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The impact of double-distilled glycerin supplementation on the quality of goat milk and cheese

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Abstract

The objective of this study was to evaluate the effects of replacing corn by double-distilled glycerin, a co-product of the biodiesel industry, on goat curd cheese in terms of physical-chemical, microbiological and sensory aspects. Twelve multiparas Saanen goats weighing 47.07 ± 2.41 kg and at 90 ± 5 days of lactation. The experimental design was completely randomized with two treatments (0 and 15% inclusion of glycerin). The physicochemical parameters analyzed were: fat (%), non-fat solids (%), protein (%), lactose (%), salts (%), relative density at 15/15 °C (gmL-1), index cryoscopic (°C), electrical conductivity (mScm-1), pH and acidity. The milk from each treatment was used to make the rennet cheese. The fat had a significant effect ($P < 0.05$) with inclusion of glycerin in the diet. The physicochemical characteristics of the cheese were influenced ($P < 0.05$) by feeding up to 15% of glycerin. Regarding sensory attributes, only firmness was influenced ($P < 0.05$) by the inclusion of glycerin in the diet of dairy goats. Bidistilled glycerin, when inserted in the diet of dairy goats at a level of 15%, causes a reduction in the lipid content of milk and cheese, consequently affecting the cheese yield, and the parameter of firmness in the sensory of the evaluated cheeses.

Keywords: glycerol; lipids; microbiological; rennet; softness.

Practical Application: Glycerin can be used in the diet without impairing the quality of cheese and milk.

1 Introduction

In raising dairy goats, a key point is the food, as it has a high cost, certain concentrates are composed of noble foods such as corn, soy and wheat (Chanjula et al., 2015). Production is directly related to feeding, the use of balanced rations is necessary to nourish the animals and maintain lactation during the period of milk production. Aiming at maximum efficiency in this activity, research and adaptation of new technologies has been intensified, including in nutrition, with the use of alternative food sources, which can replace premium foods without changing the composition and quality of goat milk and its derivatives (Agy et al., 2012; Oliveira et al., 2012; El-Shafei et al., 2020; Hadjimbei et al., 2020).

Goat milk has high nutritional value, therapeutic and dietary characteristics, in addition to high digestibility (Haenlein, 2004). Goat milk contains a high level of short-and medium-chain fatty acids with a small size of fat globules (~2.5-3 μm in diameter), while it has a low level of αs1-casein (4.5%-34% of the total protein) and a high level of β-casein (34%-64% of the total protein) (Günay et al., 2021). Moreover, goat milk also has whey proteins (WPs) at a level of 3-12 g/kg. WPs consist of β-lactoglobulin (34%-47% of the total WPs), α-lactalbumin (17%-50% of the total WPs) and serum albumin (5%-22% of the total WPs) (Alichanidis et al., 2016). The natural and healthy image and specific taste of goat milk make goat dairy products a profitable alternative (Fangmeier et al., 2019). This is because goat's milk is known for its beneficial and therapeutic effects on people who are allergic to cow's milk which, combined with nutritional and health benefits, strengthen the potential and value of goat milk and its derivatives (Popović-Vranješ et al., 2017; El-Shafei et al., 2020; Hadjimbei et al., 2020; Günay et al., 2021).

Due to the rising costs of energy-rich foods, alternative food sources such as glycerin (or glycerol) have become an important focus for the livestock industry. Glycerin is the main co-product of biodiesel production, obtained in the process of transesterification of triacylglycerols from vegetable oils or animal fats, usually using methanol and a catalyst (Chanjula et al., 2015). The glycerin generated by the process of obtaining biodiesel does not have specific legislation for its disposal, and large amounts of this co-product are being accumulated in the plants, which can harm the ecological aspect of biodiesel. So there is a need to find an appropriate and profitable destination. Thus, it has been used by several industrial sectors, mainly the pharmaceutical, cosmetics and oral hygiene industries (Milli et al., 2011). The use of this by-product of biodiesel production in animal feed is an interesting option to mitigate pollution, increase the supply of food for herds and the possibility of reducing production costs,

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in addition to reducing the environmental impact of this product on the environment (Beserra et al., 2016).

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 feed 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). There was an increase of 18%, which was one of the reasons that led us to test the level of 15% glycerin in the feeding in our study. Therefore, this work aimed to evaluate the effects of replacing corn by double-distilled glycerin, a co-product of the biodiesel industry, on goat curd cheese in physical-chemical, microbiological and sensory aspects.

2 Material and methods

2.1 Experiment site and animals

The experiment was conducted at the Federal University of Paraíba, Campus at Bananeiras-Paraíba, Brazil (altitude 552m, latitude 6° 41' 11", longitude 35° 37' 41"). Air temperature was 24.97 °C and relative humidity was 76.48% 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. Twelve multiparous Saanen goats weighing 47.07 ± 2.41 kg and at 90 ± 5 days of lactation. The experimental design was completely randomized with two treatments (0 and 15% inclusion of glycerin).

2.2 Diet

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 were: Tifton hay, milled corn, soybean meal, vitamin/mineral supplement and urea, along with the following levels of CG (99.66% glycerol): 0% (control) and 15% to corn in the diets (Table 1).

The crude energy contents of the corn and CG were 3.50 and 3.71 Mcal kg^{-1}, respectively. The animals went through a period of 15 days to adapt to the diet and three days to collect data. During the adaptation and collection periods, daily weighings of food supply and leftovers were carried out to calculate the voluntary consumption and adjust the feed supply, so as to guarantee leftovers of 10% based on dry matter (DM). Water for animal consumption was offered ad libitum, and consumption was quantified daily during the data collection period.

Table 1. 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
Calcitic limestone	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
Fiber in acid detergent. FAD (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; Monensinasódica: 100 mg.

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^{-1}) during the three days of collection of each period (all experimental period). Before milking, the goats' udders were washed with chlorinated water and dried with paper towels and then tested for mastitis (black bottom mug test). After each milking was done post-dipping, the goats' roofs were dipped in a 2% iodine solution. Milk samples from each animal were collected twice a day, at regular times, 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 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.

Physicochemical requirements for fat (%), non-fat solids (%), protein (%), lactose (%), salts (%), relative density at 15/15 °C (g mL^{-1}), cryoscopic index (°C), electrical conductivity (mS cm^{-1}) and pH were evaluated according to the Master Complete® Milk Analyzer (AKSO®, São Leopoldo, Rio Grande do Sul, Brasil), under specific technical conditions. The titratable acidity (g of lactic acid

100 mL^{-1}) was performed by titration method, being analyzed the acidity in lactic acid by the protocol 947.05 (Association of Analytical Chemists, 2010).

2.4 Formulation and cheese making process

The curd cheeses were prepared according to the technique developed by the cheese makers in the Milk and Derivatives sector, coming from Campus III, Bananeiras-PB. The following ingredients were used for cheese preparation: 10 liters of milk; 10 mL of milk yeast; 5 mL of calcium chloride; 10 mL of liquid rennet and 30 grams of sodium chloride (Lima et al., 2021).

At the end of the cheese processing, they were kept in a suitable container and relocated to the cold room located in the same processing laboratory. These, in turn, underwent the maturation process for two to three days, at a temperature of 10 ± 1 °C, as recommended by the Technical Regulation of rennet cheese identity and quality (Brasil, 2001). After this maturation period, the cheeses were vacuum packed and refrigerated until microbiological laboratory, physicochemical, and sensory analyzes.

2.5 Yield and physicochemical analysis of cheese

The food efficiency was obtained by the relation between the average production of corrected milk and the dry matter intake verified during the collection period, according to Equation 1.

$$Feed\ Efficiency\left(kg\right) = \frac{Milk\ Production\left(kg\right)}{Dry\ matter\ intake\left(Kg\right)} \quad (1)$$

Water activity (aW) was determined by the Aqualab apparatus (model 4TE – Decagon Devices, Inc., Pullman, WA). The pH was measured with the aid of the brand pH meter. Tecnal (model Tec-2). The total acidity titratable [Association of Analytical Chemists (2010), method 947.05], humidity [Association of Analytical Chemists (2010), method 925.09], ashes [Association of Analytical Chemists (2010), method 923.03], protein [Association of Analytical Chemists (2010), method 991.23], fat [Association of Analytical Chemists (2010), method 920.39] and the total dry extract (TDE) [Association of Analytical Chemists (2010), method 925.23].

2.6 Microbiological analysis of cheese

These samples were analyzed by duplicate. The microbial analysis were performed according to recommendations by the RDC Resolution Number 12, of January 2, 2001, and the samples were analyzed according to the methodology described by the American Public Health Association (2001), to determine total coliforms, thermotolerant coliforms, *viable aerobic mesophilic* bacteria, coagulase positive *Staphylococcus* and *Salmonella* sp. The analyses of total and thermotolerant coliforms were performed following the technique of the most probable number. The analysis of viable aerobic mesophilic bacteria was performed by the depth plate technique using Plate Count Agar and incubation at 35 ± 2 °C for a period of 48 h. Coagulase positive Staphylococcal analyses were performed by direct plate counting. The plates were incubated in an oven at 36°C for 48 hr, and then the plates were collected for counting the colonies. For the analysis of *Salmonella sp*. the procedure was as follows: a pre-enrichment of the samples with lactose broth and incubation at 42 ± 2 °C for 24 h was carried out, and then a selective enrichment with Tetrionate and Selenite cysteine broth followed by incubation in xylose lysine deoxycholate Agar and Enteric Agar was done.

2.7 Cheese sensory analysis

The samples were splitted into equal portions of 5 g into polystyrene plates, marked with a random 3-digit code. In order to avoid the possible effects of the order of presentation, the samples were presented to panel members following different orders (MacFie et al., 1989). Sensory analysis was performed in individual booths having controlled environmental conditions, at a temperature around 23 °C [ISO 8589, International Organization for Standardization (1988)]. The panel included nine UFPB agricultural science students who were duly selected and trained [ISO 8586-1, International Organization for Standardization (1993)]. The tests took place in two session and nine trained tasters.

For the acceptance test, a hedonic scale of 9 points was used, following the recommendations of Peryam & Pilgrim (1957): 9 (Iliked it very much); 8 (I enjoyed it); 7 (I liked it moderately); 6 (It was OK); 5 (I didn't like it/Neither did I dislike it); 4 (I didn't like it, but it wasn't too bad); 3 (I didn't like it moderately); 2 (I really didn't like it); and 1 (I hated it). The tasters described how much they liked or disliked the attributes: odor, appearance, taste, texture, and overall impression. A scale of 5 points recommended by Meilgaard et al. (2006) was used to evaluate the intention of purchasing: 1 (Certainly I would buy it); 2 (Probably I would buy it); 3 (Maybe I would buy it/Maybe I wouldn't buy it); 4 (Probably I wouldn't buy it) and 5 (Certainly I wouldn't buy it).

2.8 Statistical analysis

The animals were distributed in a crossover experimental design in a scheme of subdivided parcels 2 x 2 (two levels of dietary glycerine inclusion x two periods) with the inclusion of glycerine in the diet the main portion. Data were analyzed by the MIXED procedure of SAS 9.13 software (Statistical Analyses System, 2012), considering the effect within period as random, as well as carryover effect between the two periods. Means when significant were compared by Tukey test ($P < 0.05$). The means of the sensorial attributes were compared by the test Ryan-Einot-Gabriel-Welsch at 5% probability.

3 Results and discussion

The physicochemical variables showed no significant difference ($P > 0.05$) with inclusion of glycerin in the diet (Table 2).

The physicochemical characteristics of coalho cheese produced with goat's milk were influenced ($P < 0.05$) by feeding up to 15% of glycerin, with the exception of water activity (Table 3).

The higher than the values that favor the development of microorganism, which is 0.60, according to Franco & Landgraf (2008). High water activity values make cheese more susceptible to the development of spoilage and pathogenic microorganisms.

Table 2. Physicochemical variables of dairy goats submitted to inclusion of glycerin in the diet.

Variables	Levels of inclusion glycerin (%)		SEM	*P* value
	0.00	15.0		
Non-fat solids (%)	8.56	8.28	0.119	0.1108
Density (g/cm³)	1.030	1.029	0.000	0.5930
Protein (%)	3.14	3.14	0.063	0.9612
Lactose (%)	4.73	4.53	0.084	0.1027
Cryoscopic índex (°C)	0.53	0.53	0.008	0.6148
Salts (%)	0.70	0.67	0.009	0.0850
pH	6.44	6.44	0.010	0.7423
Conductivity	5.33	5.33	0.014	0.8966

SEM: Standard error means.

The mean value of water activity observed in this research (0.97) similar to the data found by Lima et al. (2021).

Moisture was influenced by the level of replacement of corn by double-distilled glycerin, cheeses from the diet containing double-distilled glycerin showed lower results for this variable, however, these values are close to the values of 36.0 - 54.9, presented by Technical Regulation of Identity and Quality of Coalho Cheese – Annex II (Brasil, 2001), which classifies coalho cheese in high moisture content.

The protein contents of the cheeses were altered, but the protein contents of the milks remained the same, with an average of 3.14, indicating that possibly the freezing and heating process during cheese production altered the structure of the proteins.

The total dry extract values are inversely proportional to the moisture content, since when the moisture content increases, the total dry extract, which is represented by the sum of proteins, fat, ash and others, minus moisture, decreases. In view of this fact, it can be seen that there is a variation in the moisture content of the cheeses (Table 3) which contributed to not affecting the total dry extract. According to Teixeira (2016), the control of the Total dry extract must be advocated, since the content of the total dry extract is one of the most important factors in the physicochemical characterization of cheeses. The values referring to fat in the extract were influenced by goat milk fed with double-distilled glycerin. The percentage of fat in the dry extract is one of the most expressive ways to quantify the fat contained in different types of cheeses (Brasil, 2001), indicating that the decrease in milk fat directly interfered in the values of total dry layer and defatted dry layer.

Lower pH values, which was presented at 0%, are interesting as they inhibit the proliferation of pathogenic or spoilage microorganisms. According to Balduino et al. (1999), the production of lactic acid at the beginning of fermentation reduces the pH and inhibits undesirable microorganisms such as Salmonella sp., Yersinia enterocolytic and Escherichia coli.

For the variable of cheese yield, there was a statistical difference for cheese production, indicating that the level of double-distilled glycerin, when affecting the lipid parameter, also affected cheese yield. This fact can be associated with factors such as manipulation of chemical compounds present in milk, in this case it is possible that the low lipid content found in raw material milk may have influenced the increase in the amount of milk for cheese production.

Table 3. Physicochemical variables of goat cheese fed with double-distilled glycerin.

Variables	Levels of inclusion glycerin (%)		*P* value
	0.00	15.0	
Water activity	0.97 ± 0.00	0.97 ± 0.00	0.076
Moisture (%)	55.17 ± 0.76	52.44 ± 0.16	< .0001
Protein (%)	20.35 ± 0.29	20.11 ± 0.29	0.0051
Fat (%)	13.87 ± 0.18	11.44 ± 0.16	< .0001
Degreased dry extract (%)	44.83 ± 0.77	47.56 ± 0.16	< .0001
Total dry extract (%)	30.95 ± 0.75	36.12 ± 0.21	< .0001
Ash (%)	6.13 ± 0.19	5.95 ± 0.16	0.0060
Density (g cm3)	1.03 ± 0.001	1.03 ± 0.0004	< .0001
pH	6.08 ± 0.13	6.21 ± 0.03	< .0001
Acidity (%)	0.22 ± 0.007	0.18 ± 0.007	< .0001
Lactose (%)	1.26 ± 0.16	1.36 ± 0.07	0.0214
Yield (L kg^{-1})	11.02	12.21	< .0001

As for the microbiological analysis, no microbial growth was detected in the coalho cheese produced with goat milk that received up to 15% glycerin in the diet (Table 4).

The values of the microorganisms observed were within the parameters established by the RDC 12, with adequate microbiological quality from a hygienic-sanitary point of view and could be safely consumed.

Regarding sensory attributes, only firmness was influenced ($P < 0.05$) by the inclusion of glycerin in the diet of dairy goats (Table 5).

According to the data from this research, there was no variation ($P > 0.05$) between treatments for descriptive and quantitative variables being, color, looks, homogeneity, goat milk characteristics, curd cheese aroma, curd cheese aftertaste, characteristics goat cheese type, goat cheese aftertaste, Salty, acidic, elasticity, chewiness, tenderness (Table 5). However, for the variable firmness, a statistical difference was found between treatments. The significant effect presented by the firmness variable ($P = 0.0353$) can be attributed to the decrease of lipid in the raw material milk, due to the level of substitution of glycerin by corn.

Table 4. Microbiological evaluation of rennet-type cheeses produced with milk from goats fed with double-distilled glycerin.

Microorganisms	Level of inclusion (%)	
	0.00	15.0
Coliform 35 °C (MPN.g^{-1})	< 3.0	< 3.0
Coliform 45 °C (MPN.g^{-1})	< 3.0	< 3.0
Staphylococcus spp. (CFU.g^{-1})	< 100	< 100
Salmonella spp.	Absent	Asent

Table 5. Effect of glycerin addition in the diet of dairy goats on the sensory attributes of coalho cheese.

Attributes	Levels of inclusion (%)		*P* value
	0.00	15.0	
Appearance			
Color	6.98 ± 1.11	6.85 ± 1.28	0.7591
Eyes	1.38 ± 1.38	1.84 ± 1.95	0.4515
Homogenety	7.18 ± 0.63	7.04 ± 0.82	0.6006
Characteristics of goat cheese	4.53 ± 1.71	4.12 ± 1.25	0.4363
Aroma			
Curd cheese	5.90 ± 1.89	5.73 ± 2.01	0.8013
Residual cheese like rennet	6.09 ± 1.82	5.88 ± 1.83	0.7449
Characteristic of goat cheese	5.70 ± 1.11	5.45 ± 1.41	0.5824
Flavor			
Goat cheese aftertaste	4.72 ± 1.70	3.92 ± 1.90	0.2206
Salty	4.01 ± 0.93	4.04 ± 0.86	0.9227
Acid	2.02 ± 1.60	1.74 ± 1.31	0.5912
Texture			
Elasticity	3.49 ± 1.90	3.53 ± 1.60	0.9522
Chewability	4.84 ± 1.30	4.35 ± 1.73	0.3699
Softness	5.95 ± 1.24	5.53 ± 1.57	0.4022
Firmness	4.51 ± 1.06a	3.58 ± 1.30b	0.0353

Different letters on the line differ from each other by the Ryan-Einot-Gabriel-Welsch test.

The homogeneity attribute received the highest mean of the evaluators, with mean values above 7.0. These attributes probably received these assessments due to the fact that cheese from goat's milk has a whiter color, due to the lack of carotene, since all the B-carotene is converted into vitamin A (Moreira et al., 2019), and, for not having suffered any deleterious effects during the cheese production and storage processes.

4 Conclusion

Bidistilled glycerin, when inserted in the diet of dairy goats at a level of 15%, causes a reduction in the lipid content of milk and cheese, consequently affecting the cheese yield, and the parameter of firmness in the sensory of the evaluated cheeses. Causing economic loss, since the sensory quality of firmness was impaired.

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