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Diet energy levels and temperature affect the size of the fat milk globule in dairy goats

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ABSTRACT - The study aimed to verify the effect of diet and environmental temperature on traits of milk fat globules (MFG) of goats. The experiment was conducted in climatic chambers, where we housed 12 Alpine goats with a mean age of 4.02±1.78 years, live weight of 41.8±4.59 kg, and average milk production of 2.16±0.59 kg. The animals were subjected to two different controlled temperatures, T1 = 26 °C (thermoneutral) and T2 = 34 °C (stress), and diets with different energy levels (low, medium, and high). A milk sample of each animal was collected at 6.00 h, coinciding with milking. The effect of temperature and diet was verified on MFG. The highest MFG was observed at 26 °C and medium energy diet. The MFG reached lower values with the diet of medium energy and high temperature (34 °C). On average, 35% of MFG is smaller than 2 μm, 50% is medium in size (2-5 μm), and 15% is large (>5 μm), with a maximum size of 9.57 μm. The higher prevalence of medium-sized MFG is indicative of excellent milk digestibility. The increase in dietary energy levels promoted both the fat and diameter of fat globules. The higher fat and the larger globules would positively affect the cheese-making aptitude and make it suitable for production of hard cheeses. The increase in dietary energy levels for goats promotes an increase in the diameter of fat globules and milk fat (%), essential traits to the cheese industry.

Keywords: goat, lipids, nutrition, thermal comfort

1. Introduction

Goat production is a vital livestock activity for most developing countries, concentrating in tropical and semiarid regions. The dairy goat activity has grown in these regions due to the species' adaptability to environmental changes and can provide an improvement in the nutritional level in the diet of low-income families and the general population (Ribeiro and Ribeiro, 2001).

Goat milk is recommended for people with gastrointestinal diseases or even as supplement for the elderly and malnourished (Pellerin, 2001). According to Amigo and Fontecha (2011), there has been a growing interest in goat milk and goat dairy products worldwide because of its high nutritional content and health benefits.

Although the apparent milk is a homogeneous liquid, structurally, it is a very complex mixture of components (water, fat, vitamin, lactose, among others) (Park, 2007). Milk fat is mainly composed of triglycerides and sterols. The fat forms globules that are suspended in the milk as an emulsion. Milk fat globules (MFG) have diameters ranging from 1 to 10 μm and are surrounded by a biological membrane (MFGM) (Jiménez-Flores and Brisson, 2008) that has many active compounds, such as proteins, fatty acids, peptides, and other components, which contribute to human health (Bauman et al., 2006).

The MFG vary considerably in size and number and can be affected by several factors such as species (Yao et al., 2016), breed (Martini et al., 2003; Carroll et al., 2006), diet (Mesilati-Stahy et al., 2015; Argov-Argaman et al., 2016), environmental temperature (Nguyen et al., 2016), and lactation stage (Martini et al., 2013). The importance of the size of MFG derives from the fact that it can affect milk functional and nutritional properties (Lopez, 2011) and digestibility (Ribeiro and Ribeiro, 2001). Moreover, MFG size affects the technological, sensorial properties, and nutritional quality of dairy products, as it interferes on maturity, softness, and structure of the cheese, as well as the stability of dairy products, being of great importance to industries and health (Lopez, 2011; Martini et al., 2016).

Lower total milk fat content and smaller MFG were found when cows were fed a higher energy content diet than when fed a high forage diet (Argov-Argaman et al., 2014). According to Mesilaty-Stahy et al. (2015), nutritional manipulation (through modification of the proportion of dietary concentrate:fiber components) affects the average MFG diameter recorded across raw milk by increasing fat secretion employing a specific 3.3 μm diameter of the MFG subpopulation. Besides, the results suggest that membrane remodeling is involved, probably through the enrichment of mammary epithelial cell membranes (Argov-Argaman et al., 2016; Martini et al., 2016).

The fat globules can improve the nutritional properties, such as increasing the milk membrane content and adapting milk production to specific consumer targets (Martini et al., 2016). Very few studies considered the possibility of changing milk fat globule diameter in goat species (Argov-Argaman et al., 2016). Based on the literature, we can hypothesize that the size and quantification of MFG, dry matter intake, and milk production are affected by the amount of energy in the diet and temperature.

This study aimed to verify the effect of environmental temperature and dietary energy levels on milk yield and traits of goat milk fat globules.

2. Material and Methods

The trial was conducted in two climatic chambers in Areia, Paraíba, Brazil, located in the Mesoregion of the Agreste Paraibano and Microregion of Brejo Paraibano (6°58'12" S and 35°45'15" W Gr, altitude of 620 m above sea level). According to the Köppen classification, the climate in the region is of the As’ type (hot and humid), with autumn-winter rains, with a drought period of five to six months.

The Animal Ethics Committee (protocol no. 6925281118) approved this study.

Twelve multiparous Alpine goats with a mean age of 4.02±1.78 years, live weight 41.8±4.59 kg, average daily production of 2.16±0.59 kg of milk, and 66.8±2.0 days in milk were used. All the animals were initially also identified by numbering and deworming against endo- and ectoparasites. These animals were housed in 1.5 m^2 individual metabolic barns introduced inside two climatic chambers (Figure 1), provided with feeders and drinking fountains.

The animals were placed in two climatic chambers, each with an area of 19.71 m^2, a ceiling height of 2.38 m, made of laminated steel sheets with a layer of polyurethane and interior lighting with fluorescent light. The cooling system used was SPLIT (model SAMSUNG DIGITAL, Manaus, Brazil) air conditioners with a capacity of 30,000 BTU and heating through electric resistance air heaters. Adjacent to the climate chambers was a control room with a temperature and humidity control board. For humidification after dehumidification, a commercial humidifier and dehumidifier were used, such as equipment coupled to the Full Gauge Controls® (MT-530 PLUS control system, Canoas, Brazil) configured via SITRAD software, responsible for recording and storing ambient temperature

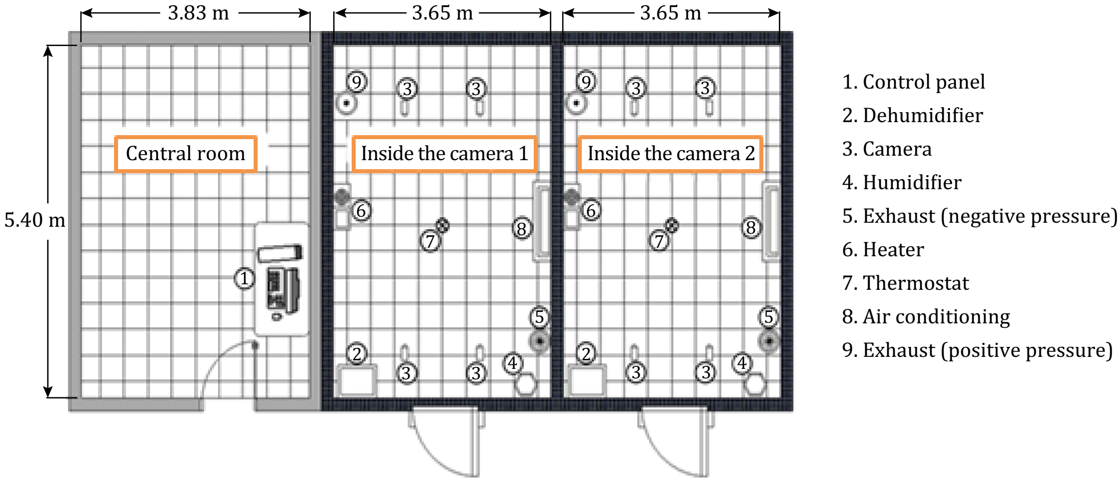

Figure 1 - Climatic chamber used in the experiment.

and relative humidity data of the air. Data acquisition was performed through a thermistor and a humidistat, both located in a permeable envelope and positioned at the height of the animals' center of mass (1.50 m).

The experimental design was a 2×3 type crossover—two temperatures and three diets: the two temperatures were 26 °C (thermoneutral) and 34 °C (stress), and three dietary energy levels (low, medium, and high). The experiment was conducted in two periods of 20 days, with 15 days of adaptation and five days of data collection. During the first period, a group of six goats was subjected to an environment with thermoneutral temperature, and a second group was subjected to the heat stress environment. The same animals were subjected to the same energy levels in the second period, but the ambient temperature was alternated.

At each period, the animals were subjected to a 24-h temperature-controlled program. The light of the climatic chamber was automatically switched off at 18:00 h and on at 6:00 h on the following morning; this procedure aimed to subject the animals to a continuous period of 12 h of light and 12 h without light. The feed given to the animals was offered as a total mixed ration, twice a day, at 8:00 and 16:00 h (Table 1).

Dietary treatments were formulated to satisfy three different energy levels as described below:

Medium-energy treatment: the diet was formulated according to the recommendations of the NRC (2007) for goats in the middle of lactation and milk yield between 1.47 and 2.3 kg day^{-1}, and average live weight of 40 kg.

Low-energy treatment: the same diet of the medium-energy treatment was offered, with 15% as a restriction in the mean dry matter intake/live weight of the medium energy treatment animals.

High-energy treatment: the diet was formulated specially for this treatment following recommendations of the NRC (2007), which increases by 15% only the metabolizable energy (ME) requirements.

Diet samples were immediately frozen for further analysis. They were thawed and pre-dried in forced air at 55 °C for 72 h, ground to a mesh size of a 1-mm sieve knife mill, and then packed into plastic bags. The determining nutrient content of ingredients and diets were according to the procedures of the Association of Official Analytical Chemists (AOAC, 2005) (Table 1). The dry matter (DM; AOAC method 934.01), crude protein (CP; Kjeldahl method, AOAC method 984.13), ether extract (EE; AOAC method 920.39), and neutral detergent fiber (NDF; method AOAC 973.18) were analyzed. Total carbohydrates were analyzed by capillary electrophoresis with ultraviolet radiation and derivatization pre-column with 250 mmol L^{-1} p-aminobenzoic acids (PABA) and 20% acetic acid at

Table 1 - Chemical composition of experimental diets

Item	Dietary energy level		
	Low	Medium	High
Ingredient (g kg^{-1} DM)			
Tifton hay (*Cynodon dactylon* (L.) Pers)	502	502	346
Ground corn	278	278	389
Soybean meal	200	200	205
Soy oil	-	-	40
Mineral nucleus[1]	10	10	10
Calcitic limestone	10	10	10
Chemical composition			
Dry matter (DM; g kg^{-1} as fed)	858	858	862
Crude protein (g kg^{-1} DM)	174	174	173
Ether extract (g kg^{-1} DM)	30	30	70
Neutral detergent fiber (g kg^{-1} DM)	462	462	365
Non-fibrous carbohydrate (g kg^{-1} DM)	272	272	336
Total carbohydrates (g kg^{-1} DM)	734	734	701
Total digestible nutrients (g kg^{-1} DM)	716	716	806
Metabolizable energy (Mcal kg^{-1} DM)	2.5	2.5	2.9
Intake			
Total digestible nutrients (g day^{-1})	907	1100	1322

[1] Guaranteed levels per kg of product: phosphorus, 70 g; calcium, 140 g; sodium, 148 g; sulfur, 12 g; magnesium, 1.320 mg; fluorine, 700 mg; zinc, 4.700 mg; manganese, 3.690 mg; iron, 2.200 mg; cobalt, 140 mg; iodine, 61 mg; selenium, 15 mg; monensin sodium, 100 mg.

40 °C. Non-fibrous carbohydrates (NFC, %) were estimated using the equation proposed by Mertens (1997): NFC = 100 − (%CP + %EE + %DM+ %NDF).

Feces sample collection was performed daily, directly from the animals' rectal ampoule (0, 2, 4, 6, 8, and 10 h after feeding). Samples were weighed, identified, and stored at −15 °C and at the end of the experimental period, they were homogenized (constituting a composite sample of animals) and pre-dried in an oven with forced circulation at 65 °C for 72 h. Indigestible NDF (iNDF) was used as an internal marker to estimate apparent nutrient digestibility and fecal output; an adult cow cannulated in the rumen was used. For iNDF analysis, 0.5 g (1 mm) of feces, orts, and feed samples were fermented *in situ* (144 h) in the cow's rumen in nylon bags. After ruminal incubation, the filter bags were washed and dried (55-60 °C for 72 h), and the incubation residues were analyzed for NDF concentrations. Fecal output was calculated by using the following equation: FE = iNDFI/iNDFF, in which: FE is the fecal output (kg day^{-1}); iNDFI is the iNDFI intake (kg day^{-1}), and iNDFF is the iNDF content in the feces (kg kg^{-1}). Estimation of total digestible nutrients (TDN) was based on the equation described by Weiss (1999): TDN = CPD + EED × 2.25 + NFCD + NDFcpD; in this equation, CPD = (CP ingested − CP feces), EED = (EE ingested – EE feces), NFCD = (NFC ingested − NFC feces), and NDFcpD = (NDFcp ingested – NDFcp feces). To calculate ME (kcal ME kg DM^{-1}), the digestible energy (DE) was initially obtained as the product between TDN content and the factor 4.409/100, considering the ME concentration of 82% of DE (Silva and Leão, 1979).

Milk production and DM intake (kg day^{-1}) were measured during the five days of data collection in each experimental period. Milk samples were collected, corresponding to 15% of the production in each milking. At the end of each period, a sample composed for each animal was made, then stored at −18 °C for later analyses. Milk fat was determined by the method 989.05 of AOAC (2000).

Milk fat globules were measured as reported by Martini et al. (2013). In brief, milk was diluted 1:100 with distilled water. After the second dilution, 500 μL of each previous sample was mixed with 50 μL of a staining solution, 0.1% of Acridine Orange dye in phosphate buffer (pH 6.8), and shaken immediately afterward. After that, 8 μL of the sample were placed in the Burker camera. We took ten photos of each sample in 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.

Data were analyzed using the PROC MIXED procedure of SAS software (Statistical Analysis System, version 9.1.3), considering the animal effect within the period as random and the carryover effect between the two periods. The means when significant were compared using the Tukey-Kramer test (P<0.05). The following model was used:

$$y_{ijk} = \mu + \alpha_i + b_j + \gamma_k + b\gamma_{jk} + \varepsilon_{ijk},$$

in which y_{ijk} = observed variable, μ = general average, α_i = period effect (i), b_j = fixed effect of environment (j), γ_k = fixed effect of diet (k), $b\gamma_{jk}$ = interaction of the effects of environment (j) and diet (k), and ε_{ijk} = residual effect of the interaction of environment (j) and diet (k) factors.

3. Results

There was no significant interaction between the effects of temperature and diet (P>0.05). So, we have presented tables (Tables 2 and 3) separately.

It was observed that DM intake was influenced (P = 0.034) by environmental temperature (Table 2). The average diameter of the MFG of goat in this study was 2.34 μm, ranging from 2.46 to 3.14 μm. The diameter of MFG was affected (P = 0.011) by the environmental temperature (Table 2) with a significant decrease (−17%) at 34 °C, compared with 26 °C.

Milk fat and MFG were influenced by the energy of the diet (P = 0.010) (Table 3). Milk fat significantly increased (+11.54%) from medium to high energy diet. The MFG diameter increased (+23%) with an increasing dietary ME of 33%. We observed influences of energy level on DM intake (P = 0.023). The highest value for DM intake was observed in the medium and high energy diet.

Table 2 - Mean and standard deviation of milk, fat, and milk fat globule (MFG) parameters of Alpine goats subjected to two temperatures

Variable	Temperature (°C)		P-value
	26	34	
Daily dry matter intake (g)	1596.41±275.59a	1364.84±255.30b	0.034
Daily milk production (kg)	2.01±0.571	2.01±0.561	0.981
Milk fat (%)	2.24±0.276	2.11±0.440	0.199
MFG diameter (μm)	2.98±0.531a	2.46±0.336b	0.011
Quantity (no. MFG×10^9)	0.96×10^9±0.311	1.0×10^9±0.203	0.759

a.b - Means followed by different letters in the same row differ from each other by Tukey-Kramer test.

Table 3 - Mean and standard deviation of milk, fat, and milk fat globule (MFG) parameters of Alpine goats subjected to three levels of metabolizable energy in the diet

Variable	Dietary energy level			P-value
	Low	Medium	High	
Daily dry matter intake (g)	1266.69±64.25b	1535.30±228.85ab	1639.90±355.69a	0.023
Daily milk production (kg)	1.91±0.261	1.98±0.773	1.95±0.563	0.656
Milk fat (%)	2.07±0.303ab	1.93±0.275b	2.34±0.462a	0.010
MFG diameter (μm)	2.53±0.311b	2.65±0.582ab	3.14±0.466a	0.010
Quantity (no. MFG×10^9)	0.89×10^9±0.391	1.2×10^9±0.295	0.84×10^9±0.382	0.423

a-b - Means followed by different letters in the same row differ from each other by Tukey-Kramer test.

4. Discussion

Thermal stress increases the need for maintenance of the animal due to the higher energy demand for maintaining homeothermia, with a deviation from the energy substrate, which would be diverted to milk production (Habeeb, 2020) as the energy requirement was met, did not interfere with milk production. Supposedly these animals, as an adaptive mechanism, used other energy sources to reduce production losses, such as the use of body reserves (Hamzaoui et al., 2013), or prioritize lipid metabolism as a way of obtaining energy in an attempt to reduce the caloric increase generated by the metabolization of nutrients.

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 and Ribeiro, 2001; Arora et al., 2013).

The DM intake decreases together with the diameter of the fat globule with increasing temperature. According to Martini et al. (2006), there is a prevalence of milk fat globules with diameter <6 μm that is positively correlated ($P<0.01$) with the percentage of DM and lipids, while the greater presence of fat globules with diameter >6 μm was associated ($P<0.01$) with higher milk production and higher percentage of lactose, defatted DM and ashes.

We did not find any effect of high temperature on fat content, differently from the study by Brasil et al. (2000), who observed a reduction of milk fat in goats subjected to high temperature for thermoregulatory mechanisms. However, some authors found no significant decrease in fat percentage for cows under heat stress (Roman-Ponce et al., 1977; Lacetera et al., 2003). Lipid is one of the main components of milk. The dominant fraction of milk fat is triacylglycerol (TAG, about 98%) present in the form of fat globules (Mansson, 2008). In addition to being an energy source, TAG composition is implicated in human health and the property of dairy products (Jensen et al., 2002; Palmquist et al., 2006). The second most important fraction of milk fat is polar lipids, which are the main structural constituents of fat globule membrane and thus play a role of emulsifier ensuring the stability of milk emulsion system (Fong et al., 2007; Sánchez-Juanes et al., 2009).

The decrease in the MFG diameter at high temperatures observed in this study is in line with Lu et al. (2018), who stated that temperature changes modify physical properties of milk fat. According to Carroll et al. (2006), the average diameter of fat globules in cows increase due to increased energy in the diet. Conversely, in the same species, lower milk fat content and smaller MFG have been found when animals were fed a higher energy content diet than a high forage diet (Argov-Argaman et al., 2014).

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). With protein, fat is an essential compound of the coagulation with rennet, and the increase in milk fat may positively affect cheese making. A larger MFG diameter has been reported to affect the texture of cheese; in particular, larger MFG make harder cheese than smaller MFG (Martini et al., 2016).

5. Conclusions

The increase in dietary energy levels of goats promotes an increase in the diameter of fat globules and fat milk yield (%), essential for the cheese industry.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: D.K.R. Azevedo, N.L. Ribeiro, R.R. Guerra and I. Altomonte. Data curation: N.L. Ribeiro and M.L.C.M. Amorim. Formal analysis: N.L. Ribeiro, R.R. Guerra, A.M.S. Sant'Ana, I. Altomonte and M. Martini. Investigation: R.G. Costa, D.K.R. Azevedo, M.L.C.M. Amorim and R.R. Guerra. Methodology: R.G. Costa, D.K.R. Azevedo, N.L. Ribeiro, M.L.C.M. Amorim, R.R. Guerra, A.M.S. Sant'Ana and M. Martini. Project administration: R.G. Costa. Software: D.K.R. Azevedo and N.L. Ribeiro. Supervision: R.G. Costa and N.L. Ribeiro. Validation: N.L. Ribeiro. Visualization: R.R. Guerra. Writing-original draft: R.G. Costa, D.K.R. Azevedo, N.L. Ribeiro, I. Altomonte and M. Martini. Writing-review & editing: R.G. Costa.

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