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Goat kids fed diets containing castor cake detoxified: II. Nitrogen balance, hepatic and renal function

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ABSTRACT. The aim of this study was to evaluate the influence of completely replacing soybean meal (SM) with castor cake detoxified (DCC) with two alkaline products on the nitrogen balance and hepatic and renal function in goat kids. Goat kids of two breeds, Saanen and Anglo Nubian, with an initial body weight of 16.2 ± 0.67 kg, and confined during the growth phase, were used. The treatments consisted of three diets: one based on SM and the other two based on castor cake detoxified with $Ca(OH)_2$ or NaOH. Twenty-four goats kids were distributed in a completely randomized design using a 3 x 2 factorial scheme (diet x breed) with four replicates per combination. The experimental period lasted for 270 days. Consumed nitrogen, fecal nitrogen, urinary nitrogen, retained nitrogen, and nitrogen balance were influenced ($p < 0.05$) by diets. There was significant effect of diets ($p < 0.05$) on creatinine, direct bilirubin, urea, alanine aminotransferase, aspartate aminotransferase and gamma-glutamyltransferase blood levels, however without any negative changes involving renal or hepatic dysfunction. Inclusion of castor cake in the diet of goats kids in confinement is an attractive option, considering that goats kids use does not cause hepatic and renal alterations, suggesting that SM can be completely replaced. NaOH DCC stands in the substitution of soybean meal, because in spite of decreasing the consumption of nitrogen provides the same retention of soybean meal.

Keywords: Anglo Nubian; blood levels; byproducts; Saanen.

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Introduction

Goats show high resistance to the toxicity of certain compounds. However, if castor cake is not completely detoxified, the presence of minimal amounts of ricin in the diet can cause hepatic and renal dysfunction, leading to decreased blood albumin and glucose levels (Menezes et al., 2012), and significant changes in serum creatinine and urea concentrations (Araújo et al., 2020a), in addition to hemoglobin reduction. Thus, evaluating the plasma levels of these parameters can be useful for monitoring possible hepatotoxic and nephrotoxic effects.

Several studies (Carrera et al., 2012; Cobianchi et al., 2012; Furtado et al., 2012; Gionbelli et al., 2014; Palmieri et al., 2016) have evaluated the effect of castor bean by-products in ruminant diets, and its implication on ruminal microbiota (Oliveira et al., 2010a). However, majority of the studies were conducted only in the initial phase of growth/development, and mostly considered only males. Furthermore, none of these studies evaluated the influence of castor cake over a long period and detoxified by alkaline solutions which have great potential in the elimination of toxic compounds (Araújo et al., 2020b). Many times, small periods of evaluation compromise the accuracy of information by under- or overestimating the productive potential during the period evaluated, reflecting directly in the productive potential of subsequent stages and the nutritional potential of the byproducts of castor oil.

In this context, residues from the biodiesel industry, especially castor cake, appear as an alternative feed for ruminants in Brazilian semiarid regions to replace soybean meal, thereby increasing its production costs. In an effort to minimize the negative effects caused by the low supply of soybean meal during

certain periods of the year, we hypothesized that castor cake detoxified with alkaline solutions is a possible substitute in the diets of these animals. Therefore, the objective of this study was to evaluate the influence of castor cake detoxified with alkaline solutions on nitrogen balance, and liver and kidney functions of female goat kids in the feedlot until the formation of dams that are able to reproduce.

Material and methods

The study was conducted at the Technological Center for Goat Milk Production of Embrapa Goats and Sheep (3º44'57,42" South and 40°20'43,50" West), in the city of Sobral-CE, Brazil. All procedures involving animals were carried out in accordance with the Ethics Committee on Animal Use regulations by the Brazilian Agricultural Research Corporation, National Goat Research Center, protocol number 005/2015.

Twenty-four goats kids (12 Saanen and 12 Anglo Nubian) with a body weight of 16.22 ± 0.67 kg were submitted to a confinement regime in individual, suspended stalls with a 5.06 m² slab floor, a 2.87 m² shaded area, equipped with drinkers, feeders and salt licks.

The treatments consisted of three diets; the first was formulated with corn and soybean meal (SM), and the other two were formulated with castor cake detoxified by either calcium hydroxide (Ca(OH)2 DCC) or sodium hydroxide (NaOH DCC), with both completely replacing soybean meal. Tifton 85 hay was used as forage. A completely randomized design with 3 x 2 (diets x breeds) factorial arrangement was used with four replicates per combination. In pre-experimental conditions, goats kids were identified, treated against ecto and endoparasites, and received a rabies vaccine.

The experimental diets were formulated based on the recommendations of the National Research Council [NRC] (2007), being isoproteic and isoenergetic with a forage:concentrate ratio of 43:57, 40:60 and 36:64 for the SM, Ca(OH)2 DCC and NaOH DCC diets, respectively. The chemical composition of the ingredients is shown in Table 1 and the proportion of the ingredients of the diets and their chemical composition based on the forage:concentrate ratio in Table 2.

Table 1. Chemical composition of the ingredients used in the experimental diets.

Item (g kg^{-1} dry matter)	Ingredient				
	Tifton 85 hay	Ground corn	Soybean meal	$Ca(OH)_2$ DCC[1]	NaOH DCC[2]
Dry matter (g kg^{-1} fresh matter)	872.50	889.20	870.20	904.20	904.80
Organic matter	911.30	965.90	956.90	867.70	855.60
Mineral matter	88.70	34.10	43.10	132.30	144.40
Crude protein	104.10	79.50	443.30	315.40	309.00
Neutral detergent insoluble protein	27.00	30.20	31.70	100.30	102.70
Acid detergent insoluble nitrogen	12.30	20.90	40.00	48.80	49.30
Ether extract	14.50	36.80	28.80	52.10	47.50
Total carbohydrates	792.80	845.70	484.70	500.10	492.60
Non-fiber carbohydrates	277.80	722.40	320.80	103.90	132.40
Neutral detergent fiber (NDF)	722.70	184.60	217.80	483.40	443.50
NDF corrected for ash and protein	607.00	120.30	143.00	250.80	196.40
Acid detergent fiber	472.20	69.00	117.90	379.20	388.70
Lignin	60.60	8.80	12.20	50.70	46.10
Total digestible nutrients [3]	546.80	848.00	822.50	620.50	627.90

[1]$Ca(OH)_2$ Castor cake: 0.90 g Na kg^{-1} DM and 22.25 g Ca kg^{-1} DM. [2]NaOH Castor cake: 29.20 g Na kg^{-1} DM and 0.63 g Ca kg^{-1} DM. [3]Calculated according to Weiss (1999).

Two alkaline products, calcium hydroxide $Ca(OH)_2$ and sodium hydroxide (NaOH) in the proportions of 90 and 60 g kg^{-1} of cake, respectively, were used to detoxify the crude castor cake, while 2000 ml of water kg^{-1} of crude castor cake was used for the dilution and efficacy of the reagents, according to recommendations of Araújo et al. 2020b. The cake was detoxified by adapting a semiautomatic mixer for homogenising the detoxifying solution. A stationary cement mixer equipped with three-phase motor was used (Fischer® MOB 400 G2) to mix the solution.

A vertical electrophoresis apparatus was used (model 2001 – Amsterdam - Pharmacia, Uppsala, Sweden) to analyse the electrophoretic profile of the proteins from the castor bean cake and identify the ricin. The SDS-PAGE was performed according to the methodology described by Laemmli and Favre, (1973).

Densitometry of the gels was performed using the Gel Analizer® application in order to confirm the disappearance of the bands, as shown in Figure 1.

Table 2. Ingredient proportions and chemical compositions of the experimental diets.

Ingredient (g kg^{-1} dry matter)	Diets		
	SM[1]	$Ca(OH)_2$ DCC	NaOH DCC
	Proportion of ingredients in the diets (g kg^{-1} DM)		
Tifton 85 hay	427.30	394.90	363.20
Ground corn	460.80	481.90	504.60
Soybean meal	57.80		
Detoxified castor cake		83.30	82.90
Soy oil	45.00	39.90	39.20
Limestone	9.10	0.01	10.10
Mineral mixture[2]	*Ad libitum*	*Ad libitum*	*Ad libitum*
Chemical composition (g kg^{-1} DM)			
Dry matter	887.70	896.10	891.80
Organic matter	942.30	897.80	938.10
Mineral matter	57.70	102.20	61.90
Crude protein	112.00	112.90	112.30
Neutral detergent insoluble protein	13.10	13.20	12.40
Ether extract	62.00	63.40	65.40
Neutral detergent fiber (NDF)	408.80	409.00	392.30
NDF corrected for ash and protein	338.00	293.60	318.00
Lignin	30.80	32.60	30.30
Total digestible nutrients[3]	664.90	658.50	663.60

[1] Soybean meal. [2] Guaranteed levels (per kg, inactive elements): calcium - 218 g; phosphorus - 71 g; sulfur - 20 g; manganese – 1.300 mg; potassium – 28.20 mg; cobalt - 30 mg; selenium – 15.30 mg; zinc - 1700 mg; copper = 710 mg. [3] Calculated according to Weiss (1999).

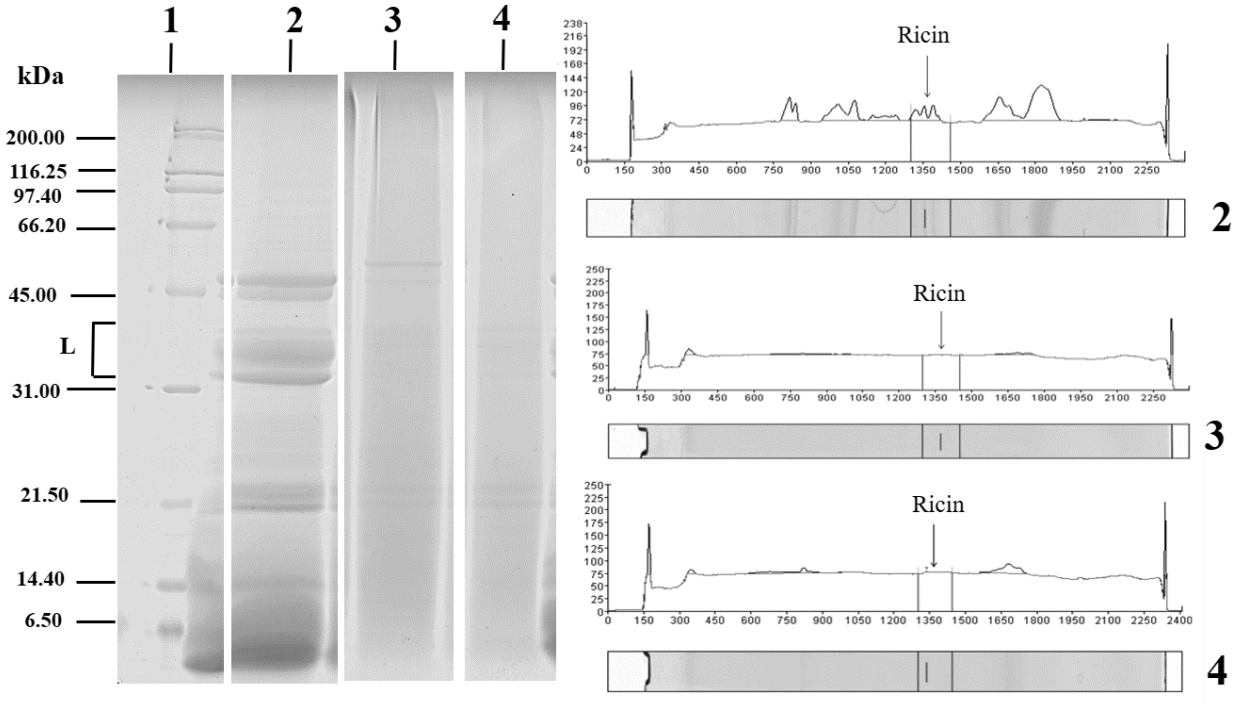

Figure 1. Electrophoretic characterization of castor cake proteins treated with different chemical products. 1: Molecular weight marker (kDa); L: Lectins; 2: Crude castor cake; 3: Detoxified castor cake $Ca(OH)_2$; 4: Detoxified castor cake NaOH.

For evaluating nitrogen balance, total urine production was estimated by the concentration of creatinine in the urine. At the end of the growth phase, spot urine samples were obtained approximately 4h after feeding, from spontaneous urination in colostomy bags (Medsonda®) with a capacity of 200 mL. Samples were prepared according to the methodology of Valadares, Broderick, Valadares Filho, and Clayton (1999) and immediately frozen. Urine production was estimated by the equation proposed by Fonseca et al. (2006), where (Equation 1):

$$\text{Urinary volume (L)} = \frac{26.05 \times \text{body weight (Kg)}}{\text{creatinine concentration in the spot sample (mg L}^{-1})} \quad (1)$$

At the end of the growth phase, feces were collected directly from the rectal bulb for five days at different times (0, 3, 6 and 9 hours after the first feeding) for a representative sampling. Furthermore, the feces samples used for digestibility tests were collected on different days, so two samplings were performed. Due to the small amount of feces collected per day, we chose to make two separate collections.

Consumed nitrogen (CN), nitrogen excreted in the feces (FN), and nitrogen excreted in the urine (UN) were determined using the micro Kjeldahl technique (method no. 954.01) of the Association of Analytical Chemists [AOAC] (2003). Nitrogen balance (NB) was calculated according to the Equation 2:

$$NB = \left(\frac{CN-(FN+UN)}{CN}\right) \times 100 \quad (2)$$

Retained nitrogen (RN) was calculated as (Equation 3):

$$RN = NB(g\ day^{-1}) - BEN(g\ day^{-1}) - \text{dermal losses } (g\ day^{-1}) \quad (3)$$

Where BEN (basal endogenous nitrogen) = 0.35 × body weight $^{0.75}$, and dermal losses = 0.018 × body weight $^{0.75}$, according to the recommendations of the Agricultural and Food Research Council [AFRC] (1993).

Blood samples were collected using 9.0 mL vacutainer tubes (Grainer Bio-One, Vacuette® Americana, SP, BRA), by puncturing the jugular vein, five days before the end of the rearing phase, and 4h after the morning feed. Two blood samples were collected from each animal; one in a tube containing an anticoagulant (EDTA) and another in a tube without the anticoagulant. The tubes with the anticoagulant were used for analyzing urea and total protein concentration, while samples without the anticoagulant were used for analyzing creatinine, total and direct bilirubin, albumin, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyltransferase (GGT) levels. To determine urea and total protein concentration, serum was obtained by centrifuging the tubes at 3,293 × g for 15 min., identified and stored in Eppendorf® mini-tubes, and frozen for analysis. Blood parameters and urine creatinine were analyzed with commercial Labtest® kits using colorimetric procedures.

Data were initially subjected to normality tests (Shapiro-Wilks) and homoscedasticity tests (Levene), and to analysis of variance by the F test when the presuppositions were met, using the following model (Equation 4):

$$Yijk = \mu + \propto i + \beta j + (\propto \beta)ijk + eijk \quad (4)$$

where:
Y_{ilk} is the dependent variable corresponding to the experimental observation;
μ is the overall mean;
αi is the fixed effect of the diets;
βj is the fixed effect of the breed;
$(\alpha\beta)_{ijk}$ is the interaction effect;
and eijk is the random error, assuming an independent normal distribution. Interaction between breed and diet was only deployed when significant at 5% probability. Statistical analyses were performed using the GLM procedure of SAS software version 9.4 (SAS Institute, 2005).

Results and discussion

Consumed nitrogen, fecal nitrogen, urinary nitrogen, retained nitrogen and nitrogen balance were influenced ($p < 0.05$) by the diets but not ($p > 0.05$) by the breed or the interaction between both factors. Goats kids that received SM-diets consumed larger amounts of nitrogen, followed by those fed $Ca(OH)_2$ DCC and NaOH DCC (Table 3).

Fecal nitrogen and urinary nitrogen were higher for goats kids fed $Ca(OH)_2$ DCC; however, urinary nitrogen of the goats kids fed this diet did not differ from that of goats fed SM, while the fecal nitrogen of goats kids consuming NaOH DCC did not differ from those fed SM-diet. Retained nitrogen was higher in goats kids that received SM-diet, and no difference was observed between diets with castor bean cake. A positive nitrogen balance was observed for all three diets, with the highest values observed for goats kids fed NaOH DCC and SM-diet, while those fed $Ca(OH)_2$ DCC presented the lowest nitrogen balance.

Table 3. Consumed nitrogen, fecal nitrogen, urinary nitrogen (UN), retained nitrogen (RN) and nitrogen balance (NB) in female dairy goats kids fed detoxified castor cake diets instead of soybean meal.

Breed	Diets			Mean	SEM[2]	P-value		
	SM[1]	$Ca(OH)_2$ DCC	NaOH DCC			Diets	Breed	D[3] x B[4]
	Consumed nitrogen (g day^{-1})							
Saanen	19.21	17.56	16.87	17.88	0.52	<0.050	0.583	0.065
Anglo Nubian	19.12	16.98	16.72	17.61				
Mean	19.17a	17.27b	16.80c					
	Fecal nitrogen (g day^{-1})							
Saanen	4.45	4.81	3.91	4.39	0.20	<0.050	0.849	0.054
Anglo Nubian	4.33	4.76	3.85	4.31				
Mean	4.39ab	4.79a	3.88b					
	Urinary nitrogen (g day^{-1})							
Saanen	2.12	2.16	1.72	2.00	0.08	<0.050	0.852	0.065
Anglo Nubian	2.09	2.20	1.78	2.02				
Mean	2.11a	2.18a	1.75b					
	Retained nitrogen (g day^{-1})							
Saanen	6.94	5.44	6.09	6.15	0.39	<0.050	0.625	0.063
Anglo Nubian	7.00	4.87	5.94	5.92				
Mean	6.97a	5.15b	6.01b					
	Nitrogen balance (% de retained N)							
Saanen	65.80	60.31	66.63	64.24	1.49	<0.050	0.960	0.071
Anglo Nubian	66.42	59.01	66.33	63.92				
Mean	66.11a	59.66b	66.48a					

[1] Soybean meal diet. [2] Standard error of the mean. [3] Diet. [4] Breed. Means within a row without common superscripts are different by the Tukey test at 5% significance.

Nitrogen balance was positive and showed a 64% higher average, in all diets evaluated in goat kids in their growth phase, indicating that the intake of nitrogen has met the nitrogenous compound requirements of animals. Recycling of urea has great importance and contributes significantly to the balance of nitrogen in ruminants, mainly in animals with low protein intake (Sinclair, Garnsworthy, Mann, & Sinclair, 2014). This response is related to higher consumed nitrogen by goats kids fed diet SM, which probably meant that the urea content of stock was higher and related to a higher nitrogen excretion in urine (Beatson, Meier, Cullen, & Eding, 2019; Lapierre et al., 2020).

Diet influenced ($p < 0.05$) creatinine, direct bilirubin, urea, ALT, AST, and GGT levels (Table 4); no influence ($p > 0.05$) was observed on total bilirubin, total proteins, albumin and alkaline phosphatase. No effect of the breed or of the interaction between diet and breed was found. Higher creatinine levels were found in goats kids fed $Ca(OH)_2$ DCC. Direct bilirubin, ALT and AST levels were higher in goats kids fed SM-diet. No difference in GGT levels was observed between SM and $Ca(OH)_2$ DCC fed goats kids or between the latter and those fed NaOH DCC.

For renal and hepatic parameters, higher levels of creatinine were observed in goats kids fed $Ca(OH)_2$ DCC. High creatinine is a good indicator of glomerular dysfunction in ruminants; however, the concentrations found do not indicate any type of nephropathy, since according to Kaneko, Harvey, and Bruss (2008), values between 0.7 and 1.5 mg dL^{-1} are normal in goats.

Direct bilirubin level is related to the renewal of red blood cells and is a good indicator of liver problems, which, similar to creatinine values, was observed to be normal for this category (0.00 to 0.15 mg dL^{-1}). The highest direct bilirubin concentrations were observed in SM-fed goats, which may be related to higher consumed nitrogen and higher metabolic rate of nitrogenous compounds, which in turn increase the blood circulation rate, thus decreasing the lifespan of the red blood cells and increasing the production of bilirubin from hemoglobin. Solaiman, Gurung, McCrary, Goyal, and McElhenney (2008) evaluated the effect of inclusion of cottonseed meal in the diet of Nubian goats kids, and found no effects on total bilirubin and alkaline phosphatase levels; however, there was an increase in serum creatinine and total protein levels in the serum.

The highest values of blood urea were observed in SM-fed goats kids; a value that is slightly above the reference values of 10 to 20 mg dL^{-1} (Kaneko et al., 2008). It is important to remember that blood urea levels are mainly affected by the consumed nitrogen content. In this context, we found that higher levels of blood urea in goats kids fed SM were a consequence of the high consumed nitrogen content rather than of the existence of liver disease, since the ammonia produced during nitrogen metabolism in the rumen, which is not used by microorganisms, is absorbed by the ruminal wall.

Table 4. Creatinine (CRE), direct bilirubin (DB), total bilirubin (TB), total proteins (TP), albumin (ALB), urea (URE), alkaline phosphatase (AP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyltransferase (GGT) in female dairy goats kids fed detoxified castor bean cake diets instead of soybean meal.

Breed	Diets			Mean	SEM[2]	P-value		
	SM[1]	$Ca(OH)_2$ DCC	NaOH DCC			Diets	Breed	D[3] x B[4]
	CRE (mg dL^{-1})							
Saanen	0.87	1.18	0.90	0.98	0.06	<0.050	0.096	0.656
Anglo Nubian	0.79	1.00	0.70	0.84				
Mean	0.83b	1.09a	0.81b					
	DB (mg dL^{-1})							
Saanen	0.13	0.09	0.13	0.12	0.03	<0.050	0.710	0.563
Anglo Nubian	0.14	0.08	0.07	0.10				
Mean	0.14a	0.08b	0.10b					
	TB (mg dL^{-1})							
Saanen	0.53	0.51	0.53	0.52	0.06	0.877	0.719	0.679
Anglo Nubian	0.53	0.59	0.49	0.54				
Mean	0.53	0.55	0.51					
	TP (g dL^{-1})							
Saanen	5.74	6.37	6.21	6.10	0.37	0.408	0.627	0.114
Anglo Nubian	5.25	6.18	7.92	6.32				
Mean	5.50	6.27	6.95					
	ALB (g dL^{-1})							
Saanen	1.99	1.79	2.20	1.99	0.09	0.210	0.115	0.367
Anglo Nubian	2.12	2.12	2.24	2.17				
Mean	2.05	1.97	2.21					
	URE (mg dL^{-1})							
Saanen	22.34	18.87	18.49	19.90	0.87	<0.050	0.249	0.297
Anglo Nubian	20.78	20.17	15.64	18.69				
Mean	21.67a	19.52ab	17.06b					
	AP (IU L^{-1})							
Saanen	4.41	5.10	4.62	4.71	0.72	0.765	0.612	0.547
Anglo Nubian	4.89	4.91	5.56	5.14				
Mean	4.65	5.33	4.74					
	ALT (IU L^{-1})							
Saanen	16.50	11.50	19.75	12.91	1.08	<0.050	0.051	0.803
Anglo Nubian	23.00	15.75	12.66	17.54				
Mean	19.75a	13.62b	11.57b					
	AST (IU L^{-1})							
Saanen	114.34	63.35	67.89	81.86	4.97	<0.050	0.703	0.648
Anglo Nubian	109.84	71.98	65.89	84.08				
Mean	112.09a	67.66b	67.03b					
	GGT (IU L^{-1})							
Saanen	71.01	51.90	61.76	61.56	3.55	<0.050	0.475	0.721
Anglo Nubian	70.41	59.24	63.84	64.56				
Mean	70.71a	62.65ab	55.57b					

[1] Soybean meal diet. [2] Standard error of the mean. [3] Diet. [4] Breed. Means within a row without common superscripts are different by the Tukey test at 5% significance.

Greater liver activity was observed in goats kids fed SM, as they presented higher levels of ALT, AST, and GGT. According to Kaneko et al. (2008), healthy animals have ALT and AST levels ranging from 6 to 19 IU L^{-1} and 66 to 230 and IU L^{-1}, respectively.

Oliveira et al. (2010b) evaluated the inclusion of castor bran, castor cake detoxified with calcium hydroxide and undetoxified castor cake in sheep diets and found ALT and AST values within the reference values, recommended for this species, although they were unable to detoxify the material 100%. Nevertheless, the authors in this study only included 15% of the above-mentioned by-products in the animals' diet.

Despite the variations, all these enzymes were within the standard limits for animals of this category except for GGT, in which case the goats kids fed SM-diet showed no difference from the goats kids fed diets based on $Ca(OH)_2$ DCC, but were superior to those fed NaOH DCC. The enzyme GGT that is found in cell membranes, is involved in the transfer of amino acids, mainly in the hepatocytes (Kaneko et al., 2008), and is therefore, high levels a good indicator of hepatic lesions in ruminants. Animals that consume the castor bean cake containing ricin, present high levels of GGT (Araújo et al., 2020a). Thus, it was demonstrated that offering DCC to animals not cause any type of lesion in the liver and kidneys.

Conclusion

The castor cake detoxified by alkaline solutions in replacement of soybean meal proved to be a viable alternative in the feeding of goats in growth, because it does not affect the functionality of the liver and kidney. NaOH DCC stands in the substitution of soybean meal, because in spite of decreasing the consumption of nitrogen provides the same retention of soybean meal.

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