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SUMMARY – The objective of this study was to evaluate the effects of partial replacement of conventional roughages with an ensiled-mixture made up of crude olive cake, orange pulp and wheat straw on fatty acid composition of ewe's milk. Thirty-six lactating Comisana ewes at about 150 days in milking, were divided into three homogenous groups (grass, G; silage 100, S100; and silage 50, S50) of 12 animals. Ewes of group G were allowed to graze pasture during the day and received hay plus concentrate on their return to the stall. The other two experimental groups were fed a mixed-diet containing silage, hay and concentrate. The silage was a mixture (% as fed) of 30% crude olive cake, 65% orange pulp and 5% wheat straw. The two silages differed only in the level of stone removal from the olive cake (100% in S100 and 50% in S50). The trial lasted 70 days preceded by a 21-day adaptation period to the experimental diets. Every 14 days individual milk samples of the complete morning milking were collected and analysed for fatty acid composition. In milk from S100 and S50 groups, the C18:0, C18:1 t9, C18:1 c9, C18:1 t11, C18:2 t9-c12, C20:0 and C20:3 n6 fatty acids increased significantly, while C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C14:1, C15:0, C16:0, C18:2 c10-t12, C18:2 c9-t11 (CLA) and C18:3 n3 fatty acids decreased significantly in comparison to the G group. This trend has determined a significant decrease of the short (C6-C11) and medium-chain (C12-C17) fatty acids, an increase in UFA, in particular MUFA, and an improvement of the UFA/SFA ratio in milk of the S100 and S50 groups. The n-6 PUFA content was significantly higher in the milk of the S100 and S50 groups than in G group, while the n-3 PUFA one was significantly higher in the milk of group G. As a consequence, n-6/n-3 PUFA ratio was significantly higher in milk from S100 and S50 groups in comparison to G group.

Keywords: Ewes, olive cake, orange pulp, fatty acid, wheat straw, silage.

RESUME – *"Effets de l'ensilage de tourteau d'olive, pulpe d'agrumes et paille de blé sur la composition en acides gras du lait de brebis Comisana". L'objectif de cette étude était d'évaluer les effets du remplacement partiel de fourrages conventionnels par un mélange ensilé formé de tourteau d'olive brut, pulpe d'oranges et paille de blé sur la composition en acides gras du lait de brebis. Trente-six brebis Comisana en lactation à environ 150 jours de traite ont été divisées en trois groupes homogènes de 12 animaux (herbe, G ; ensilage 100, S100; et ensilage 50, S50). Les brebis du groupe G pouvaient pâturer pendant la journée et recevaient du foin et concentré lorsqu'elles revenaient à la bergerie. Les deux autres groupes expérimentaux recevaient une alimentation mixte contenant de l'ensilage, du foin et du concentré. L'ensilage était un mélange (% tel que distribué) de 30% de tourteau d'olive brut, 65% de pulpe d'oranges et 5% de paille de blé. Les deux ensilages différaient uniquement par le niveau d'élimination des noyaux du tourteau d'olive (100% dans S100 et 50% dans S50). L'essai a duré 70 jours précédés d'une période d'adaptation de 21 jours aux régimes expérimentaux. Tous les 14 jours, des échantillons individuels de lait de la traite complète du matin étaient prélevés et analysés pour connaître la composition en acides gras. Dans le lait des groupes S100 et S50, les acides gras C18:0, C18:1 t9, C18:1 c9, C18:1 t11, C18:2 t9-c12, C20:0 et C20:3 n6 augmentaient significativement, tandis que les acides gras C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C14:1, C15:0, C16:0, C18:2 c10-t12, C18:2 c9-t11 (CLA) et C18:3 n3 baissaient significativement comparés au groupe G. Cette tendance a entraîné une diminution significative des acides gras à chaîne courte (C6-C11) et moyenne (C12-C17), une augmentation des UFA, en particulier MUFA, et une amélioration du rapport UFA/SFA dans le lait des groupes S100 et S50. La teneur en PUFA n-6 était significativement supérieure dans le lait des groupes S100 et S50 par rapport au groupe G, tandis que la teneur en PUFA n-3 était significativement supérieure dans le lait du groupe G. Comme conséquence, le rapport PUFA n-6/n-3 était significativement supérieur dans le lait des groupes S100 et S50 par rapport au groupe G.*

Mots-clés : *Brebis, tourteau d'olive, pulpe d'oranges, acides gras, paille de blé, ensilage.*

Introduction

The agro-industrial by-product are residual of the plant production and industrial processes. Owing to oil crisis and increase of production cost and, consequently, of raw materials, would be useful to exploit residual of the vegetable productions for animal nutrition.

Especially in the Mediterranean area, the agro-industrial by-products have a considerable importance for animal feeding, also considering the nutritional characteristics of the available forage resources, often obtained in unfavourable climatic conditions.

A rational use of these by-products leads to two important advantages: the first one of economic order, reducing feeding costs of the farm; the second of environmental order. Indeed, the use of by-product in animal feeding can reduce, in part, the problem of the impact on the ecosystem consequent to their disposal.

Another important aspect concerns the qualitative characteristics of by-products and the effects on meat or milk produced by animals eating them.

The use of olive cake for ewe feeding and its effect on milk fatty acid composition has been studied (Chiofalo *et al*., 2004). In the trial of Chiofalo *et al*. (2004), the ewes received a diet containing 20% of olive cake (partly destoned); the final result of this trial showed, mainly, a high level of monounsaturated fatty acid in milk of ewes that received a diet containing 20% of olive cake.

The objective of this experiment was to study the effect of using a silage made up only with by-products (olive cake, citrus pulp and wheat straw) on ewe milk fatty acid composition.

Materials and methods

Thirty-six lactating Comisana ewes at about 150 days in milking were divided into three homogenous groups (grass, G; silage 100, S100; and silage 50, S50) of 12 animals. Ewes of G group grazed a sward sown (the pasture was composed primarily of Leguminosae and Graminaceae species) for about 4 hours per day and received hay ad libitum plus 600 g/d concentrate on their return to the stall. The other two experimental groups were fed a mixed-diet containing silage, the same hay and the same concentrate. The silage was a mixture (% as fed) of 30% crude olive cake, 65% orange pulp and 5% wheat straw. The two silages differed only in the level of stones removal from the olive cake (100% in S100 and 50% in S50). The trial lasted 70 days preceded by a 21-day adaptation period to the experimental diets. Every 14 days individual milk samples of the complete morning milking were collected and subjected to fatty acid analyses.

Milk fatty acids were determined by gas-chromatograph as fatty acids methyl esters (FAMEs). Milk fatty acid composition was measured according to the modified procedure of Sukhija and Palmquist (1988) as described by Tice *et al*. (1994). Fatty acids were expressed as g/100 g of methyl esters.

Statistical analysis

A t-test was done to compare the effects of ewes production system on milk fatty acids. Single animals were considered as experimental units.

Results and discussion

Milk fatty acids composition is reported in Table 1. The short-chain saturated fatty acids caproic (C6:0), caprylic (C8:0) and caprinic (C10:0) were higher ($P<0.01$, $P<0.001$ and $P<0.001$ respectively) in the milk from ewes of the G group than from the other two groups. Moreover these fatty acids were significantly higher in the milk from ewes given silage 50 than in the milk from 100 group.

The saturated fatty acids (SFA) lauric (C12:0), myristic (C14:0) and palmitic (C16:0) were higher in the milk from animals of the G group ($P<0.001$, $P<0.001$ and $P<0.01$ respectively) than in the milk of two silage groups. Stearic (C18:0) acid was higher in both silage groups than in milk from G group. Moreover, this fatty acids was significantly higher in the milk from ewes given silage 100 than in the milk from ewes of silage 50 group.

Table 1. Effect of ewe feeding system on milk fatty acid composition (g/100g of methylesters)

	G	S100	S50	SEM	*P*-value
Caproic acid (C6:0)	3.27a	1.44b	2.60a	0,251	0.006
Caprylic acid (C8:0)	3.63a	1.09c	1.86b	0.232	0.000
Capric acid (C10:0)	12.48a	3.01c	4.65b	0.778	0.000
Undecanoic acid (C11:0)	0.75a	0.22b	0.37b	0.053	0.000
Lauric acid (C12:0)	7.22a	2.29b	2.99b	0.406	0.000
Tridecanoic acid (C13:0)	0.41a	0.15b	0.13b	0.028	0.000
Myristic acid (C14:0)	15.34a	8.51b	9.69b	0.598	0.000
Myristoleic acid (C14:1)	0.38a	0.25b	0.27b	0.019	0.009
Pentadecanoic acid (C15:0)	1.48a	0.7b	0.7b	0.072	0.000
Palmitic acid (C16:0)	26.41a	24.17b	23.8b	0.356	0.003
Palmitoleic acid (C16:1)	1.36	1.23	1.23	0.036	0.241
Heptadecanoic acid (C17:0)	0.46	0.50	0.45	0.026	0.724
cis-10-Heptadecenoic acid (C17:1)	0.28	0.27	0.25	0.011	0.591
Stearic acid (C18:0)	6.06c	12.12a	10.40b	0.547	0.000
Elaidic acid (C18:1n9t)	2.28b	3.75a	3.2a	0.195	0.004
Oleic acid (C18:1n9c)	13.44b	34.32a	31.74a	1.734	0.000
Vaccenic acid (C18:1n11t)	0.42b	0.98a	0.88a	0.056	0.000
Linolelaidic acid (C18:2n6t)	0.39	0.36	0.41	0.013	0.384
C18:2 t9-c12	0.24b	0.31a	0.35a	0.013	0.001
C18:2 c9-t12	0.49a	0.2b	0.18b	0.028	0.000
Linoleic acid (C18:2n6c)	1.24b	2.28a	2.12a	0.099	0.000
CLA c9-t11	0.24a	0.16b	0.10b	0.019	0.004
Arachidic acid (C20:0)	0.21b	0.43a	0.34a	0.024	0.000
g-Linolenic acid (C18:3n6)	0.14	0.08	0.18	0.019	0.111
cis-11-Eicosenoic acid (C20:1)	0.04	0.08	0.09	0.012	0.267
Linolenic acid (C18:3n3)	0.72a	0.37b	0.33b	0.043	0.000
cis-8,11,14-Eicosatrienoic acid (C20:3n6)	0.04b	0.10a	0.12a	0.012	0.016
cis-11,14,17-Eicosatrienoic acid (C20:3n3)	0.05	0.08	0.04	0.008	0.142
Arachidonic acid (C20:4n6)	0.25	0.31	0.25	0.019	0.386
cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3)	0.12	0.11	0.11	0.012	0.851
Lignoceric acid (C24:0)	0.07	0.07	0.09	0.012	0.660
cis-4,7,10,13,16,19-Docosahexaenoic acid (C22:6n3)	0.08	0.08	0.08	0.008	0.988
SFA	77.79a	54.7b	58.08b	1.933	0.000
PUFA (Polyunsaturated fatty acids)	4.00	4.43	4.26	0.119	0.340
MUFA (Monounsaturated fatty acids)	18.2b	40.87a	37.66a	1.883	0.000
w6 PUFA	2.79b	3.64a	3.61a	0.112	0.001
w3 PUFA	0.97a	0.63b	0.55b	0.048	0.000
w6/w3	3.26b	6.27a	6.69a	0.362	0.000
SFA/UFA	3.58a	1.22b	1.45b	0.198	0,000

a,b: values in the same row with different letters are significantly different.

Therefore, the use of citrus pulp, olive cake and wheat straw silage determined a significant decrease of the short and medium chain fatty acids (C6:0-C16:0) in ewes milk. The fatty acids that raise plasma cholesterol (in humans) are the saturated fatty acids (lauric, myristic and palmitic), and it is interesting to notice that these fatty acids are significantly lower in the two experimental groups.

Among the monounsaturated fatty acids, myristoleic (C14:1) acid was higher ($P<0.01$) in the milk from ewes of G group; no significant differences for palmitoleic acid (C16:1) has been observed.

Elaidic (C18:1 9-trans), oleic (C18:1 9-cis) and vaccenic (C18:1 11-trans) acids were higher in the milk from ewes of both silage groups ($P<0.01$, $P<0.001$ and $P<0.001$, respectively) in comparison to milk from grass fed ewes.

The level of MUFA was higher ($P<0.001$) in milk from ewes given silage than in milk of ewes from G group; oleic (C18:1 cis 9) acid represent the fatty acid that mainly influenced this value. This was strictly correlated with the high level of this fatty acid in the diet for silage groups (Table 2). Furthermore a part of oleic acid derived from the activity of Δ^9 desaturase on stearic acid (Uchiyama *et al*., 1967).

Table 2. Fatty acid profile (g/100g of methylesters) of feed consumed by ewes of the S50 and S100 groups

	D100	Unifeed D100
Palmitic acid (C16:0)	7.47	14.14
Heptadecanoic acid (C17:0)	3.19	3.37
Stearic acid (C18:0)	2.11	1.84
Oleic acid (C18:1n9c)	65.36	54.35
Vaccenic acid (C18:1n7t)	1.29	1.53
Linoleic acid (C18:2n6c)	12.80	17.22
g-Linolenic acid (C18:3n6)	1.92	1.20
Linolenic acid (C18:3n3)	5.86	6.36
SFA	12.77	19.35
PUFA	20.58	24.77
MUFA	66.65	55.88
w6 PUFA	14.72	18.42
w3 PUFA	5.86	6.36
w6/w3	2.51	2.90
SFA/USA	0.15	0.24
	D50	Unifeed D50
Palmitic acid (C16:0)	13.46	12.83
Heptadecanoic acid (C17:0)	0.70	3.41
Stearic acid (C18:0)	2.99	2.79
Oleic acid (C18:1n9c)	60.39	56.02
Vaccenic acid (C18:1n7t)	1.96	1.18
Linoleic acid (C18:2n6c)	16.05	16.52
g-Linolenic acid (C18:3n6)	1.81	1.38
Linolenic acid (C18:3n3)	2.63	5.86
SFA	17.16	19.03
PUFA	20.49	23.76
MUFA	62.35	57.21
w6 PUFA	17.86	17.90
w3 PUFA	2.63	5.86
w6/w3	6.78	3.05
SFA/USA	0.21	0.24

Integration of silage that included olive cake characterised by a high MUFA content, determined a significant increase of this type of fatty acid and decrease the saturated ones. As a consequence, increase the unsaturated/saturated ratio but without change in the long chain polyunsaturated fatty acid (PUFA) percentage, despite the control group and the two experimental groups showed significant differences in the rates of linoleic and linolenic acids. Indeed linoleic (C18:2 n-6) acid was higher (P<0.001) in milk from ewes of both silage groups, whereas linolenic (C18:3 n-3) acid was higher (P<0.001) in milk from ewes of the G group. This latter value was due to the different production system. In fact ewes of the G group were allowed to pasture and had fresh grass and they have a high concentration of linolenic (C18:3 n-3) acid, mainly with young pasture (Chilliard *et al*., 2001). The milk of ewes from G group was richer in n-3 PUFA (P<0.001) compared to milk from ewes of silage groups, whereas the rate of n-6 PUFA was higher (P<0.01) in the milk from ewes of S50 and S100 groups and consequently n-6/n-3 ratio was lower (P<0.001) in the milk from ewes allowed pasture.

In our trial there were no differences in milk fatty acid composition among the two silage groups.

Conclusions

The use of olive cake, citrus pulp and wheat straw silage in dairy ewes feeding reduced the level of milk saturated fatty acids that are considered to raise plasma cholesterol, and increase the rate of monounsaturated fatty acids, mainly oleic acid, which is interesting in human feeding for it beneficial effects on blood cholesterol and on health (Keys *et al*., 1965; Williams, 2000).

However, it will be important to study in depth regard it effect on the rate n-6/n-3.

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