Fatty acid profile of milk from Nordestina donkey breed raised on Caatinga pasture

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SUPPLEMENTARY FILE

Materials and methods

Sampling

The donkey mares were kept on a farm located in the municipality of Felipe Guerra, in the state of Rio Grande do Norte, Brazil (5° 37' 20'' South, 37° 38' 57'' West). The grazed on the native vegetation of the semiarid Caatinga biome during the day and were housed and their diets supplemented with sorghum silage and ground corn at night. Foals were maintained with their dams except for a four hour period before milking. Donkey mares were milked by hand once a day at 05 am and then released to pasture. Twenty-four multiparous donkey mares of the Nordestina breed, aged between from 4 and 10 years, were selected for milk sampling collection. Milk samples were collected from 5 animals with ≈30 days in milk (DIM), 5 animals with ≈60 DIM, 5 animals with ≈90 DIM, and 9 animals with DIM ranging from 120 to 180 days. Milk samples used for fat determination were refrigerated (4 ± 1 ºC) without preservatives, and those used for fatty acid (FA) determination were freeze-dried and stored at -20 ºC until analysis.

Fat content analysis and determination of FA

Milk fat content was determined by infrared spectroscopy using a Bentley Dairy Spec FT® (Bentley Instruments, Chaska, Minnesota, USA) calibrated for donkey milk. The milk FA profiles were determined from direct transesterification of freeze-dried milk samples according to the procedures described by Molkentin and Precht (2000) but complemented with an acidic transesterification step. Briefly, 50 mg of dry milk solids was dissolved in 1 mL of n-hexane, containing 19:0 as the internal standard. Then 0.2 mL of KOH (2 mol/L in methanol) was added followed by vigorous shaking for 3 min and the mixture was left to stand for 1 h. Thereafter, 2 mL of HCl 1.25 M in methanol was added and tubes were placed in a 50 ºC water bath for 10 min and left to cool. Finally, 1 mL of water and 1 mL hexane were added and the tubes vortexed, centrifuged and the organic phase collected, dried with anhydrous sodium sulphate and transferred to gas chromatography (GC) vials.

The FA methyl esters were analysed in a gas chromatographer (Varian 430, California, USA) equipped with a flame ionization detector and a 100 m SP-2560 capillary column (Supelco, Bellefonte, USA). The FA was identified by comparison with FA methyl ester standards (ME19-Kit, Supelco, Bellefonte, USA) and expressed as area percentage.

The atherogenic index (AI) was computed according to Ulbricht and Southgate (1991) as: $AI = (12{:}0 + 4 \times 14{:}0 + 16{:}0) / [\Sigma \text{ MUFA} + \Sigma \text{ (n−6 PUFA)} + \Sigma \text{ (n−3 PUFA)}]$

Reference

Ulbricht, T LV and Southgate, DAT (1991) Coronary heart disease: seven dietary factors. *The Lancet*, 338, 985-992.