction du taux butyrique avec l'inclusion de WCF. Elle était de 8,2; 7,95; 7,69 and 7,1 pour les groups recevant respectivement 0, 10, 20 et 30% de WCF (P<0,05). La teneur en protéines du lait a été maximale avec le group recevant 10% WCF (6,4%) et minimale avec celui ayant 30%WCF (6,0% ; P<0,05), car la teneur en caséine du lait a été la plus faible avec le groupe recevant 30%WCF (-0,3% par rapport aux groupes avec 0 et 10% de WCF; P< 0,05), tandis que la teneur en protéines solubles a été similaire pour tous les groupes (moyenne: 1,17%). Tout au long de la période expérimentale, on a observé une diminution de la production de lait standard (6%FCM) avec toutes les rations (-35% par semaine), mais il a été plus prononcé pour les groupes recevant 20% et 30% WCF que pour les autres. En conclusion, l'incorporation de WCF pourrait contribuer à diminuer la dépendance vis à vis des céréales pour les brebis à haute production sans affecter les paramètres de production laitière ; mais d'autres études relatives aux effets de WCF sur la condition corporelle et sur la capacité des animaux en lactation à mobiliser leurs réserves doivent être faites.*

Mots-clés. *Sous-produits – Agrumes – Brebis laitières – Production de lait – Taux butyreux – Taux azoté – Céréales – Pulpe de betteraves.*

I – Introduction

Mediterranean countries are responsible for 24% of World citrus production, generating many tonnes of residue in high citrus production areas. Feeding citrus surplus to small ruminants, characteristic livestock of the Mediterranean area, could be a practice that would diminish dependence on grains and contribute to reducing the environmental problems linked to their elimination.

The most frequently used citrus by-product in feeding small ruminants is citrus pulp obtained from the juice industry, being ensiled or dry commercialised. Recently Piquer *et al*. (2009a) and Piquer et. al. (2009b) suggested a different rumen fermentative behaviour for the whole citrus fruits (WCF) coming from unmarketable fruits and surplus compared with ensiled citrus pulp (ECP).

Feed of Mediterranean livestock, as could be Manchega and Guirra ewes, has traditionally been complemented with the wide range of agro-industrial by-products of this typically agricultural area, including WCF, although, to the authors' knowledge, no references about the effect of their use on milk yield and composition of the animals frequently given these products are available. So, the aim of the present work was to evaluate the possible effect of partial or total substitution of cereal grain and sugar beet pulp by WCF in total mixed rations (TMR, described as a mixture of both roughage and processed ingredients, formulated and mixed to supply the livestock requirements, in a form that precludes selection) on the milk yield, composition and somatic cell count (SCC) of milking Manchega and Guirra ewes.

II – Material and methods

1. Whole citrus fruits and experimental rations

The genus *Citrus* includes several fruits (oranges, tangerines, lemons, grapefruits…). In the present experiment, a variety of "Clementines" (*Citrus clementina*) was used, usually classified as a mandarin sort, called "Clemenules". The fruits had a high amount of water (843 g/kg), their dry matter (DM) being rich in soluble sugars (529 g/kg DM) and high fermentative fibres (266 g hemicelluloses + pectin/kg DM), but poor in proteins (72 g/kg DM). This composition permits the fruits to show a similar estimated net energy for lactation as main cereal grains used in ruminant feeding (1.25 UFL per kg DM).

Experimental rations were diets containing fresh WCF at 0, 10, 20 and 30% replacing dry-rolled barley and pelleted beet pulp on a DM basis (Table 1).

Rations were formulated to be iso-energetic (approx. 2.1 UFL/kg DM), presenting a similar non-fibrous carbohydrates content (181 g NFC/kg DM) throughout the substitution of starch for soluble sugars, and iso-protein (approx. 250 g PDI/kg DM), by means of a slightly greater inclusion of soybean meal as the WCF level increased. The four rations presented the same amount of forage (1 and 0.26 kg/day of alfalfa hay and barley straw, respectively). Chemical analyses of ingredients and TMR were performed following the methods of the Association of Official Analytical Chemists (AOAC, 1991) for DM, ash, ether extract (EE), crude protein (CP) and crude fibre (CF), and of Van Soest *et al*.(1991) for neutral detergent (NDF), acid detergent (ADF) and lignin detergent (ADL) fibre fractions, with a thermostable amylase pre-treatment. Starch was hydrolysed by a two-step enzymatic procedure, using a thermostable amylase followed by amyloglucosidase (Tecator, application note 85/86), and the resulting glucose was measured by the hexokinase glucose-6-phosphate dehydrogenase/NADP system (Boheringer). Total soluble sugars were analysed using the Fehling reagent according to the method described by Matissek *et al*. (1998). Pectins were extracted from the citrus samples with ethanol (Yu *et al*., 1996), the galacturonic acid content of extracts being analysed by the m-hydroxydiphenyl method (Kintner and Van Buren, 1982), while the pectin content was calculated as the galacturonic acid content multiplied by 3 (Garleb *et al*., 1991). Non-fibrous carbohydrates (NFC) were calculated as the addition of starch and soluble sugars.

Table 1. Ingredients and nutrient composition of the experimental rations

	0% WCF	10% WCF	20% WCF	30% WCF
Ingredients (g DM/day)				
Dry-rolled barley grains	660	440	220	0
Dried pelleted beet pulp	22	134	67	0
Whole citrus fruits (WCF)	0	250	500	750
Soya ben meal (44%CP)	220	257	293	330
Alfalfa hay	1000	1000	1000	1000
Barley straw	260	260	260	260
Molasses	30	30	30	30
Vitamin/mineral mixture†	74	74	74	74
Chemical composition (g/kg day)				
Dry matter (DM; g/kg)	871	656	556	436
Ash	87.9	88.2	95.5	98.0
Crude protein (CP)	144	150	164	162
Ether extract (EE)	20	18	20	18
Neutral detergent fibre (NDF)	463	386	368	373
Acid detergent fibre (ADF)	260	220	214	235
Acid detergent lignin (ADL)	82	73	64	82
Starch (St)	152	103	53	1
Soluble sugars (SS)	29	78	128	180
Non-fibrous carbohydrates (NFC††)	181	181	181	181
Energy value (UFL††† kg-1 DM)	2.1	2.1	2.1	2.1
PDIE†††	252	252	250.9	250.7
PDIN†††	250.9	248.1	245	242.2

† Contains (g/kg): retinol, 0.2; cholecalciferol, 0.003; a-tocopherol, 0.27; $FeSO_4$ $7H_2O$, 1; $CuSO_4$ 5 H_2O, 0.17; ZnO, 1.5; $CoSO_4$ 7 H_2O, 0.03; MgO, 36.1; MnO, 0.67; KI, 0.04; Na_2SeO_3, 0.01; $CaCO_3$ $2H_2O$, 400; NaCl, 133; S, 6.7; antioxidant (BHT), 8.3.

†† NFC caldulated as St + SS content.

††† Estimated using equations of the Institute National de la Recherche Agronomique (1989).

2. Animals

Experimental procedures were approved by the Committee on Animal Use and Care at the Polytechnic University of Valencia, and follow the codes of practice for animals used in experimental works proposed by the EU (2003).

A total of 48 lactating ewes in their first (n=14) or second lactation (n=34) from the Animal Science Department of the Polytechnic University of Valencia (Spain) were used in the present trial. The animals belonged to 2 different Mediterranean breeds, Guirra (n=24) –rustic native breed of medium size– and Manchega (n=24) ewes –mixed aptitude breed of greater size– with a balanced distribution of breeds in the first and second lactation. After parturition, a 42 day rearing period was allowed. After weaning, ewes were milked twice daily (08:30 and 17:30 h) with a milking machine. Weeks 7 and 8 after parturition were used as a 2-week pre-experimental period. After this time, the ewes were split into 4 groups of 12 animals equally balanced by breed, parturition number and productive level during the pre-experimental period, the body condition score of the ewes being similar (approx. BCS=3). Until this point, all the animals received the same ration for lactating ewes similar to 0% WCF ration. An additional adaptation period to the experimental ration of 1 week was allowed before data and sample collection (week 9). Data and sample acquisition were obtained once a week (Wednesday) at morning (08:30 h) and afternoon (17:30 h) milking.

At these times, milk yield was recorded and milk samples were collected for composition analysis. Experimental rations were offered twice daily (10:00 and 17:00 h). Milk samples were automatically analysed (MilkoScan FT120, A/S Foss Electric, Hillerød, Denmark) for total solids (TS), fat, total protein, casein, soluble protein, lactose and useful dry extract (UDE) from samples obtained for each animal at each of the morning and afternoon milkings. As no different effect of the treatments between the morning and afternoon records were observed, statistical analysis was performed with the mean daily records.

3. Statistical analysis

The effect of the inclusion of WCF in the ration of Guirra and Manchega ewes on milk yield, composition was determined by the following statistical model: Yijklm = m + Trti +Brj + Wkk + NLl + Em(Trti × Brj × NLl) + Trti×Brj + Trti×Wkk+ Brj×Wkk + Trti×Brj×Wkk + cov + eijklm

where: Yijklm = dependent variable; μ = general mean; Trti = fixed effect of the experimental treatment (0, 10, 20 and 30% of WCF); Brj = fixed effect of the breed (Guirra or Manchega); Wkk = fixed repeated effect of the experimental week (2 to 9); NLl = fixed effect of the lactation number (1 or 2); Em(Trti × Brj × NLl) = random effect of the ewe nested within experimental treatment, breed and lactation number; cov = pre-experimental records of depend variables were included as a covariate; eijklm = residual error.

The statistical analyses were carried out with the Mixed Procedure from SAS program (SAS, 1996), following a repeated measures design that takes into account the variation between animals and covariation with them. All results presented in the tables are given as Least Square Means. Covariance structures of mixed procedure were objectively compared using the most severe criteria (Schwarz Bayesian criterion), as suggested by Littell *et al*. (1998). DM intake of ewes was not statistically analysed, as only mean daily intake of the group was controlled.

III – Results and discussion

Although the daily ration offered to each group was the same for the different dietary treatments (2.44 kg/DM day), the mean feed intake of groups were 2.07, 2.18, 2.13 and 2.15 kg DM/day for the 0, 10, 20 and 30% WCF rations respectively, the values obtained for the three WFC groups were 5% higher than for the non-WCF group. Generally, citrus by-product feedstuff does not seem to affect intake of diet for ruminants in which it is included (Bampidis and Robinson, 2006).

The effect of WCF inclusion in TMR of milking ewes on the daily milk production and composition is presented in the Table 2. Daily milk yield recorded at the morning milking was similar for the different experimental TMR (mean: 563 ml/day), but milk yield of ewes on the 30%WCF group was significantly higher (+49 ml/day; $P<0.05$) at the evening milking than those observed for the other groups. Total milk yield values observed for 30% WCF group were 12% higher than those controlled with the other groups. These differences were mainly due to Manchega ewes which showed a clearly greater milk yield with the 30% compared with 0% WCF ration (+192 ml/day; $P<0.05$). Although, Guirra ewes presented a similar total milk yield independently of the experimental ration. Most of the works, where dry or ensiled citrus pulps was included as partial or total replacement of cereal grains in the concentrates, reported a decrease on the values of the milk yield in cows (Broderick *et al*., 2002) and ewes (Fegeros *et al*., 1995; Volanis *et al*., 2004). This reduction in milk yield has been related to a dietary pectin/digestible carbohydrate rate rise that could induce an acetate/propionate rate increase (Broderick *et al*., 2002). However, results were to the contrary when citrus by-products rich on soluble sugars were used. Wing *et al*. (1988), evaluating the possible use of citrus molasses distillers solubles (rich in soluble sugars) to partially replace corn grain at 90 g/kg DM in Holstein cow TMR, reported increase of both DM intake and milk yield (+14% of total milk yield), without any negative effect on the milk composition and body weight of the cows.

Table 2. Effect of inclusion of fresh whole citrus fruits (WCF) in the ration of milking ewes on daily milk production and composition

	Experimental treatment LS means†				P-value††						
	0%WCF	10%WCF	20%WCF	30%WCF	Trt	Br	0 vs 30%	Wk	TrtxWk	TrtxBr	Cov
Daily milk yield (ml/day)											
Morning milking	552±51	552±36	542±46	604±46	0.30	0.02	0.21	<0.01	0.05	0.56	<0.01
Evening milking	307^{a}±29	297^{a}±20	298^{a}±26	350^{b}±26	0.03	<0.01	0.07	<0.01	0.12	0.37	<0.01
Total	849±76	820±52	839±67	935±68	0.12	<0.01	0.16	<0.01	0.16	0.48	<0.01
6% FCM†††	1117±98	1080±68	1051±87	1075±87	0.88	0.06	0.6	<0.01	<0.01	0.59	<0.01
Milk composition (%)											
Total solids	19.5^{b}±0.6	19.6^{b}±0.4	18.8^{ab}±0.5	18.3^{a}±0.5	0.01	0.4	0.01	<0.01	<0.01	0.09	<0.01
Fat	8.21^{b}±0.48	7.95^{b}±0.33	7.69^{ab}±0.44	7.18^{a}±0.44	0.03	0.42	<0.01	<0.01	<0.01	0.06	<0.01
Protein	6.21^{ab}±0.22	6.44 ^{b}±0.16	6.13^{a} ±0.2	6.00^{a} ±0.2	0.05	0.94	0.24	<0.01	0.03	0.14	<0.01
Casein	5.14^{b}±0.16	5.23^{c}±0.11	4.98^{ab}±0.14	4.90^{a} ±0.14	0.03	0.88	0.05	<0.01	<0.01	0.16	<0.01
Soluble protein	1.20±0.06	1.18±0.04	1.16±0.05	1.14±0.05	0.56	0.70	0.21	<0.01	0.02	0.17	<0.01
Lactose	4.45±0.09	4.47±0.06	4.40±0.08	4.51±0.08	0.25	0.91	0.36	<0.01	0.21	<0.01	<0.01
Useful dry extract††††	14.7^{b}±0.6	14.4^{b}±0.4	14.0^{ab}±0.6	13.4^{a} ±0.6	0.03	0.57	< 0.01	<0.01	<0.01	0.02	<0.01
BCS††††† at 17th wk of lactation	2.48±0.11	2.37±0.09	2.48±0.09	2.41±0.09	0.79	0.01				0.9	

† Treatments were whole citrus fruits (WCF) included as 0, 10, 20 and 30% of the total mixed ration DM.

†† Trt: Treatment effect; 0 *vs* 30%: contrast of 0% versus 30% WCF; Wk: Effect of the experimental week; Trt x Wk: Interaction of the treatment with the experimental week; Trt x Br: Interaction of the treatment with the ewe breed (Guirra or Manchgea); Cov: Effect of the covariate (pre-experimetnal records).

††† 6% fat-corrected milk.

†††† Useful dry extract: calculated as the addition of milk protein and fat.

††††† Body condition score.

The fat content of milk was linearly (P<0.05) reduced by the inclusion of WCF in the ration. In the present trial, a direct substitution of barley grain and sugar beet pulp by WCF was performed (slightly corrected with soyabean meal), which mainly supposed chemical change of cereal starch for soluble sugars and hemicelluloses for pectin. Hall *et al*. (1998) observed that citrus pulp presented similar volatile fatty acid production, but lower acetate/propionate rate than the sugar beet pulp (1.16 *vs* 1.51, respectively) after 24h *in vitro* fermentation. These results could support the increase in milk yield and the reduction in milk fat content observed in our experiment. In contrast, the whole substitution of the cereal grains by dry citrus pulp (Ben-Ghedalia *et al*., 1989) or WCF (Piquer *et al*., 2009a) in the diets of sheep have both been related with a clear acetate/propionate rate increase (from 3.5 to 4.1 or 4.8, respectively) and a total volatile fatty acid reduction (10 and 11%, respectively) in the ruminal liquor measured at different intervals of the day. However, these differences in VFA production and profiles observed seem to be related to differences in the rate and pattern of fermentation. NFC of the experimental diets was the same (181 g/kg DM) but, while soluble sugars are quickly and completely fermented in the rumen (Piquer *et al*., 2009b), an important part of the starch (depending on the type and level of cereal and their processing; Waldo, 1973) leaks from the stomachs and is digested at intestinal level, e.g. Ben-Ghedalia *et al*. (1989) observed that 20% of the barley starch leaks from the rumen fermentation.

Milk protein content was maximum for ewes on 10% WFC ration (6.4%) and minimum for 30% WCF group (6.0%; P<0.05), due to the lower milk casein content of ewes given 30% WCF ration (-0.3% compared with 0 and 10% WCF groups; P<0.05), while soluble protein content was similarly maintained for all the groups (mean: 1.17%). The introduction of citrus by-products feedstuff in the TMR of ruminants has not been frequently related to milk protein content changes in cows nor ewes, and when it was reported the reduction was very low (-1 g/kg; Broderick *et al*., 2002; Volanis *et al*., 2004) as in the present work (-2 g/kg between 0 and 30% WCF groups). In any case, the values for total milk protein yield were instead greater for the 30% WCF group (52.3, 52.8, 51.4 and 56.1 g of milk protein per day for 0, 10, 20 and 30% WCF groups, respectively; P<0.05).

The lactose content of the milk was not significantly affected by the type of ration. UDE (P<0.05) and TS content (P<0.01) of the milk were significantly reduced by the WCF inclusion in the ration.

As a consequence of the milk yield and composition changes, mean 6% fat-corrected milk (6%FCM) was similar independently of the experimental ration used (mean: 1080 ml/day). However there are a breed effect and values for 6% FCM were higher for Manchega than for Guirra ewes (+9%; P=0.06). This could be due by the higher daily milk potential of Manchega ewes, in fact most Spanish ewes breeds (including Manchega) have traditionally been classified as animals with medium or medium-high dairy potential, although Rodríguez *et al*. (2005) classified Guirra ewes as a breed with medium or medium-low dairy potential.

The dietary treatment did not affect the body condition score of the ewes at the end of the experiment (BDS=2.44), but the body condition score of Manchega ewes was significantly higher than that observed for Guirra ewes at the end of the experiment (+0.27 point; P<0.01).

As mentioned above, a non-negligible amount of starch leaked into the rumen, supposing an interesting glucose contribution for the animal at intestinal level. Therefore, lower 6%FCM reduction of Guirra ewes with high WCF rations could be an answer to their lower glucose contribution, reducing the fat content of the milk to avoid body condition deterioration. Guirra breed is characterised by greater rusticity and lower body weight and dairy potential than Manchega breed (Rodríquez *et al*., 2005), which could explain their lower BCS at the end of the experimental period. Van Horn *et al*. (1975) studying the effects of high corn grain and high dry citrus pulp TMR on lactating dairy cow performance and milk composition, observed that feed intake, milk yield and milk protein content were similar among treatments, but body weight was higher in cows fed the high corn diet at the en of the 84 day experiment.

IV – Conclusions

In conclusion, the use of WCF, rich on soluble sugars, as an alternative to cereal grain and beet pulp in milking ewe rations, leads to different results to those observed for the most frequently used citrus by-product in small ruminants (ensiled citrus pulp). From a practical point of view, and considering the results obtained in the present work, WCF (at 20 to 30% DM) could contribute to diminishing dependence of high-milking ewes on grains, without affecting the milk output and reducing the ration cost in citrus production areas, but they must be included cautiously (no more than 10% DM) in low body condition animals.

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