http://dx.doi.org/10.1590/1678-4162-11210

Arq. Bras. Med. Vet. Zootec., v.72, n.1, p.136-144, 2020

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Metabolic stress and reproductive features in post-partum goats supplemented for a long period with detoxified castor meal as the source of dietary nitrogen

[*Estresse metabólico e características reprodutivas em cabras pós-parto suplementadas por longo período com mamona desintoxicada como fonte de nitrogênio na dieta*]

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ABSTRACT

This study evaluated the effect of detoxified castor meal on the reproductive performance, metabolic stress, milk production, and kid development in peripartum goats. The diet of the animals were with (DCM, n= 20) or without (WDCM, n= 21) detoxified castor meal during the entire gestation and until weaning, 60 days post-birth. No differences were observed in the gestation period, litter size, rate of multiple births, and mortality between the two groups. The postpartum plasma concentrations of progesterone remained below 1ng/mL in all animals, thus, confirming the absence of active corpora lutea. The thickness of sternum adipose tissue and loin area, levels of urea and cholesterol, milk production, and daily weight gain in the kids were low in the DCM group when compared to those in the WDCM group (P< 0.05). To conclude, the use of detoxified castor meal in peripartum goats resulted in lower level of performance in the kids because of reductions in the amount of milk received from their mothers during lactation. In addition, the diet containing detoxified castor meals was not efficient in recovering from the loss of stored body reserves able to initiate the recovery of the cyclic activity of the goats.

Keywords: Ricinus communis L., postpartum, lactation, goat kids, progesterone

RESUMO

Este estudo avaliou o efeito da torta de mamona desintoxicada na reprodução, no estresse metabólico, na produção de leite e no desenvolvimento de cabritos no periparto de cabras. Um grupo foi alimentado com torta de mamona (DCM, n=20), e o outro (WDCM, n=21) não recebeu tal suplemento , durante a gestação até o desmame, 60 dias pós-parto. Não foram observadas diferenças significativas no período de gestação, no número de cabritos, na taxa de partos múltiplos e na mortalidade entre os dois grupos. Em todos os animais, a concentração plasmática de progesterona ficou abaixo de 1ng/mL, confirmando a ausência de atividade lútea. A espessura da gordura subcutânea do esterno e da área de olho-de-lombo, a concentração de ureia e colesterol, a produção de leite e o ganho de peso dos cabritos foram menores no grupo DCM (P<0,05). Conclui-se que o uso de torta de mamona desintoxicada no periparto de cabra resultou em cabritos mais leves devido à redução na produção de leite das matrizes e as cabras não retornaram ao cio, pois não recuperaram a massa corporal.

Palavras-chave: Ricinus communis L*, pós-parto, lactação, cabritos, progesterona*

INTRODUCTION

Ruminant production in tropical regions, especially in semi-dry areas such as North Eastern Brazil, requires strategies to optimize alternative food sources, aiming at reducing the costs of animal food supplements. Seasonal fluctuations in hay availability and quality, which is associated with low grain production, are the limiting factors in animal production in these areas (Kawas *et al*., 1999). However, with the growing importance of biodiesel as a source of energy worldwide, new opportunities for

Recebido em 6 de fevereiro de 2019
Aceito em 3 de julho de 2019
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ruminant production have been developed using sub-products derived from oleaginous oil extraction (Oliveira *et al*., 2013).

Castor oil plant (*Ricinus communis* L.) is known for its ability to adapt to sandy soils and resistance to drought and it has become an important resource for biofuel production in North Eastern Brazil (Severino *et al*., 2005). However, the protein ricin is found in the endosperm of castor seeds and remains in castor meal and castor meal even after the oil extraction process (Aslani *et al*., 2007). This protein inhibits eukaryotic protein synthesis and is considered one of the most potent plant toxins in nature (Aslani *et al*., 2007). For this reason, the fate of the sub-products of castor oil plant processing is of concern, particularly in terms of its environmental impact. Despite the presence of ricin, studies have demonstrated that alkaline treatment promote the inactivation of potentially toxic compounds (Souza *et al*., 2018) and can be used as an alternative dietary source for ruminants due to its high protein value.

Castor oil plant residues remaining after oil extraction have been routinely used as organic manure due to the presence of toxic compounds such as ricin (Severino *et al*., 2005). According to Vieira *et al*. (2011), detoxified castor meal, when used for 70 days, improves the digestion in sheep in the finishing stages of production. Menezes *et al*. (2012) did not observe alterations in the liver enzymes of sheep fed for 20 days with detoxified castor meal; in addition, replacing 45% of soy meal with detoxified castor meal improves the ruminal environment. In goats, non-detoxified castor de-oiled cake with ricin concentrations up to 50mg/kg of the residue may be used for long periods, at levels corresponding to 15% of the diet, completely substituting soybean cake, without compromising the meat traits (Silva *et al*., 2015).

In addition, in recent studies our laboratory has demonstrated that detoxified castor meal could be used as a long-term alternative source of protein in sheep without affecting preantral or antral folliculogenesis (Silva *et al*., 2013), and in goats without affecting the reproductive performance, as well as the embryonic and early fetal development, and the blood metabolites (Silva *et al*., 2015). However, changes in the plasma levels of IgG and the mRNA levels of HSP70 were detected, as well as a lower rate of transferable embryos in animals fed non-detoxified castor meal. Despite these studies, it is still unknown if detoxified castor meal can be used for prolonged periods as an alternative protein source for the adequate nutrition of peripartum goats.

It is well known that in goats the body condition at kidding as well as the feeding plan applied during the postpartum are the main factors responsible for the performance of the kids (Celi *et al*., 2008). Females with larger reserves of body fat in the postpartum period have higher circulating levels of non-esterified fatty acids and use larger amounts of these reserves for the synthesis of milk fat in relation to those with smaller deposits of fat during the lactation phase which can affect the weight of the young at weaning (Žujovic *et al*., 2011). In addition the administration of adequate nutrition during the postpartum period allows for the optimization of food intake of does which in turn reduces the extent of the negative energy balance.

The aim of this study was to evaluate the effects of using detoxified castor meal on the body condition, activity of corpora lutea, metabolic stress, and milk production of peripartum goats, as well as in the development of kids.

MATERIALS AND METHODS

Castor meal was obtained from the factory Brasil óleo de Mamona (BOM), which is located in Salvador – Bahia, Brazil. Detoxification was carried out according to the method described by Silva *et al*. (2015). The absence of ricin after the detoxification process was verified by assaying the total proteins of samples of castor de-oiled cake, following the method of Bradford (1976) and Protein bands were quantified in volume units (area vs. intensity) following the method detailed by Oliveira *et al*. (2015).

All procedures were previously approved by the Ethics Committee from the State university of Ceará for the experimental use of animals (Protocol n. 09230950-0). The experiment was conducted at the Padre João Piamarta Farm in Itaitinga-CE (4°01′ S and 38°31′ W) from April to November. The area is characterized by a constant photoperiod regimen, and has a warm, tropical, sub-humid climate with 2 distinct seasons: rainy from January to May and dry from June to December. The mean annual rainfall and temperature are 1,416.4mm and 26–28°C, respectively.

Forty-one mixed-breed, cyclic, and pluriparous goats were separated into 2 homogeneous groups (mean±SEM) regarding body weight and condition (41.2±1.8kg and 2.6±0.1, respectively). The goats were housed in 2 pens that were separated by a central feed alley, where mineral salt and water were provided *ad libitum*. Each pen measured 40 × 50m and contained a 40 × 3m open front shelter. The feed alley and the front shelter were composed of clay and concrete, and faced an east-west direction.

The animals were obtained from the same farm. The health and reproductive characteristics of all animals were checked throughout the entire course of gestation. The does received 2 different diets composed of a mixture of guinea grass (*Panicum maximum* var. *Mombasa*) hay and isoenergetic (74% of TDN) and isonitrogenous (14% CP on DM basis) concentrates with a different source of nitrogen (Table 1). In the first group, without detoxified castor meal (WDCM n= 20), the animals received soybean meal as the nitrogen source in the concentrate. In the second group, detoxified castor meal (DCM, n= 21), the animals were fed with detoxified castor meal, which replaced soybean meal as the nitrogen source in the concentrate. The formulation of the diets was based on the nutritional requirements (Nutrient…, 2007) for gestation and lactation of adult non-dairy does. The animals were fed the respective diets twice a day (at 07:00 and 15:00), from 20 days before mating until 60 days after parturition (when the kids were weaned). After parturition, the kids remained with the goats and were weaned until 60 days of age. The kidding period lasted 26 days.

Table 1. Ingredient and chemical composition of the concentrate -based supplements

Ingredient (% DM)	Diet			
	WDCM	DCM	Guinea grass hay	Detoxified castor meal
Ground corn	81.8	79.6	-	-
Soybean meal	12.1	-	-	-
Detoxified castor meal	-	14.5	-	-
Urea	1.0	1.0	-	-
Vitamin mineralized premix	4.3	4.1	-	-
White salt	0.8	0.8	-	-
Composition (% DM)	Concentrate - based supplements			
Organic matter	97.2	97.1	92.8	82.4
Crude protein	15.0	15.0	6.8	36.1
Ether extract	3.1	3.5	1.2	2.9
Ash	2.8	2.9	7.2	17.6
Neutral detergent fiber	-	-	69.9	38.4
Acid detergent fiber	-	-	40.5	30.6

DM: dry matter; WDCM: without detoxified castor meal; DCM: detoxified castor meal.

At birth and every 7 days up to weaning, the weight and body condition score of the mother and kid goats were evaluated according to Morand-Fehr and Hervieu (1999). In the same period, ultrasounds of the sternum and loin regions were performed according to Teixeira *et al*. (2008), with minor modifications. In the sternum region, the transducer was placed perpendicular to the sternum at the third sternebra for the measurement of the loin area and subcutaneous sternal fat thickness (mothers). In the lumbar region, the transducer was placed perpendicular to the longissimus dorsi length, between the 12th and 13th ribs, for the measurement of the loin area (kids). Ultrasound measurements in the kids were performed from day 14 post-birth until weaning. Ultrasonographic measurements were obtained using a B mode ultrasound instrument (Chisson D600 VET, Chisson Medical Imaging Co. Ltda., China), coupled to a 5.0MHz linear transducer and measured in triplicate using the software program ImageJ® (Image J, National Institutes of Health, Millersville, USA).

Milk production was measured twice a week using the weigh-suckle-weigh technique, from the third day post-partum until weaning (60 days post-partum), following the method described by Celi *et al*. (2008). In summary, the day before the measurement, all kids were isolated from dams at 16:00. At 08:00 the following day, each kid was weighed before and after being allowed to suckle the dam. Suckling periods did not exceed 30min. The difference between pre- and post-suckling weight was recorded as the estimated milk production of the dam.

Between birth and weaning, milk samples were collected weekly and stored at -20ºC until analysis using an automatic milk analyzer Lactoscan SA® (Milkotronic LTD, Nova Zagora, Bulgaria). The percentages of lipids, proteins, lactose, minerals, and non-fat solids were determined.

Blood samples were collected at partum and every 5 days until weaning. Blood was centrifuged at 3000rpm for 15min, and the obtained plasma was stored in the freezer at -20°C. The plasma concentrations of glucose, urea, creatinine, protein, total bilirubin, albumin, cholesterol, triglycerides, and glutamic oxaloacetic transaminase (GOT) were obtained by spectrophotometric assays in an automated biochemical analyzer (Labmax 240, Labtest®), using a commercial kit (Labtest®, Lagoa Santa, Brazil). NEFA concentrations were determined by the enzymatic–colorimetric method (Randox Laboratories, UK), using a commercial kit (NEFA Randox® Laboratories, Crumlin, Co. Antrim, Northern Ireland, UK).

The presence of corpora lutea in the ovaries was determined using progesterone concentrations. A corpus luteus (CL) was considered to be functional when at least 2 consecutive samples showed progesterone levels of over 1ng/mL. A CL was considered to be non-functional when less than 1ng/mL progesterone was detected (Rodrigues *et al*., 2011). The progesterone assay was performed by a microparticle enzyme immunoassay (MEIA) (Abbott Diagnostics Axsym® System), using a commercial kit (Axsym P_4, Abbott, Tokyo, Japan). The test was sensitive to within the level of 0.2ng/mL, while the intra- and inter-assay coefficient of variation was 7.9 and 3.3%, respectively.

Data was subjected to analysis of variance (ANOVA) using the GLM procedures. The ANOVA model for weights, BCS, reproductive attribute, and milk traits, included effect of diet (WDCM and DCM), type of parturition (simple and multiple), and the interaction of diet versus the type of parturition. Data regarding litter size and the number of weaned kids/does were previously log10 transformed. For kids, the factors used for body weight analysis included diet, type of parturition, sex (male and female), and the interaction of diet versus the type of parturition and diet versus sex. For the milk quality parameters, progesterone and the plasma metabolite concentration and for the daily weight gain of kids, the tested factors included diet, interval of assessment considered (time), type of parturition, and the interaction of diet versus time and diet versus the type of parturition.

The ultrasonography results were analyzed by GLM procedures for repeated measures ANOVA. The factors tested in does (loin area and sternal fat thickness) included diet, type of parturition and the interaction of diet versus the type of parturition. For kids (loin area), the ANOVA model included diet, type of parturition, sex, and the interaction of diet versus the type of parturition and diet versus sex. Images of the ultrasonography structures used for the measure (1, 2, and 3) were the repeated measures. For the kidding, twinning and mortality rates, effect of group and type of parturition were analyzed by the Kruskal-Wallis ANOVA test. Comparisons of means were determined by the student *t*-test (2 groups) or Duncan's multiple range test (more than 2 groups). Comparison of numbers was performed using the chi-squared test.

RESULTS AND DISCUSSION

No differences in live weight and body condition of the goats resulting from diet or type of birth were observed (Table 2). A significant reduction ($P< 0.05$) in the live weight and body condition of the does from birth to weaning was observed in both diets. The decrease in goat body mass associated with lactation is believed to be due to the mobilization of fat and muscle tissues (Eknaes *et al*., 2006). A significant reduction in sternal fat thickness and loin eye area from birth to weaning was observed in the WDCM group ($P< 0.05$) (Table 2). In contrast, the animals in the DCM group showed a smaller loin area and a decrease in sternal fat thickness only at birth.

Table 2. Body weight (BW), body condition (BC), sternal subcutaneous fat thickness (SSCFT), loin area (LA), reproductive parameters, milk traits and metabolites in goats fed diets without detoxified castor meal (WDCM) and with detoxified castor meal (DCM). Values are given as means±SEM

Attributes	Diet		p – value				
	WDCM	DCM	Diet	TP	Time	D x TP	D x T
No. of does exposed	20	21					
BW at parturition, kg	41.3±2.6	38.2±1.6	0.32	0.17	-	0.99	-
BC at parturition	2.5±0.1a	2.4±0.1a	0.66	0.61	-	0.55	-
BC at weaning	1.9±0.1b	1.8±0.1b	0.42	0.87	-	0.99	-
SSCFT, mm	7.9±0.1	6.0±0.1	0.01	0.11	0.03	0.01	0.77
LA, cm^2	4.2±0.1	3.4±0.1	0.01	0.09	0.01	0.72	0.88
Reproductive attributes							
Gestation length, days	148.4±0.7	146.9±1.1	0.20	0.12	-	0.31	-
Kidding rate, % (n/n)	90.0 (18/20)	95.2 (20/21)	0.52	0.41	-	-	-
Litter size	1.4±0.1	1.4±0.1	0.78	-	-	-	-
Twinning rate, % (n/n)	44.4 (8/18)	40.0 (8/20)	0.78	-	-	-	-
Mortality, % (n/n)	15.4 (4/26)	17.9 (5/28)	0.84	0.35	-	-	-
Kids weaned/doe	1.2±0.1	1.1±0.2	0.81	0.01	-	0.48	-
Kid weight at weaning/doe, kg	10.2±1.2	9.2±0.8	0.41	0.01	-	0.92	-
Milk Traits							
Daily milk yield, g/day	944.0±31.4	883.5±29.6	0.01	0.01	0.01	0.01	0.42
Peak, week	3.8±0.3	4.2±0.3	0.57	0.63	-	0.08	-
Milk yield at peak, g	1485.9±0.1	1439.1±0.1	0.19	0.01	-	0.25	-
Lactation persistency *, %	43.7±4.7	29.4±3.5	0.01	0.10	-	0.07	-
Lipids, %	2.8±0.2	3.4±0.4	0.28	0.46	0.01	0.09	0.89
Protein, %	3.1±0.2	3.6±0.3	0.22	0.61	0.01	0.90	0.24
Lactose, %	4.1±0.2	5.2±0.3	0.01	0.76	0.01	0.87	0.61
Salts, %	6.2±0.5	8.5±0.9	0.20	0.23	0.01	0.47	0.18
Solids-non-fat, %	8.2±0.6	9.3±0.7	0.30	0.86	0.01	0.99	0.32
Metabolites							
Glucose, mg/dL	49.8±0.9	51.3±0.9	0.14	0.15	0.01	0.65	0.65
Creatinine, mg/dL	0.8±0.03	0.8±0.03	0.82	0.06	0.58	0.58	0.23
Protein, g/dL	6.1±0.1	6.2±0.1	0.14	0.15	0.36	0.78	0.13
Albumin, g/dL	2.1±0.1	1.9±0.03	0.02	0.53	0.86	0.52	0.99
Bilirubin, mg/dL	1.1±0.1	1.2±0.1	0.76	0.19	0.80	0.59	0.26
TGO, U/L	67.4±2.3	67.3±1.6	0.99	0.19	0.67	0.86	0.56

The differences in stored body reserves, as well as in the extent of fat and muscle loss during the peripartum period, are due to a negative energy balance. In this study, the negative balance affected the plasma concentrations of various metabolites such as NEFA, urea, and cholesterol (Figure 1). As expected, a decrease in plasma NEFA concentrations in both groups was observed at birth, whereas those in the WDCM groups showed an increase ($P< 0.05$) between days 5 and 20 post-kidding. Nevertheless, this mobilization tends to decrease in the presence of a negative energy balance (Butler, 2000). Thus, in this study, the reduction in stored body reserves at partum in the DCM group led to a decrease in urea and cholesterol levels ($P< 0.05$) between day of birth and day 20 post-kidding when compared to that of the WDCM group (Figure 1). On the other hand, both metabolites increased at weaning.

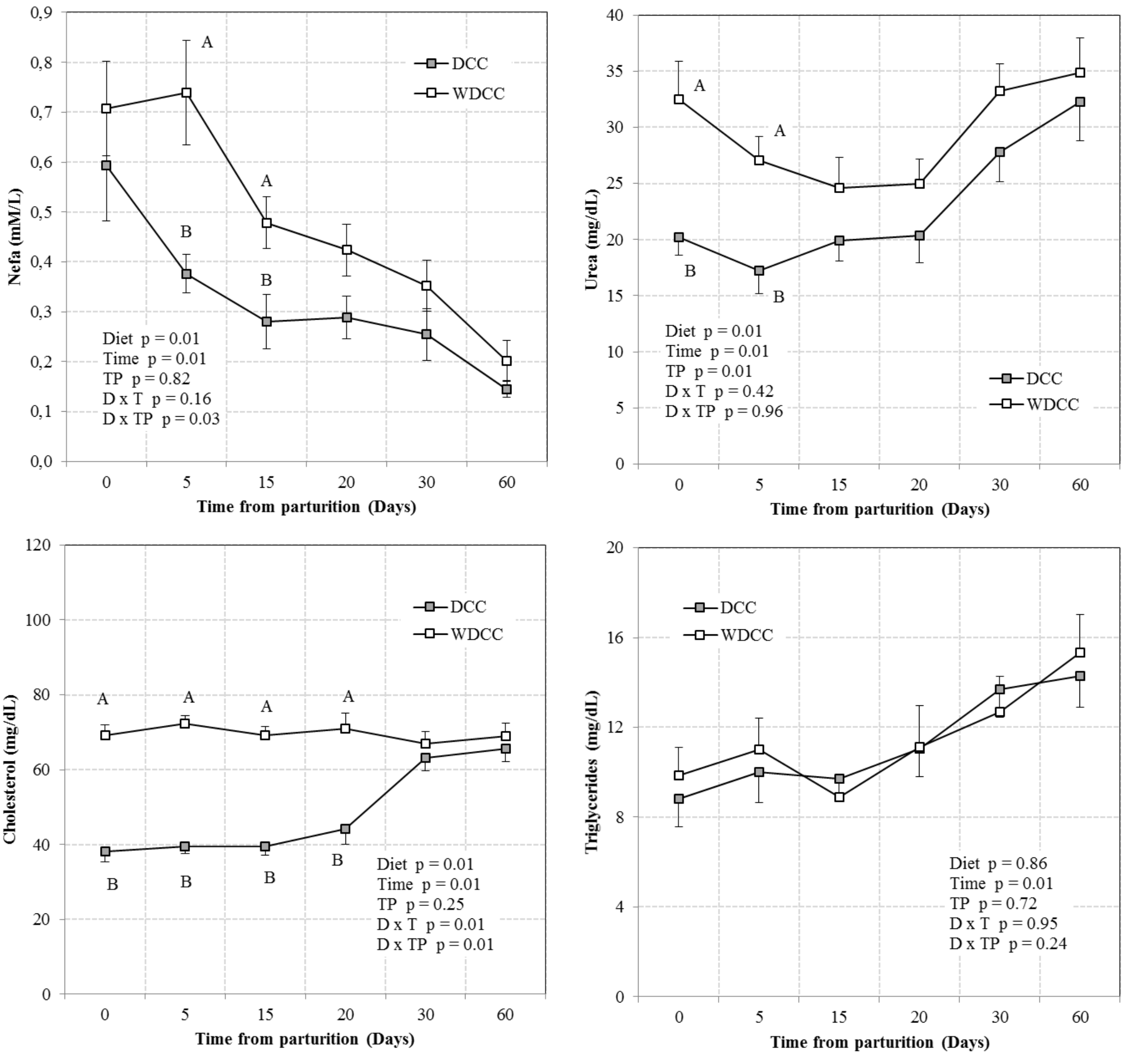

Figure 1. Plasma concentrations of urea, NEFA, urea, cholesterol and triglycerides in goats fed diets without detoxified castor meal (WDCM) and with detoxified castor meal (DCM). A, B P< 0.05 comparison between diets. ANOVA results for the effects of diet, time, type of parturition and the interaction diet *vs.* time, and diet vs. type of parturition are represented in the figure. Values are given as means±SEM.

No significant changes in liver (protein, bilirubin, albumin and GOT) and kidney function metabolites (urea) were observed during the experimental period. Similar results were previously described for sheep (Menezes *et al*., 2012) and does (Silva *et al*., 2015) fed with diets containing detoxified castor meal. Thus, the results obtained in this study are within the reference range, which indicates that the experimental diets provide sufficient nutrients for the metabolic requirements of the animals.

The results of this study showed that the diets were not significantly advantageous in terms of reproductive performance. No differences in gestation period, litter size, number of multiple births, and mortality were observed between groups (Table 2). In addition, both groups showed no indication of a corpora lutea during the entire experiment. For all animals, plasma concentrations of progesterone were <1ng/mL. In goats, the restart of the cyclic activity is dependent on the extent of the negative energy balance, which in turn depends on the capacity of the animal to recover from the loss of stored body reserves, which was not observed in this study. At weaning, goats from both groups showed a body score of <2, which was equivalent to a mean loss of 6kg per animal. According to Chagas *et al*. (2007), the absence of follicular development and ovulation peripartum may be attributable to the inhibition of the hypothalamic-pituitary-ovarian axis, which is a consequence of the high negative energy balance. As a result, gonadotropin-releasing hormone (GnRH) production, as well as the synthesis and release of gonadotropins (FSH and LH) and estradiol, are compromised, leading to a scenario of anovulation and anestrus (Scaramuzzi *et al*., 2006).

Milk production was higher in the WDCM group (Table 2), regardless of the type of birth, simple or multiple. For animals fed with detoxified castor meal, the decrease in milk production was significantly higher between the lactation peak and weaning. The type of parturition affected daily milk production, as well as the mean milk production during lactation. All the qualitative parameters of milk (Table 2) were diminished ($P< 0.05$) in the WDCM group between days 1 and 30 post-kidding. The DCM group showed similar results; however, only that of lactose was statistically significant. Nutritional changes in milk during the first weeks after partum are well known and correspond to an increased milk yield that precedes the lactation peak (Goetsch *et al*., 2011). In this study, the animals in the WDCM group showed an increased capacity in mobilizing stored nutrients for milk production compared to the DCM group. This mobilization reflects increased lipolysis and decreased lipogenesis in fat tissues at the start of lactation, which may be attributable to changes in endocrine and metabolic characteristics (Bell, 1995). Some studies have shown a positive correlation between decreased body score and increased milk production in dairy cattle (Roche *et al*., 2009).

The weight of the goat kids at birth (Table 3) was not significantly affected by the diet. However, at weaning, the kids from the WDCM group had an accumulated difference of over 1kg compared to those in the DCM group. Thus, despite the fact that both diets led to a significant increase in the kids' weight between birth and weaning ($P< 0.05$), the daily weight gain was higher in the WDCM group. This discrepancy in the *in vivo* performance of the kids was undoubtedly due to the increase in milk production.

Table 3. Body weight (BW), daily weight gain, loin area (LA) and loin subcutaneous fat thickness (LSFT) in kids derived from goats fed diets without detoxified castor meal (WDCM) and with detoxified castor meal (DCM). Values are given as means±SEM

Attributes	Diet		p-value						
	WDCM	DCM	Diet	Time	TP	Sex	DxT	DxTP	DxS
No. of kids exposed	26	28							
Kids *in vivo* performance									
BW at birth, kg	2.8±0.1a	2.8±0.1a	0.42	-	0.02	0.57	-	0.78	0.19
BW at weaning, kg	7.8±0.5b	6.7±0.4b	0.41	-	0.07	0.61	-	0.70	0.37
Daily weight gain, g	78.6±4.1	61.6±3.1	0.01	0.03	0.02	0.68	0.52	0.85	0.28
Carcass predictors									
LA at 14 days, cm^2	2.2±0.05a	2.1±0.05a	0.54	-	0.01	0.94	-	0.61	0.08
LA at weaning, cm^2	2.9±0.08b	2.6±0.07b	0.76	-	0.01	0.09	-	0.56	0.03

The qualitative and quantitative traits of carcass components in small ruminants are influenced by various factors such as race, age, sex and diet. Some studies have shown that detoxified castor meal can replace soybean meal without causing changes in carcass characteristics in ovine (Menezes *et al*., 2016). However, the use of detoxified castor bean meal in place of soybean meal can contribute to the reduced production and milk composition in cows (Cobianchi *et al.*, 2012) and reduced the digestibility of dry matter, organic matter, crude protein, and total carbohydrates in finishing animals (Silva *et al*., 2011).

It is suggested that treated castor meal replacing soybean meal does not harm animal performance, mainly in relation to reproduction (Silva *et al*., 2015). This effect may be due to the nature of the diets ingested, the pH of the ruminal environment and the relationships established between ruminal microbiota (Karnati *et al*., 2007). The results in the literature are conflicting and have not yet been elucidated as a cause of depression in goat milk production. Alkaline treatment is efficient to inactivate the ricin molecule through protein denaturation (Souza *et al*., 2018). However, the impact of this chemical process on other molecules and the long-term use of ruminal microorganisms is unclear.

CONCLUSION

In conclusion, the use of detoxified castor meal for long periods led to deterioration in the performance of kids during lactation due to low milk production. In addition, the diet containing detoxified castor meals was not efficient in recovering from the loss of stored body reserves able to initiate the recovery of the cyclic activity of the goats. Further studies are needed to evaluate effects of using this detoxified residue in animal feeding.

ACKNOWLEDGMENTS

Financial support was received from Edital CNPq/MAPA/SDA nº. 064/2008 – ref. nº. 578189/2008-9. L.M. Silva was the recipient of a doctoral scholarship from CNPq (n°. 551634/2010-3). Rondina D. is a senior investigator for CNPq/Brazil.

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