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Glycerin diet affects the size of the fat globule and the fatty acid profile of goat's milk

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Abstract

The objective of this study was to evaluate the production, fat and fatty acids of milk as well as the diameter, classification and quantity of the milk fat globules (MFG) of goats consuming a diet with 15% of crude glycerin. Twelve multiparous Saanen goats weighing 40 ± 6 kg and 90 ± 5 days of lactation were used. The experimental design was completely randomized with two treatments (0 and 15% inclusion of glycerin). In this way, each milk sample is classified according to the percentage of milk fat globules that were included in these three size categories. For the variables milk production, fat, diameter, medium and large fat globules and amount of globules showed a significant effect of the inclusion of glycerin in the diet ($p < 0.05$). Fifteen fatty acids were found, mostly saturated. For caprylic, palmitic, and linoleic fatty acids, there was no significant influence of the inclusion of glycerin ($p > 0.05$). Can recommend the use of 15% double-distilled glycerin in the feeding of lactating goats increased milk production, the amount of fat, increased the size of the milk fat globule. Regarding the saturated and polyunsaturated fatty acids, they kept the level of 15% of glycerin.

Keywords: lipids; monounsaturated fatty acids; nutrition; polyunsaturated fatty acids; small fat globule.

Practical Application: The use of crude glycerin in the diet of dairy goats increases the diameter of milk fat globules.

1 Introduction

There is considerable interest in the use of alternative foods that can replace part of the concentrate provided, to reduce the cost of milk production without impairing the consumption and performance of the animals. Among the main agro-industrial byproducts with the potential to be used in the feeding of ruminants, currently, those from the production of biodiesel stand out (Lage et al., 2010).

The researchers supplemented dairy cows' diets with glycerin in purified (Carvalho et al., 2011) and crude (Boyd et al., 2013) form. When crude glycerin (82.6% glycerol) was added up to 15.6% in diets for medium-yield cows, no changes was observed in milk production and quality (Harzia et al., 2013), evidencing no difference in results of studies using crude or purified glycerin, as reported by Omazic et al. (2013). These experiments indicate that purified or crude glycerin can be fed up to 15% of dietary dry matter to lactating cows without harmful effects.

In a research carried out with dairy goats using in their feed the addition of four increasing levels of glycerin (0, 6, 12 and 18%), it was observed that the physicochemical characteristics of the milk did not show any significant difference ($p > 0.05$) with except for fat, which showed low concentration at levels of 12 and 18%. Thus, we used the same animals in a separate experiment to analyze the 15% level of glycerin addition (Lima et al., 2021). Studies carried out on bovine milk have reported that the amount of single FAs trend to change based on the dimensions of the secreted milk fat globules (MFGs) (Martini et al., 2006). However, these relationships have been scarcely investigated in other species such as sheep (Martini et al., 2008, 2010) and goats (Argov-Argaman et al., 2016). Modifying the MFG size could have a significant impact on the nutritional value, technological and organoleptic characteristics of milk (Martini et al., 2008) and could provide products with specific dietary features. T

he work has as hypothesis to evaluate the use of crude glycerin in the diet of milk goats is able to modify the fat globules and the fatty acid profile of milk. The objective of this study was to evaluate the production, fat and fatty acids of milk as well as the diameter, classification and quantity of the fat globules in the milk of goats consuming a diet with 15% of crude glycerin

2 Material and methods

2.1 Experiment site and animals

This project was submitted to the Ethics Committee on Animal Use (CEUA) of the Federal University of Paraíba and approved according to protocol no. 052/2017.

The experiment was conducted at the Federal University of Paraíba, Campus at Bananeiras - Paraíba, Brazil (altitude 552 m,

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latitude 6° 41' 11", longitude 35° 37' 41"). The air temperature was 25 °C, and relative humidity was 76.5% in the stalls.

This project was submitted to the Ethics Committee on Animal Use (CEUA) of the Federal University of Paraíba and approved according to protocol no. 052/2017. We used twelve multiparous Saanen goats weighing 40 ± 6 kg and at 90 ± 5 days of lactation. The experimental design was completely randomized with two treatments (0 and 15% inclusion of glycerin). The animals spent 14 days to adapt to the diet.

2.2 Diets

During the adaptation and collection periods, daily weightings of food supply and leftovers were carried out to calculate the voluntary consumption and adjust the feed supply to guarantee leftovers of 10% based on a dry matter. Water for animal consumption was offered *ad libitum*, and consumption was quantified daily during the data collection period.

The diets were adjusted to meet the requirements of the National Research Council (2007) for lactating goats producing 2.0 kg of 4% fat milk day^{-1}, with a bulk ratio of 55:45 forage: concentrate. The experimental diet was offered ad libitum as a complete mixture at 07:30 a.m. and 04:30 p.m. The ingredients used in the diet were: tifton hay, ground corn, soybean meal, vitamin/mineral supplement, urea and glycerin (Table 1). The gross energy contents of corn and GC were 3.50 and 3.71 Mcal kg-1, respectively.

2.3 Milk production and physicochemical analysis of milk

Milking was performed manually, throughout the experiment, occurring twice a day at the times of (6:00 a.m. and 3:00 p.m.), including adaptation periods and data collection, and the dairy control was performed by weighing. Milk (kg/day) during the three days of collection of each period (all experimental period).

Table1. Percentage and bromatological composition of experimental diets.

Ingredient (g kg^{-1} DM)	Levels of inclusion (%)	
	0.00	15.0
Glycerin	0.00	15.0
Soybean meal	9.50	9.50
Ground corn	33.5	18.0
Tifton hay	55.0	55.0
Urea	0.00	0.50
Mineral supplement[1]	1.50	1.50
Calciticlimestone	0.50	0.50
Chemical composition		
Dry matter, DM (g kg^{-1} as fed)	882	836
Crude protein, CP (g kg^{-1} DM)	114	98.7
Ethereal extract, EE (g kg^{-1} DM)	12.8	18.8
Neutral detergent fiber, NDF (g kg^{-1} DM)	905	674
Acid detergent fiber, ADF (g kg^{-1} DM)	282	267
Metabolizable energy, ME (Mcal/kg DM)	3.62	3.58

[1]Composition of mineral supplement. per kg: P: 70 g; Ca: 140 g; Na: 148 g; S: 12 g; Mg: 1.320 mg; F: 700 mg; Zn: 4.700 mg; Mn: 3.690 mg; Fe: 2.200 mg; Co: 140 mg; I: 61 mg; Se: 15 mg; monesinasodic: 100 mg.

Milk samples from each animal were collected twice a day, at regular times (6:00 a.m. and 3:00 p.m.), during the three days of data collection of each period respecting the proportion of milk milked (morning/afternoon).

Vials and glassware were sanitized at 105 °C for one h, to avoid contamination by milk residues from the previous milking. The samples of the morning production were conditioned in a refrigerated environment (4 °C) to be later mixed to the milk samples of the afternoon, forming a sample composed of goat per day. From the whole milk milked per animal (kg day-1), an aliquot of 200 mL was taken (with the participation of the samples proportional to the morning and afternoon milking), for analysis of the physicochemical characteristics. After being placed in identified plastic bottles, the samples were slowly pasteurized at 65 °C for 30 minutes (Brasil, 2001) and finally frozen at -4 °C (in a freezer) for further analysis.

Physical-chemical analysis of fat (%) was the Analyzer of Master Complete® Milk (AKSO®, São Leopoldo, Rio Grande do Sul, Brazil), under specific technical conditions.

2.4 Fat globule analysis

The milk was diluted with distilled water (1980 µL distilled water: 20 µL milk). Milk fat globules were measured as reported by Martini et al. (2013). After dilution, 500 µL of each sample were mixed with 50 µL of dye in staining solution (0.1%) of Acridine Orange in phosphate buffer (pH 6.8) and shaking immediately after After that, it was taken eight µL of the sample and place it in the Burker camera. It was taken ten photos of each sample to a 40x objective through the microscope (Olympus BX53F) with Olympus Camera (DP73) (Martini et al., 2013). The program used to make measurements and ascertain the amount of MFG was the Olympus cellSens Dimension. The frequency distribution of the measured milk fat globules was evaluated according to their size. In essence, they were divided into three categories of fat globules: small globules with a diameter < 2.5 µm, medium size globules with a diameter of 2.5 to 5.5 µm, and large globules with a diameter of > 5.5 µm. In this way, each milk sample was classified according to the percentage of milk fat globules that were included in these three size categories adapted from Scolozzi et al. (2003).

2.5 Analysis of fatty acids

Twelve samples composed of milk were used, each weighing 30 g weighed on a semi-analytical scale (WTB 3000, RADWAG), soon after they were placed in the Freeze Dryer (L101, LIOTOP) for 24 hours, for complete removal of water. The lyophilized samples were taken to the Laboratory of Chromatography and Atomic Absorption Spectrometry. For the analysis of fatty acids, a gas chromatograph was used (TraceTM 1310 Gas Chromatograph, THERMO FISHER SCIENTIFIC).

The Chromatograpf was coupled in a flame ionization detector using a mixture of Synthetic Air 5.0 and oxygen to assist the flame, with a capillary column (SPTM-2380) 60 meters x 0.25 mm (d.i.) x 0.20 µm thick. Nitrogen was used as the carrier gas at a flow rate of 1 mL/min. The initial temperature of the oven was 40 °C and remained for 2 min. The temperature of the

column was increased by 10 °C/2 min until reaching 180 °C, where it remained for 30 min, after the temperature of 180 °C, every 2 min an additional 10 °C increased until reaching the final temperature of 240 °C for 30 min, totaling 82 minutes of running. The temperature of the Split / Splitless injector remained at 250 ° C, and the detector at 260 °C. 1.0 µL aliquots of the sample was injected. The chromatograms were recorded using XcaliburTM software (THERMO FISHER SCIENTIFIC).

Fatty acids were identified by comparing the retention times of the methyl esters of the samples with Supelco 37 standards - Component FAME Mix in Dichloromethane (FATTY ACID METHYL ESTERS C4-C24). The results of fatty acids were quantified by normalizing the areas of methyl esters and expressed as a percentage of area.

2.6 Statistical analysis

The experimental design was completely randomized in a factorial scheme (2x2), with two treatments in two periods, according to the mathematical model below (Equation 1):

$$Y_{ijk} = \mu + T_i + P_j + TP_{ij} + e_{ijk} \; ; \; Y_{ijk} \; = \quad (1)$$

Observed value of the studied variables, relative to each level j of crude glycerin; µ = Constant of the characteristic; T_i = Treatment effect i (i = 1, 2); Pj = Effect of the period j (j = 1, 2); TP_{ij} = Effect of interaction treatment x period; e_{ijk} = Random error associated with each observation.

The data were analyzed using the PROC MIXED procedure of the SAS 9.2 software (SAS, 2001), considering the animal effect within the period as random, as well as the carryover effect between the two periods. The means when significant were compared using the Tukey-Kramer test ($p < 0.005$).

3 Results and discussion

For the variables milk production, fat, diameter, medium and large fat globules and amount of globules showed a significant effect of the inclusion of glycerin in the diet ($p < 0.05$) (Table 2).

The production of milk, fat and diameter showed high values at the 15% level of glycerin, while the medium, large and quantity globules showed low values at the 15% level. This result indicates that with the decrease of these variables mentioned, an increase in milk digestibility may occur, making it a better activity food functional in the body (Martini et al., 2013). The MFG diameter of the goats has an average range of 2.2 to 2.8 µm, according to the study by Salari et al. (2016). The goat MFG has a smaller size than that of cows, ranging from 3.5 to 5.5 µm (Martini et al., 2016). According to Martini et al. (2009), more than 90% of goat MFG is smaller than 5 µm. The small diameter of MFG probably presents the best digestive parameters due to the greater surface area of exposure to lipase action, which may facilitate milk digestibility compared to cow milk (Ribeiro & Ribeiro, 2001; Arora et al., 2013).

Diets with higher forage:concentrate ratio (40:60 vs. 60:40) as well as isoenergetic and isoproteic diets resulted in an increase in the percentage of fat in sheep milk, but did not affect the mean diameter of the fat globule (Martini et al., 2012). Based on the literature, we can hypothesize that MFG size is affected by the dietary content of both energy and temperature and is related to fat in milk. In this regard, we also observed that the increase in the diameter is associated with the increase of milk fat, as reported in cows (Carroll et al., 2006; Martini et al., 2017).

Fifteen fatty acids were found, mostly saturated. For caprylic (C8: 0), palmitic (C16: 0), and linoleic (C18: 2n6) fatty acids, there was no significant influence of the inclusion of glycerin ($p > 0.05$) (Table 3).

One of the most interesting aspects of goat milk, according to Sampalayo et al. (2007), concerns the nature of fat, because, in addition to being its main nutrient, it is responsible for the origin of pleasant or unpleasant odors and the formation of flavor in milk. The peculiar characteristic of goat milk is due to the presence of short-chain fatty acids (caproic - C6:0, caprylic - C8:0, capric C10:0), with levels twice as high as in cow's milk, making them chemically and sensory distinct (Costa et al., 2009). These fatty acids are responsible for the sensory characteristics of goat milk and its derivatives, as a more pronounced flavor than milk from other species. The milk goat, the proportion of these fatty acids, is higher than in other milk (Costa et al., 2009; Amigo & Fontecha, 2011). Undecanoic acids (C11:0) are present in milk and dairy products in reduced amounts of less than 1% (Rossell, 1991).

For butyric acid C4:0 decreased with inclusion of 15% glycerin (from 1.83 to 1.39, $p < 0.05$) was observed in the treatment without the inclusion of glycerin, to 1.39 ± 0.54, at 15% of inclusion of glycerin, with effect significant ($p < 0.05$),

Table 2. Mean and standard deviation of milk, fat and milk fat globule (MFG) parameters of dairy goats submitted to inclusion of glycerine in the diet.

Variables	Levels of inclusion (%)		*P* value
	0.00	15.0	
Milk production (kg)	1.31 ± 0.34b	1.68 ± 0.76a	<. 0001
Fat (%)	2.25 ± 0.27b	3.26 ± 0.76a	<. 0001
Diameter MFG (µm)	2.76 ± 0.96b	3.02 ± 0.89a	<. 0001
-Small globule: < 2.50 µm (%)	56.17 ± 5.27a	60.40 ± 8.46a	0.6526
- Medium globule: 2.50-5.00 µm (%)	38.15 ± 5.20a	31.92 ± 4.20b	0.0056
- Large globule: > 5.00 µm (%)	5.68 ± 1.80a	7.68 ± 2.01b	0.0466
Quantity (nº MFGx10^9)	1.5 x 10^9a	1.2 x 10^9b	0.0466

Means followed by different letters, differ by the t test.

Table 3. Lipid profile (%) present in the milk of goats fed double-distilled glycerin.

Variable	Levels of inclusion (%)		*P* value
	0.00	15.0	
C4:0	1.83 ± 0,06a	1.39 ± 0.07b	< 0.0001
C6:0	2.48 ± 0,03a	2.14 ± 0.002b	< 0.0001
C8:0	2.79 ± 0,08	2.85 ± 0.07	0.1990
C10:0	9.43 ± 0,21b	10.70 ± 0.93a	0.0104
C11:0	0.20 ± 0,04b	0.29 ± 0.03a	0.0034
C12:0	4.17 ± 0,11b	6.36 ± 0.41a	< 0.0001
C14:0	11.74 ± 0,70b	13.30 ± 1.16a	0.0179
C14:1n9	0.18 ± 0,03b	0.34 ± 0.02a	< 0.0001
C15:0	0.74 ± 0,05b	1.30 ± 0.18a	< 0.0001
C16:0	30.08 ± 2,15	32.63 ± 2.72	0.1027
C16:1n7	1.21 ± 0,07b	1.47 ± 0.06a	0.0001
C17:0	0.46 ± 0,04b	0.55 ± 0.03a	0.0001
C18:0	11.18 ± 0,86a	8.24 ± 0.47b	0.0066
C18:1n3	27.79 ± 0,74a	23.25 ± 2.18b	0.0007
C18:2n6	2.47 ± 0,18	2.67 ± 0.20	0.1058
SFA	75.15 ± 2,38	79.80 ± 5.40	0.0829
MUFA	29.20 ± 0,74a	25.06 ± 2.16b	0.0013
PUFA	2.48 ± 0,17	2.66 ± 0.21	0.1351
Omega3 (w3)	27.79 ± 0,74a	23.25 ± 0.18b	0.0007
Omega6 (w6)	2.47 ± 0,18	2.67 ± 0.20	0.1058
AI	1.36 ± 0,04b	1.81 ± 0.12a	< 0.0001
TI	0.48 ± 0.01b	0.58 ± 0.04a	< 0.0001

SFA+ saturated fatty acids, MUFA=monounsaturated fatty acids, PUFA=polyunsaturated fatty acids, AI= atherogenicity índices, TI= thrombogenicity index; Means followed by different letters, differ by the t test.

as bidistilled glycerin was included in the animals' diet. The reduction of short and medium-chain fatty acids may indicate that there was interference in the ruminal activity by the fat offered, as these come from the new synthesis and, therefore, from the molar concentration of acetate resulting from the digestion of fibers in the rumen (Sampalayo et al., 2007).

Among saturated fatty acids, the highest concentrations of lauric acid C12:0 (P = 0.0002) and myristic C14:0 (P = 0.001) were found in the milk of goats fed with double distilled glycerin. According to the literature, the fatty acid with the greatest negative and most undesirable effect is myristic (Souza et al., 1998). However, myristoleic acid (C14:1) has a significant effect (p <0.05) diverging directly from the observed data Pellegrini et al. (2012), who demonstrated that goat's milk does not contain this acid.

Consequently, the values of oleic acid (C18:1) were significant; the treatment with 15% of double-distilled glycerin showed an average lower than the treatment of 0% glycerin. There was an effect of the treatments for the concentration of stearic acid (C18:0) (P = 0.0066). Fernandes et al. (2010), suggest that, even when there is a difference between food systems for C18:0 content, as this is a neutral fatty acid, it does not favor or harm any of the tested systems. Then, this fatty acid is of great importance for the health (Mcdonald et al., 2011), since its transformation into oleic acid (C18:1) is rapid, decreasing the concentration of total cholesterol in humans (Moloney et al., 2001). Regarding total saturated fatty acids (SFA), there was no significant difference between treatments (P = 0.0829), indicating that these acids remained with constant concentrations, regardless of the concentration of glycerin. Even though the diets are different, it did not change the amount of saturated fatty acids present in milk, probably due to the need to maintain a favorable environment for ruminal microorganisms with the reduction of the toxic effects of unsaturated fatty acids through biohydrogenation (Julia et al., 2013).

The results found showed a significant reduction (p < 0.0013) for monounsaturated fatty acids (MUFA), these results were proportional to the decrease in the presence of C18:1n3, which also had a significant effect (p < 0.05). Due to these results, the presence of omega 3 in milk was also reduced (p < 0.0007). Some studies report the importance of omega 3 in controlling and combating several diseases (Chowdhury et al., 2012; Julia et al., 2013), indicating that the reduction in omega-three may decrease the consumption of this milk. For linoleic acid (C18:2n6) of the milk under study, there was no influence of the diet containing bidistilled glycerin.

The opposite effect to that observed in the values of omega 3, were observed for polyunsaturated fatty acids (PUFA). For the presence of omega-six fatty acids, 15% containing milk presented higher mean values of 2.66 for PUFA and meant 2, 67. These results presented make positive the use of glycerin in the feeding of lactating goats. The index increased from 1.36 ± 0.04 data regarding the control treatment to 1.86 ± 0.12 of the 15% treatment as glycerin was included in the diet. However, the results for the thrombogenicity were not verified significant

effect ($p > 0.05$). The results are not suitable for technical parameters and consumption, when relating nutrition, as the decrease in these indices improves the nutritional value of milk. Atherogenicity and thrombogenicity indices indicate the potential to stimulate platelet aggregation, that is, the lower the values of AI and TI, the greater the amount of anti-atherogenic fatty acids present in certain oils and fat, consequently, the greater the potential for prevention. to the onset of coronary heart disease (Tonial et al., 2010)

4 Conclusions

Can recommend the use of 15% double-distilled glycerin in the feeding of lactating goats increased milk production, the amount of fat, increased the size of the milk fat globule. Regarding the saturated and polyunsaturated fatty acids, they kept the level of 15% of glycerin.

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