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Ruminants

Effect of the addition of protected fat from palm oil to the diet of dairy sheep

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ABSTRACT - The objective of this study was to evaluate the effect of protected fat from palm oil on body weight and milk production and composition of lactating Lacaune ewes. Four treatments (0, 20, 40, and 60 g kg^{-1} as fed) of protected fat from palm oil were used to feed nine animals in each group. Isoproteic and isoenergetic diets were formulated and adjusted for each animal after milk weighing. Corn silage was used as roughage on the same proportion of concentrated to feed all animals. Milk samples were collected weekly for chemical analyses up to the seventh week of the lactation period and every other week after that until the end of the lactation period (182 days). Increased levels of protected fat in the diet of lactating ewes resulted in lower body weight gain and poor animal body condition. The production peak showed differences and was higher in the treatment of 40 g kg^{-1} of palm oil, but milk production during lactation was higher in animals that received 60 g kg^{-1} of protected fat supplementation. Animals fed 60 g kg^{-1} of protected fat showed the highest milk yield. We observed a positive linear effect on milk fat content, whereas the effect on milk protein, lactose, and nonfat solids was linearly negative after dairy sheep fed protected fat from palm oil. There were no changes in the amount (kg) of milk fat, protein, lactose, total solids, and nonfat solids in any of the treatments. Therefore, protected fat from palm oil is efficient to increase milk production and fat content.

Key Words: chemical composition, dairy sheep breed, milk production

Introduction

In the South, Southeast, and Midwest regions of Brazil, there has been a significant increase in the production of dairy sheep due to several favorable factors such as fertile soil, mild climate, and topography, as well as a new niche of consumers searching for different, flavorful, and healthy foods like sheep milk and its derivatives (Bianchi et al., 2014a).

Sheep milk is primarily intended for cheese production since it may yield twice as much cheese compared with cow or goat milk (Brito et al., 2006). Therefore, sheep milk should be considered for processing into cheese and derivatives (Bencini and Pulina, 1997). During lactation, there is a high nutritional demand, and consequently, it is necessary to provide balanced diets, considering that a scarce supply may lead to lower productivity and changes in milk composition (Natel, 2010). After birth, ewes with high milk production may show pronounced and long-lasting negative energy balance, which may interfere with milk production and composition, which could affect animal health (Bencini and Pulina, 1997; Ribeiro et al., 2004), forcing farmers to give diets with high energy density.

Many oils can be used as sources of fatty acids in the form of protected fat (Bona-Filho et al., 1994; Zhang et al., 2006; Sanz Sampelayo et al., 2007; Afonso et al., 2008), including palm oil. For instance, *Elaeis guineensis* oil contains approximately 50% of saturated and 50% of unsaturated fatty acids. Palm oil is rich in tocotrienol, a form of vitamin E beneficial to health in general (Bianchi et al., 2014a). The use of fat is an effective method to increase dietary energy density. On the other hand, the addition of fat may reduce fiber digestion, which can be toxic to ruminal microorganisms (Palmquist and Mattos, 2006). For this reason, the use of calcium salts of fatty acids (protected fat) may be an option since it is partially hydrogenated at normal ruminal pH, only getting dissociated at acidic pH in the abomasum, thus, providing a high amount of energy into

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the bloodstream of the animal (Schauff and Clark, 1992). Therefore, the objective of the study was to evaluate the effect of diets supplemented with protected fat from palm oil on milk production and composition as well as on body weight of Lacaune sheep. In addition, we also evaluated the lactation curve of these animals since it shows the production profile.

Material and Methods

The experiment was conducted in Chapecó, Santa Catarina – Brazil (27°02'10" S latitude, 52°39'27" W longitude, and 674 m of altitude); the area presented average temperatures and rainfall of 19.6 °C and 942 mm, respectively, during the experimental period (June to December 2012). In the current study, we used 36 lactating Lacaune ewes, 2-4 years of age, on their second or third lactation, with one or two offsprings. All lambs were separated from their mothers at seven days of age, when they were artificially fed. After three days of weaning, milk production and body weight were recorded, and all animals were separated into four treatments based on their body weight, age, parity order, number of lambs, and milk production. The treatments consisted of 0, 20, 40, and 60 g kg^{-1} of protected fat from palm oil in the concentrated feed (Table 1), being the composition of the diet shown in Table 2. The amount of concentrate provided was adjusted every week, according to milk production and body weight.

The animals were restrained twice a day (07:00 and 18:00 h) in pens for individual feeding with concentrate containing protected fat, followed by corn silage (50:50). The amount of concentrate given was adjusted according to the volume of milk produced by each animal and its body weight (up to 1.4 kg per day), which decreased towards a minimum of 0.6 kg/day at the end of the experiment, when body weight was also reduced. Each animal also received 2.5 kg of corn silage in every meal, which was plenty since we always found leftovers that were collected and weighed.

Table 1 - Composition of the experimental concentrates and diets given to sheep during lactation

Item	Level of calcium soap (g kg^{-1} as fed)			
	0	20	40	60
Ingredient				
Ground corn	510	410	300	190
Soybean meal	430	430	410	400
Wheat bran	20	100	210	310
Calcium soap (palm oil)	0	20	40	60
Vitamin and mineral	25	25	25	25
Calcitic limestone	10	10	10	10
Sodium bicarbonate	5	5	5	5
Nutrient (g kg^{-1} as fed)				
Dry matter	887	890	892	895
Crude protein	215	219	218	219
Crude fat	22.7	27.3	28.9	29.4
Total digestible nutrients	734	739	737	738

Each treatment contained nine animals housed in pens of 24 m^2. Automated milking was performed twice a day (05:00 and 17:00 h), and ten days postpartum, all milk produced was weekly individually weighed up to seven weeks using a specific equipment (True-test®, Auckland, New Zealand). In the subsequent weeks, milk samples were collected every other week until the end of lactation, totaling 18 weeks (182 days of lactation). Immediately after sampling, milk samples were placed into plastic bottles containing Bronopol (2-bromo-2-nitro-1, 3-propanediol) and sent under refrigeration to an official laboratory in Concórdia, Santa Catarina, Brazil.

Analyses of sheep milk composition (fat, protein, lactose, total solids, and nonfat dry extract) were performed using an infrared analyzer (Bentley 2000®), previously used to test bovine milk. The reliability of the equipament to test sheep milk was checked and confirmed, i.e., the correlation between the results from this equipment and conventional methods was higher than 90% for all the parameters studied, without any significant differences between both methods (P>0.05) (Penna, 2011).

All animals were weighed using an electronic scale, their body condition was scored, and the volume of milk produced was registered. The methodology to evaluate body condition score (BCS) was previously described by Cañeque et al. (1989), assigning values of 1 to 5 with 0.25 increase, in which 1 was for extremely thin and 5 for obese ewes.

To compose the lactation curve, each day of the lactation period (182) was considered one observation. Data from milk production were fitted on a nonlinear regression, and production peak, day of peak production, and lactation persistency (in months) were considered variables of the curve, by means of the proposed model of Wood (1963) with the following equation 1:

$$Y_{ijk} = a_k t_{ijk}^{b_k} e^{-c_k t_{ijk}}, \qquad \text{Equation (1)}$$

in which, i is the animal (1, 2, ... , N); j is the time: 1, 2, ... , J; k is the treatment (1, 2, ... , K); y is the milk production (L/day); a is the initial production of milk (L); b is the rate of increase of production to peak; c is the rate of production decline after peak (persistency factor); t is the day of lactation.

To estimate the production peak (*pp*), day of peak production (*dpp*), and persistency (*s*) in months, we used the parameters of Wood's curve (equation 2, 3 and 4). For the Bayesian model, it was considered that the observations followed a normal distribution, i.e.,

Table 2 - Chemical composition of feed used in the experimental diets for lactating ewes

Ingredient (g kg^{-1} as fed)	Corn silage	Corn ground	Soybean meal	Wheat bran	Calcium soap (palm oil)
Dry matter	313.8	887.2	895.2	896.3	972.6
Crude protein	73.6	91.2	445.6	154.7	-
Total digestible nutrients	667.6	863.4	848.6	654.9	1884.8
Crude fat	35.6	36.1	16.6	24.6	848.7
Neutral detergent fiber	498.5	89.0	138.0	365.2	-
Acid detergent fiber	237.1	28.9	81.6	138.5	-

$y_i \sim N(f(t_i),\tau)$ being $f(t_i)$ in (1) and standard deviation $\sigma = \tau^{-1/2}$ (parameterization OpenBugs). We considered the prior noninformative distributions for all model parameters given in (1), considering the investigated treatments: $a \sim Gamma(10^{-2},10^{-2})$; $b \sim Unifom(0,1)$; $c \sim Unifom(0,1)$; and $\tau \sim Gamma(10^{-3},10^{-3})$.

$$pp = a\left(\frac{b}{c}\right)^{b} e^{-b},\ dpp = \frac{b}{c} \text{ and } s = -(b+1)\ln(c)$$

Equations (2, 3, and 4, respectively)

Multiple comparisons between posterior distributions from the averages of the parameters of interest were made to compare treatments. Significance was considered at 5% level for treatments that showed credibility intervals for mean differences that did not include zero, obtaining marginal posterior distributions for all parameters through Brugs package of the program R (R Development Core Team, 2014). It generated 1,100,000 values in a Markov Chain Monte Carlo process, considering a sampling discart of 100,000 initial values; thus, the final sample obtained every 100 jumps contained 10,000 values. The convergence of the chains was verified by the CODA package of the program R, according to the criterion of Heidelberger and Welch (1983).

This study had a randomized experimental design with four treatments and nine replicates, considering each animal as one repetition. Data from animal performance, milk composition, and production were subjected to analysis of variance using SAS software (Statistical Analysis System, version 8.1) at a significance level of 5% according to the statistical model (Equation 5). Initialy, we searched for interactions between treatment and lactating period for all variables. As a result, we found that fat content was the only variable with this type of interaction, and it was analysed for all milking periods (initial, intermediate, and final). Since the other variables did not show interaction, they were evaluated as one single milking period.

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}, \quad \text{Equation (5)}$$

in which, Y_{ij} is the response variable in the j; μ is the overall average; T_i is the fixed effect of the i treatment; and ε_{ij} is the random error. It is assumed that $\varepsilon_{ij} \sim \text{NID}(0,\sigma^2)$.

When the factors were significant, they underwent a polynomial regression testing. However, when the regression was not significant, multiple comparisons of means were performed by Tukey test at 5% probability (P<0.05), using the MIXED procedure of SAS with the model in Equation 6.

$$Y_{ij} = \mu + T_i + W_j + T \times W_{ij} + \varepsilon, \quad \text{Equation (6)}$$

in which Y_{ij} is the observation ij; μ is the general average; T_i is the effect of treatment i; W is the effect of lactation week j; $T \times W$ is the effect of the interaction between treatment i and week j; and ε is the random error. The week factor was considered a repeated measure in the model.

Results

The addition of protected fat showed effect (P<0.0001) on sheep body weight (Table 3), i.e., animals that received 20 g kg^{-1} protected fat were heavier. It was also noted that the average daily intake of dry matter was 2.24 kg per animal, representing 34.8 g kg^{-1} day^{-1} of the body weight. There were differences in body condition score (P<0.05), similarly to body weight.

Initially, all four treatments showed similar milk production (a) (1.85 kg of milk) (Table 4). There was no significant increase in the production peak (b), even though there was a slight tendency to be higher in animals fed 40 and 60 g kg^{-1} protected fat. There was no statistical difference regarding the day of peak production (dpp) with an average of 17 days of experiment, but the study began when the animals were at 10 days of lactation (postpartum), so the real production peak was reached at 27 days, i.e., in the fourth week of lactation (Table 4). The rate of decline in milk production after peak (c) was similar for both groups (P<0.005). The production peak (pp) showed differences and was higher in the treatment of 40 g kg^{-1} palm oil. Based on these parameters, lactation curve was composed (Figure 1). We emphasize that milk production during lactation was higher in animals that received 60 g kg^{-1} as fed of protected fat supplementation (Figure 2).

Table 3 - Body weight (kg), body condition score (BCS), and dry matter intake of sheep fed different levels of protected fat

Variable	Level of protected fat concentrate (g kg^{-1} as fed)				Mean
	0	20	40	60	
Initial body weight (kg)	57.91	56.60	55.28	55.05	56.21
Mean body weight (kg)	67.77±0.75a	66.70±0.74a	63.58±0.74b	62.64±0.74b	65.17
Final body weight (kg)	76.51	74.44	68.78	69.28	72.25
Initial BCS	1.56	1.72	1.78	1.61	1.67
Mean BCS	2.78±0.03a	2.77±0.03a	2.81±0.03a	2.67±0.03b	2.75
Final BCS	4.52	4.38	4.27	4.05	4.31
Dry matter intake (mean)					
kg/animal/day	2.24	2.25	2.12	2.32	2.24
Body weight (g kg^{-1} d^{-1})	33.50	34.20	33.80	37.40	34.80

a,b - Means followed by different letters in the same line differ by Tukey test (P<0.05).

Table 4 - Bayesian estimates (mean ± standard deviation) for parameters of the lactation curve

Protected fat (g kg^{-1} as fed)	Parameter/Bayesian estimate					
	a	*b*	*c*	*pp*	*dpp*	*s*
0	1.85±0.16a	0.08±0.033a	0.005±0.0008a	2.13±0.07ab	16±5a	5.76±0.11a
20	1.83±0.13a	0.07±0.026a	0.005±0.0006a	2.09±0.05b	16±4a	5.82±0.09a
40	1.88±0.13a	0.09±0.025a	0.005±0.0006a	2.25±0.05a	18±3a	5.78±0.08a
60	1.86±0.14a	0.10±0.029a	0.005±0.0007a	2.24±0.06ab	19±4a	5.81±0.09a

a - initial production of milk (L); *b* - rate of production increase up to the peak; *c* - rate of production that declined after peak (persistency factor); *pp* - production peak (L); *dpp* - day of peak production; s - persistency in months.
a,b - Different letters in the same column indicate significant differences between the mean of treatments by comparing Bayesian with 95% credibility.

The fat content in the milk from sheep fed different levels of palm oil showed an interaction between treatment and period (P<0.01). In the initial third, which comprises the early lactation (1 to 60 days), there was a linear effect (P<0.01), that is, increased level of protected fat caused an increase in fat content (Figure 2). In the second stage, in the intermediate third of the lactating period (61 to 120 days), the fat content had a quadratic effect (P<0.05), with decreased fat content in treatments with 20 and 40 g kg^{-1} palm oil (Figure 2). In the final third of the lactation period (121 to 182 days), the fat content showed a linear effect (P<0.05), similarly to early lactation, i.e., animals that received higher levels of palm oil showed higher milk fat content (Figure 2). Milk fat content throughout lactation (182 days) also showed a linear effect (P<0.05).

The addition of protected fat showed a linear effect (P<0.01) on protein content, demonstrating a negative correlation (decreased protein content when increased levels of palm oil was used) (Figure 3). Lactose content also showed a linear effect (P<0.01), indicating that higher levels of fat lead to decreased levels of lactose (Figure 3). Similarly, defatted dry extract content (Figure 3) showed a linear profile (P<0.001), e.g., increased levels of palm oil, decreased levels of defatted dry extract content. The percentage of total solids in milk had no significant effect (P>0.05), with a mean of 167.6 g kg^{-1}. No significant effect (P>0.05) on total yield of solids (Table 5) was observed.

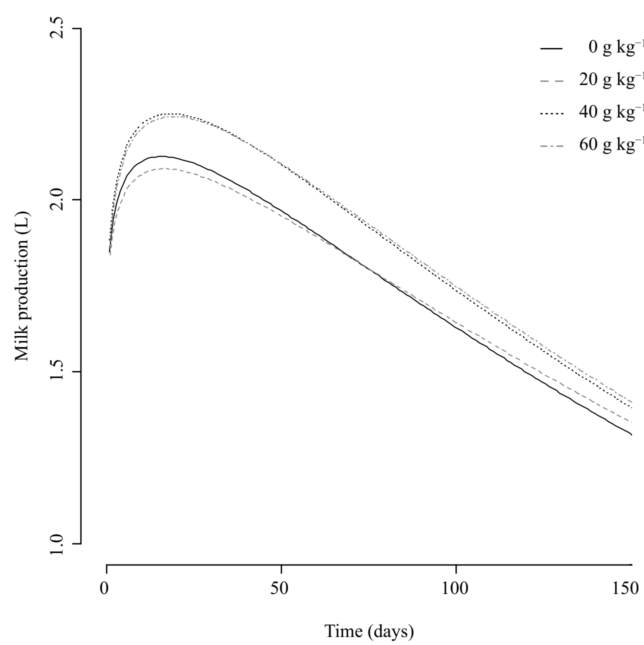


Figure 1 - Lactation curve adjusted for different levels of protected fat (0, 20, 40, and 60 g kg^{-1}).

Discussion

Animals that received the highest levels of protected fat (40 and 60 g kg^{-1}) had lower body weight and the worst BCS throughout the lactation period, possibly because these animals had greater milk production throughout lactation. Therefore, weight loss during the first four weeks

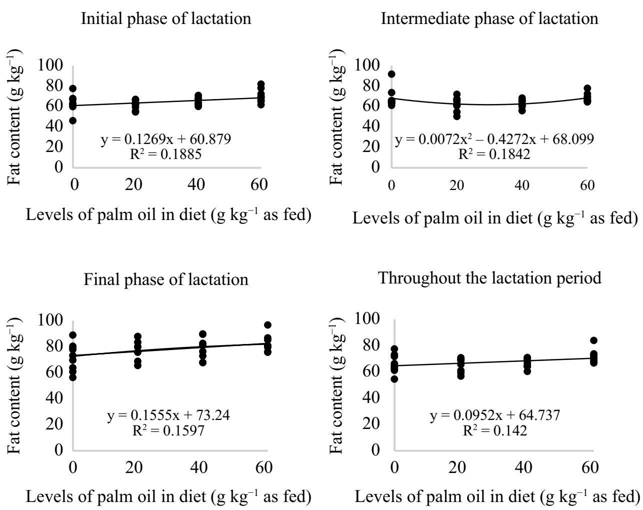
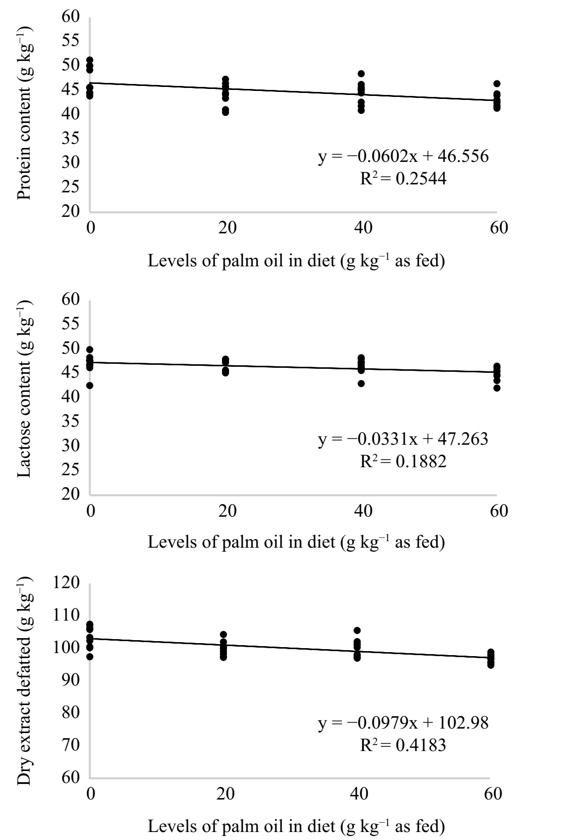


Figure 2 - Levels of fat in the milk of sheep fed different levels of protected fat in the initial, intermediate, and final phases and throughout the lactation period of 182 days.

of lactation is usually minimal, but it may be more severe with the lactation persistency (Snowder and Glimp, 1991). In our study, there was no body weight loss, only a lower body weight gain in those animals that produced more milk and received higher amounts of protected fat.

Another contributing factor to lower body weight in sheep fed higher levels of protected fat might be related to the fact that their milk has higher amounts of fat; thus, these animals demanded larger quantities of nutrients to synthesize fatter milk to use less tissue deposition. According to the literature, increased amount of milk production and milk fat content might be possible due to sheep weight loss (Barnes et al., 1990). Another study using 0, 30, 60, and 90 g kg^{-1} protected fat for goats showed a decrease in weight gain (110.3, 67.5, 53.3, and 39.6 g, respectively) (Teh et al., 1994), similarly to those found in this study.

The lactation curve showed a difference in milk production at the lactation peak, that is, sheep that received higher amounts of palm oil showed higher peaks, resulting in greater milk production throughout lactation since the rate of decline after the peak was the same for all treatments. In this study, the lactation peak occurred approximately at day 27 postpartum, a fact also shown by Bencine and Pulina (1997), in which the lactation peak was reached between the third and the fifth week of lactation. Another study showed that milk production peak in confined sheep with a suckling lamb occurred after 30 days of lactation; however, females with twins showed lactation peak at 21 days of lactation (Cardellino and Benson, 2002). Mixed system of sheep milk production (lamb suckling + milking until 45 days of age) showed production peak in the seventh week of lactation (Stradioto, 2007). In this case, according to the



No different total dry extract was observed.

Figure 3 - Protein, lactose, and defatted dry extract content of milk from sheep fed different levels of protected fat during lactation.

authors, the peak may have been slowed by the effect of lamb suckling, which may have hampered milk production.

The mean and total volume of milk produced throughout the lactation period was greater in animals that received higher amounts of protected fat (40 and 60 g kg^{-1}). The fat content in milk throughout lactation increased linearly with higher amounts of palm oil. Fontaneli (2001) reported that milk fat is composed almost entirely by triglycerides (98% of total fat). These triglycerides are synthesized in the epithelial cells of the mammary gland, and fatty acids composing these triglycerides may be derived from lipids present in the blood or by the *de novo* synthesis in the epithelial cells. Thus, there is a great uptake of triglycerides from the bloodstream by the mammary gland, which is used for the synthesis of fat that will be milk secreted. It is worth highlighting that sheep that received palm oil in the diet (40 and 60 g kg^{-1}) had an increase in seric triglycerides (Bianchi et al., 2014b), which may explain the numerical increase in milk fat of sheep that received 60 g kg^{-1} palm oil in the diet. Cannas et al. (2002) reported that the use of protected fat for sheep caused an increase in milk production and fat content. However, these findings occur much faster for fat content than for milk production.

Table 5 - Total production of fat, protein, lactose, total dry extract, and defatted dry extract in milk of Lacaune sheep fed different levels of protected fat during the lactation period (182 days)

Variable (kg)	Level of protected fat concentrate (g kg^{-1} as fed)				Mean	CV (%)	Effect
	0	20	40	60			
Fat	21.23	20.93	22.76	24.42	22.33	19.01	ns
Protein	15.23	14.32	15.35	14.58	14.87	19.34	ns
Lactose	15.70	15.38	16.39	15.59	15.76	20.75	ns
Total dry extract	55.31	53.63	57.85	57.54	56.08	19.32	ns
Defatted dry extract	33.98	32.69	34.94	33.24	33.71	19.84	ns

CV - coefficient of variation; ns - no significant effect (P>0.05).

Filgueiras Neto and Moura (2012) reported that high-producing dairy cows may have severe ruminal pH decrease, even when well fed with balanced diets. This is more commonly seen when protected fat from soybean is used, compared with protected fat from palm oil. The reason for that relies, in part, on the amount of polyunsaturated fatty acids that is much lower in palm oil than in soybean, and its pKa is also lower even when the ruminal pH is bellow normal, allowing the release of fatty acids in the rumen with low dissociation, and without any effect on ruminal microorganisms. Therefore, there is no severe impact on the biohydrogenation of fats in the rumen and, consequently, on milk fat content. Due to these factors, it is possible to assure that protected fat from palm oil is safer since it ensures similar energy input compared with fat from soybean oil and decreases the risk of lower levels of fat in milk (Filgueiras Neto and Moura, 2012).

The protein content in milk showed a linear decrease after an increase in the levels of palm oil. This fact may be due to a dilution factor since the total volume of milk also increased, and consequently, the total protein production during lactation (kg protein) showed no significant difference, demonstrating that the use of higher levels of protected fat to feed lactating ewes did not affect the total amount of protein secreted in milk. According to the literature, there is a negative correlation between milk production and milk composition. Therefore, when sheep produced more milk volume, the fat and protein concentrations were reduced, and this is also valid for breed of high and low production (Bencine and Pulina, 1997).

According to Chilliard et al. (2003), milk protein production is related to nutritional factors, energy intake being the major factor. We did not anticipate changes in the levels of protein, given that the diets were formulated to meet animal production needs. Replacing the sources of fermentable carbohydrates in the rumen with unsaturated fat leads to lower production of volatile fatty acids and, overall, to a lower production of microbial protein. The reduced production of these fatty acids in the rumen leads to greater gluconeogenesis from amino acids by decreasing the concentration of protein in milk (Wu and Huber, 1994). In the present study, we observed a decrease in protein levels of milk from animals that received higher amounts of protected fat. There are two possible explanations for that: increased milk production leads to lower protein levels, and a dilution event may have ocurred (Table 5), with no changes on protein (kg); the second explanation was mentioned above, with a pH drop with dissociation of protected fat, and release of unsaturated fat.

This effect was evidenced by Fernandes et al. (2008), who reported that the use of certain types of additional fat increased milk production, as well as the percentage of fat in milk, but at the same time, also decreased the percentage of protein. When there is no replacement of carbohydrates for lipids in the rumen, and when lipids are supplied in very large amounts, there is a toxic effect on microorganisms of the rumen, causing reduction in microbial growth and, thus, an effect on the transport of amino acids to the mammary gland. Therefore, the protein content of milk may decrease with the deficiency of one or more amino acids (Santos et al., 2001).

Lactose content in milk from sheep decreased as they were fed higher amounts of protected fat. This might be explained by the dilution of this compound as a consequence of a higher milk production. Fontaneli (2001) reported that glucose is the major precursor of lactose in milk and contributes with approximately 60 to 70% for the synthesis of this component; the rest of the glucose is used by the secretory cells for protein synthesis, and glycerol or other precursors, for the synthesis of fat. Authors reported that the main precursor of animal glucose is propionic acid, mainly produced by the fermentation of non-fibrous carbohydrates in the rumen (Antunes and Rodriguez, 2006). Thus, the inclusion of protected fat has little effect on propionate production, not interfering with the synthesis of glucose by the liver, and subsequently lactose by the mammary gland. As observed in our study using isocaloric diets, the way to increase the levels of protected fat was to decrease ground corn, a source of non-fiber carbohydrate, thus reducing the production of propionic acid, a precursor of glucose, thereby reducing lactose in milk. This study also found that the treatments caused a linear effect ($P<0.0001$) on solids nonfat. This decrease in dry fat extract in relation to increased levels of palm oil is because it is mainly composed of protein and lactose, demonstrating similar patterns between compounds.

The total solid content (fat, protein, lactose, and minerals) in milk showed no significant differences between groups since fat content is inversely proportional to the levels of protein and lactose, which was found in milk of all groups. Likewise, total production of solids did not change among groups, and a possible reason for this is that the values were calculated by multiplying milk production and solids. The amount (kg) of protein, lactose, and solids nonfat showed no significant changes since their concentration were inversely proportional to milk production. We expected that the total production of fat would show a significant increase because fat content showed a positive linear effect, and milk production followed the same trend, but this fact did not occur, probably due to the high coefficient of variation (19.01%) found for this variable.

Conclusions

The addition of low amounts (20 g kg^{-1}) of protected fat from palm oil in the diet of dairy sheep causes a positive effect on weight gain and body condition. Moreover, milk production and fat content are higher in sheep that receive 60 g kg^{-1} of protected fat. On the other hand, protein, lactose, and solids nonfat are lower in milk of ewes fed protected fat.

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