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Short communication

Physicochemical composition of the milk and cheese yield of sheep supplemented with concentrate based on corn grain or whole cottonseed

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ABSTRACT

This study aimed to evaluate the effects of nutritional supplementation with concentrate based on corn grain or whole cottonseed on the milk yield, physicochemical composition of the milk and the cheese yield from lactating sheep. Twelve Lacaune x Santa Ines ewes were kept on irrigated pasture of Tifton 85 (*Cynodon dactylon*) and were distributed in replicated 3 × 3 Latin Square design. The animals were allocated into three experimental groups for 63 days of supplementation, as follows: Pasture (composed of Tifton 85 grass without supplementation), Corn (concentrated mixture containing ground corn grain and Tifton 85 pasture) and Cottonseed (concentrated mix containing whole cottonseed and Tifton 85 pasture). The milk from the Cottonseed group showed higher values ($P < 0.05$) of non-fat solids, protein, minerals, density, freezing point when compared to the Pasture and Corn groups, but there was no difference ($P > 0.05$) for fat content and milk yield among Pasture, Corn and Cottonseed groups. Cheese yield in the Cottonseed group was 30 % higher than the cheese yield in the Corn group ($P > 0.05$). The inclusion of 50 % of whole cottonseed in concentrate supplementation of crossbred ewes increases the physicochemical composition of the milk, and this may improve the cheese yield of manufactured cheeses.

1. Introduction

The sheep production play an important role in agricultural development and human nutrition in developing countries (Haenlein, 2007). The Northeastern region of Brazil stands out in sheep farming, as it owns approximately 65.7 % of the national herd and is the second largest producer of sheep milk (538,000 liters/year) in the country (IBGE / SIDRA, 2017).

Sheep milk has high levels of protein, fat, minerals and vitamins, standing out for its high nutritional value, when compared to the milk of other domestic species (Sampelayo et al., 2007; Balthazar et al., 2017). However, sheep milk is rarely consumed *in natura* (Blagitz et al., 2013) and it is entirely destined for the manufacture of various products, especially cheese. Due to its high total solids content, sheep milk is excellent for producing dairy products, such as cheese and yoghurt (Ribeiro et al., 2007; Balthazar et al., 2017).

One of the strategies for producing dairy sheep in tropical regions is the use of irrigated and cultivated pastures. However, the exclusive use of pasture is not enough to meet the nutrient demands of lactating animals, therefore, a nutrition supplementation favors the intake of nutrients, improves milk yield, quality of cheese and the reproductive performance (Santos et al., 2017).

Corn is an important feed nutrient and it is widely used for sheep nutritional supplementation, as it has a high content of total carbohydrates (85 %), with significant concentrations of starch, being equivalent to 70 % of dry matter (Rooney and Pflugfelder, 1986). In spite of that, corn grain has become costly, as the supply of grains from Brazil to the foreign market has increased its value. An alternative to replace corn in animal supplementation is the use of whole cottonseed as a nutrient for sheep supplementation. Cottonseed is an excellent source of protein and energy, as it has about 24.9 % of crude protein; in addition, it is rich in fiber, energy in the form of lipids, showing 96 % of total digestible nutrients (Rogério et al., 2004). A correct sheep supplementation enables high milk yield, whereas the milk should have maximum levels of protein and fat, which are the main ingredients that determine the quality of the cheese produced (Balthazar et al., 2017).

There is no information on the effects of nutritional supplementation with cottonseed in concentrate for dairy sheep in the semi-arid region of

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Table 1
Ingredient proportion (% of dry matter - DM), chemical composition and *in vitro* dry matter digestibility of the forage and concentrates based on ground corn and whole cottonseed for lactating ewes.

Ingredients/Nutrients		Corn	Cottonseed
Ground corn, % DM		88.0	47.9
Whole cottonseed, % DM		-	50.0
Soybean meal, % DM		9.5	1.0
Urea, % DM		1.5	0.1
Mineral salt mix*		1.0	1.0
Total		100	100
	Chemical composition		
% DM	**Pasture**	**Corn**	**Cottonseed**
Dry matter[a]	28.97	91.60	91.38
Ash, %DM	6.26	1.51	3.20
Crude protein, %DM	14.76	16.49	16.55
Neutral detergent fiber, %DM	74.83	25.94	34.66
Acid detergent fiber, %DM	35.54	7.28	12.38
Ether extract, %DM	1.92	4.14	11.26
In vitro dry matter digestibility, %DM	55.90	70.86	65.62

Composition of commercial mineral salt mix for sheep: Calcium (g/kg): 202.0; Phosphorus (g/kg): 45.0; Magnesium (g/kg): 10,0; Sulfur (g/kg): 8,0; Chlorine (g/kg): 240.0; Sodium (g/kg): 156.0; Cobalt (mg/kg): 35.0; Copper (mg/kg): 150.0; Iodine (mg/kg): 40.0; Manganese (mg/kg): 2000; Selenium (mg/kg): 15.0; Zinc (mg/kg): 2500; Iron (mg/kg): 1.300; Fluorine (mg/kg): 450.0.
a As feed.

Northeastern Brazil. We hypothesized that nutritional supplementation with whole cottonseed can substitute ground corn as energy source and that cottonseed might increase the cheese yield. Therefore, the present study aimed to evaluate the effects of supplementation with concentrate based on corn grain or whole cottonseed during the lactation period on the physicochemical composition of the milk and the cheese yield of Lacaune x Santa Ines crossbred ewes.

2. Materials and methods

2.1. Location and Ethical aspects

The study was carried out at the Experimental Field of Embrapa Semiárido, the Brazilian Agricultural Research Corporation, located in the municipality of Petrolina, Pernambuco, Brazil (09°09' South, 40°22' West and 365 m altitude). Average annual rainfall in the region is 567 mm, mean annual temperatures vary from 24.2 to 28.2ºC, and annual relative humidity varies from 66 % to 71.5 %.

All procedures were carried out in accordance to the guidelines for ethics and animal well-being and approved by the Committee for Ethics in the Use of Animals (CEUA) of Embrapa Semiárido, under protocol number 05/2017.

2.2. Experimental design

For the experiment, 12 Lacaune x Santa Inês crossbred ewes were selected and held on irrigated Tifton 85 pasture. All animals were healthy, with single lamb deliveries, and in eleventh week of lactation (77 days after parturition) in the beginning of the study, with ages varying from 1.5 to 2 years. The initial body condition score was 2.75 (scale from 1 to 5) and the initial body weight was 43.1 ± 4.3 Kg. The animals were previously identified, weighed, treated for endo and ectoparasites and kept on pasture of irrigated Tifton 85.

The animals were distributed in replicated 3 × 3 Latin Square experimental design, which was three experimental groups and three periods, with four sheep in each experimental group, similarly to what was done by Vicente et al. (2020). The experimental period lasted for 63 days, divided in three periods of 21 days each (16 days of adaptation and five days of sampling for physicochemical composition of the milk). The animals were allocated into three experimental groups: i) Pasture: exclusive grazing of Tifton 85 grass (*Cynodon dactylon*), and without concentrated supplementation; ii) Corn: concentrated mix containing ground corn grain and access to Tifton 85 pasture; and iii) Cottonseed: concentrated mix containing whole cottonseed and access to Tifton 85 pasture.

Feed supplementation with corn or cottonseed mixes were offered every morning in the amount of 500 g/sheep/day in fresh matter (0.45 Kg DM/day) and the proportion of ingredients and chemical composition of concentrates are presented in Table 1. In order to determine dry matter and nutrient intakes the sheep were kept in individual pens (1.5 m x 2.0 m) for three hours after milking, with water and mineral salt *ad libitum*, and then led to the paddocks, remaining there until next milking day. Concentrate leftovers were sampled and weighted. The sheep remained in each paddock for 21 days, corresponding to each experimental period, with fixed stocking rate.

In each paddock, 40 kg of nitrogen/ha was applied in the form of urea. The grazing interval of the paddocks was 30 days. The average heights and pasture mass for pre-grazing and post-grazing were 27.6 cm and 14.58 cm, and 4250 kg of DM/ha and 2750 kg of DM/ha, respectively.

2.3. Laboratory analysis, fecal production, digestibility and dry matter and nutrient intake

The *in vitro* dry matter digestibility (IVDMD) of the concentrates and the pasture (Table 3) were carried out following the adapted methodology of Tilley and Terry (1963). To estimate pasture dry matter intake (DMI pasture) the following formula was used: DMI = FP/1- pasture IVDMD* 100; being: DMI = dry matter intake; FP = fecal production; IVDMD = *in vitro* dry matter digestibility.

Forage intake was estimated using titanium dioxide (TiO_2) as external marker to estimate fecal production. The TiO_2 was supplied at 2.0 g/day through an esophageal tube for 15 days, being 10 days of adaptation and five days of sampling. Fecal samples were collected directly from the rectal ampoule, in the morning, before offering concentrated supplements. Fecal samples were pre-dried (55 °C until constant weight), and they constituted a composite sample by treatment, being stored at − 20°C. Fecal production was estimated by the equation: FPtit (g DM/day) = supplied TiO_2 (g/day)/(% TiO_2 in feces/DM 105 °C); being: FPtit = fecal production obtained through titanium dioxide; supplied Tit. = quantity of TiO_2 administrated orally; DM = dry matter 105 °C.

To estimate the total DMI for supplemented groups, it was considered the fecal production from concentrate and pasture intakes, by the amount of concentrate ingested and determining the digestibility of the supplement. Concentrate DMI was determined by subtracting the amount of concentrate offered daily and the leftovers. Fecal production from concentrate was estimated as follow: FP = concentrate DMI – (concentrate DMI x concentrate IVDMD), while the estimative of FP from pasture were performed as follow: from pasture = total FP – FP from concentrate.

The intake of nutrients (CP, NDF) was determined as the difference between total nutrients in the consumed feed and the total nutrients in the leftovers. Chemical analyses (forage, concentrates and leftovers) were carried out using methods described by the Association of Official Analytical Chemists (Aoac, Association of Official Analytical Chemists, 2016) for dry matter (DM; method 967.03), ash (method 942.05), crude protein (CP; method 981.10) and ethereal extract (EE; method 920.29). The content of neutral detergent fiber (NDF) and the acid detergent fiber (ADF) were determined as described by Van Soest et al. (1991) (Table 1).

2.4. Physicochemical composition and milk production

The evaluation of yield and physicochemical composition of sheep milk was carried out individually as well as collectively, for each of the three experimental groups (Pasture, Corn and Cottonseed). To

Table 2
Concentrate intake, pasture intake, total dry matter intake and digestibility of ewes Lacaune x Santa Inês supplemented with different energy sources.

Variable	Pasture	Corn	Cottonseed	SEM	P value
Initial body weight (kg)	43.30	42.30	41.90	1.24	0.5359
Final body weight (kg)	43.03	42.50	43.90	1.19	0.1260
Concentrate DMI (kg/day)	0.00c	0.29b	0.37a	0.03	0.0496
Pasture DMI (kg/day)	1.12[a]	0.79b	0.86b	0.08	0.0045
Total DMI (kg/day)	1.12	1.08	1.23	0.07	0.1152
Crude protein intake (kg/ day)	0.17b	0.18b	0.22a	0.01	0.0015
Neutral detergent fiber intake (kg/day)	0.83	0.75	0.85	0.04	0.2852

DMI = Dry matter intake; P = Probability; SEM = Standard error of the mean. Mean with different letters (a, b, c) in the same row differ ($P < 0.05$).

Table 3
Milk production and physicochemical composition of the milk of crossbred ewes Lacaune x Santa Inês supplemented with different energy sources.

Milk composition	Pasture	Corn	Cottonseed	SEM	P value
Milk production (g/ewe/ day)[a]	346.70	377.10	417.90	20.69	0.3561
Fat (%)	6.50	6.50	7.36	0.60	0.2593
Non-fat solids (%)	11.50b	12.00b	13.10a	0.41	0.0036
Protein (%)	4.26b	4.47b	4.87a	0.15	0.0037
Density (Kg/ m^3)	38.30b	40.50b	43.90a	1,37	0.0067
Freezing Point (°C)	0.82b	0.87b	0.97a	0.04	0.0039
Minerals (%)	0.93b	0.98b	1.06	0.03	0.0038

[a] Corrected for 6.5 % fat and 5.8 % protein. P = Probability; S.E.M = Standard error of the mean. Mean with different letters (a, b) in the same row differ ($P < 0.05$).

determine milk yield, the ewes were milked individually in the morning using a mechanical milking machine for five consecutive days. For complete milk ejection, 3.0 IU oxytocin was given intramuscularly, 10 min before milking.

The milk yield was corrected for 6.5 % fat and 5.8 % protein, using the equation described by Pulina et al. (2005): FCM (6.5) = M (0.37 + (0.097 x F)) and FPCM (6.5; 5.8) = M (0.25 + (0.085 x F) + (0.035 x P)), where: FCM, FPCM = Fat (and protein) corrected milk; M = Milk yield (Kg); F = Milk fat content (%) and P = Milk protein content (%).

The milk from each animal was weighed, and then 100 mL individual milk samples were collected, placed in identified Falcon tubes, and stored at − 5°C for posterior analysis. To determine the physicochemical composition of the sheep milk, a milk analyzer Master Classic, brand AKSO Brasil, calibrated for sheep, was used, where all analyses for fat, non-fat solids, density, freezing point, minerals and proteins were performed with the milk samples obtained from each animal.

2.5. Cheese yield analysis

The coalho type cheese was hand-produced on the local farm, where all procedures were adopted for hygiene and quality, slow pasteurization of milk, curd formation and separation of whey after the coagulation of the milk.

To calculate the cheese yield, three samples of 10 liters per experimental group was used, which makes a total of 30 liters of milk per group that was converted into cheese. Each sample of 10 liters/group was collected within 7 days (360 mL of milk x 4 sheep x 7 days). Cheese production was obtained by calculating how many liters of milk were needed to produce each kilogram of cheese, as described by Silveira and Abreu (2003).

2.6. Statistical analysis

A quadruple 3 × 3 Latin Square design was used, with 12 animals and 36 observations for each variable. Initially, data were submitted to the Kolmogorov-Smirnov normality test, and afterwards an analysis of variance (ANOVA) was carried out. The ANOVA, followed by a Tukey test, was used to compare the effects of the experimental groups on the variation of body weight, yield and physicochemical composition of the milk, and for dry matter and nutrients intakes. The samples of cheese yield were analyzed by Fisher test. The online statistical program SAS® OnDemand for Academics (SAS INSTITUTE, 2020) was used, and the differences were considered significant when $P < 0.05$.

3. Results and discussion

The hypothesis that whole cottonseed could substitute corn as energy source and that whole cottonseed could increase the cheese yield of Lacaune x Santa Ines sheep and was accepted.

There were no significant differences ($P < 0.05$) among experimental groups for initial and final weight of the ewes (Table 2). Therefore, ingredients from Pasture only and feed supplementation were sufficient to meet the energy demands of the animals and reduce the negative energy balance during lactation. Total DMI was similar among experimental groups (Table 2) and it was not increased by concentrate supplementation of corn mix or cottonseed mix. This can be explained by a substitution effect, which supplemented groups showed a lower ingestion of forage compared to the Pasture group. The concentrate DMI and crude protein intake was greater ($P < 0.05$) in Cottonseed group when compared to Corn and Pasture groups (Table 2). The lower crude protein intake ($P < 0.05$) in the Pasture compared to the Cottonseed group is explained by the crude protein composition of pasture (14.76 %) compared to Cottonseed group (16.55 %) (Table 1).

The results showed that there were no differences ($P > 0.05$) for milk production between the experimental groups (Table 3), most probably because there were no differences in Total DMI between treatments (Table 2). Although the supplemented ewes ingested 290 g and 370 g of concentrate/day (Table 2), by the corn and cotton groups, respectively, there was an effect of substitution of forage by the consumption of concentrate, leading to a lower forage intake by the supplemented animals. On the other hand, non-supplemented ewes ingested a greater amount of forage, which presented good nutritional value, with 14.76 % of protein and 55.90 % of IVDMD (Table 1), favoring the supply of nutrients. In addition, the similar intake of nutrients, such as NDF, which represented a large dry matter intake by the animals in the different treatments also contributed to the lack of difference in milk production.

According to Angeles-Hernandez et al. (2020), the significant decrease in milk production in ewes fed exclusively with pasture may be related to the higher fiber content and the lower amount of non-structural carbohydrates such as starch and other sugars, when compared to concentrate-based diets, which does not corroborates with data found in this study. In our study, it was expected that milk production would be reduced in the Pasture group, since there was a higher quantity of NDF (74.8 %) and ADF (35.5 %) and a lower amount of ether extract (1.9 %) compared to the Corn and Cottonseed groups, but there were no differences between treatments in milk production (Table 3). In comparison to the present study, Vasconcelos et al. (2017) assessed the milk yield of Rabo Largo sheep during a period of 10 weeks, and they observed a greater milk production, which was 1.0 kg milk/ewe/day at the peak milk production. The ewes supplemented with leucaena hay and ground corn grain obtained greater amounts of milk when compared with non-supplemented ewes kept on Tifton 85 grass (Vasconcelos et al., 2017).

The results showed that the nutritional supplementation modified the physicochemical composition of the milk (Table 3). The milk from the Cottonseed group showed higher concentrations ($P < 0.05$) of non-fat solids, protein, density, freezing point and minerals when compared to the Pasture and Corn groups (Table 3). In the physicochemical analysis of the sheep milk, the fat content was the only parameter that did not differ among the treatments. The similarity in fat content among the

Table 4
Cheese yield from milk of Lacaune x Santa Inês crossbred ewes supplemented with different energy sources.

Samples*	Cheese yield (Kg of cheese/10 L of milk)			SEM	P value
	Pasture	Corn	Cottonseed		
1. (10 L /group)	2.50	2.50	3.00	0.17	0.60
2. (10 L /group)	2.10	2.00	2.40	0.12	0.60
3. (10 L /group)	0.93	1.00	2.10	0.38	0.60
Cheese yield (kg/30 L)	**5.53**	**5.50**	**7.50**	0.66	0.34
Liters for 1.0 kg of cheese	5.42	5.45	4.00	0.48	0.50

* Each sample with 30 liters of milk (10 L/group), totaling 90 liters of milk in 3 samples.

experimental groups (Table 3) may be explained by several factors. First, a similar DMI that allowed a similar supply of nutrients among treatments. Second, there was a similar NDF intake, ranging from 750 to 850 g/day (Table 2), demonstrating a high proportion of roughage. Another point to be considered is that most of the ingested NDF came from forage, which has the capacity to stimulate chewing, rumination, maintenance of ruminal pH and milk fat content (Nudda et al. 2014). Probably, the similarity in the ingested nutrients was not enough to change the proportion of acetate and butyrate fatty acids, and the modification in the short-chain fatty acids profile in the rumen is considered one of the main factors modified by the diet with capacity to change the levels of fat in milk (Nudda et al. 2014).

Nevertheless, the fat content in sheep milk may vary significantly according to the compounds in diets provided to sheep, and the fat content in the milk is normally increased with supplementation with fat-supplemented diets (Sampelayo et al., 2007). Supplementation of non-structural carbohydrates, smaller amount of forage and high level of concentrate in the diet reduces the production of acetate and butyrate, which are the main precursors of these fatty acids and of the fat content in the milk (Sampelayo et al., 2007). Therefore, in this study, the milk fat content was expected to increase in the Pasture group, due to the higher amount of NDF and ADF (Table 1).

In the present study, the protein content and density was higher in the milk of ewes supplemented with cottonseed (Table 3). This may be explained by the higher levels of protein intake in the Cottonseed group (Table 2). The proteins content in milk are synthesized from protein supplementation and the rapidly degraded carbohydrates in the rumen, which promote the synthesis of microbial protein through the diet, providing the animal with a supply of metabolizable protein, to increase production of milk and protein content in the milk (Uddin et al., 2015). In addition, sheep milk contains a large amount of total solids, proteins and it has a high density and low freezing point (Park et al., 2007). Therefore, results from the present study show the importance of protein and energy from cottonseed in milk composition (Rogério et al., 2004).

Although there was no significant difference, the animals in the Cottonseed group achieved the highest cheese yield ($P > 0.05$), producing 7.5 kg of cheese from 30 liters of milk. The cheese yields form Pasture and Corn groups were lower compared from Cottonseed group, which was used an average of 5.4 liters of milk to produce 1.0 kg of cheese (Table 4). The cheese yield in the Cottonseed group was about 30 % higher ($P > 0.05$) than the cheese yield in the Pasture and Corn groups. This higher cheese yield from animals supplemented with cottonseed may be explained by greater composition of non-fat solids, protein and density in the milk of Cottonseed group when compared to the other experimental groups (Table 3**).** In the present study, the cheese yield was similar to the one reported by Emediato et al. (2009), who offered Bergamacia sheep with cottonseed based meal and ground corn, and they observed a yield of 5.91 L milk/Kg cheese for "Prato" type cheese (Brazilian soft cheese) and a yield of 7.34 L milk/Kg cheese for Roquefort type cheese.

The inclusion of 50 % of whole cottonseed in concentrate supplementation of Lacaune x Santa Ines crossbred ewes increases the physicochemical composition of the milk (protein, non-fat solids, density and minerals), and these may improve the cheese yield of manufactured cheeses.

Declaration of Competing Interest

The authors have no conflict of interest.

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