Effect of extruded linseed on sarda donkey milk quality

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ABSTRACT

The aim of this work was to evaluate the effect of extruded linseed in donkey diet on milk yield, milk composition and milk fatty acids (FA) profile. Eight Sarda donkeys were allocated to two groups homogenous for milk yield (0.354 ± 124 kg/d), days in milking (123 ± 41 d), and body weight (110 ± 15 kg). The first group was fed a control diet (CON), while the second group was fed the control diet supplemented with 100 g/day per head of extruded linseed (ELS). The trial lasted 7 weeks, with 2 weeks of adaptation and 5 weeks of experimentation. Data were analysed using a linear mixed model, considering the diet, sampling time and their interaction as fixed effects and animals as random effect. Milk yield and milk chemical composition were not affected by the diet. The diet led to some variations of FA profile. Long-chain FA, unsaturated FA and polyunsaturated FA were higher in ELS compared to control group. These results reflected the trend of some individual FA, in particular the concentration of alpha-linolenic acid. This FA was affected by diet, sampling time and their interaction and, at the end of the trial, the average value was double in ELS than CON. Dietary linseed reduced thrombogenic and atherogenic indices and increased hypo-to hypercholesterolemic ratio of milk. In conclusion, extruded linseed can be used in donkey diet to improve the milk FA profile, in terms of polyunsaturated FA and nutritional indices, which could have a positive impact on human health.

HIGHLIGHTS

- The valorisation of donkey milk could be a good strategy to preserve donkey native breed.
- Extruded linseed, rich in alpha-linolenic acid, improves fatty acid profile of donkey milk, increasing PUFA and decreasing SFA.
- Linseed supplementation affects positively milk nutritional indices.

Introduction

For centuries donkeys have played an important role in human societies as working and transporter animals. In some developed and technologically advanced societies they suffer for unused and abandon, with loss of genetic diversity because of the extinction risk of some breeds. Recently, some studies (Bornaz et al. 2009; Liu et al. 2020; Cesarani et al. 2021) focussed on species different from cattle, often named as minor dairy species, but that are of great economic and social importance for several regions worldwide. Donkey has regained interest in developed societies for the use in donkey-assisted therapy, social recreational activities, and milk for human nutrition and cosmetics applications. In particular, donkey milk has been used as alternative to cow milk in children suffering of cows’ milk protein allergy or intolerance (Tesse et al. 2009; Polidori et al. 2015; Sarti et al. 2019), because of the similarity to human milk for casein content, whey protein and lactose (Vincenzetti et al. 2017). Moreover, donkey milk has also other important and peculiar properties, that makes this product very interesting from a nutritional and healthy point of view. The content in lysozyme (1 g/L), a protein with high antimicrobial properties, is higher than in ruminants (<0.6 mg/L) and human milk (0.4–0.7 g/L) (Salimei et al. 2004; Claesys et al., 2014; Gridneva et al. 2021); high levels of this enzyme, together with other antimicrobial agents (lactoperoxidase and lactoferrin), makes this milk unique for fresh consumption, as a preservative in cheese making, and for use in the innovative production of mixed cheeses (Galassi et al. 2012; Cosentino et al. 2022).

A problem of using donkey milk, especially in children nutrition, is related to its low-fat content. Donkey...
milk has usually three times less fat percentage compared to human milk, corresponding to a lower energy supply (Altomonte et al. 2019). For this reason, in children below six months, with a diet based exclusively on donkey’s milk, the fat levels would need to be supplemented, e.g. by adding vegetable oils (Altomonte et al. 2019). Despite the low-fat content, the milk fat triglycerides are characterised by a high PUFA content (10–15% of total fatty acids, FA; Martini et al. 2014; Blasi et al. 2008) compared to ruminant (ranging from 3 to 6.5% of total FA; Blasi et al. 2008; Borreani et al. 2013; Correddu et al. 2019) and humans’ milks (Chiofalo et al. 2011). This is because in non-ruminants’ milk, the PUFA content mirrors the fatty acids composition of the diets, whereas in ruminant the biohydrogenation activity deeply modifies the dietary fatty acids. The Sarda donkey is a breed originating and living in the Island of Sardinia (Italy); the animals are bred in extensive farming system browsing and grazing on natural wooded and shrubs pastures, with fresh vegetal sources only during spring season. During the summer season, fat supplementation could be a feeding strategy to increase the energy density of the diets and to enhance the nutritional quality of the milk fat. Extruded linseed, characterised by high alpha-linolenic acid (C18:3 n3, ALA), has been found a valuable feeding strategy to enhance the concentration of omega-3 fatty acids in cow (Oliveira et al. 2021) and goat milk (Cosentino et al. 2021). Our hypothesis is that supplementing donkey with extruded linseed will enhance the FA profile of milk by reducing the saturated fatty acid content and increasing the unsaturated fatty acid content.

Materials and methods

Animals and dietary treatments

All procedures were carried out in accordance with the European Union legislation for the protection of animals used for scientific purposes (2010/63/EU Directive). This research was conducted in a private farm rearing 25 Sarda donkeys, an Italian breed native to the island of Sardinia. For the experiment, eight donkeys were assigned to two experimental groups (4 animals each) homogeneous for body weight (BW, 110 ± 15 kg), milk production (0.354 ± 0.123 kg) and days in milking (DIM, 123 ± 41 d). The two groups were randomly assigned to one of the experimental diets (Table 1): a control diet (CON) and a diet including 100 g/day per head of extruded linseed (ELS). The experiment lasted 7 weeks, with 2 adaptation weeks and 5 experimental weeks of sampling. During the adaptation period the animals grazed on dry range-land and were fed a commercial concentrate (1200 g/day per head) and hay (3000 g/d per head). In the diet of animals belonging to the ELS group, 100 g of concentrate were replaced by 100 g/day per head of extruded linseed. Animals and diets were managed as follows: in the morning (h 8:00) the foals were separated from the mothers that were fed 400 g of concentrate (350 g, + 50 g of extruded linseed for the ELS group); the milking took place at h 11:00 and 200 g of concentrate were given; after that, foals were rejoined with their mothers; in the evening (h 17:00) donkeys received other 600 g (550 g, + 50 g of extruded linseed for ELS only). Fresh water was always available. Considering that the trail was carried out on summer season, no pasture was available and the whole diet was as explained above.

Samples collection and analysis

Samples of the feed ingredients were collected and analysed for chemical composition. The dry matter (DM) content was determined by oven-drying at 105 °C for 24 h; neutral detergent fibre (NDF) and acid detergent lignin (ADL) were determined following the method of Van Soest et al. (1991), using an Ankom220 fibre analyser (AnkomTMtechnology, Fairport, NY, USA); NDF was measured using heat stable amylase and expressed exclusive of residual ash (aNDFom); ADL: acid detergent fibre; ADL: acid detergent lignin determined by solubilisation of cellulose with sulphuric acid; CP: crude protein; EE: ether extract.

Table 1. Ingredients and chemical composition of the diets supplied to lactating donkeys.

| Ingredients offered, g/d | Concentrate | Linseedb | Hay | CON | ELS |
|--------------------------|-------------|----------|-----|-----|-----|
| Dry matter, g/d          | 90.17       | 91.1     | 90.92| 3809.6| 3810.6|
| NDF                      | 23.71       | 24.0     | 69.43| 564.4| 564.4|
| ADF                      | 10.42       | 12.0   | 46.55| 362.9| 363.2|
| ADL                      | 2.21        | 3.1    | 8.17 | 64.8 | 65.0 |
| CP                       | 16.38       | 26.4   | 4.94 | 81.9 | 84.3 |
| EE                       | 4.42        | 34.7   | 2.00 | 26.9 | 34.1 |
| Ash                      | 8.18        | 4.4    | 7.8  | 79.1 | 78.2 |

aCON: control diet; ELS: diet including 100 g/d of extruded linseed.
bLinseed: extruded linseed (Linoies, CortalExtrasoy) SpA, Padova, Italy; fatty acid profile of linseed (g/100 g of total FA) = C16:0 (5.5); C18:0 (4.5); C18:1 cis-9 (18.0); C18:2 n-6 (15.0); C18:3 n-3 (56.3).

C16:0: fatty acid profile of linseed (g/100 g of total FA) = C16:0 (5.5); C18:0 (4.5); C18:1 cis-9 (18.0); C18:2 n-6 (15.0); C18:3 n-3 (56.3).
(proc. 920.39; AOAC. 2005) and ash by using a muffle at 550 °C (proc. 942.05; AOAC. 2000).

Individual milk samples were collected weekly, and each sample was split into 2 aliquots: one was immediately analysed for the determination of fat, protein, casein, lactose, and MUN contents, using a MilkoScan 6000 instrument (Foss Electric, Hillerød, Denmark), for somatic cell count (SCC) using a Fossomatic 360 instrument (Foss Electric), and for pH; the second aliquot was kept at −20°C before the gas chromatographic (GC) analysis.

Fat extraction and fatty acid analysis

Fat extraction was performed following Nudda et al. (2005) with some modifications. Briefly, 0.4 mL of ammonia (25%) and 2 mL of ethanol (95%) were added to 2 g of milk. Then 4 mL of hexane were used to extract the fat content. The mixture was shaken on a vortex mixer for 1 minute, centrifuged at 3000 xg for 15 minutes, and the supernatant was collected. After the addition of 2 mL of ethanol, a second extraction was conducted using and 4 mL of hexane. The samples were vortexed and centrifuged as described above and the upper layer was collected. A third extraction was performed using 4 mL of hexane following the same conditions of the previous extractions; then, the supernatant was collected. The fatty acid methyl esters (FAME) were prepared following the FIL-IDF standard procedure (FIL-IDF 1999). Briefly, the extracted fat (11–14 mg) was methylated using 500 μL of sodium methoxide (0.5 N) and then vortexed for 2 minutes to shake the solution. Then, 1 mL of internal standard, composed by valerate and tridecanoic methyl esters (0.4 mg/ml in hexane), was added to each sample. The mixture was vortexed for 1 minute and then the upper part was moved to a vial for GC analysis.

A 7890A GC system (Agilent Technologies, Santa Clara, CA, USA) with a CP-Sil88-fused silica capillary column SP™-2560 (100 m × 0.25 mm ID, 0.20-μm film, Supelco, Bellefonte, PA, USA) was used to determine the FA composition. The instrument was provided with 7693 Autosampler (Agilent Technologies, Santa Clara, CA, USA) and a flame ionisation detector (FID). Helium, with a flow rate of 1 ml/min and a pressure of 28 psi, was used as carrier gas. The initial column temperature, maintained for 4 min, was 45°C that increased by 13°C/min to achieved 175°C. This temperature was kept for 27 min and increased by 4°C/min until 215°C. The detector and the injector had a temperature of 250°C. Split ratio was 1:80. The area of the FAME was identified using OpenLABCDS GCChemStation Upgrade software data system (Revision C.01.04, Agilent Technologies Inc., Santa Clara, CA, USA). The retention time of samples fatty acid and those of methyl standard were compared to identify the peaks of the samples, as described by Nudda et al. (2005). The individual fatty acids were expressed in g/100 g of the total FAME and divided into the following corresponding groups:

- SFA= ∑ of individual saturated fatty acids
- UFA= ∑ of individual unsaturated fatty acids
- MUFA= ∑ of individual monounsaturated fatty acids
- PUFA= ∑ of individual polyunsaturated fatty acids
- TFA= ∑ of individual trans fatty acids
- OCFA= ∑ of individual odd-chain fatty acids
- BCFA= ∑ of individual branched-chain fatty acids
- OBCFA= ∑ of individual odd- and branched-chain fatty acids
- SCFA= ∑ of individual short-chain fatty acids (from C4:0 to C10:0)
- MCFA= ∑ of individual medium-chain fatty acids (C11:0 to C17:0)
- LCFA= ∑ of individual long-chain fatty acids from (C18:0 to C22:6)
- PUFA 3-n= ∑ of individual n-3 fatty acids
- PUFA 6-n= ∑ of individual n-6 fatty acids
- CLA= ∑ of individual conjugated linoleic acids

The nutritional properties were also evaluated using atherogenic index (AI) and trombogenic index (TI), calculated according to Ulbricht and Southgate (1991), with some modification suggested by Nudda et al. (2013): AI = [(12:0 + (4 × 14:0) + 16:0)/(PUFA) + (MUFA)]; TI = [(14:0 + 16:0)/(0.5 × MUFA) + (0.5 × n-6) + (3 × n-3) + (n-3: n-6)]; and hypcholesterolemic to hypercholesterolemic ratio (h:H), calculated in accordance with Fernández et al. (2007): [(sum of 18:1cis-9, 18:1cis-11, 18:2 n-6, 18:3 n-6,18:3 n-3, 20:3 n-6, 20:4 n-6, 20:5 n-3, 22:4 n-6, 22:5 n-3 and 22:6 n-3)/(14:0 + 16:0)].

Statistical analysis

Data were analysed with a linear mixed model using the PROC MIXED procedure of SAS (SAS institute, 1992), as follow:

\[ y_{ijkl} = \mu + \text{diet}_i + \text{time}_j + (\text{diet} \times \text{time})_{ij} + \text{animal}_k + e_{ijkl} \]

where: \( y = \) response variable; \( \mu = \) overall mean;
Table 2. Milk composition of donkeys belonging to the two experimental groups: CON (control diet) ELS (linseed diet).

| Group* | CON | ELS | SEM | Group | Time | G × T | p Value |
|--------|-----|-----|-----|-------|------|-------|---------|
| Milk yield, kg/d | 0.339 | 0.263 | 0.019 | .342 | .096 | .093 |
| Fat, % | 0.36 | 0.43 | 0.026 | .563 | .130 | .108 |
| Protein, % | 1.52 | 1.49 | 0.020 | .632 | .046 | .290 |
| Casein, % | 1.25 | 1.27 | 0.017 | .780 | .043 | .470 |
| Lactose, % | 6.39 | 6.75 | 0.086 | .317 | .198 | .786 |
| Log SCC, ×1,000 cells/mL | 1.04 | 0.92 | 0.094 | .724 | <.001 | .262 |
| Bacterial load, ×1,000/mL | 18.70 | 7.70 | 6.373 | .400 | .107 | .433 |
| Urea, mg/dL | 19.38 | 25.89 | 1.156 | .057 | <.001 | .990 |
| Freezing point, ‘H’ | 0.53 | 0.54 | 0.002 | .600 | .002 | .808 |
| pH | 6.88 | 6.97 | 0.026 | .402 | .053 | .597 |

*CON: control diet; ELS: diet including 100 g/d of extruded linseed.

diet = the fixed effect of the i dietary treatment (i = 2; CON and ELS); time = the fixed effect of the j week of sampling (j = 5; 1, …, 5); diet × time = the fixed effect of the ij interaction between dietary treatment and week of sampling; anim = the random effect of the k animal (k = 8); e = the vector of the random residuals.

Results

Composition of diets

The chemical composition of the diets and of dietary ingredients used in this experiment are reported in Tables 1. Including extruded linseed to the diet increased the fat content of the ELS diet. The lipid composition was characterised by high proportion of alpha-linolenic acid (18:3 c9,c12,c15; n-3), which exceeded 50% of the total FA.

Milk yield and composition

Milk yield and composition were not significantly affected by the dietary ELS (p > .05, Table 2). Sampling time influenced several milk components (p < .05). The temporal evolution of fat, protein, casein, lactose, and SCC during the trial is reported in Figure 1. The fat content was very low in both groups (0.36% and 0.43% for CON and ELS, respectively) and it did not change during the experiment. The percentage of milk protein, casein and lactose increased during the trial, achieved the peak at the third week (Figure 1). The SCC kept low values until the 4th week of the trial, after that an increase of this parameter was recorded, partly due to a concentration effect and, partly, to one jennet that achieved a higher value (2.8) than the others (mean of 1.59).

Fatty acids profile

Dietary administration of ELS influenced milk FA composition, as showed in Table 3. In particular, some short- and medium-chain fatty acids (C9:0, C15:0 and C16:0) were reduced by ELS supplementation (p < .05). A significant diet × sampling time interaction was found for C18:0 and C18:3n3 (stearic acid, SA and alpha linolenic acid, ALA). The concentration of SA was very similar between the two groups throughout the trial, except for the 2nd sampling showing higher value for CON (Figure 2). The ALA content (Figure 3) at the beginning of the trial showed a similar value between the two groups and then doubled in ELS group during the experimental period compared to CON group (15.76 vs 7.83 g/100g of FA).

The dietary ELS affected also the LCFA, SFA, PUFA, UFA and OCFA groups. In particular, SFA decreased and PUFA increased in ELS group. The inclusion of linseed in the diet of donkeys resulted in a reduction of n6/n3 ratio (p < .05), TI (p < .05) and AI (p = .07), and in the increase of h/H ratio (Table 4).

The sampling time influenced all FA groups (p < .001) and some nutritional indexes. The SCFA and MCFAs decreased and, consequently, LCFA increased during the trial (Figure 4). The nutritional indexes TI, AI decreased and h:H increased during the sampling time. A significant interaction diet × sampling time was also observed for BCFA and OBCFA (p < .05) which were lower in ELS than CON in the middle part of the trial (Figure 5).

Discussion

Milk composition

The fat content of donkey milk is generally low and related to the lactation period (Martemucci and D’Alessandro 2012). The fat content recorded during the trial was consistent with previous research carried out with the Littoral-Dinaric breed (Ivanković et al. 2009) and consistent with the fat content on Martina Franca breed at the end of lactation that was 0.42% (Martemucci and D’Alessandro 2012). The fat content was lower than values reported in donkeys at the beginning of lactation that in Martina Franca breed reached the 0.77% (Martemucci and D’Alessandro 2012). Salimei et al. (2004) reported no difference on the fat concentration among breeds (Martina Franca and Ragusana breed) and during different lactation periods. The protein content in milk observed in our trial was lower than the values reported in other studies on milk jennet which averaged about 1.8% (Salimei et al. 2004;
Giosuè et al. 2008; Cosentino et al. 2016) but in line with values reported previously in animals in late lactation stage (1.41%; Ivanković et al. 2009). Casein values followed the protein trend. The variations in protein and casein concentrations recorded in this study confirmed the decrease of these components during the lactation period, probably to meet the different protein request of young animals, even if the experimental period was too short to highlight more evident differences. The increase of SCC at the end of the trial could be partially due to the progress of the lactation. In fact, the highest value of SCC was found at the beginning and at the end of lactation (Beghelli et al. 2009).

**Milk fatty acid profile**

Donkey milk has a specific fatty acid profile, very different from ruminant milk (Massouras et al. 2017). In particular, a lower concentration SFA of and higher concentration of PUFA in donkey milk than ruminant milk can be observed, with important consequences on the nutritional quality of fat milk. According to the literature, these large differences are related to the different physiology between ruminant and non-ruminant herbivores, and, in particular, to the lack of biohydrogenation processes that in ruminants markedly modifies the fatty acid profile of ingested diets.
However, variation in FA profile of donkey milk have been found in literature, due to dietary ingredients, variations of body condition, farming system and breed (Salimei et al. 2004; Valle et al. 2018). In this trial the inclusion of linseed in the diet was effective to increase C18:3n-3 concentration; this suggests that, under these dietary and experimental conditions, an adaptation-time is necessary. To date, there is a lack of information in literature about the effect of donkeys increased the ALA concentration in milk.

The evolution of ALA during the trial evidenced that linseed was effective to increase C18:3n-3 concentration only after 2 weeks of administration; this suggests that, under these dietary and experimental conditions, an adaptation-time is necessary. To date, there is a lack of information in literature about the effect of

### Table 3. Fatty acid composition of donkeys’ milks belonging to the two experimental groups: CON (control diet) ELS (linseed diet).

| Fatty acid (g/100 g of FAME) | Group<sup>b</sup> | p Value |
|-----------------------------|-------------------|---------|
|                             | CON ELS SEM Group | Time G × T |
| C6:0                        | 0.18 0.13 0.013 0.157 | <.001 .689 |
| C8:0                        | 2.58 2.12 1.071 2.199 | .003 .452 |
| C9:0                        | 0.14 0.10 0.006 0.017 | .044 .244 |
| C10:0                       | 6.32 4.93 0.298 0.104 | .001 .736 |
| C11:0                       | 0.91 0.61 0.068 0.347 | .156 .171 |
| C12:0                       | 6.64 4.88 0.351 0.131 | .003 .855 |
| anteisoC13:0                | 0.17 0.09 0.020 0.440 | .665 .339 |
| C14:0                       | 6.06 4.50 0.313 0.188 | .000 .743 |
| C14:1c9                     | 0.33 0.18 0.043 0.467 | .649 .326 |
| C15:0                       | 0.36 0.29 0.015 0.046 | <.001 .171 |
| isoC16:0                    | 0.14 0.11 0.007 0.248 | <.001 .285 |
| C16:0                       | 20.63 18.27 0.363 0.014 | .000 .283 |
| C16:1c7                     | 0.30 0.34 0.014 0.379 | .001 .708 |
| anteisoC17:0                | 0.14 0.09 0.008 0.162 | <.001 .194 |
| C16:1c9                     | 2.52 1.96 0.228 0.569 | <.001 .397 |
| C17:0                       | 0.35 0.27 0.017 0.144 | <.001 .467 |
| C17:1c6+c7                  | 0.14 0.16 0.005 0.462 | <.001 .960 |
| C17:1c9                     | 0.30 0.25 0.012 0.074 | .032 .303 |
| C18:0                       | 3.64 3.03 0.170 0.117 | <.001 .001 |
| C18:1c9                     | 0.17 0.15 0.005 0.200 | .000 .799 |
| C18:1t12                    | 0.49 0.77 0.148 0.087 | <.001 .001 |
| C18:1c9                     | 17.15 18.55 0.508 0.247 | <.001 .581 |
| C18:1t11                    | 0.93 0.92 0.033 0.891 | .000 .130 |
| C18:2n6                     | 19.68 21.18 0.587 0.421 | <.001 .552 |
| C20:0                       | 0.09 0.07 0.003 0.038 | .002 .066 |
| C20:1t11                    | 0.19 0.16 0.006 0.029 | .003 .211 |
| C18:3n3                     | 7.79 14.35 0.709 0.004 | <.001 .015 |
| C22:0                       | 0.02 0.02 0.001 0.036 | 0.397 .504 |
| C20:3n3                     | 0.16 0.23 0.009 0.017 | <.001 .151 |
| EPA                         | 0.02 0.02 0.001 0.470 | <.001 .827 |
| C24:0                       | 0.02 0.02 0.001 0.834 | .834 .890 |
| DPA                         | 0.06 0.07 0.003 0.194 | <.001 .711 |
| DHA                         | 0.01 0.01 0.001 0.516 | .007 .317 |

<sup>a</sup>EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid.

<sup>b</sup>CON: control diet, ELS: diet including 100 g/d of extruded linseed.

### Table 4. Groups, ratio and indices of fatty acids from donkeys’ milks belonging to the two experimental groups: CON (control diet) ELS (linseed diet).

| Fatty acid (g/100 g of FAME)<sup>a</sup> | Group<sup>b</sup> | p Value |
|------------------------------------------|-------------------|---------|
|                                          | CON ELS SEM Group | Time G × T |
| SCFA                                     | 9.26 7.31 0.410 0.117 | .001 .642 |
| MCFAs                                    | 39.49 32.44 1.090 0.107 | <.001 .251 |
| LCFA                                     | 51.25 60.25 1.288 0.042 | <.001 .376 |
| SFA                                       | 48.73 39.83 1.290 0.016 | <.001 .339 |
| MUFA                                      | 22.90 23.76 0.612 0.546 | <.0001 .440 |
| PUFA                                      | 28.37 36.41 1.089 0.020 | <.001 .153 |
| OCFAs                                     | 51.27 60.17 1.290 0.016 | <.001 .339 |
| BCFA                                      | 1.76 1.28 0.071 0.085 | <.0001 .059 |
| OBCFA                                     | 0.77 0.59 0.031 0.014 | <.001 .002 |
| n6:n3                                     | 2.53 1.87 0.071 0.033 | <.001 .008 |
| PUFA6                                     | 20.23 21.63 0.589 0.449 | <.0001 .551 |
| PUFAs                                     | 8.04 14.68 0.717 0.004 | .001 .015 |
| n6:n3                                     | 2.80 1.54 0.157 0.043 | .270 .116 |
| CLA                                       | 0.04 0.04 0.003 0.269 | .059 464 |
| TFA                                       | 0.78 1.04 0.147 0.146 | <.001 <.0001 |
| AI                                        | 1.05 0.71 0.054 0.072 | <.001 .357 |
| h:H                                       | 0.62 0.36 0.033 0.044 | <.001 .025 |
| Ti                                        | 1.77 2.50 0.105 0.018 | <.001 .167 |

<sup>a</sup>SCFA: short-chain fatty acids, sum of the individual fatty acids from C4:0 to C10:0; MCFAs: medium-chain fatty acids, sum of the individual fatty acids from C11:0 to C17:0; LCFA: long-chain fatty acids, sum of the individual fatty acids from C18:0 to DHA; SFA: sum of the individual saturated fatty acids; UFA: sum of the individual unsaturated fatty acids; MUFA: sum of the individual monounsaturated fatty acids; PUFA: sum of the individual polyunsaturated fatty acids; TFA: trans fatty acids, sum of the individual trans fatty acids, except CLA isomers; OCFAs: sum of the individual odd-chain fatty acids; BCFA: branched-chain fatty acids, sum of iso- and anteiso-FA; OBCFA: odd- and branched-chain fatty acids, sum of odd- and iso-, and anteiso-FA; PUFAs: sum of individual n-3 and n-6 fatty acids, respectively; CLAs: sum of individual conjugated linoleic acids; Ti: thrombogenic index; AI: atherogenic index; h:H: hypocholesterolemic to hypercholesterolemic ratio.

<sup>b</sup>CON: control diet, ELS: diet including 100 g/d of extruded linseed.
extruded linseed on milk fatty acid profile of donkey milk. However, the increase in ALA concentration in milk of ELS group was expected, as previously observed in other monogastric species such as swine (Yao et al. 2012) and extensively reported in ruminants, such as sheep (Mele et al. 2007; Correddu et al. 2016), goats (Nudda et al. 2006) and cows (Chilliard et al. 2009; Hurtaud et al. 2010). However, the extent of the linseed effect in donkey was higher compared to that observed in ruminants. Indeed, in monogastric herbivorous the milk content of LC-PUFA is largely dependent on the dietary fatty acid composition, due to the absence of the unsaturated FA biohydrogenation occurring in ruminants (Sjaastad et al. 2010). As an example, dietary linseed allows to reach ALA concentration in milk of 2.00, 2.06 and 2.74 g/100g of FA in cow, goat and sheep, respectively (Chilliard et al. 2013; Ferlay et al. 2013; Serra et al. 2018), whereas, in the present work, this FA reached the concentration of 14.4 g/100g of FA, in agreement to that observed by Falaschini et al. (2009) in Romagnola breed fed extruded linseed, and by Valle et al. (2018) in donkeys fed a diet with 50% of pasture and 50% of hay. Pasture essences contain high levels of ALA and contribute to reach high levels of this FA in the milk of grazing animals (Nudda et al. 2014). Accordingly, Valle et al. (2018) observed that increasing proportions of pasture in the diet of donkeys resulted in increased concentration of ALA, that reached value of about 20 g/100g of total FA in donkeys fed exclusively on pasture.

The increased level of ALA, and consequently of PUFA n3, and the concomitant reduction of SFA concentration (e.g. C16:0) lead to an improvement of the nutritional quality of milk from donkey fed ELS. This was confirmed by the improvement of some nutritional indexes as the reduction of n6/n3 ratio and AI and TI indexes, and the increase of h/H ratio. That is a positive feature according to the position of the Academy of Nutrition and Dietetics, which recommends an increased consumption of n3 fatty acids in adults’ diets (Vannice and Rasmussen 2014). The n6:n3 ratio is an index used to evaluate the nutritional value of fats, and the Food and Agriculture Organisation of the United Nations and the World Health Organisation (FAO/WHO 1993) recommended that the value should not exceed 4.0, considering that large intakes of n-6 PUFA can decrease the formation of anti-inflammatory mediators originating from the n-3 PUFA (Simopoulos 1999). The mean value observed in the present study

Figure 4. Variation in the milk content of short-chain fatty acids (SCFA), medium-chain fatty acids (MCFA) and long-chain fatty acids (LCFA) during the experimental period.
were below this recommendation (mean of 2.17), and it decreased in the ELS group, proving the beneficial effect of dietary extruded linseed on milk quality. The AI, TI and h/H are indicators used to assess the nutritive values of foods (Nantapo et al. 2014; Attia et al. 2017). The reduction of AI and TI and the increase of h/H in the ELS group compared to CON suggests a positive effect of dietary linseed in improving the quality of donkey milk, in terms of health-related properties. Another interesting result was observed for C18:0. In this study C18:0 was decreased in ELS group, but the mean content (3.32%) was higher than that achieved in previous investigations on donkey milk, ranging from 0.68 to 1.99% (Martemuccia and D’Alessandro 2012; Massouras et al. 2017; Valle et al. 2018). Differently from our trial, in these last works the animals were reared under semi extensive systems, with high pasture availability, and a consequently diet richer in PUFA and poorer in SFA (i.e.: C18:0), compared to a concentrate-based diet. On the other hand, the mean of C18:0 was lower compared to that reported for ruminants, in which ranged from 6 to 12% of total FA (Hurtaud et al. 2010; Correddu et al. 2016; 2017). In general, the low C18:0 in donkey milk, compared to ruminant milk, could arise, partly, from the low content of this FA in the diet, but mainly by the lack of PUFA biohydrogenation, as this FA represents the last product of the enzymatic reduction of dietary PUFA (namely linoleic acid and alpha-linolenic acids) operated by ruminal microorganisms; moreover, the activity of the Δ9-desaturase in the mammary gland, converting C18:0 into C18:1c9, could be also involved, as previously reported in ruminants (Schennink et al. 2008; Sánchez et al. 2010).

The lower of BCFA, OBCFA and OCFA concentrations observed in ELS group compared to CON agreed with the effect of oilseeds supplementation in the diet of ruminants (Vlaeminck et al. 2006; Correddu et al. 2016). However, the interpretation of this result, in the present work, is quite difficult because the literature about the variation of these FA in equines milk is very poor. Instead, the biological mechanism involved in the process of the formation OBCFA is well known in ruminants (Valle et al. 2018). This group of fatty acids is mainly of microbial origin and is found in bacterial membrane lipids. High concentrations of these FA have been retrieved in the milk of sheep and goats (Nudda et al. 2021). In ruminants, dietary oilseeds containing high amount of long chain fatty acids can lead to a

Figure 5. Temporal evolution of odd-chain fatty acids (OCFA), branched-chain fatty acids (BCFA) and odd- and branched-chain fatty acids (OBCFA) in milk of donkey fed diet without supplementation (CON) and diet including linseed (ELS).
modification the relative abundance of specific ruminal bacterial population (Vlaeminck et al. 2006), as a consequence of the toxicity of UFA on different strains of bacteria (Maia et al. 2007; 2010). The content of these FA in donkey milk is likely related to the abundant gastrointestinal microbiota activity (Liu et al. 2019) that characterises the monogastric herbivorous. However, the decrease of OBCFA concentration in ELS milk could be due to the depressive effect of the linseed UFA on the intestinal microflora, as several bacteria found in the rumen were found also in the equine gastrointestinal microbiome (e.g. Ruminococcus flavescens, Ruminococcus albus, Fibrobacter succinogenes and Butyrivibrio sp.); for this reason, a similar regulation mechanism of the microbiota in the rumen and in equine intestinal compartment was suggested (Valle et al. 2018). The high content of milk OBCFA is of nutritional interest, especially the C15:0 and C17:0 that have been inversely associated to cardiovascular disease (Jenkins et al. 2015) and to the incidence of type 2 diabetes (Imamura et al. 2018). In a recent in vivo study, Venn-Watson et al. (2020) showed C15:0 as an active dietary fatty acid that attenuates inflammation, anaemia, dyslipidemia, and fibrosis in vivo.

Conclusions

The supplementation of extruded linseed, as source of PUFA n3, to lactating donkeys is able to double the concentration of PUFAn3 in milk and to improve significantly the nutritional quality of milk fat. The results evidenced that, in the management condition of this trial, the response to lipid supplementation is slightly delayed probably because the adaptation of gastrointestinal microbial population to the unsaturated lipid supplementation. Finally, to the high content of unsaturated FA, represented mainly by oleic, linoleic and linolenic acid is associated an interesting concentration of branched and odd chain FA. However, further studies are necessary to find feeding strategies able to increase also the concentration of milk fat in the different stages of lactation.

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Data availability statement

The data that support the findings of this study are available from the corresponding author, [FC], upon reasonable request.

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