Anim. Res. 53 (2004) 67–78
© INRA, EDP Sciences, 2004
DOI: 10.1051/animres:2003049

Original article

Composition and characteristics of ass's milk[1]

Elisabetta SALIMEI[a*], Francesco FANTUZ[b], Raffaele COPPOLA[c], Biagina CHIOFALO[d], Paolo POLIDORI[b], Giorgio VARISCO[e]

[a] Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Università degli Studi del Molise, via De Sanctis, 86100 Campobasso, Italy
[b] Dipartimento di Scienze Veterinarie, Università degli Studi di Camerino, via Circonvallazione 93, 62024 Matelica (MC), Italy
[c] Dipartimento Scienze e Tecnologie Agro Alimentari e Microbiologiche, Università degli Studi del Molise, via De Sanctis, 86100 Campobasso, Italy
[d] Dipartimento di Morfologia, Biochimica, Fisiologia e Produzioni Animali, Università degli Studi di Messina, Polo Annunziata, 98016 Messina, Italy
[e] Isituto Sperimentale Zooprofilattico della Lombardia e dell'Emilia, via Bianchi n. 9, 25124 Brescia, Italy

(Received 14 November 2002; accepted 10 October 2003)

Abstract – Ass's milk yield and chemical composition were investigated in two lactations at d 28, 45, 60, 75, 90, 105, 135 and 150 after parturition. Milk yield per milking (averaging 740 mL) of 6 asses of Martina Franca (n = 3) and Ragusana (n = 3) breeds, which quickly adapted to the milking machine procedures, increased in the second (or third) milking per day but was unaffected by stage of lactation or breed. Year of lactation, stage of lactation, breed and milking time did not influence the gross composition of the milk, except for fat and protein contents. The overall average protein percentage (1.72 $g \cdot 100\ g^{-1}$ of milk), showing a significant negative trend throughout lactation, was characterised by low casein (47.3% of crude protein) and whey protein contents (36.9% of crude protein). A specific whey protein profile was also found, being the relative percentage on total whey protein 4.48%, 6.18%, 29.85%, 21.03% and 22.56% for respectively lactoferrin, serum albumin, β-lactoglobulin, lysozyme and α-lactoalbumin. The low average fat content of ass's milk (0.38 $g \cdot 100\ g^{-1}$ of milk), showing a high individual variability, was significantly affected by the year of the study; the lipid fraction was also characterised by high levels of linoleic (average 8.15 $g \cdot 100\ g^{-1}$ of total fatty acids) and linolenic acid (average 6.32 $g \cdot 100\ g^{-1}$ of total fatty acids). The ash content of ass's milk (0.39 $g \cdot 100\ g^{-1}$ of milk) was constant throughout the experimental period and showed high levels of Ca and P, the ratio of which, ranging between 0.93 and 2.37, was on average 1.48.

ass's milk / protein / fatty acids / minerals

Résumé – **Composition et caractéristiques du lait d'ânesse.** La production et la composition chimique du lait d'ânesse ont été évaluées sur deux lactations successives à 28, 45, 60, 75, 90, 105,

[1] Some of these results were presented at the 4th Congress of the Società italiana di ippologia, Campobasso, Italy, July 2002.
* Corresponding author: salimei@unimol.it

135 et 150 jours après la mise bas. Au total, 6 ânesses de race Martina Franca (n = 3) et de race Ragusana (n = 3), rapidement adaptées à la traite mécanique, ont été utilisées. La production laitière par traite et par jour (en moyenne 740 mL) a été supérieure lors de la seconde (ou troisième) traite. Elle n'a été affectée ni par le stade de lactation ni par la race. L'année de lactation, le stade de lactation, la race et l'heure de traite n'ont pas influencé la composition brute du lait sauf ses teneurs en lipides et en protéines. Le pourcentage de protéines (moyenne de 1,72 g·100 g^{-1} de lait) a eu tendance à diminuer significativement tout au long de la lactation, et a été caractérisé par une teneur réduite en caséine (47,3 % des matières azotées) et en protéines sériques (36,9 % des matières azotées). Un profil particulier des protéines sériques a été mis en évidence, avec un pourcentage relatif par rapport aux protéines sériques totales de 4,48, 6,18, 29,85, 21,03 et 22,56 %, respectivement, pour la lactoferrine, la sérum albumine, la β-lactoglobuline, le lysozyme et l'α-lactalbumine. La faible teneur du lait en matières grasses (0,38 g·100 g^{-1}) a été caractérisée par une variabilité individuelle élevée. La fraction lipidique a présenté des niveaux élevés d'acide linoléique (moyenne de 8,15 g·100 g^{-1} d'acides gras totaux) et linolénique (moyenne de 6,32 g·100 g^{-1} d'acides gras totaux). Le contenu en cendres (0,39 g·100 g^{-1} de lait) n'a pas varié au cours de la période expérimentale et a été caractérisé par des valeurs élevées de Ca et de P, avec un rapport Ca/P compris entre 0,93 et 2,37 et une valeur moyenne de 1,48.

lait d'ânesse / protéines / acides gras / minéraux

1. INTRODUCTION

Cow's milk protein intolerance is the most frequent food intolerance in infancy, occurring in between 0.3 and 7.5% of the infant population [6]. In such cases, when breast feeding is not possible, a cow's milk free diet often resolves symptoms, although some infants can present intolerance to the foods used as alternatives [7], including formulas containing soy or hydrolysed protein [26]. Recent clinical studies confirm ass's milk feeding as a safe and valid treatment of most complicated cases of multiple food intolerance [7]. However, information on ass's milk composition [11, 26, 37, 43] is more limited than that on mare's milk [18, 19, 29, 30, 33, 38], which has also been studied as an infant food [5, 15].

In addition, Carroccio et al. [6] suggest the use of ass's milk, though enriched with medium-chain triglycerides, in a cow's milk free diet in infancy because of its better palatability than semielemental milk formulas, its similar composition to human milk and its hormonal peptides, which stimulate the functional recovery and development of the intestine. Besides peptides providing growth factors and protective factors, substances with bioactive properties are also found among the lipids in milk [35]: the role of dietary fats in food-related allergic symptoms requires particular attention, since the pivotal role of fat-derived inflammatory substances is now acknowledged [28].

On the basis of the advantages recognised in infant nutrition of the use of ass's milk, dietary and therapeutic properties of which have been known since ancient times [37], the present study was carried out to examine the quantitative and qualitative characteristics of ass's milk and their variability, from newly machine-milked animals. Besides the chemical composition and some hygiene parameters, nitrogen and lipid fractions were studied.

2. MATERIALS AND METHODS

2.1. General (animals, housing and feeding)

Six pluriparous asses (3 Martina Franca and 3 Ragusana breed) were used to provide milk samples in a study carried out over two consecutive lactations; two asses (1 Martina Franca and 1 Ragusana), not confirmed gravid, were replaced in the second year of the trial. The animals, stabled with their foals in boxes provided with a large external paddock, had never been milked before, and tested negative for glanders, brucellosis and equine infectious anæmia. In addition, the experimental animals underwent preliminary examinations and serological and laboratory evaluation to make sure they were in a healthy condition.

Both the investigated lactations lasted 150 days. For each lactation asses were machine milked at d 28, 45, 60, 75, 90, 105, 120, 135 and 150 after parturition. The pilot milking machine was a wheeled trolley type with a sheep cluster; from the results of studies on dairy mares [42], operating parameters were set at vacuum level 42 kPa, pulse ratio 50% and pulse rate 120 cycles·min^{-1}.

During the first year of the study the animals were milked three times per day (at 12:00, 15:00 and 18:00 h) and in the second year twice a day (at 12:00 and 15:00 h), since no significant differences in milk yield and composition were observed between the two afternoon milkings during the first year of the trial. Foals were physically separated from the dams 3 h before the first milking, following Doreau's [17] observations in milking nursing mares.

According to their body condition status measured on a 0 to 5 scale [31], which ranged between 3 and 3.5 at foaling, asses were fed a similar diet over the two investigated lactations, consisting of 10 kg meadow hay (CP 9%; EE 1.8%, DE 6.8 MJ·kg^{-1}, as fed) and 2.5 kg grain-based commercial concentrate (CP 15%, EE 2.2%, DE 11.5 MJ·kg^{-1}, as fed), divided into two daily meals.

2.2. Measurements, sampling and laboratory analyses

Individual milk yield was recorded for each milking; at the same time, individual milk samples were taken, split into aliquots and appropriately preserved and stored until analysis.

Refrigerated samples (4 °C) were analysed by IR (Milkoscan 605, Foss Italia) calibrated according to FIL-IDF [22] for fat, crude protein (as N × 6.38) and lactose content. Dry matter (DM) content was measured following drying of weighted samples at 110 °C and ash content was determined gravimetrically after ashing at 530 °C overnight; pH was measured potentiometrically, and titratable acidity was determined by the AOAC method [1]. Gross energy (kJ·kg^{-1}) was calculated with the coefficients reported by Perrin [39], i.e. 9.11 for fat, 5.86 for protein and 3.95 for lactose. Milk hygiene and the healthy condition of the udder were monitored respectively by total bacteria (Bactoscan, 8000) and somatic cell count (Fossomatic 360) of individual samples.

During the second lactation (d 28, 45, 60, 75, 90, 105, 120, 135, 150), bulk milk samples of the two daily milkings were analysed for calcium, sodium, potassium and magnesium by atomic absorption spectrophotometry, phosphorus by spectrophotometry and chloride by a potentiometric method.

On frozen individual samples (20 mL, –80 °C) from the second lactation (d 28, 45, 60, 75, 90 and 105), the non-protein N (NPN), casein and whey protein contents were determined by Kjeldahl's method on milk, acid whey at pH 4.6 and 12% TCA filtrate of milk respectively for total N (TN), non-casein N (NCN) and NPN [2], from which casein (6.38 × (TN-NCN)) and whey protein (6.38 × (NCN-NPN)) contents were calculated.

More thorough analysis of the protein fraction of individual milk samples (d 90 and 105 of the second lactation) was carried out by SDS–PAGE according to the methods described by Pagliarini et al. [38]. Separation of casein and whey protein fractions was carried out on the basis of isoelectric precipitation and sensitivity to temperature according to the method described by

Ochirkhuyag et al. [36]. Briefly, casein was obtained from skimmed milk by isoelectric precipitation (pH 4.6) at 22 °C or 4 °C (45000 *g*, 30 min). The supernatant resulting from the centrifugation at 4 °C was then warmed up to 30 °C and centrifuged again (45 000 *g*, 30 min) to obtain another small casein pellet. Proteins were identified on the basis of molecular weight (molecular weights of markers were 97.4, 66, 42.7, 31, 21.5 and 14.4 kDa; BioRad) and by comparing the migration pattern with that of mare's milk [4, 38]. To determine the relative proportion of different whey proteins, a semi-quantitative analysis on SDS-PAGE was performed and the separating gels were scanned and analysed using Quantiscan software (Biosoft, USA).

Lyophilised samples of bulk ass's milk from the second lactation (d 75, 90, 105, 120) were subjected to lipid extraction and the fatty acid methyl esters (1 μL), prepared by direct transesterification [9], were separated by gas chromatography [8]. Fatty acid composition of feeds was also analysed as described by Chiofalo et al. [8].

2.3. Statistical analysis

Quantitative data on the production of milk and its major constituents, along with the somatic cell count (SCC) and total bacteria count (CFU), were analysed by analysis of variance for a nested experimental structure (proc. GLM; SAS Inc., Cary, NC USA):

$$Y_{ijklmn} = \mu + LAC_i + STAGE_j + BREED_k + ANIMAL(LAC\ BREED)_{ikl} + HOUR(LAC)_{im} + e_{ijklmn},$$

where LAC_i is the year of lactation, $STAGE_j$ is the day of lactation, $BREED_k$ is the breed, $ANIMAL_l$ is the subject nested in the year of lactation and breed, $HOUR_m$ the milking time nested in the year of lactation. Significance was declared at $P < 0.05$. The post hoc Scheffè test was applied to test the significance of differences throughout lactation.

3. RESULTS AND DISCUSSION

3.1. Yield per milking

The experimental asses quickly adapted to the milking machine routines; over the entire experimental period, average milk yield per milking was 740 mL (± 32.3 mL, SEM) being significantly higher ($P < 0.001$) during the second year of the study (606.5 mL vs. 854.3 mL). Milk yield per milking did not vary significantly during lactation (Fig. 1). However**,** the average milk yield of the morning milking was found to be statistically lower than that observed for the afternoon milkings (549.2 mL vs. 949.3 mL; $P < 0.001$). The observed higher milk production during the middle part of the day, also reported by other authors in dairy [16 and unpublished data] and nursing mares [24], supports the hypothesis of a coadaptation of the dam to the suckling rhythm and activity patterns of the foal [24], although such a circadian rhythm for suckling was not noted in free-ranging horses (Doreau, personal communication). The asses' breed did not significantly affect milk yield.

3.2. Milk composition

Overall means of gross composition, standard error of the means together with minimum and maximum values are reported in Table I.

The low dry matter content of ass's milk (Tab. I) was consistent with the values reported in the literature for equid's milk [37, 38]; day of lactation and the other investigated factors of variability, breed, year of lactation and milking times, did not influence the dry matter content of the milk (Fig. 2a).

The observed average protein content (Tab. I), consistent with data reported for ass's milk by Oftedal and Jenness [37] and reviewed in mares' milk by Doreau et al. [19], was not significantly affected by milking times, breed or year of lactation.

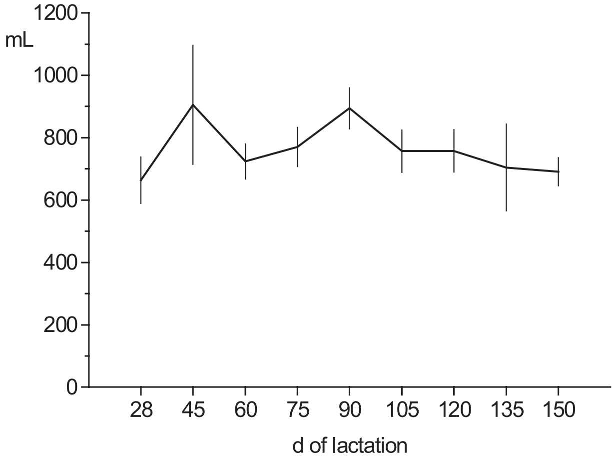

Figure 1. Milk yield per milking during lactation (mean values ± SEM).

On the other hand, protein content varied significantly during lactation (Fig. 2b), as also noted by others in a study on nursing Haflinger mares' milk [30].

The average fat content of ass's milk (Tab. I) was similar to values observed in mare's milk [18, 19, 44], and the wide variability of the fat content was also consistent with previous observations in mare's milk [18, 41]. In particular, fat content, unaffected by breed and milking time effects, was significantly lower during the second investigated lactation (0.20 g·100 g^{-1} milk vs. 0.45 g·100 g^{-1} milk). No significant differences during lactation were observed in the individually variable fat content of the milk (Fig. 2c). Similarly, data on mare's milk did not show marked variations in lipid content from d 40 to 150 of lactation [30].

Table I. Chemical composition of ass's milk (g·100 g^{-1} of milk).

	Mean	SEM	Min.	Max.
Dry matter	8.84	0.07	8.45	9.13
Fat	0.38	0.04	0.10	1.40
Protein	1.72	0.02	1.25	2.18
Lactose	6.88	0.02	6.03	7.28
Ash	0.39	0.02	0.36	0.44

The high lactose content detected (Tab. I) was consistent with values reported for mare's milk [29, 30]; the lactose content of ass's milk was unaffected by breed, milking time, year and stage of lactation.

Calculated gross energy content of ass's milk, on average 1708 kJ·kg^{-1} (± 19.9 kJ·kg^{-1}, SEM), was found to be slightly lower than values reported for mare's milk [30]. Neither stage of lactation nor the other investigated factors of variability influenced the energy content of the milk. According to Oftedal and Jenness [37], the observed low energy content of ass's milk is related to the large amounts of milk secreted to meet the nutritional requirements of the foal for its rapid growth.

The intense neonatal pace of foal growth also requires an adequate mineral content in milk: in this regard, the ashable content of ass's milk (Tab. I), consistent with data on mare's milk [30, 43], was unaffected by year and stage of lactation, breed and milking time.

The concentrations of macro-elements in ass's milk (Tab. II) were also consistent with data reported in the literature for equid's milk [13, 18, 43]; regarding the renal load of solutes of ass's milk, observed values of mineral composition were closer to human milk than other milks except for

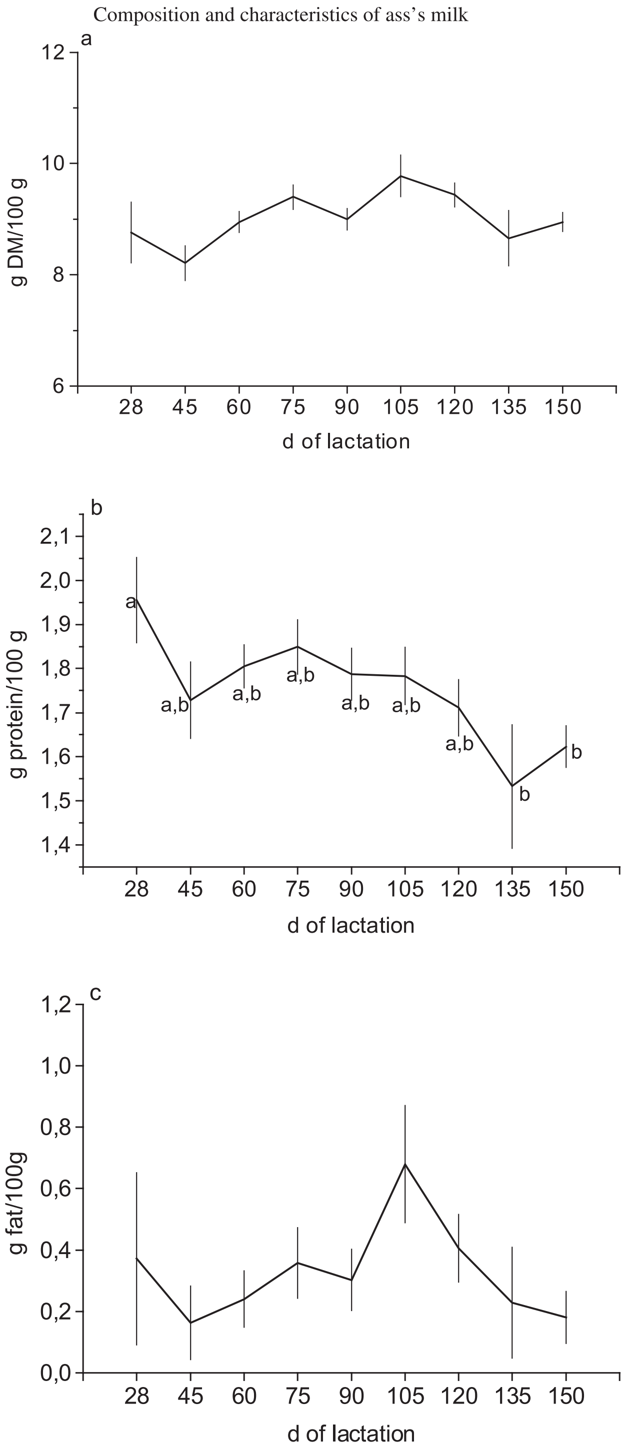

Figure 2. Ass's milk contents (mean values ± SEM) during lactation: (a) dry matter; (b) crude protein; (c) fat.

Table II. Average mineral composition of ass's milk (mg·kg^{-1} of milk).

Macroelements	Mean	SEM	Min.	Max.
Ca	676.7	62.8	360	1140
P	487.0	29.2	320	650
K	497.2	57.6	244	640
Na	218.3	26.2	100	268
Mg	37.3	4.52	17	48
Chloride	336.7	55.5	140	500

the higher absolute levels of calcium and phosphorus [3]. However, ass's milk Ca/P ratio, ranging between 0.93 and 2.37, averaged 1.48 (± 0.12, SEM), which lies between the lower values of cow's milk and the higher values of human milk [38].

Of the physicochemical characteristics of ass's milk, the pH value, ranging between 6.63 and 7.60, did not significantly vary during the investigated lactation periods, in agreement with data reported for mare's milk [30], nor was it influenced by the other investigated factors, as also observed for titratable acidity.

Average pH (7.18 ± 0.03 SEM), higher than that of cow's milk [44], was associated with a low average titratable acidity (2.72 °SH ± 0.13 SEM): these data, consistent with findings on mare's milk [38], may be explained by the lower casein and phosphate contents than in cow's milk.

Positive results for ass's milk hygiene and mammary health were also observed: both the average somatic cell count and the total bacteria in ass's milk were in fact very low (3.68 log SCC·mL^{-1} ± 0.048, SEM; 4.46 log CFU·mL^{-1} ± 0.076, SEM) particularly when compared with the Council Directive 92/46/EEC on milk for human consumption. As a probable effect of a more accurate milking hygiene, it is important to note that total bacterial count significantly improved not only throughout the investigated lactation period (5.2 log CFU·mL^{-1} at d 45 vs. 4.38 log CFU·mL^{-1} at d 150) but also throughout the study (year 1: 4.70 log CFU·mL^{-1} vs. year 2: 4.2 log CFU·mL^{-1}). The overall low microbial count has also been linked to the high content of lysozyme [9], one of the milk components with useful biological properties.

3.2.1. Nitrogenous fractions

Results for the nitrogenous components of ass's milk (Tab. III) show an average NPN content (0.29 g·100 g^{-1} milk) very close to the values for human and mare's milk [29]. The nutritional and biological significance of this milk fraction is still far from being completely understood, but seems to be related to the development of the newborn infant [20].

As Table III shows, the average casein content of ass's milk (47.3% of crude protein) was lower than that reported for mare's milk at the beginning of lactation [33]. In addition, the observed casein level (Tab. III) was intermediate between human and ruminant milk casein [45], while whey protein content was similar to that observed in mare's milk [19, 29].

Casein, whey protein and NPN contents did not vary during lactation, and were not significantly affected by breed or milking time.

As regards the protein content, it is interesting to note that patients who tolerated ass's milk and to a lesser degree mare's milk [5, 7, 15, 23] experienced intolerance to goat's or sheep's milk: this effect may be due to specific levels of major allergenic components of the milk.

Table III. Average composition of nitrogenous fractions of ass's milk (g·100 g^{-1} of milk).

	Mean	SEM	Min.	Max.
NPN (N × 6.38)	0.29	0.01	0.18	0.41
Casein	0.87	0.03	0.64	1.03
Whey protein	0.68	0.02	0.49	0.80

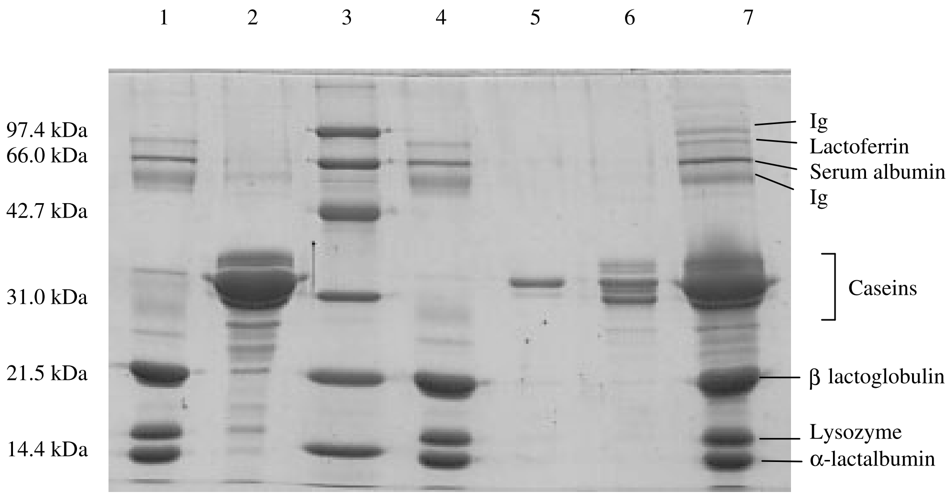

Figure 3. SDS-PAGE of ass's skimmed milk and protein fractions. 1: whey protein fraction soluble at pH 4.6 and 22 °C; 2: casein precipitated at pH 4.6 and 22 °C; 3: mol. weight markers; 4: resulting whey protein fraction after the first centrifuging at 4 °C and the second at 30 °C; 5: residual casein precipitated at 30 °C but soluble at 4 °C; 6: casein obtained by the first centrifuging at 4 °C; 7: skimmed milk (See Materials and Methods for details).

Results obtained by SDS-PAGE (Fig. 3) showed a pattern similar to that reported in the literature for mare's milk [4, 38]. By comparing protein migration patterns with those previously published for mare's milk [4, 38] it was possible to identify the following whey proteins: lactoferrin (approx. M_r 75 kDa), serum albumin (approx. M_r 67 kDa), β-lactoglobulin (approx. M_r 19 kDa), lysozyme (approx. M_r 17 kDa) and α-lactalbumin (approx. M_r 12 kDa). Determined by semi-quantitative analysis, the relative percentages of total whey protein of the above proteins were respectively 4.48%, 6.18%, 29.85%, 21.03% and 22.56%. Based on the literature [14], the two bands with molecular weights of approximately 107 and 60 kDa (respectively 4.68% and 6.81% of total whey protein) should be due to immunoglobulins.

The casein fraction, of approximate molecular weight in the range 27 to 33 kDa, showed a different sensitivity to temperature, as also observed for mare's milk [36]. A residual fraction still soluble at pH 4.6 and 4 °C was obtained (Fig. 3, lane 5) by second centrifuging at 30 °C of the supernatant obtained at 4 °C. The presence in ass's milk of α_s-like casein, β-like casein, κ-like casein and γ-like caseins has been reported [21, 27].

Among potentially allergenic milk components, it must be noted that the observed percentage of β-lactoglobulin was much lower than that in bovine milk, where β-lactoglobulin can account for up to 50% of total whey protein [44]. Moreover, β-lactoglobulin levels in ass's milk were equal to or lower than in mare's milk [19, 29, 33]. Other authors found a low β-lactoglobulin content in mare's milk compared with bovine or even ass's milk [10]. These findings, together with the low casein content, are probably related to the hypoallergenic characteristics reported for both ass's milk and mare's milk [5, 7, 15, 26]; β-lactoglobulin is in fact the probable major milk allergen in infants and small children, whereas casein is considered the predominant allergen in adults [6]. However, the occurrence of genetic variants for ass's milk lysozyme and β-lactoglobulin is reported in the literature [25].

A major difference in whey protein composition between mare's and ass's milk is evident when lysozyme percentage is considered: the percentage of lysozyme in ass's whey protein (21.03%) was in fact much higher than in mare's milk [19, 29, 33], whereas only traces were found in bovine milk [44]. The large amount of lysozyme in ass's milk is confirmed by Civardi et al. [10] and Coppola et al. [11]. According to these last authors, ass's milk represents an optimal growth medium for certain strains of useful lactic acid bacteria [11]. It must be noted that lysozyme can also be considered as an indirect "bifidogenic factor" [34].

Table IV. Average fatty acid composition of ass's milk (g·100 g^{-1} of total fatty acids).

Fatty acid	mean	SEM	Fatty acid	mean	SEM
$C_{4:0}$	0.60	0.14	$C_{10:1}$	2.20	0.08
$C_{6:0}$	1.22	0.11	$C_{12:1}$	0.25	0.05
$C_{7:0}$	Trace		$C_{14:1}$	0.22	0.01
$C_{8:0}$	12.80	0.29	$C_{16:1\ n\text{-}7}$	2.37	0.28
$C_{10:0}$	18.65	0.45	$C_{17:1}$	0.27	0.02
$C_{12:0}$	10.67	0.25	$C_{18:1\ n\text{-}9}$	9.65	0.35
$C_{13i:0}$	0.22	0.03	$C_{20:1\ n\text{-}11}$	0.35	0.05
$C_{13:0}$	3.92	0.45			
$C_{14i:0}$	0.12	0.02	$C_{18:3\ n\text{-}3}$	6.32	0.51
$C_{14:0}$	5.77	0.17	$C_{18:4\ n\text{-}3}$	0.22	0.05
$C_{15i:0}$	0.07	0.01	$C_{20:3\ n\text{-}3}$	0.12	0.01
$C_{15:0}$	0.32	0.01	$C_{20:4\ n\text{-}3}$	0.07	0.01
$C_{16i:0}$	0.12	0.01	$C_{20:5\ n\text{-}3}$	0.27	0.01
$C_{16:0}$	11.47	0.29	$C_{22:5\ n\text{-}3}$	0.07	0.01
$C_{17i:0}$	0.20	0.04	$C_{22:6\ n\text{-}3}$	0.30	0.04
$C_{17:0}$	0.22	0.02			
$C_{18:0}$	1.12	0.12	$C_{18:2\ n\text{-}6}$	8.15	0.47
$C_{20:0}$	0.12	0.01	$C_{18:3\ n\text{-}6}$	0.15	0.01
$C_{22:0}$	0.05	0.01	$C_{20:2\ n\text{-}6}$	0.35	0.05

3.2.2. Lipid fraction

Results of the study of the fatty acid composition of ass's milk (Tab. IV) show the highest concentration of saturated fatty acids (67.6 g·100 g^{-1} ± 1.39, SEM), consistent with data on mare's milk [32, 38]. However, when a limited number of asses reared at pasture was studied, a different milk fatty acids profile was found, with an unsaturated/saturated fatty acid ratio of approximately 1 [40].

More specifically, butyric ($C_{4:0}$), caproic ($C_{6:0}$) and lauric ($C_{12:0}$) acid contents were consistent with mare's milk data (Tab. IV); lower caprylic ($C_{8:0}$) and capric acid ($C_{10:0}$) contents were observed in mare's milk [19]. Among the fatty acids of nutritional interest and weakly represented in ass's milk, the observed concentrations for myristic acid ($C_{14:0}$) and stearic acid ($C_{18:0}$) (Tab. IV) were similar to the values for mare's milk [12, 29], while a lower palmitic acid ($C_{16:0}$) content was observed here [12, 19, 29]. As reviewed by Doreau and Boulot [18] the relatively high amounts of fatty acids with 16 and fewer carbons in mare's milk suggest a biosynthetic pathway common to ruminants but not monogastrics.

Among monounsaturated fatty acids (15.8 g·100 g^{-1} ± 0.5, SEM), levels of both palmitoleic ($C_{16:1\ n-7}$) and oleic acid ($C_{18:1\ n-9}$) (Tab. IV) were lower than values observed in ass's milk by others, in different experimental conditions [40], but they lay within the wide range of variation reported for equine milk [19].

Ass's milk shows a high PUFA content (Tab. IV), with an n–3:n–6 series ratio of 0.86. In particular, linoleic ($C_{18:2\ n-6}$) and linolenic acid ($C_{18:3\ n-3}$) contents, consistent with data reported for mare's milk [19, 42] were slightly lower than those observed by Pinto et al. in ass's milk [40]. The PUFA class also showed small amounts of eicosapentaenoic (EPA, $C_{20:5\ n-3}$) and docosahexaenoic acids (DHA, $C_{22:6\ n-3}$), both present only as traces in mare's milk [42].

In this regard, the dietary fat fraction, the effects of which are described for mare's milk fat [18, 19], showed an unsaturated fatty acid content of 56.9 $g \cdot 100\ g^{-1}$ of total fatty acids, and PUFA levels for n-3 and n-6 series were 17.6 $g \cdot 100\ g^{-1}$ and 23.4 $g \cdot 100\ g^{-1}$ of total fatty acids, respectively.

4. CONCLUSIONS

Throughout the experimental period, milk yield, obtained by asses that quickly adapted to the milking machine, was not affected by stage of lactation but differences were observed between the two investigated years and among daily milkings.

Dry matter, lactose and ash contents of ass's milk were not influenced by the investigated factors of variability, i.e. breed, day and year of lactation or milking times.

On the other hand, the overall low fat content of milk showed a decrease in the second year of the study when the milk fatty acid profile was found similar to that of mare's milk, except for EPA and DHA. Further work is needed to clarify the possible role of nutrition, physiology and machine milking management on quantitative and qualitative characteristics of ass's milk lipid fraction.

Protein percentage of ass's milk significantly declined from the beginning of the study (d 28 of lactation) to d 135; on the other hand casein, whey protein and NPN contents did not significantly change during lactation.

Ass's milk thus resembles mare's milk in its gross composition, but electrophoretic profiles of whey protein depicted a different presence of lactoferrin, serum albumin, β-lactoglobulin, lysozyme and α-lactoalbumin. In particular, the low casein and β-lactoglobulin contents are probably related to the hypoallergenic characteristics reported in the literature for ass's and mare's milk. However, a major difference between mare's and ass's milk was found in the lysozyme content, the level of which was quite high in ass's milk, where a low microbial count was also noted.

As a practical implication of the study, it must be noted that the wider use of ass's milk could provide an economic justification for breeding asses and preserving their natural environment, i.e. marginal and hilly areas, with positive effects on animal biodiversity, helping to protect certain ass breeds from extinction in industrialised countries.

ACKNOWLEDGEMENTS

The authors thank Dr. A. Marano and Dr. R. Belli Blanes (DVM, ASL Salò, Brescia, Italy) and Giuseppe Borghi and his docile asses (Azienda Montebaducco, Salvarano di Quattro Castella, Reggio Emilia, Italy) for their patient cooperation. Research supported by University of Molise (Italy) and M.I.U.R. – Cofinanziamento PRIN 2003.

REFERENCES

[1] AOAC, Official Methods of Analysis, 14th ed., Association of Official Analytical Chemists, Washington, DC, 1984.

[2] Aschaffenburg R., Drewry J., New procedures for the routine determination of the various non-casein proteins of milk, Proceedings of the 15th International Dairy Congress, London, United Kingdom, 1959, pp. 1631–1637.

[3] Belli Blanes R., Il latte di asina a confronto con il latte umano, caprino bovino e le formule commerciali, Convegno "L'asino: attualità e prospettive dell'impiego in campo medico, zootecnico ed alimentare", Mondello (PA), Italy, May 25th, 2001.

[4] Bonomi F., Iametti S., Pagliarini E., Solaroli G., Thermal sensitivity of mares' milk proteins, J. Dairy Res. 61 (1994) 419–422.

[5] Businco L., Giampietro P.G., Lucenti P., Lucaroni F., Pini C., Di Felice G., Iacovacci P., Curadi C., Orlandi M., Allergenicity of mare's milk in children with cow's milk allergy, J. Allergy Clin. Immunol. 105 (2000) 1031–1034.

[6] Carroccio A., Cavataio F., Iacono G., Cross-reactivity between milk proteins of different animals, Clin. Exp. Allergy 29 (1999) 1014–1016.

[7] Carroccio A., Cavataio F., Montaldo G., D'Amico D., Alabrese L., Iacono G., Intolerance to hydrolysed cow's milk proteins in infants: clinical characteristics and dietary treatment, Clin. Exp. Allergy 30 (2000) 1597–1603.

[8] Chiofalo B., Biondi L., Ziino M., Pennisi P., Salvo F., Priolo A., Variazione della composizione acidica del latte di pecore Comisane alimentate con un unifeed secco, Riv. Ital. Sci. Alim. 25 (1996) 261– 266.

[9] Christie W.W., Preparation of ester derivatives of fatty acids for chromatographic analysis, in: Christie W.W. (Ed.), Advances in lipid methodology-2, The Oily Press LTD, Dundee, Scotland, 1993, pp. 69–111.

[10] Civardi G., Curadi M.C., Orlandi M., Cattaneo T.M.P., Giangiacomo R., Capillary electrophoresis (CE) applied to analysis of mare's milk, Milchwiss. 57 (2002) 515–517.

[11] Coppola R., Salimei E., Succi M., Sorrentino E., Nanni M., Ranieri P., Belli Blanes R., Grazia L., Behaviour of Lactobacillus rhamnosus strains in ass's milk, Ann. Microbiol. 52 (2002) 55–60.

[12] Csapò J., Stefler J., Martin T.G., Makray S., Csapò-Kiss Zs., Composition of mares' colostrum and milk. Fat content, fatty acids composition and vitamin content, Int. Dairy J. 5 (1995) 393–402.

[13] Csapò-Kiss Ks., Stefler J., Martin T.G., Makray S., Csapò J., Composition of mares' colostrum and milk. Protein content, amino acid and contents of macro- and micro-elements, Int. Dairy J. 5 (1995) 403–415.

[14] Curadi M.C., Orlandi M., Greppi G.F., Toppino P.M., Barzaghi S., Cattaneo T.M.P., Identification of protein fractions in mares' colostrum and milk, Milchwiss. 55 (2000) 446–449.

[15] Curadi M.C., Giampietro P.G., Lucenti P., Orlandi M., Use of mare milk in pediatric allergology, Proceedings of 14th Congress ASPA (Associazione Scientifica di Produzioni Animali), Firenze, Italy, 2001, pp. 647–649.

[16] Dell'Orto V., Salimei E., Bontempo V., Fantuz F., Toppino P.M., Contarini G., Locci F., Dairy mares' milk: I. Yield and composition of milk and relation with some plasma metabolites, J. Dairy Sci. 77 (Suppl. 1) (1994) 347.

[17] Doreau M., Le lait de jument, INRA Prod. Anim. 4 (1991) 297–302.

[18] Doreau M., Boulot S., Recent knowledge on mare production: a review, Livest. Prod. Sci. 22 (1989) 213–235.

[19] Doreau M., Gaillard J.L., Chobert J.M., Léonil J., Egito A.S., Haertlé T., Composition of mare and donkey milk fatty acids and protein and consequences on milk utilisation, Proceedings of 4° Convegno Soc. Ital. Ippolog., Campobasso, Italy, 2002, pp. 51–71.

[20] Emmett P.M., Rogers I.S., Properties of human milk and their relationship with maternal nutrition, Early Hum. Dev. 49 (Suppl.) (1997) S7–S28.

[21] Fantuz F., Vincenzetti S., Polidori P., Vita A., Polidori F., Salimei E., Study on protein fractions of donkey milk, Proceedings of 14th Congress ASPA (Associazione Scientifica di Produzioni Animali), Firenze, Italy, 2001, pp. 635–637.

[22] FIL-IDF, Détermination des teneurs en matière grasse laitière, protéines et lactose, Norme FIL-International, 141B, FIL, Bruxelles, 1996.

[23] Gall H., Kalveram C.M., Sick H., Sterry W., Allergy to the heat-labile proteins of alpha-lactalbumin and beta-lactoglobulin in mares milk, J. Allergy Clin. Immunol. 97 (1996) 1304–1307.

[24] Glade M.J., Effects of dietary yeast culture supplementation of lactating mares on the digestibility and retention of the nutrients delivered to nursing foals via milk, Equine Vet. Sci. 11 (1991) 323–329.

[25] Herrouin M., Mollé D., Fauquant J., Ballestra F., Maubois J.L., Léonil J., New genetic variants identified in donkey's milk whey proteins, J. Prot. Chem. 19 (2000) 105–115.

[26] Iacono G., Carroccio A., Cavataio F., Montaldo G., Soresi M., Balsamo V., Use of ass's milk in multiple food allergy, J. Pediatr. Gastroenterol. Nutr. 14 (1992) 177–181.

[27] Iametti S., Sessa F., Bonomi F., Greppi G.F., Feligni M., Enne G., Biochemical methods for the characterisation of mare and donkey casein, IDF Bulletin, issue Milk protein polymorphism, 1998, pp. 268–274.

[28] Kaila M., Salo M.K., Isolauri E., Fatty acids in substitute formulas for cow's milk allergy, Allergy 54 (1999) 74–77.

[29] Malacarne M., Martuzzi F., Summer A., Mariani P., Protein and fat composition of mare's milk: some nutritional remarks with

reference to human and cow's milk, Int. Dairy J. 12 (2002) 869–877.

[30] Mariani P., Summer A., Martuzzi F., Formaggioni P., Sabbioni A., Catalano A.L., Physicochemical properties, gross composition, energy value and nitrogen fractions of Haflinger nursing mare milk throughout 6 lactation months, Anim. Res. 50 (2001) 415–425.

[31] Martin Rosset W., L'alimentation des chevaux, INRA Édition, Paris, 1990.

[32] Martuzzi F., Summer A., Catalano A., Barbacini S., Mariani P., Il contenuto in acidi grassi polinsaturi del grasso del latte di cavalla prodotto nelle prime settimane di lattazione, Proceedings of 52nd Congress of the Società Italiana Scienze Veterinarie, Silvi Marina, Teramo, Italy, 1998, pp. 537–538.

[33] Martuzzi F., Tirelli A., Summer A., Catalano A.L., Mariani P., Ripartizione delle sieroproteine nel latte dei primi due mesi di lattazione in giumente Sella Italiano, Riv. Soc. Ital. Ippologia 6 (2000) 21–27.

[34] Modler H.W., Bifidogenic factors – sources, metabolism and applications, Int. Dairy J. 4 (1994) 383–407.

[35] Molkentin J., Bioactive lipids naturally occurring in bovine milk, Nährung 43 (1999) 185–189.

[36] Ochirkhuyag B., Chobert J.M., Dalgalarrondo M., Haertlé T., Characterization of mare caseins. Identification of α_{s1}- and α_{s2}-caseins, Lait 80 (2000) 223–235.

[37] Oftedal O.T., Jenness R., Interspecies variation in milk composition among horses, zebras and asses (Perissodactyla: Equidae), J. Dairy Res. 55 (1988) 57–66.

[38] Pagliarini E., Solaroli G., Peri C., Chemical and physical characteristics of mares' milk, Ital. J. Food Sci. 4 (1993) 323–332.

[39] Perrin D.R., The caloric value of milk of different species, J. Dairy Sci. 25(1958) 215–220.

[40] Pinto F., Lestingi A., Caputi Jambrenghi A., Marsico G., Vonghia G., Conservazione e valorizzazione dell'asino di Martina Franca: influenza dell'integrazione alimentare su alcuni aspetti quanti-qualitativi del latte. I. Indagine preliminare, Proceedings of 4th Congress on Biodiversity, Alghero, Sassari, Italy, 1998, pp. 1173–1176.

[41] Salimei E., Bontempo V., Dell'Orto V., Nutritional status of the foals related to the age and to mares' feeding, Pferdeheilkunde 12 (1996) 245–248.

[42] Salimei E., Cattaneo M., Chiofalo B., Dell'Orto V., Exploitation of mare's milk by polyunsaturated fatty acids enrichment, in: Enne G., Greppi G.F. (Eds.), Food & Health: role of animal products, 1996, pp. 223–227.

[43] Schryver H.F., Oftedal O.T., Williams J., Cymbaluk N.F., Antczak D., Hintz H.F., A comparison of the mineral composition of milk of domestic and captive wild equids (*Equus przewalski, E. zebra, E. burchelli, E. caballus, E. asinus*), Comp. Biochem. Physiol. 85A (1986) 233–235.

[44] Solaroli G., Pagliarini E., Peri C., Composition and nutritional quality of mare's milk, Ital. J. Food Sci. 1 (1993) 3–10.

[45] Travia L., Significato biologico e nutrizionale del latte nell'alimentazione dell'uomo, Il Latte 9 (1986) 358–371.