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Concentrate with calcium salts of fatty acids increases the concentration of polyunsaturated fatty acids in milk produced by dairy goats

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ABSTRACT

Feeding rumen-inert fat, such as calcium salts of fatty acids (CSFA), has dual benefits as it increases milk yield and improves the fat composition in milk. However, there is a shortage of information on the effect of CSFA on milk yield and composition in grassland Saanen goats. Thus, this study aimed to evaluate the effects of CSFA in the concentrate of lactating grassland Saanen goats on milk yield, composition, quality, and fatty acid composition and to determine the best response to the addition of CSFA. Five multiparous Saanen goats (five years old) were distributed in a 5 × 5 Latin square design with five treatments (0%, 1.5%, 3.0%, 4.5% and 6.0% CSFA), and four primiparous Saanen goats (three years old) were distributed in a 4 × 4 Latin square design with four treatments (0%, 1.5%, 3.0% and 4.5% of CSFA); the goats had an average of 78 ± 10 days in lactation at the start of the experiment. Each period lasted 21 days, including 14 days for adaptation and seven days for data collection. Pelleted concentrate was composed of ground corn, soybean meal, a mineral–vitamin supplement for goats, salt and CSFA at the levels of inclusion. For grazing goats, an area with Stargrass (*Cynodon nlemfuensis*) was used. The addition of CSFA to the concentrate of grassland Saanen goats had no effect on milk yield, milk components such as fat, protein, lactose and totals solids, or milk quality (acidity and somatic cell counts) in multiparous or primiparous goats. However, the concentration of fatty acids was modified. The concentration of capric (10:0) and myristic (14:0) fatty acids decreased linearly with increased inclusion of CSFA in the concentrate. There was a quadratic effect on medium-chain and long-chain fatty acids and omega-3 (n-3) in the milk of multiparous Saanen goats following treatment. The inclusion of CSFA in the diet of primiparous goats had a positive linear effect for linoleic fatty acid (18:2 n6c), conjugated linoleic acid, omega-6 (n-6) and polyunsaturated fatty acids, whereas the concentration of medium-chain fatty acids showed a negative linear effect. In conclusion, CSFA in the concentrate of grassland primiparous goats showed positive responses on the fatty acid composition of goat milk, increasing the polyunsaturated fatty acid concentration.

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1. Introduction

The consumption of goat milk in Brazil is driven by two factors: goat milk is considered a functional food, and goat milk is used as a raw material for the production of fine cheeses. The functionality of goat milk is due to the better fat digestibility of goat milk, as well as the lower concentration of allergenic proteins and reduced allergenicity of goat milk in relation to cow milk (Silanikove et al., 2010).

The composition and concentration of proteins is mainly altered by genetic factors, with few effects of diet on milk proteins contents (Amills et al., 2012). However, the milk's fat composition can be easily altered by diet with changes in the amount of fat and fatty acids. Thus, strategies to add sources of fat to the diet of ruminants are a way of modifying the composition of milk fat by increasing the concentration of fatty acids beneficial to human health, such as conjugated linoleic acid (CLA), omega-3, and omega-6 (Novello et al., 2010).

According to Chilliard et al. (2007) feeding dairy ruminants with the addition of unsaturated fatty acids in the ration such as oleic acid (18:1) and linoleic acid (18:2) has been shown to be an efficient strategy to modify milk's fatty acid content. However, the presence of unsaturated fatty acids in the rumen is known to inhibit ruminal microbial activity and fermentation (Yang et al., 2009).

The saponification of long chain fatty acids, typically derived from soybean or palm oils, with calcium ions results in calcium salts, a type of rumen-inert fat with high levels of fatty acids such as palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1) and linoleic acid (18:2). Thus, feeding of calcium salts of fatty acids (CSFA), which are inert in the rumen, can enhance the energy density of the ration and has minimum effects on ruminal fermentation. Increased availability of rumen-inert fatty acids serves a dual benefit, as it increases milk yield and improves the fat composition of milk (Shingfield et al., 2009; Titti, 2011; Souza et al., 2014) There is considerable plasticity in terms of ruminant milk fatty acid composition which can be changed by nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids (Chilliard and Ferlay, 2004).

However, there is a shortage of information on the use of rumen-inert fat on milk yield and composition in grassland Saanen goats. Thus, this study aimed to evaluate the addition of CSFA in the concentrate of multiparous and primiparous lactating grassland Saanen goats on milk yields, composition, quality, fatty acid composition and to determine the best response to the addition of CSFA through the evaluation of the cost and net profits of the concentrate.

2. Materials and methods

2.1. Goats and experimental treatments

The experiment was conducted at an experimental farm at the State University of Maringá, southern Brazil. Five multiparous Saanen goats (five years old and on their third lactation; body weight 57 ± 2.7 kg) and four primiparous Saanen goats (three years old; body weight 54 ± 1.8 kg) were sued, with average of 78 ± 10 days in lactation at the start of the experiment. Goats were distributed in two Latin square designs with an experimental period of 21 days, including 14 days for adaptation and seven days for data collection. The multiparous Latin square design was 5 × 5 with five treatments (0%, 1.5%, 3.0%, 4.5% and 6.0% CSFA) and for the primiparous a 4 × 4 Latin square design with four treatments (0%, 1.5%, 3.0% and 4.5% CSFA) was used. Goats remained in the pasture for approximately seven hours per day (8:00 am to 3:30 pm) and were housed in individual pens in the evening and overnight. The goats had free access to water in the pasture and pens.

Goats were milked manually twice daily (7:30 am and 3:30 pm) and the milk yield of individual goats was measured using an electronic balance at each milking. After the afternoon milking, goats were fed with the concentrate.

Pelleted concentrate was composed of ground corn, soybean meal, a mineral–vitamin supplement for goats, salt and rumen-inert fat in the form of calcium salts of long-chain fatty acids (CSFA) from a commercially available product derived from soybean oil (Lactoplus® from Dalquim Chemical Industry Ltd.; with 1.94 g g^{-1} total digestible nutrients, 820 g kg^{-1} ether extract, 100 g kg^{-1} calcium, 260 g kg^{-1} oleic acid and 420 g kg^{-1} linoleic acid); in five levels of inclusion (0%, 1.5%, 3.0%, 4.5% and 6.0% the concentrate) (Table 1). The amount of concentrate offered the goats was established at 1 kg day^{-1} as feed, or about half of the estimated nutritional requirements of Saanen goats (NRC, 2007) with a body weight of 60 kg and a milk yield of 3.0 kg day^{-1} with 3.5% fat.

For grazing goats, an area of one hectare (1 ha) was used with the subtropical forage grass Stargrass (*Cynodon nlemfuensis*) maintained by continuous stocking with 339.2 g kg^{-1} dry matter, 108.7 g kg^{-1} crude protein, 656.0 g kg^{-1} neutral detergent fiber and 0.59 g g^{-1} *in vitro* dry matter digestibility. The grassland was fertilized and corrected through physical and chemical analysis of soil and grass demand. Fertilizer was applied at an N–P–K ratio of 8 kg ha^{-1} of nitrogen, 67 kg ha^{-1} of phosphorus and 70 kg ha^{-1} of potassium (200 kg of N–P–K fertilizer 4–20–20; 150 kg of single superphosphate and 50 kg of potassium chloride) in September 2011, which was distributed by throwing 30 days before the input of goats. The grazing period was from 8 October 2011 to 20 January 2012.

The fatty acid composition of the concentrate and forage used are presented in Table 2.

2.2. Sample collection and analyses

Milk samples were collected on the 15th day of each period from each goat from two consecutive milkings and pooled on a yield basis.

For the chemical composition determination, milk samples were stored at 4 °C with a preservative (2-bromo-2-nitropropane-1,3-diol) until analyzed for fat, protein, lactose and total solids by infrared spectroscopy (Bentley model 2000; Bentley Instrument Inc., Chaska, MN). Milk somatic cell counts (SCC) were obtained using an electronic counter (Somacount 500, Chaska, MN), which was calibrated for cow milk analysis. At the same time, milk acidity was measured using Dornic solution, according to method no. 947.05 (AOAC, 1998).

Another two milk samples were collected and frozen at −20 °C without the addition of preservatives; one was used to analyze the milk urea nitrogen, and the other one to determine the milk fat composition.

Milk samples were centrifuged for 30 min at 3000 × *g* at 4 °C and the serum was separated and frozen at −20 °C for subsequent analyses. The concentration of milk urea nitrogen was analyzed using a commercial kit (urea-PP kit category 427; Gold Analisa Diagnostica®) on a spectrophotometer (Shimadzu UV-1601 UV–visible Spectrophotometer®) at 600 nanometers wavelength.

Milk fat was extracted by centrifugation (Murphy et al., 1995) and the transesterified according to method no. 5509 (ISO, 2000) with KOH/methanol and n-heptane. Thereafter, the methyl ester composition of fatty acids were measured by gas chromatography (Trace GC Ultra, Thermo Scientific, USA) equipped with an auto sampler, a flame ionization detector at 240 °C and a fused-silica capillary column (100 m long, 0.25 mm internal diameter and 0.20 μm film thickness, Restek 2560®).

Fatty acids were quantified as g.100 g^{-1} lipids, compared to the retention time of methyl ester fatty acids from the sample standard tricosanoic acid methyl ester (23:0) (Sigma–Aldrich®, Brazil). The column parameters were as follows: the initial column temperature of 65 °C was maintained for 8 min; the temperature was then increased at a rate of 50 °C/min to 170 °C; this temperature was maintained for 40 min and then increased at a rate of 50 °C/min to 240 °C and maintained for 28.5 min. The injector and detector temperatures were 220 and 245 °C, respectively. The gas flow was 1.5 ml/min for hydrogen (carrier gas), 30 ml/min for N_2 (auxiliary gas), 35 ml/min for H_2 and 350 ml/min for compressed air. With a microliter syringe, 2 μl of the samples were injected with a split ratio of 1:100. Fatty acid peaks were identified by comparison with the retention times of pure methyl ester standards (Sigma–Aldrich®, Brazil).

The milk yield was corrected to 4.0% fat according to the NRC (2007) using the NRC (2001) equation: FCM (4.0%) = (0.4 × MY) + (15 × ((FY × MY)/100)); where FCM: fat corrected milk to 4.0% of fat (kg day^{-1}), MY: milk yield (kg day^{-1}) and FY: fat yield (kg day^{-1}).

The net energy of milk (NE_{milk}), *i.e.* the energy contained in the milk, is equivalent to the sum of the heats of combustion of individual milk components (fat, protein and lactose), calculated according to the equation (NRC, 2001): VE_{milk} (Mcal/kg) = (0.0929 × Fat%) + (0.0547 × Protein%) + (0.0395 × Lactose%).

Sampling of the forage, for the chemical analysis and manual separation of morphological components (leaf blades, stems and sheaths, dead material), were collected once in each experimental period (October 9 2011, November 1 2011, November 23 2011, December 14 2011, January 3 2012); to ensure random sampling, one 1.0 m^2 wire square was thrown eight times in the paddock and the grass was cut 15 cm above the ground. Samples of forage used to determine total forage mass were cut close to the soil. The sward height was measured with a wooden ruler graduated