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Fresh forage in dairy ass's ration: effect on milk fatty acid composition and flavours

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RIASSUNTO – Il foraggio fresco nella razione dell'asina in lattazione: effetto sulla composizione in acidi grassi e aromi del latte. *Lo studio degli effetti della base foraggiera su alcuni aspetti qualitativi del latte è stato condotto su 8 asine in lattazione, di razza Martina Franca, divise in due gruppi: CTR (fieno+mangime) e TRT (erba+mangime). La prova ha avuto una durata di 63 d. Sugli alimenti e sul latte sono stati determinati gli acidi grassi, mediante GC, e i flavours, mediante SPME-GC/MS. Nel latte le maggiori differenze sono state riscontrate: per i PUFA (CTR = 21,56% vs. TRT = 30,51%; P=0,001), in particolare per i PUFA- ω 3 (CTR = 9,31% vs. TRT = 18,28%; P<0,001) imputabili ai livelli di acido linolenico, raddoppiati per il gruppo TRT (16,33%) rispetto al CTR (8,43%); per gli indici Aterogenico (CTR = 1,11 vs. TRT = 0,80; P=0,010) e Trombogenico (CTR = 0,68 vs. TRT = 0,32; P=0,001) e per i terpeni (CTR = 0,17% vs. TRT = 0,69%; P=0,002) responsabili del caratteristico flavour di erba verde del latte.*

Key words: fresh forage, ass's milk, fatty acids, flavours.

INTRODUCTION – The first studies on the quali-quantitative aspects of ass's milk have focussed the attention towards nutrition of human infants affected by multiple food intolerance (Carroccio *et al.*, 2000). More recently, its use is studied also in cardiopathology prevention, in neonatal cerebral growth and in the regulation of the immuno-inflammatory system, where the fatty acids are directly implicated (Chiofalo *et al.*, 2003). Nevertheless, the researches on the breeding conditions of the donkeys, in relation to a good production activity, must still give complete responses as regards the asses' feeding and the milk nutritional quality. In this context the effect on the milk acidic and aromatic composition in lactating asses fed with fresh forage was studied.

MATERIAL AND METHODS – The research was carried out on 8 Martina Franca asses bred in an organic farm. The animals were divided into two groups of 4 each one, CTR and TRT, homogeneous for milk yield (1576±560 mL/d), day of lactation (154±56 days) and body weight (300±35 kg). The asses, stabled with their foals in boxes provided with a large external paddock, received the daily ration (on average: dry matter intake = 9.5 kg/d, Crude Protein = 10% DM and Digestible Energy = 8.5 MJ/kg DM) consisting of 8 kg/head of meadow hay (Dry Matter = 90.6%, on a DM basis: 8.3% Crude Protein, 62.8% Neutral Detergent Fibre) for the CTR group, of 20 kg/head of meadow fresh herbage (Dry Matter = 21%, on a DM basis: 12.3% Crude Protein, 49.4% Neutral Detergent Fibre) and 3 kg/head of meadow hay for the TRT group; all the animals received daily 2.5 kg/head of commercial concentrate (Dry Matter = 89.3%, on a DM basis: 15.6% Crude Protein, 36.2% Neutral Detergent Fibre). The chemical composition of feeds, sampled every 21 days, was determined

(A.O.A.C., 2000). The trial lasted for 63 days, preceded by a 15-day adaptation period which consisted of a gradual administration of the experimental diet to the asses of TRT group. During the trial the BCS of the animals was 3.1 (± 0.25), measured on a 0-5 scale (Martin-Rosset, 1990). Every 21 days the asses were machine milked twice after 3-3.5 hours of separation of the foals from the mothers, as described by Salimei *et al.* (2004). The acidic composition of feed and of individual milk samples was determined (Chiofalo *et al.*, 2004) by GC-FID and the atherogenic and thrombogenic indices were calculated using equations proposed by Ulbricht and Southgate (1991). The quali-quantitative aromatic profile (Mondello *et al.*, 2004) was investigated by Solid Phase Microextraction coupled with GC/MS and the results expressed as percentages of the relative peak areas. The results, expressed as g/100 g fatty acids, were subjected to the statistical analysis ANCOVA (SAS, 2001), using the following model: $y_{ij} = \mu + a_i + b \cdot x_{ij} + e_{ij}$; where a_i = effect of diet, x_{ij} = days of lactation.

RESULTS AND CONCLUSIONS – Table 1 shows the acidic composition of the feedstuffs. The different PUFA values between meadow hay and fresh forage are considerable; particularly, the higher $\omega 3$ -PUFA content of the fresh forage is due to the higher α -linolenic percentage in fresh forage (39.53 ± 3.75 g/100 g fatty acids) than that in hay (14.13 ± 2.72 g/100 g fatty acids).

Table 1. Fatty Acid class composition of feedstuffs (g/100 g fatty acids, Mean \pm SD).

	Meadow Hay	Meadow Herbage	Concentrate
Saturated (SFA)	39.55 \pm 4.48	23.63 \pm 2.69	51.69 \pm 0.16
Monounsaturated (MUFA)	17.33 \pm 2.06	10.72 \pm 1.62	36.27 \pm 0.11
Polyunsaturated (PUFA)	43.12 \pm 4.55	65.66 \pm 4.31	12.04 \pm 0.04
$\omega 3$ -PUFA	14.33 \pm 2.69	39.85 \pm 4.00	0.52 \pm 0.03
$\omega 6$ -PUFA	28.79 \pm 4.61	25.81 \pm 0.30	11.53 \pm 0.01

The PUFA percentages in the milk of the TRT group (Table 2), unlike those of the SFA and MUFA, were higher in relation to the fresh forage administration. In the monogastric herbivorous, the content of milk long-chain fatty acids is correlated to the dietary fatty acid composition, because of the absence of the fatty acid hydrogenation in the digestive tract before the absorption (Doreau *et al.*, 2002): these results are consistent with findings by Salimei *et al.* (2004), that reported a lower level of PUFA and consequently a different fatty acid composition in milk of Ragusana and Martina Franca asses fed with hay and concentrate rather than fresh herbage.

Table 2. Milk fatty acid composition in relation to the diet (g/100 g fatty acids, LSM \pm SE).

	CTR group	TRT group	SE	P
Saturated (SFA)	55.33	49.29	1.88	0.042
Monounsaturated (MUFA)	23.11	20.20	0.81	0.024
Polyunsaturated (PUFA)	21.56	30.51	1.55	0.001
$\omega 3$ -PUFA	9.31	18.28	0.95	<0.001
$\omega 6$ -PUFA	12.25	12.23	0.81	0.990

Also the higher PUFA- $\omega 3$ content in milk for TRT group (Table 2), could be explained by the higher α -linolenic acid content in the fresh forage than that in the hay (see above). Indeed, α -linolenic acid resulted twofold higher in TRT group (16.33 ± 2.48 g/100 g fatty acids) than that in the CTR group (8.43 ± 3.0 g/100 g fatty acids), according to results in Doreau *et al.* (2002) on milk from mares fed herbage or hay. On the other hand, no effect seems to be related to the long-chain fatty acid mobilisation from adipose tissue since asses, at the beginning of the trial, were in intermediate-late lactation. The level of linoleic acid, the most represented fatty acid of the $\omega 6$ series, has shown similar percentages in the milk of both groups (CTR = 11.23 ± 2.71 and TRT = 11.24 ± 1.85

g/100 g fatty acids; $P=0.887$). Table 3 shows the most significant ratios among the milk acid classes; the $\omega 3/\omega 6$ and the UFA/SFA ratios were significantly higher in TRT group, according to the higher milk content of both $\omega 3$ -PUFA and UFA (*i.e.* MUFA+PUFA) classes in this group. Consequently, the milk Atherogenic and Thrombogenic indices (Table 3), that are correlated to pathological phenomena such as the formation of atheroma and/or thrombosis, resulted significantly lower, especially for the TRT group, than those observed in the milk of both polygastric species and Ragusana and Martina Franca asses fed hay and concentrate (Salimei *et al.*, 2004); these data confirm the interest towards this product as “functional food”.

Table 3. $\omega 3/\omega 6$, UFA/SFA ratios and Quality Indices of the milk (LSM \pm SE).

	CTR group	TRT group	SE	P
$\omega 3/\omega 6$	0.78	1.53	0.08	<0.001
Unsaturated/Saturated (UFA/SFA)	0.81	1.07	0.08	0.035
Atherogenic Index (AI)	1.11	0.80	0.07	0.010
Thrombogenic Index (TI)	0.68	0.32	0.06	0.001

The aromatic profile of the fresh forage was, as expected, richer than that of the hay (41 components identified *vs.* 15), with a greater quantity of carbonylic compounds (herbage = 47.08%; hay=6.35%) and alcohols (herbage = 32.71%; hay = 0%) and lower percentages of terpenes (herbage = 2.24%; hay = 19.57%), which are, as well-known (Urbach, 1998), scarce in the lowland pasture (Salvarano di Quattro Castella is 600 m a.s.l.). Among the classes of fatty acids, esters, alcohols, ketones, aldehydes, terpenes, thirty flavour components were identified in the milk. Some of them (*e.g.*: terpenes and fatty acids) could be derived from the feeding regime (CTR = 55.49% and TRT = 51.76%; $P=0.054$), others, as aldehydes, alcohols, ketones (CTR = 7.96% and TRT = 9.77%; $P=0.039$), could be also due to biochemical modifications occurring during the digestive processes of the animal as reported by Urbach (1998) in polygastric species. Among the milk flavours with a distinctive sensorial character, were identified: alcohols and aldehydes C_6 and C_9 (CTR = 4.21% and TRT = 4.33%; $P=0.833$), that account for the “green” aromatic notes of milkfat, so as β -pinene, β -myrcene, limonene, p-cymene and γ -terpinene (CTR = 0.17% and TRT = 0.69%; $P=0.002$), responsible for the characteristic “green-grassy” flavour of the milk. The phenethyl alcohol, aromatic alcohol with floral notes, was identified only in the milk of TRT group (0.22%). Data obtained from the aroma investigation could be supported by further studies based on the sensorial analysis, in order to evaluate the olfactory threshold of the compounds determined.

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