# ASS'S MILK:
# EXPLOITATION OF AN ALIMENTARY RESOURCE

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**Abstract**
Authors report the results obtained in a study on the acidic fraction of Ragusana ass's milk, with a particular attention to the essential (EFA) and polyunsaturated (PUFA) fatty acids of the ω3 and ω6 series, considering their nutritional and therapeutic properties. Milk samples were analysed using High Resolution Gas Chromatography (HRGC). The results, with regard to the saturated (67.57%), monounsaturated (15.82%) and polyunsaturated (16.60%) classes and to the unsaturated/saturated ratio (0.48), showed a different acidic pattern from ass's milk of other breeds but a similar ω3/ω6 ratio (0.86). Data point out the interest about the cognitive aspect and the characterisation of the milk of this animal, contributing to the knowledge on this topic.
The results confirm the biological value of the ass's milk and suggest the potential economic value of the exploitation of the asinine breeding. As an alternative food, ass's milk could in fact satisfy the dietetic and/or healthy requirements, designing new perspective to this species and its habitat, mostly represented by «marginal» areas.

## INTRODUCTION

In the last years a negative trend is observed in Italy in some autochthonous asinine populations [1]: on this regard, a considerable numeric contraction is occurring in Sicily mainly for «Ragusana» donkeys but also for «di Pantelleria» donkeys, used once as breeders for both improving other asinine breeds and in the mule production in Italy and in foreign Countries. The diffusion of the donkeys in Sicily date back to ancient times, probably because of the oro-pedoclimatic conditions of the Region and their adverse environment, where the donkey was the most qualified means of labour and transportation for its qualities of rusticity, sobriety, patience and resistance against fatigues. On the other hand, in the minor Islands of Sicily, the asinine presence is usually considered only a marginal agricultural activity, being underestimated the zootechnic role of its production and its genetic potential. Donkeys represent in fact a versatile species with low maintenance costs and, besides the above-mentioned qualities, they can supply milk with nutritional and therapeutic properties known since ancient times, according to the historian Erodoto (V century BC), and today revalued by the

modern alimentary trends, which shift towards alternative products [2]. On this regard, some studies showed that ass's milk, animal production with both compositional and organoleptic characteristics rather similar to human milk, can represent the best nutriment for infants with food allergies during the first months of life, which often are refractory patients to others treatments [3]. Ass's milk could support an efficient cerebral growth and the development of a normal and complete immunitary system in the neonate [4]. Besides its use in infancy, some Authors point out ass's milk role in the osteogenesis process [5], as well as in the arteriosclerosis therapy, in the rehabilitation of the patients with coronary hearth diseases, or in the premature senescence [6] and the hypocholesterolemic diets [7].

Therefore the knowledge of the quantitative and qualitative characteristics of ass's milk production is important not only for the evalutation of the nutritional requirements of the dam and of its foal, but also could hold a remarkable practical and economic interest in human feeding (paediatric and/or geriatric diets) as well as in the pharmaceutical industry. The various zootechnic opportunities of reconsideration for this animal could then contribute in preserving the asinine biodiversity

In consideration of these important functions, aim of this study was to investigate on the acidic composition of Ragusana asses' milk, giving particular attention to ω3 and ω6 polyunsaturated fatty acid (PUFA) levels for their important role in relation not only to coronary heart diseases [8] but also in relation to brain development in neonates [9] as well as in the modulation of immune-inflammatory system in the human [10].

## MATERIALS AND METHODS

The study was carried out on individual milk samples from 4 multiparous Ragusana asses, clinically healthy and at the 110 day of lactation. The animals, stabled in boxes provided with an external common paddock, were fed twice a day with hay (10 kg/head/d) and concentrate (2.5 kg/head/d). Water was provided free choice.

Average milk yield was 770 mL/milking (± 100 mL, s.d.) and its average fat content was 0.6% (± 0.08%). Asses were machine milked, by a wheeled trolley type with a sheep cluster, modified appropriately and just utilised during previous successful trials [2, 11]; milk samples were immediately cooled at 4°C and divided in parts of 100 mL which were frozen at –20°C until analyses. Lyophilised samples were subjected to the extraction by Soxhlet with petroleum ether/ ethyl ether (1:1, vol/vol) for 5 hours to determine the total lipids [12].

The qualitative and quantitative composition of fatty acids in milk and feedstuffs was determined by gas chromatography on the methyl esters, prepared by direct transesterification [13].

*Gachromatograhic analysis*. The fatty acid methyl esters, 1 µl, were separated by HRGC, using a SHIMADZU 17A gas chromatography equipped with a software CLASS-VP to elaborate the data, a flame ionization detector (FID) and a fused

silice capillary column OmegaWax 250 (SUPELCO), internal diameter 0.25 mm, film thickness 0.25 μm, and length 30 m. The oven temperature was programmed: for the analyses of milk samples, from 50° to 240°C, with a linear increment of 8°C/min, initial isotherm of 5 min. and final isotherm of 40 min, and for the analyses of feed from 100° to 200°C, with a linear increment of 4°C/min. The injector and detector temperatures were 250°C and 270°C respectively. Identity of peaks was determined by comparison of retention times to standard references mixtures.
Data obtained were processed by mathematical elaboration to calculate the average and the standard deviation.

## RESULTS

In table 1 the average composition of fatty acid classes in feeds is reported. Table 2 and 3 show the fatty acids pattern in relation to the unsaturation grade and the values of the saturated, monounsaturated and ω3 and ω6 polyunsaturated classes identified in the milk; in table 4 the ω3/ω6 PUFA and Unsaturated/Saturated ratios in the milk are reported. Fatty acid content has been expressed in percentage of total of identified fatty acids.
The milk fatty acids identified were (table 2):

- Saturated linear fatty acids from $C_4$ to $C_{22}$;
- Monounsaturated fatty acids from $C_{10}$ to $C_{20}$;
- Polyunsaturated fatty acids from $C_{18}$ to $C_{22}$, of which 3 of ω6 series and 7 of ω3 series, containing from 2 to 6 double bonds.

### Saturated fatty acids

Milk saturated fatty acids found in greater quantity were: caprilic ($C_{8:0}$ = 12.80%), caprinic ($C_{10:0}$ = 18.65%) and palmitic ($C_{16:0}$ = 11.47%) acids (table 2). The levels of these acids disagree with those observed in Martina Franca asses' milk [6], probably because of the different experimental methodologies (breed, stage of lactation, milking system and management, etc.) Among the fatty acids of nutritional interest and found in modest amounts, the highest concentrations were observed for the miristic acid ($C_{14:0}$ = 5.77%) (tab. 2), while the lowest levels for the stearic acid ($C_{18:0}$ = 1.12%) was similar to the those (1.55%) observed in mare's milk [14, 15]. Compared to monounsaturated and polyunsaturated classes (table 3), saturated milk fatty acids were the most represented (67.57 %): these results, consistent with those reported for equine's milk (61%) [16], were found lower than those in ovine (73%) [17] and caprine (77%) [12] milk. According to Others' observations on mare's milk, the relatively high content of fatty acids with 16 and less carbons could be due to a synthesis of these fatty acids from acetate and 3-hydroxybutirate, as in ruminants, and not from glucose, as monogastrics [15].

## Monounsaturated fatty acids

Monounsaturated fatty acid found in greater quantity was oleic acid ($C_{18:1\omega9}$ = 9.65%) (table 2) that, nevertheless, is about a third of that reported for Martina Franca breed [6]. The caproleic ($C_{10:1}$) and palmitoleic ($C_{16:1\omega7}$) acids, showed lower quantity (2.20% and 2.37% respectively) (table 2) than milks of different asinine breeds, however these acids reach higher levels in comparison with those of the different asinine races, and the highest levels in comparison with those of other animal species [6, 17]. The sum of the monounsaturated percentages was 15.82% (table 3), lower than that of each other species both non-ruminant and ruminant [18].

## Polyunsaturated fatty acids

This class showed a similar distribution of the ω3 (7.45%) and ω6 PUFA (8.65%) (table 3). Among these acids, the linolenic ($C_{18:3\omega3}$ = 6.32%) and the linoleic ($C_{18:2\omega6}$ = 8.15%) acids (table 2) were the most represented components of the polyunsaturated class, which reached the value of 16.60% (table 3). Fatty acid levels of this class in comparison with those of other animal species, except mare, showed the higher values. Among the essential fatty acids, the linolenic and linoleic acids show higher values than those observed in the ruminants ($C_{18:3\omega3}$ = 0.7-1.%) ($C_{18:2\omega6}$ = 1.8-2.1%) [17], but are comparable with those ($C_{18:3\omega3}$ = 5-19%) ($C_{18:2\omega6}$ = 5-10%) found in mare's milk [7, 15]. In monogastric herbivora, like equids, the amount of unsaturated long chain fatty acids in milk is related to the amount consumed with forages: the absence of hydrogenation of fatty acids in the digestive tract before absorption, as occurring in ruminants, can in fact explain the high content in linoleic and linolenic acids [19].
The UFA (unsaturated fatty acid = mono and polyunsaturated) content was 32.42%, i.e. lower than that reported by Pinto [6] in Martina Franca asses' milk (50.69%) but higher than that observed in ruminants' milk (19-26%) [12, 17].

## Ratios

The ω3/ω6 polyunsaturated fatty acids ratios was 0.86 (table 4) rather similar to that reported in literature by Pinto (0.85) [6] and to that observed in the equine species (0.84) [2]; however these species showed higher ratio than that found in ruminants' (0.44-0.55) [17] and human's milk (0.07) [6]. The unsaturated/saturated ratio in Ragusana ass's milk (0.48) was lower than that observed in Martina Franca asses (1.03) [6], similar to mare's milk (0.65) [16], and higher than that found in the ruminants' milk (0.26-0.38) [12, 17].
The significant content of the ω3 polyunsaturated fatty acids could have an interesting role on the outcome of the grafts, on some kinds of tumor, on the

bodily and neuropsychic development [20]. Besides, the considerable percentage of medium chain fatty acids influences the vasodilation phenomena [21] and, together with the short chain fatty acids, determine an increase of the anti-oxidative defences of the organism [22]. Nevertheless, the low levels of docosaesaenoic acid ($C_{22:6\omega3}$) observed in ass's milk, suggest that dietary supplement with this acid is advisable. In fact the considerable presence of the DHA in the membranes of neurons, of the external portion of retinic receptor cells and of the spermatozoon acrosome determines a normal development and maturation of the nervous tissues as well as of the reproductive apparatus [23].

Tab. 1 – Average composition of fatty acid classes in the feed (% of total)

| | **Hay** | **Concentrate** |
|---|---|---|
| **Saturated fatty acids** | 47.8 | 27.7 |
| **Unsaturated fatty acids** | 52.2 | 72.3 |
| **ω3 series** | 21.7 | 4.1 |
| **ω6 series** | 18.3 | 40.2 |

Tab.2 –Fatty acids composition (%) of ass's milk (mean±SD).

| **Fatty acids** | **mean±SD** | **Fatty acids** | **mean±SD** |
|---|---|---|---|
| **Saturated** | | **Monounsaturated** | |
| $C_{4:0}$ | 0.60±0.29 | $C_{10:1}$ | 2.20±0.16 |
| $C_{6:0}$ | 1.22±0.22 | $C_{12:1}$ | 0.25±0.10 |
| $C_{7:0}$ | Tracce | $C_{14:1}$ | 0.22±0.05 |
| $C_{8:0}$ | 12.80±0.59 | $C_{16:1\omega7}$ | 2.37±0.57 |
| $C_{10:0}$ | 18.65±0.91 | $C_{17:1}$ | 0.27±0.05 |
| $C_{12:0i}$ | 10.67±0.49 | $C_{18:1\omega9}$ | 9.65±0.70 |
| $C_{13:0r}$ | 0.22±0.05 | $C_{20:1\omega11}$ | 0.35±0.10 |
| $C_{13:0}$ | 3.92±0.90 | **Polyunsaturated ω3** | |
| $C_{14:0r}$ | 0.12±0.05 | $C_{18:3\omega3}$ | 6.32±1.02 |
| $C_{14:0}$ | 5.77±0.33 | $C_{18:4\omega3}$ | 0.22±0.10 |
| $C_{15:0r}$ | 0.07±0.01 | $C_{20:3\omega3}$ | 0.12±0.05 |
| $C_{15:0}$ | 0.32±0.05 | $C_{20:4\omega3}$ | 0.07±0.01 |
| $C_{16:0r}$ | 0.12±0.05 | $C_{20:5\omega3}$ | 0.27±0.05 |
| $C_{16:0}$ | 11.47±0.59 | $C_{22:5\omega3}$ | 0.07±0.01 |
| $C_{17:0r}$ | 0.20±0.08 | $C_{22:6\omega3}$ | 0.30±0.08 |
| $C_{17:0}$ | 0.22±0.05 | **Polyunsaturated ω6** | |
| $C_{18:0}$ | 1.12±0.24 | $C_{18:2\omega6}$ | 8.15±0.94 |
| $C_{20:0}$ | 0.12±0.05 | $C_{18:3\omega6}$ | 0.15±0.03 |
| $C_{22:0}$ | 0.05±0.01 | $C_{20:2\omega6}$ | 0.35±0.10 |

Tab.3 – Fatty acid classes (%) of milk samples (mean±SD).

| | **mean±SD** |
|---|---|
| **Saturated** | 67.57±2.78 |
| **Monounsaturated** | 15.82±0.95 |
| **Polyunsaturated ω3** | 7.45±1.15 |
| **Polyunsaturated ω6** | 8.65±1.07 |
| **Polyunsaturated** | 16.60±2.33 |

Tab.4 – Fatty acid ratios of ass's milk (mean±SD).

| | **mean±SD** |
|---|---|
| **Ratio ω3/ω6** | 0.86±0.07 |
| **Ratio Unsat./Sat.** | 0.48±0.06 |

## CONCLUSIONS

Compared with ruminants' milk, the considerable presence of unsaturated fatty acids found in ass's milk make it very useful in the prevention of the cardiovascular, auto-immune and inflammatory diseases. The ω3 polyunsaturated fatty acids content, characteristic constituents of the fish oils, can counteract the above-mentioned pathologies through the synthesis of antiinflammatory, antiaggregant and non immunosuppressant substances, like lipidic mediators (eicosanoids), prostaglandins ($PGE_3$) and leukotrienes ($LTB_5$), and proteic mediators (cytokines), interleukins ($IL_4$, $IL_{10}$, $IL_{13}$, $IL_{1ra}$), tumor necrosis factor, etc.

The different levels of the milk fatty acids observed among asinine breeds seem very interesting for the cognitive aspect but also in the prospect of a further characterisation of the milk of this animal species. On this regard, in depth studies are required on the relationship between ass's nutrition and milk composition, particularly for fat fraction, the most variable milk constituent.

Besides the protein pattern, ass's milk for its peculiar fatty acids composition opens up very interesting horizons for the human nutrition, involving donkeys breeding for the productive aspect and the animal biodiversity and representing a cultural reminiscence, testimony of the past, common inheritance to preserve just like animal welfare and environment.

## REFERENCES

1) M.C. Pagano, S. Pelosi, M. Franchi, M. Scialandrone. «Parametri biologici e morfologici in asini di Martina Franca». *Riv. SIDI*, 3, 47-51,1999.

2) E. Salimei, M. Cattaneo, B. Chiofalo, V. Dell'Orto «Exploitation of mares' milk by polyunsaturated fatty acids enrichment». *Proceedings of the 31° Simposio Internazionale di Zootecnia «Food and health: Role of Animals Products*», Milano 13 settembre, 223-227, 1996.
3) G. Iacono, A. Carroccio, F. Cavataio, G. Montalto, M. Soresi, V. Balsamo. «Use of ass' milk in multiple food allergy». *J. Paed. Gatroenterol. Nutr.*, 14, 177-181, 1992.
4) F. Chiarelli «Allergia alle proteine del latte vaccino nell'età pediatrica. Possibile ruolo del latte d'asina» *www.geocities.com/eugeniomilonis/latteasina.htm,* (2001).
5) R. Wolter. «Osteocondrosi e alimentazione nel cavallo». *Riv. SIDI*, 2, 2, 27-32, 1996.
6) F. Pinto F. from G.F. Sportelli. «Latte equino, alternativa molto interessante». *Informatore Zootecnico*, 10, 57-59, 1998.
7) V. Dell'Orto, E. Salimei, V. Bontempo, F. Fantuz, P.M. Toppino. «Produzione e composizione di latte equino: osservazioni sperimentali». *Proceedings of the S.I.S.Vet.*, XLVII, 3, 2073-2077, 1993.
8) J. Kinsella, K. Shane Broughton, J. Whelan. «Dietary unsaturated fatty acids: interactions and possible needs in relation to eicosanoid synthesis». *J. Nutr. Biochem.*, 1, 123-141, 1990.
9) A.C. Van Houwelingen, J. Dalby Sorensem, G. Hornstra, M.M. Simonis, J. Boris, N.J. Secher. «Essential fatty acid status in neonates after fish oil supplememntation during late pregnancy». *Br. J. Nutr*., 74, 723-731, 1995.
10) G. Ballarini. «Nutrizione e immunità». *O.D.V*., 2, 31-42, 1990.
11) E. Salimei. «Caratteristiche quali/quantitative della produzione del latte d'asina: risultati sperimentali». *Conv. L'asino: Attualità e prospettive dell'impiego in campo medico, zootecnico ed alimentare*, Palermo 25 maggio 2001.
12) B. Chiofalo, M. Ziino, F. Salvo «Studio della composizione acidica del latte di capre autoctone siciliane, di differenti età, analizzato nel corso dell'intera lattazione». *Riv. Ital. di Scienza dell'Alimentazione*, 24, 2, 405-411, 1994.
13) W.W. Christie. *«Preparation of Ester Derivatives of fatty acids for chromatographic analysis – Advances in Lipid Methodology-Two».* Ed. The Oily Press LTD: Dundee, Scotland, 1993.
14) J. Csapó, J. Stefler, T.G. Martin, S. Makray, Zs. Csapó-Kiss. «Composition of Mares' Colostrum and Milk. Fat Content, Fatty acid Composition and Vitamin Content». *Int. Dairy Journal*, 5, 393-402, 1995.
15) M. Doreau, S. Boulot. «Recent knowledge on mare milk production : a review». *Liv. Prod. Sci*., 22, 213-235, 1989.
16) F. Martuzzi, A. Summer, A.L. Catalano, S. Barbacini, P. Mariani. «Il contenuto in acidi grassi polinsaturi del grasso del latte di cavalla prodotto nelle prime settimane di lattazione». *Proceedings of the S.I.S.Vet.*, LII, 537-538, 1998.
17) B. Chiofalo, L. Biondi, M. Ziino, P. Pennisi, F. Salvo, A. Priolo.. «Variazione della composizione acidica del latte di pecore Comisane alimentate con un unifeed secco». *Riv. Ital. di Scienza dell'Alimentazione*, 25, 3, 261-266, 1996.

18) B. Chiofalo. «Gli acidi grassi nel latte di asina: molecole bioattive di grande interesse». *Conv. L'asino: Attualità e prospettive dell'impiego in campo medico, zootecnico ed alimentare*, Palermo 25 maggio 2001.
19) T.C. Jenkins, H.G. Bateman, S.M. Block S.M.. « Butylsoyamide increase unsaturation of fatty acids in plasma and milk of lactating dairy cows». *J. Dairy Sci.,* 79, 585-590, 1996.
20) G. Caramia, M. Cocchi, N. Frega. «Recenti progressi in nutrizione». *Progress in nutrition*, 2, 2, 25-41, 2000.
21) R.P. White, A.M. el-Bauomy, W.B. Wood. from L. Pizzoferrato «Antiossidanti naturali nei prodotti lattiero-caseari» *Caseus*, 3, 3, 46-49, 1998.
22) B. Cestaro. *«Per una vita inossidabile. Apporti nutrizionali del latte e derivati».* ETASLIBRI RCS Medicina, Milano, 1994.
23) M. Cocchi. «Acido docosaesaenoico (DHA) e funzione visiva». *Progress in nutrition*, 2, 2, 43-51, 2000.