

## FAT COMPOSITION OF MARE'S MILK WITH REFERENCE TO HUMAN NUTRITION

### COMPOSIZIONE LIPIDICA DEL LATTE DI CAVALLA IN FUNZIONE DELL'ALIMENTAZIONE UMANA<sup>(1)</sup>

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#### SUMMARY

Dietary fat is a very important factor influencing human condition, particularly in cardiovascular diseases. The quality of health would really be improved not only by a general reduction in fat dietary intake, but with a change in saturated/unsaturated fatty acids ratio, favourable to the second ones. Mare milk seems to summarize all these peculiarities. Milk samples from 22 Haflinger multiparous mares, belonging to the same stud-farm, were collected at 30, 60, 90 and 105 days of lactation in order to evaluate the fatty acids content's variation, saturated/unsaturated ratio and, particularly, linoleic/ $\alpha$ -linolenic ratio. Palmitic, oleic and linoleic fatty acids showed highest amounts during lactation. Linoleic acid was 10.89% on total fatty acids in 30 days samples, with a not significant increase at 60 (11.21%) and significant decrease at 90 (8.35%) and 105 days (8.54%). The other essential fatty acid,  $\alpha$ -linolenic, significantly increased from 5.56% on total FA in the first month until 6.29% at 60 days, 6.26% at 90, and 6.66% at 105 days. Eicosapentaenoic acid and docosahexaenoic acid were present only as traces. Linoleic/ $\alpha$ -linolenic ratio was about 2:1 in 30 days samples and pointed out a progressive decrease, with a light increasing linolenic acid amounts during lactation. Saturated/unsaturated ratio was slightly favourable to the first ones as a ratio between 1.2:1 and 1.3:1. Summarising, linoleic/ $\alpha$ -linolenic ratio in our samples could be interesting for human nutrition, specially in low EFA content diets, and ideal for pre-term infant's diets, suggesting also an application in the adult man.

Key words: mare milk, human nutrition, fatty acids.

#### RIASSUNTO

La ricerca è stata condotta su campioni di latte provenienti da 22 cavalle pluripare di razza Avelignese e prelevati nella prima fase di lattazione (105 giorni). Lo scopo è stato quello di esaminare i contenuti in acidi grassi, il rapporto acidi grassi saturi/insaturi ed in particolare i contenuti ed il rapporto tra livelli di acido linoleico (LA) e  $\alpha$ -linolenico (ALA). La quota di acido linoleico è risultata di 10,89% sulla totalità degli acidi grassi nei campioni relativi al trentesimo giorno di lattazione, mentre abbiamo assistito

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ad un leggero decremento verso la fine del periodo di osservazione. L'ALA è aumentato significativamente fino ai prelievi operati a 105 giorni. EPA (acido eicosapentaenoico) e DHA (acido docosaesaenoico) sono stati rilevati solo in tracce in tutte le campionate. Il rapporto LA/ALA è risultato di circa 2:1 nei campioni relativi al prelievo iniziale (30 giorni) e si è ridotto progressivamente, prevalentemente a causa dell'aumento quantitativo del secondo, durante il corso della lattazione. La proporzione tra acidi grassi saturi ed insaturi è leggermente favorevole ai primi con un rapporto oscillante tra 1.2:1 e 1.3:1.

Parole chiave: latte equino, nutrizione umana, acidi grassi.

## INTRODUCTION

Dietary fat is a very important factor influencing human health, particularly in cardiovascular diseases. The quality of health would really be improved not only by a general reduction in fat dietary intake, but with a change in saturated/unsaturated fatty acids ratio, favourable to the second ones (Noble, 1999). About cow's milk, ruminal biohydrogenation results in very low polyunsaturated fatty acids (PUFA) levels and also the use of vegetable oils to enhance milk PUFA levels produced poor results (Glavert, 1990; Kempster, 1990). Instead, mare's milk seem to contain  $\alpha$ -linolenic (ALA) and linoleic (LA) acids amounts, usually called EFA (essential fatty acids) and respectively precursors of  $\omega$ -3 and  $\omega$ -6, higher than in cow's milk (Csapò et al., 1995), although long-chain polyunsaturated fatty acids (LCPUFA) contents seem to be limited. From  $\alpha$ -linolenic (C18:3n-3) and eicosapentaenoic (C20:5 n-3, EPA) acids derive prostaglandins, like PGI, with vasodilatory effects, tromboxans, like TXA, with vasoconstrictive effects, and docosahexaenoic acid (C22:6n-3, DHA). From linoleic acid (C18:2 n-6) derives many other prostaglandins (PGI<sub>2</sub>) and tromboxans with different influences on the circulatory system (Gibney, 1993; Calder, 1996).

One of the most interesting topic belongs to perinatal period. During pregnancy, in fact, DHA and AA (arachidonic acid) cross the placenta to the fetus. Postnatally these PUFA are contained in breast milk, including precursors and metabolites, but most infant formulae contain only the precursor essential fatty acids,  $\alpha$ -linolenic acid and linoleic acid, from which formula-fed infants must respectively synthesize their own DHA and AA. The absence of LCPUFA in for-

mulae may be further compromised by inhibition of incorporation of endogenously produced LCPUFA caused by the high concentrations of LA in this kind of food. Formula-fed infants have shown less DHA and AA in their erythrocytes than those fed breast milk, and so infant formulae containing only LA and ALA may not be effective in meeting their full EFA requirements, although various combination of LA and ALA concentrations are still being evaluated (Clark et al., 1992).

At present, the question if infants may benefit from LCPUFA-unsupplemented formulae with higher  $\alpha$ -linolenic acid content is always open and recent studies demonstrate that pre-term infants are able to form AA and DHA, even if this synthesis seems extremely low (Giovannini et al., 1998). Moreover EFA show the possibility to affect allergic inflammation through the modulation of prostaglandin and leucotriene production, the inhibition of cellular activation and cytokine secretion as well as the alteration of the composition and function of the epidermal lipids barrier. A deficit of n-6 EFA leads an inflammatory skin condition in both animals and humans (Uauy, Hoffman, 2000).

In atopic dermatitis we can find a low blood EFA concentration, although recently it has been established that there is no deficit of linoleic acid in this eczema. Concentration of linoleic acid, instead, tends to be elevated in blood, milk and adipose tissue of patients with atopic eczema. This suggests a reduced conversion of linoleic acid to  $\alpha$ -linolenic acid (ALA), so that the administration of  $\alpha$ -linolenic acid in human diet has been found to improve the clinically assessed skin condition, the objectively assessed skin roughness, and blood catecholamine concentration (Olivry et al., 2001). Mare's milk fat composition, in addition to the protein fraction characteristics, suggests that this product is more similar to human milk than cow's (Godovac-Zimmermann et al., 1987; Businco et al., 2000; Horrobin, 2000) and, more, the consideration that it could be utilized in cow's milk allergic children diets, as a substitute (Pagliarini et al., 1993; Horrobin, 2000), supports its potential utilization in paediatric dietetics and, generally, in human nutrition.

The purpose of our studies concerns the evaluation of mare's milk quality, since some our current researches seem indicate variability not only connected with breed but also with administered feedstuff; by this way Haflinger leaded us to believe interesting qualitative cha-

racteristics in consideration of the diffusion, potential productivity as related to live weight, relatively easy handling and management of this horse breed.

Aim of this work belongs to the study of fatty acids variations, specially essential fatty acids, in Haflinger's milk samples during the first period of lactation. We consider saturated/unsaturated and linoleic/ $\alpha$ -linolenic ratio in regard to a concrete qualitative mare's milk fat evaluation.

## MATERIALS AND METHODS

Milk samples from 22 Haflinger multiparous mares, belonging to the same stud-farm, were collected at 30, 60, 90 and 105 days of lactation; samples were collected with the same procedure adopted in a previous trial (Orlandi et al., 2002). All mares were fed with the same diet consisting of 4-5 kg concentrate (60% barley, 25% oats, 15% corn), *ad libitum* medium hay and fresh pasture, whose chemical composition is reported in Table I.

**Tab. I.** Chemical composition of administered diets (% d.m.) and LA, ALA amounts (% total lipids).

	Hay	Concentrate	Pasture
Dry matter	89.42	90.01	36.74
Crude protein	4.95	12.58	11.00
Ether extract	2.00	3.00	1.60
Crude fibre	34.52	6.57	24.37
N-free extract	53.33	74.43	54.27
Ash	5.20	3.42	8.76
C18:2 $\omega$ -6 (LA)	3.37	26.76	6.36
C18:3 $\omega$ -3 (ALA)	3.40	2.17	15.25

Samples were frozen at -20°C until chemical investigations were performed. FA milk composition was performed according to Roese-Goettlieb extraction (FIL-IDF: 1D-1996) by using capillary gas chromatographic investigation for methyl esters (FAME), with a HP 23

cis/trans polar column, length 30 m (i.d. 0.32 mm.; d.f. 0.25 mm), according to FIL-IDF: 182-1999 method (CE Instruments, GC8000). Gas-chromatographic analysis was also carried out for animal feed-stuff, using 100 m x 0.25 mm capillary column, d.f. 0.25 mm. The profile of fatty acids considered includes from C8:0 until C24:1.

MANOVA with repeated measures for linoleic and  $\alpha$ -linolenic acids to evaluate significative differences at different collection times was performed by JMP (SAS Inst., 1994).

## RESULTS AND DISCUSSION

In the Table I the composition of diets administered to the mares is reported. In the Table II gas-chromatographic composition of any of main FA in mare milk samples (mean  $\pm$  SD) is presented.

**Tab. II.** Main FA amounts (% , total FA) in milk samples at different collection times.

	30 d	60 d	90 d	105 d
C16:0	24.12	23.48	27.14	26.84
C18:1 $\omega$ -9	19.54	20.14	18.21	18.85
C18:2 $\omega$ -6 (LA)	10.89	11.21	8.35	8.54
C18:3 $\omega$ -3 (ALA)	5.56	6.29	6.26	6.66
EPA	0.02	0.02	0.04	0.03
DHA	0.03	0.04	0.04	0.03
Tot. Sat.	55.30	54.72	56.16	55.72
Tot. Unsat.	44.70	45.31	43.84	44.28

Palmitic, oleic and linoleic fatty acids show highest amounts during lactation. Linoleic acid (Tab. III) was 10.89% on total fatty acids in 30 days samples, with a not significative increase at 60 (11.21%) and significative decrease at 90 (8.35%) and 105 days (8.54%). The other essential fatty acid,  $\alpha$ -linolenic, significantly increases from 5.56% on total FA in the first month until 6.29% at 60 days, 6.26% at 90, and 6.66% at 105 days. These EFA amounts are very higher in mare's milk than in cow's, as suggested by Malacarne et al. (2002) that found structural and functional peculiarities in

mare's milk so that it could be more suitable for human nourishment than cow's. LA and ALA are required in human nutrition because they can't be produced and could be extremely important for formula-fed infants. Doreau (1994) confirms that composition of the diet, particularly forage/concentrate ratio doesn't greatly modify milk yield of mares fed *ad libitum*, but an increase in the proportion of concentrates results in a decrease in milk fat and protein content changing also FA composition, as decrease of  $\alpha$ -linolenic acid amount and contemporary increase of linoleic's. On this way we can explain LA decrease and contemporary ALA increase during our Haflinger mares lactation. In the Table II we observe that arachidonic acid, EPA and DHA are present only as traces.

**Tab. III.** LA, ALA amounts (% total FA), LA/ALA and Sat/Unsat FA ratio in milk samples at different collection times.

	30 d	60 d	90 d	105 d
C18:2 $\omega$ -6 (LA)	10.89 a	11.21 a	8.35 b	8.54 b
C18:3 $\omega$ -3 (ALA)	5.56 a	6.29 b	6.26 b	6.66 b
LA/ALA	1.98	1.78	1.44	1.29
Sat/Unsat FA	1.24	1.21	1.28	1.25

a, b:  $p < 0.05$ .

In the Table III linoleic/ $\alpha$ -linolenic and saturated/unsaturated FA ratio at different collection times are reported. We observe a linoleic/ $\alpha$ -linolenic ratio as about 2:1 in 30 days samples and a progressive decrease, with an increasing  $\alpha$ -linolenic acid amounts during lactation. We must remark that the high intake of linoleic acid doesn't inhibit the conversion of  $\alpha$ -linolenic to DHA (Sauerwarld et al., 1996). The premature infant with a very low birth weight is capable of synthesizing arachidonic from linoleic acid and EPA and DHA from  $\alpha$ -linolenic (Carnielli et al., 1996), although LA amounts could inhibit the incorporation of endogenously produced LCPUFA (Clark et al., 1992). Microsomes from infant liver are ever capable of desaturating linoleic and  $\alpha$ -linolenic acids (Poisson et al., 1993). On this way, mare's milk EPA and DHA low content couldn't be a problem for

children nutrition. Some other Authors stated also that the contents in brain and nerve tissues probably indicated a remarkable thirst for long chain n-3 and n-6 fatty acids for human fetal development and suggested the necessity of including these acids in formulas for pre-term infants (Crawford, 1993). Saturated/unsaturated ratio is interesting for human nutrition, showing an average ratio between 1.21:1 and 1.28:1. Unsaturated FA are found in high proportion in the structural lipids of cell membranes, particularly those of the central nervous system and their accretion primarily occurs during the last three months of pregnancy and in the first year of life (Clandinin et al., 1980).

## CONCLUSIONS

On the basis of our research, we can affirm that mare's milk presents large EFA amounts, especially linoleic, and that's probably partially connected with the diet. Concentrations of these FA are interesting and required because they can't be produced by human body. The linoleic/ $\alpha$ -linolenic ratio in our samples could be interesting for human nutrition, specially in low EFA content diets, and ideal for pre-term infant's diets, since their liver is probably capable to transform them in EPA, DHA and AA. An application could also be suggested in the adult man, because PUFA have similar action as statins: both enhance endothelial nitric oxide synthesis, inhibit the production of pro-inflammatory cytokines, lower cholesterol levels, prevent atherosclerosis and are of benefit in coronary heart diseases (Das, 2001). Moreover the action of the statins turns to enhance the conversion of linoleic and eicosapentaenoic acid to their long chain derivatives. Saturated/unsaturated FA ratio is moreover favourable to the second ones, with an increasing ALA amount during lactation. Further studies are regarding milk contents in mare's from different breeds and feeding diets, with the purpose of obtaining more information about EFA contents and their variability.

## REFERENCES

BUSINCO L., GIAMPIETRO P.G., LUCENTI P., LUCARONI F., PINI C., DI FELICE G., IACOVACCI P., CURADI M.C., ORLANDI M. (2000). Allergenicity of mare's

- milk in children with cow's milk allergy. *J. All. and Clinic. Immun.*, 105 (5): 1031-1034.
- CALDER P.C. (1996). Effects of fatty acids on dietary lipids on cells of immune system. *Proc. Nutr. Soc.*, 55: 127-150.
- CARNIELLI V.P., WATTIMENA J.J., LUIJENDIJK I.H.T., DESENHART H.J., SAUER P.J.J. (1996). The very low birth weight premature infant is capable of synthesizing arachidonic and docosahexaenoic acids from linoleic and  $\alpha$ -linolenic. *Pediatr. Res.*, 10: 169-174.
- CLANDININ M.T., CHAPPELL J.E., LEONG S., HEIM T., SWAYER P.R., CHANCE G.W. (1980). Intrauterine fatty acids accretion rates in human brain: implications for fatty acids requirements. *Early Hum. Dev.*, 4: 121-129.
- CLARK K.J., MAKRIDES M., NEUMANN M.A., GIBSON R.A. (1992). Determination of the optimal ratio of linoleic acid to  $\alpha$ -linolenic in infant formula. *J. Pediatr.*, 120: 151-158.
- CRAWFORD M. (1993). The role of essential fatty acids in neural development: implications for perinatal development. *Am. J. Clin. Nutr.*, 57: 703-710.
- CSAPÒ J., STEFLER J., MARTIN T.G., MAKRAY S., CSAPÒ-KISS Z. (1995). Composition of mare's colostrum and milk. Fat content, fatty acids composition and vitamin content. *Int. Dairy J.*, 5: 393-401.
- DAS U.N. (2001). Essential fatty acids as possible mediators of the actions of statins. *Prostaglandins Leukot. Essent. Fatty Acids*, 65 (1): 37-40.
- DOREAU M. (1994). Le lait de jument et sa production: particularites et facteurs de variation. *Lait*, 74 (6): 401-418.
- GIBNEY M.J. (1993). Fish oils in human health. Recent advances in animal nutrition in Australia, D.J. Farrell ed., Univ. of New England, Armidale, N.S.W. 2351.
- GIOVANNINI M., RIVA E., AGOSTONI C. (1998). The role of dietary polyunsaturated fatty acids during the first 2 years of life. *Early Hum Dev.*, 53: 99-107.
- GLAVERT H.P. (1992). Fatty acids in foods and their implications, M. Dekker, New York.
- GODOVAC-ZIMMERMANN J., SHAW D., CONTI A., MC KENZIE H. (1987). Identification and the primary structure of equine  $\alpha$ -lactalbumin B and C. *Biol. Chem. Hoppe Seyler.*, 368: 427-433.
- HORROBIN D.F. (2000). Essential fatty acid metabolism and its modification in atopic eczema. *Am. J. Clin. Nutr.*, 71 (1): 367-372.
- KEMPSTER A.J. (1990). Reducing fat in meat animals. Elsevier Sci. Pub.
- MALACARNE M., MARTUZZI F., SUMMER A., MARIANI P. (2002). Protein and fat composition of mare's milk: some nutritional remarks with reference to human and cow's milk. *Int. Dairy J.*, 12: 869-877.
- NOBLE R.C. (1999). Animal supplies of omega-3 fatty acids for human nutrition. *Progress in nutrition*, 1 (3-4): 4-13.
- OLIVRY T., MARSELLA R., HILLIER A. (2001). The ACVD task force on canine atopic dermatitis: are essential fatty acids effective? *Vet. Immunol. Immunopathol.*, 20, 81 (3-4): 347-362.
- ORLANDI M., GORACCI J., CURADI M.C. (2002). Essential fatty acids (EFA) in Haflinger and Thoroughbred mare's milk. *Ann. Fac. Med. Vet. Pisa*, LV: 319-325.



- PAGLIARINI F., SOLAROLI G., PERI C. (1993). Chemical and physical characteristics of mare's milk. *Ital. J. Food Sci.*, 332-333.
- POISSON J.P., DUPUY R.P., SARDA P., DESCOMPS B., RIEU D., VARCE M., CRASTOS DE PAULET A. (1993). Evidence that liver microsomes of human neonates desaturate essential fatty acids. *Biochim. Biophys. Acta.*, 167: 109-113.
- SAUERWALD T.U., HACHEY D.L., JENSEN C.L., CHEN H., ANDERSON R.E., HEIRD W.C. (1996). Effect of dietary  $\alpha$ -linolenic acid intake on incorporation of docosahexaenoic and arachidonic acids into plasma, phospholipids of term infants. *Lipids*, 31: 131-135.
- UAUY R., HOFFMAN D.R. (2000). Essential fat requirements of pre-term infants. *Am. J. Clin. Nutr.*, 71 (1): 245-250.