Productive performance and efficiency of utilization of the diet components in dairy cows fed castor meal treated with calcium oxide[1]

Juliana Variz Cobianchi[2], André Soares de Oliveira[3], José Maurício de Souza Campos[4], Amanda Vasconcelos Guimarães[5], Sebastião de Campos Valadares Filho[6], Flávio Pereira Cobianchi[7], Thalita Evani Silva de Oliveira[8]

[1] Supported by CNPq and FINEP.
[2] Mestre em Zootecnia/UFV, Viçosa-MG, fellow student by CNPq.
[3] Instituto de Ciências Agrárias e Ambientais da UFMT, Campus Sinop, Sinop-MT. Researcher at INCT-Ciência Animal/CNPq.
[4] Universidade Federal Rural de Pernambuco, Unidade Acadêmica de Garanhuns. Researcher at CNPq.
[5] Estudante de doutorado em Zootecnia/UFLA, Lavras-MG, Brazil.
[6] Departamento de Zootecnia, Universidade Federal de Viçosa, Campus Viçosa.
[7] Laticínios Bela Vista, Bela Vista de Goiás-GO, Brazil.
[8] Estudante de Medicina Veterinária/UNIVIÇOSA, Viçosa-MG, Brazil.

ABSTRACT - The effect of replacing of 0; 0.33; 0.67 and 1.0 (kg/kg) of soybean meal (SBM) by undecorticated castor seed meal treated with calcium oxide (CMT - 60 g/kg) was evaluated on performance and efficiency of nutrient utilization in dairy cows. Sixteen Holstein and crossbred cows were distributed in four 4 × 4 latin squares. Animals received concentrated feed at a ratio of 1 kg for 3 kg of milk produced, in the natural matter. The diets had the same amount of nitrogen (150.4 g crude protein/kg DM), containing 325.6 g of concentrated feed/kg DM. There was no effect on the serum concentration of transaminase and the animals showed no clinical symptoms of intoxication by ricin. The intake of DM, crude protein (CP) and non-fibrous carbohydrates (NFC) reduced from 0.67 replacement of SBM by CMT. The intake of neutral detergent fibers corrected for ash and protein (NDFap) increased from 0.33 replacement of SBM with CMT. Although the digestibility of dietary components decreased from 0.33 replacement, the intake of digestible components only reduced from 0.67 replacement. Because of the reduction of digestible energy, the synthesis of microbial CP and the utilization efficiency of rumen-degradable protein for the synthesis of microbial CP reduced with full replacement of SBM by CMT. Milk yield, milk composition, daily variation of body weight and the efficiency of utilization of the nutrients for the synthesis of N in milk reduced from 0.67 replacement of SBM by CMT. Castor seed meal treated with calcium oxide can replace up to 0.33 of SBM (50 g/kg DM diet DM) in the diet of dairy cows with an average milk production of 20 kg/day.

Key Words: digestibility, intake, milk production, nitrogenous compounds, ricin, *Ricinus communis* L.

Introduction

The expected growth of the participation of biodiesel in the world energy matrix has generated opportunities for the ruminant production because of the possibility of utilizing byproducts resulting from the process of extraction of oil from oilseeds. Among the cultures available, the castor oil seed (*Ricinus communis* L.) stands out for the potential of exploitation in regions not included in the economic development process and by the greater intensity in the use of labor force.

Despite the potential of utilization in the feeding of dairy cows (Robb et al., 1974; Matos, 1976), the castor seed meal and cake have been utilized exclusively as organic fertilizers, due to the presence of a powerful toxin (ricin), which reduces the competitiveness in relation to the other oilseeds (Oliveira et al., 2010a; Oliveira et al., 2010b).

Ricin is a protein found mainly in the endosperm of castor seeds and irreversibly inactivates eukaryotic ribosomes, inhibiting the synthesis of protein (Olsnes et al., 1974; Endo & Tsurugi, 1988). It is composed of two subunits of different biological functions. Subunit A (36 kDa) inactivates specifically and irreversibly the eukaryotic ribosomes, impeding the protein synthesis; subunit B (29 kDa), in turn, is bound to the cell membrane and to subunit A, and allows the entrance of the latter into the cytosol by endocytosis (Olnes et al., 1974; Endo & Tsurugi, 1988).

Although some detoxification methods have been known since the end of the decade of 1940 (Borchers, 1949; Kodras et al., 1949; Gardner et al., 1960), only recently has some more conclusive study been developed, in which only the use of autoclave (15 psi, 90 min) or the treatment with calcium oxide or calcium hydroxide (60 g/kg castor meal) provoked complete denaturation of the toxin (Oliveira et al., 2007).

The procedure of detoxification with calcium oxide suggested by Oliveira al. (2007) showed to be operationally simple, and with great economic viability. However, before recommending its use for animal feeding, studies of validation must be conducted, covering the evaluation of markers of nutritional efficiency, health and animal performance, which are still scarce for dairy cows. It has been hypothesized that undecorticated castor meal treated with 60 grams calcium oxide/kg (CMT) can be utilized in replacement of soybean meal (SBM) in diets for dairy cows without affecting the utilization efficiency of the diet components or productive performance or causing alterations in the liver functions.

Therefore, the objective of this study was to evaluate the effect of replacing SBM by CMT (0; 0.33; 0.67 and 1.00 kg/kg, on a dry matter basis) on the serum concentration of liver enzymes, the intake and digestibility of the components of the diet, productive performance and metabolism of nitrogenous compounds (N) in dairy cows with average milk yield of 20 kg/day.

Material and Methods

The experiment was conducted at the Unidade de Ensino, Pesquisa e Extensão, Departamento de Zootecnia of Universidade Federal de Viçosa. Sixteen purebred and crossbred Holstein cows (milk yield of 20.3±7.3 kg/day; body weight of 540±64 kg; 100±73 days of lactation) were distributed in four 4 × 4 Latin squares according to the period of lactation, balanced for residual effect. At the end of the experiment, all cows presented fewer than 150 days of gestation.

The experiment was comprised of four 21-day periods; the first 15 days were utilized for animals to adapt to the diets, and the rest, for data collection. Animals were fed four experimental diets referring to four levels of replacement of SBM by CMT: 0; 0.33; 0.67; and 1.0 kg/kg, on a DM basis.

The inactivation of the ricin from the undecorticated castor seed meal (CM) was performed by denaturation with alkaline treatment, utilizing a CaO solution (agricultural micro-sprayed quicklime, containing 90% total oxide, Mineração Belocal LTDA, São José da Lapa – Minas Gerais, Brazil), at the ratio of 1 kg in 10 liters of water, making up a dose of 60 grams of lime per kg of CM, in the natural matter basis, as recommended by Oliveira et al. (2007). After mixing the CM with the lime solution, the material remained still for a period of twelve hours (overnight), and then was dried in the sun on a cemented yard for 48 hours.

Diets were formulated to contain the same amount of nitrogen, with 153.6 g crude protein (CP)/kg DM, so as to meet the nutritional requirements of a cow weighing 540 kg body weight, producing milk at 25 kg/day and with 35 g fat/kg milk (NRC, 2001). The mixture containing nine parts of urea and one part of ammonium sulfate was utilized to adjust the levels of crude protein of the diet. The animals received concentrate feed at the ratio of 1 kg for each 3 kg of milk produced, in the natural matter basis, which corresponded to a supply of diets containing 325.6 g concentrate/kg DM. Corn silage was utilized as the only source of forage (Table 1).

After previous analysis of the CP content of feedstuffs (Table 1), their proportion in the experimental diets was elaborated as described in Table 2. The mixture of

Table 1 - Chemical composition of the ingredients utilized in the experimental diets

Items	Feedstuffs				
	Corn silage	Ground corn grain	Soybean meal	Wheat bran	CMT[1]
Dry matter (DM), g/kg	240.1	884.4	877.8	888.6	908.4
Organic matter, g/kg DM	952.7	986.3	937.8	950.5	853.1
Ether extract, g/kg DM	27.1	35.4	8.5	6.4	6.9
Crude protein, g/kg DM	80.8	90.1	506.3	179	357.4
Rumen-degradable protein, g/kg DM[2]	63.3	27.4	254.1	151.6	156.7
Non-protein nitrogen, g/kg TN[3]	719.4	160.7	94	224.2	199.4
NDIN, g/kg TN	208.8	148.2	136.6	154.7	356
ADIN, g/kg TN	162.4	6.7	19.5	20	67.5
NDF corrected for ash and protein, g/kg DM	523.5	115.3	143.7	372.6	342.6
Non-fibrous carbohydrates, g/kg DM	321.3	745.4	390.4	281.4	146.2
Acid detergent fiber (ADF), g/kg DM	355.7	18.5	76.3	108.7	295.3
Lignin H_2SO_4, g/kg DM	44.7	19.5	17.2	25.5	46.9
Cutin, g/kg DM	28.6	0.3	6.7	18.1	212.4
Indigestible NDF, g/kg DM[3]	301.3	18.3	42.1	162.8	403.4
Indigestible ADF, g/kg DM[3]	166.8	11.9	4.6	95.6	339.9

[1] CMT - castor seed meal treated with 60 g calcium oxide/kg, according to Oliveira et al. (2007).
[2] Estimated according to the NRC (2001).
[3] Obtained after *in situ* rumen incubation for 264 hours (Casali, 2006).
TN - total nitrogenous compounds; NDIN - neutral detergent insoluble nitrogen; ADIN - acid detergent insoluble nitrogen.

macrominerals was balanced to meet the total nutritional requirements, according to the NRC (2001).

The concentrated feed was mixed in the beginning of each period, and each feedstuff was sampled. Animals were managed in individual tie-type stalls, where they received feed *ad libitum* twice daily, at 7h00 and 16h00. In the collection period, twice daily, the quantities of corn silage supplied and leftovers were weighed and sampled, and the amount of concentrate feed supplied was weighed. Samples were stored at -15 ºC for subsequent chemical analyses.

Feces were collected daily from the rectal ampulla once daily, at 8h00, 10h00, 12h00, 14h00 and 16h00, from the 16th to the 20th days of each experimental period. Daily samples of feces from each animal, in each period, were stored at -15 ºC for subsequent drying and chemical analyses.

Samples of corn silage and feces were dried in forced-ventilation oven (60 ºC for 72 hours) and, along with those of feedstuffs, processed in knife mill with 1 mm mesh sieve for chemical analyses, and with 2 mm mesh for *in situ* rumen incubation. Composite samples were made from the air-dried daily samples of feces from each animal, in each period, for subsequent chemical analysis.

The determination of the concentration of ricin in the non-treated castor seed meal (CM) and that treated with calcium oxide (CMT) was done through separation of fractions A (36 kDa) and B (29 kDa) by electrophoresis gel over 10% polyacrylamide in denaturing condition (SDS-PAGE) according to the methodology proposed by Laemmli (1970) and by densitometric analysis of the crude protein extracts according to Oliveira et al. (2010a).

The analyses of the contents of DM, crude protein (total nitrogen × 6.25), acid detergent fiber (ADF) and lignin (H_2SO_4 720 g/g) were conducted according to methods described in Silva & Queiroz (2002). The quantification of cutin was done through hydrolysis of the residue of ADF with H_2SO_4 720 g/g, followed by oxidation of the residue of hydrolysis with $KMnO_4$ and burning in a muffle at 550 ºC, as described by Van Soest (1994). For the analysis of the concentration of neutral detergent fiber (NDF), samples were treated with thermostable alpha-amylases without sodium sulfite, corrected for the ash residue (Mertens, 2002) and for the residue of nitrogenous compounds (Licitra et al., 1996). Analyses of NDF and ADF were conducted in Ankom® extraction chambers with TNT (non-woven textile) bags (Valente, 2010) of dimensions 5 × 5 cm, keeping an

Table 2 - Proportion of ingredients and average chemical composition of the diets containing different levels of replacement of soybean meal by treated castor seed meal[1]

Itens	Level of replacement of soybean meal by castor seed meal (kg/kg)			
	0.00	0.33	0.67	1.00
Corn silage, g/kg DM	674.4	674.4	674.4	674.4
Ground corn grain, g/kg DM	89.3	89.3	89.3	89.3
Wheat bran, g/kg DM	67	63.5	60.2	56.8
Soybean meal, g/kg DM	148.8	99.3	49.6	0
Treated castor seed meal, g/kg DM	0	49.6	99.3	148.8
Urea and ammonium sulfate (9:1), g/kg DM	0.9	4.3	7.7	11.2
Mineral mix, g/kg DM	13	13	13	13
Sodium chloride, g/kg DM	4.7	4.7	4.7	4.7
Potassium chloride, g/kg DM	0.9	0.9	0.9	0.9
Sulfur flower, g/kg DM	0.9	0.9	0.9	0.9
Chemical composition				
Dry matter (DM), g/kg	317.5	317.3	317.1	316.8
Organic matter, g/kg DM	933.8	926.4	919.0	911.5
Ether extract, g/kg DM	23.1	23.0	22.9	22.8
Crude protein, g/kg DM	152.3	153.2	154.1	154.9
Rumen-degradable protein, g/kg DM	95.6	99.0	102.6	106.1
Non-protein nitrogen, g/kg TN	346.1	408.5	470.6	532.0
NDIN, g/kg TN	162.3	179.6	196.7	213.5
ADIN, g/kg TN	69.7	73.8	77.9	82.0
NDF corrected for ash and protein, g/kg DM	409.7	418.3	426.9	435.5
Non-fibrous carbohydrates, g/kg DM	350.2	338.8	327.5	316.1
Acid detergent fiber (ADF), g/kg DM	260.2	270.7	281.2	291.7
Lignin H_2SO_4, g/kg DM	36.2	37.5	38.9	40.3
Cutin, g/kg DM	21.5	31.7	41.8	52.0
Indigestible NDF, g/kg DM	222.0	239.4	256.8	274.1
Indigestible ADF, g/kg DM	120.6	136.9	153.3	169.6

[1] Castor seed meal treated with 60 g calcium oxide/kg, according to Oliveira et al. (2007).
NDIN - neutral detergent insoluble nitrogen; ADIN - acid detergent insoluble nitrogen; NDF - neutral detergent fiber; ADF - acid detergent fiber.

average ratio of 14 mg of DM/cm² of fabric and 100 mL of neutral detergent/g of air-dried sample. The quantification of non-protein nitrogen (NPN) of the feedstuffs was done according to Licitra et al. (1996).

The rumen-degradable protein content of the feedstuffs was calculated utilizing the equation $RDP = a + (b \times kd)/(kd + kp)$, in which a (g/g) = soluble fraction, represented by the NPN fraction in the TN; b (g/g) = potentially degradable insoluble fraction, represented by the fraction of rumen potentially-degradable N of the TN (NDIN-ADIN); kd = degradation rate of fraction b in time t, utilizing the values obtained by Oliveira (2008); kp (h^{-1}) = the passage rate, calculated according to the equations described in the NRC (2001). The RUP content (g/g) was calculated as $1000 - RDP$ (g/g), in which RDP = rumen-degradable protein; and RUP = rumen-undegradable protein. The intake values of RDP and RUP were calculated by multiplying the DM intake by the RDP and RUP contents in the diet, respectively.

The contents of non-fibrous carbohydrates corrected for ash and protein (NFC_{ap}) were calculated as proposed by Hall (2000), but adapted, as follows: $NFC_{ap} = 100 - [(CP - CP$ from urea + urea in the diet$) + NDF_{ap} + EE + Ash]$. The total digestible nutrients (TDN) were calculated with adaptations to the method described by Weiss (1999), through the following equation: TDN (g/kg) $= DCP + DNDF_{ap} + DNFC_{ap} + 2.25DEE$, in which DCP = digestible crude protein; $DNDF_{ap}$ = digestible neutral detergent fiber; $DNFC_{ap}$ = digestible non-fiber carbohydrates; and DEE = digestible ether extract.

The total amount of fecal DM excreted was estimated by the concentration of indigestible acid detergent fiber (iADF), obtained after rumen incubation of the feedstuffs, leftovers and feces in polyester bags (Ankom®, filter bag 57) for a period of 264 hours, according to Casali (2006).

Cows were milked mechanically twice daily, with milk production recorded from the 15th to the 21st days of each experimental period. Through a device attached to the milking machine, a sample of approximately 300 mL of milk was collected on the 18th and 19th days, in the morning and afternoon milking sessions, with composite samples made for each day according to milk yield. Two aliquots were taken from each sample: the first aliquot (50 mL) was conditioned in plastic bottles with preservative (Bronopol®), kept at between 2 and 6 ºC and sent to the Laboratory of Analyses of Milk Quality of Embrapa Gado de Leite for determination of the contents of lactose, fat, total solids and nonfat total solids of the milk, according to the methodology described by the IDF (1996); the second aliquot was deproteinized with trichloroacetic acid (10 mL of milk mixed with 5 mL trichloroacetic acid at 250 g/L),

filtered through filter paper, determining the content of total nitrogen in the filtered solution (Silva & Queiroz, 2002) and storing the rest at -15 ºC for subsequent analysis of allantoin. Milk yield (MY) corrected for 35 g fat/kg of milk (MYC) was calculated according to Sklan et al. (1992).

On the seventh day of adaptation and at the end of each experimental period, animals were weighed individually for evaluation of variation of weight. Their weights corresponded to the averages of two weighing sessions, conducted before the supply of feeding and after milking sessions.

Blood samples were collected on the 18th day by coccygeal venipuncture, utilizing test tubes with anticoagulant (EDTA). Samples were immediately centrifuged at 5,000 rpm for 15 minutes, and then plasma samples were taken and conditioned in eppendorf tubes and frozen at -15 ºC for subsequent analyses of urea nitrogen concentration and measurement of liver enzymes gamma glutamyl transpeptidase (GGT), alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST).

Spot samples of urine were obtained from all cows on the 17th day of each experimental period, during urination stimulated by vulva massage at three times: zero, four and eight hours after morning feeding. The urine was filtered and aliquots of 10 mL from each time were collected and diluted immediately in 40 mL sulfuric acid at 0.072 N, to avoid bacterial destruction of the purine derivatives and precipitation of the uric acid, and stored at -15 ºC for subsequent analyses of total nitrogen, urea, allantoin (AL), uric acid (UA) and creatinine. Immediately before analyses, samples from each time were thawed, centrifuged at 2,000 xg for 15 minutes, and then composite samples were formed (10 mL for each time) per cow in each period.

The analysis of the serum levels of GGT, AST and ALT were conducted via optimized kinetic method UV (commercial kits). Analyses of AL in the urine in the milk were done by colorimetry, according to Fujihara et al. (1987), described in Chen & Gomes (1992). Analyses of urea were accomplished by the enzymatic-colorimetric system by the urease method, utilizing commercial kits (Labtest Diagnóstica S.A.). Analyses of uric acid in the urine were done via the Trinder enzymatic method, with the aid of commercial kits (Labtest Diagnóstica S.A.). The analyses of creatinine in the urine were done by the endpoint method with picrate and acidifier, utilizing commercial kits (Labtest Diagnóstica S.A.).

The daily urinary volume was estimated by dividing the daily urinary excretions of creatinine by the observed values of creatinine in the urine. The daily urinary excretion of creatinine was estimated from the proposition of 24.05 mg/kg live weight (LW) of creatinine (Chizzotti et al., 2008).

The total excretion of purine derivatives (PD) was calculated by the sum of the amounts of allantoin and uric acid excreted in the urine and the amount of allantoin excreted in the milk, expressed in mmol/day. Absorbed purines (AP, mmol/day) were calculated from the excretion of PD (PD, mmol/day), through the equation: $PD = 0.85*AP+0.512*LW^{0.75}$, in which 0.85 is the recovery of absorbed purines as purine derivatives (Verbic et al., 1990) and $0.512*LW^{0.75}$ is the endogenous contribution to the excretion of purines obtained for dairy cows (Gonzalez-Ronquillo et al., 2003).

The synthesis of microbial nitrogenous compounds in the rumen (micN g/day) was calculated in function of AP (mmol/day), by the equation: micN = (70*AP)/(0.83*0.116*1000), in which 70 accounts for the content of N in the purines (mg N/mmol); 0.83, the digestibility of microbial purines; and 0.116, the N-purine:total N ratio in the bacteria (Chen & Gomes, 1992).

The balance of nitrogenous compounds (NB) was obtained by the difference between the total nitrogen ingested (Ning) and excreted in the feces (N-feces), in the urine (N-urine) and in the milk (N-milk). The determination of the total nitrogen in the feces and in the urine was done according to the technique described in Silva & Queiroz (2002).

The dataset was subjected to tests of conformity of homogeneity of variance (Cochran) and normality (Lilliefors), confirming the normality and homoscedasticity of the data. They were subjected to analysis of variance utilizing the mixed model (PROCMIXED, Statistical Analysis System, 9.2):

$$Y_{ijkl} = \mu + S_i + T_j + (P/S)_{ik} + (C/S)_{il} + S \times T_{ij} + e_{ijkl},$$

In which Y_{ijkl} = observation in cow 1, in period k, subjected to treatment j, in latin square i; μ = overall mean; S_i = effect of latin square i, in which i = 1, 2, 3; T_j = effect of treatment j, in which j = 1, 2, 3, 4; $(P/S)_{ik}$ = effect of period k, within latin square i, in which k = 1, 2, 3, 4; $(C/S)_{il}$ = effect of cow 1, within latin square i, in which l = 1, 2, 3, 4; $S \times T_{ij}$ = effect of interaction between latin square i and treatment j; e e_{ijkl} = random effect, associated with each observation, assumption of NID (0; σ^2). All the effects were considered fixed, except for $(C/S)_{il}$; and e_{ijkl}, considered random.

The test of Williams (Williams, 1971) was applied, specifically for comparison of means of quantitative nature. Probability level of 0.05 was adopted for type I error. The results were presented as means of the minimum squares.

Results and Discussion

The substitution of SBM by CMT modified the composition of the nitrogenous compounds (N) and of the carbohydrate fraction of the diet (Table 2). The fractions of non-protein N (NPN), neutral detergent insoluble N (NDIN) and acid detergent insoluble N (ADIN) increased in 54, 32 and 18% with the total replacement of SBM by CMT. The elevation of the NPN fraction occurred due to the greater inclusion of the mixture of urea and ammonium sulfate in the diet. In spite of the enlargement of the N fractions associated with the carbohydrates and lignin of the cell wall (NDIN and ADIN), which are considered of low availability to the rumen microorganisms and those of low intestinal digestibility (Van Soest, 1994; Detmann et al., 2006; Henriques et al., 2007), the greater increase of the NPN fraction enabled an increase of 11% in the content of RDP in the diet with the total replacement of SBM by CMT.

The NDF content increased 6%, and that of NFC reduced 10% with the total replacement of SBM by CMT. Moreover, the contents of lignin and cutin increased in 11% and 142%, which contributed to increase the acid detergent indigestible fraction in the diet in 41% with the total replacement of SBM by CMT. The majoritarian participation of cutin in the NDFap of CMT (62% in the NDFap) was similar to the value observed by Oliveira et al. (2010b), of 63.82% of the NDFap, and indicates elevated presence of the hulls of the castor seed (Van Soest, 1994). Cutin is the main non-phenolic fraction of crude lignin, composed of polymers of esters of hydroxy long-chain fatty acids and alcohols, present in the epidermis of plant tissues, promoting superficial protection to them. Although it does not form bonds with carbohydrates, for being indigestible, it is a barrier to the entrance of rumen microorganisms, reducing the extent of digestion (Van Soest, 1994).

The presence of the two subunits of ricin and of other proteins soluble in buffer pH 3.8 was verified in CM, indicating that although the process of oil extraction with solvent is capable of destroying the toxin (Kim, 2011), it still presents residues, requiring procedures for detoxification. The effectiveness of the alkaline treatment can be evaluated by the difference in the intensity of the subunits of ricin (35 kDa and 29 kDa) in the polyacrylamide gel (SDS-PAGE). In this sense, it could be verified that the alkaline treatment provoked complete denaturation of the ricin (Figure 1). According to the densitometric analysis, the content of ricin in the CM was reduced from 1,004.6 to 73.9 mg/kg DM of meal with the alkaline treatment, with detoxification effectiveness of 92.6%, confirming the results verified by Oliveira et al. (2007; 2010a).

According to Oliveira et al. (2010a), with the alkaline treatment, the pH values (12.5) of CM surpass the isoelectric point value of ricin of 5.2 to 5.5, making the net charge of protein negative, provoking electrostatic

repulsion, breakage of hydrogen bonds which maintain the tridimensional structure and consequently, denaturation of the protein. Denaturation represents extreme alterations in the tridimensional structure of a protein which does not involve breakage of peptide bonds and is almost always linked to loss of function. In addition to loss of function, hydrophobic groups are exposed during denaturation, resulting in decrease in the solubility of protein in aqueous solutions. Thus, the disappearance of ricin subunits indicates absence of their solubility to buffer pH 3.8 (optimal value of ricin extraction, according to Waller & Negi (1958)), because of changes to the denatured state of the toxin when subjected to the alkaline treatment (Oliveira et al., 2010a).
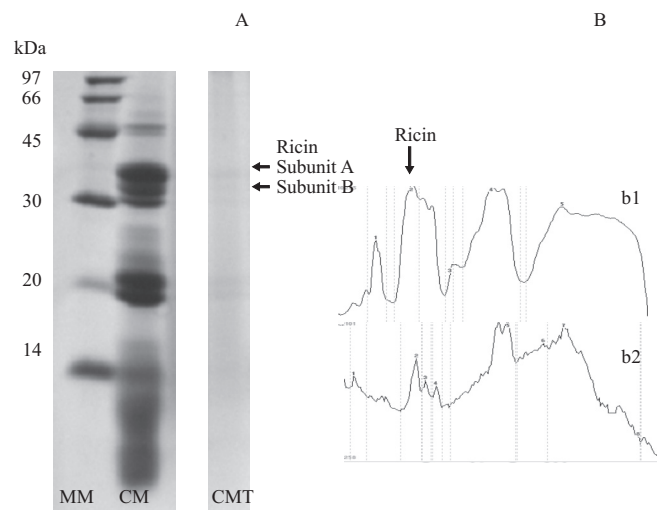
Although the ricin intake increased (P<0.05) from the replacement of 0.33 kg/kg of SBM by CMT (maximum value of 0.311 mg ricin/kg body weight), there was no effect (P>0.05) on the serum levels of the enzymes gamma glutamyl transpeptidase (GGT), alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) (Table 3). These results indicate absence of clinic effect of intoxication by ricin, for the serum levels of GGT, ALT and AST constitute markers of liver function which elevate at the occurrence of liver injury and are associated with the occurrence of intoxication by ricin in rats and sheep (Kumar et al., 2003; Aslani et al., 2007). Besides, the values of enzymes observed are within the standards established as normal for the species (Radostits et al., 2002). The main reason for the absence of intoxication in the animals is because of the alkaline treatment of CM, which promoted ricin ingestion at levels below the level considered tolerable for ruminants of 2.37 (Oliveira et al., 2010b) and 3.06 mg/kg body weight (Diniz et al., 2010).

The DM intake was not affected (P>0.05) up to 0.33 kg/kg replacement of SBM by CMT, but reduced (P<0.05) from 0.67 kg/kg replacement (Table 4). The decrease in DM intake may be associated with the greater content of NDF and NDF intake (P<0.05), and, especially, with the greater indigestible fraction of NDF and CMT in relation to SBM, due to the higher content of cutin of the CMT (Table 1).

MM - molecular weight marker between 14 and 97 kDa. Densitometric analysis of the gel (SDS-PAGE) of the CM (b.1) and of CMT (b.2) [B].
The ordinate axis represents the unit of relative density, and the abscissa axis shows the protein fractions expressed in unit of relative molecular mass (from the highest to the lowest value). The ricin area is indicated by the arrow.

Figure 1 - Polyacrylamide gel (SDS-PAGE) for the evaluation of the effectiveness of the treatment with castor seed meal (CM) with 60 g CaO/kg natural matter (CMT) in the disappearance of ricin (subunit A with 35 kDa and subunit B with 29 kDa) [A].

Basically, the enlargement of the indigestible fraction of NDF enhanced the effect of rumen fill, once the disappearance of this fraction is affected only by the passage, whereas the disappearance of the potentially digestible fraction of the NDF (NDF-iNDF) is affected by passage and digestion (Allen & Mertens, 1988; Oliveira et al., 2011).

Crude protein and NFC intake also reduced (P<0.05) from 0.67 kg/kg of replacement (Table 4), due to the decrease in DM and reduction in the dietary content (only for NFC). The consumption of rumen-degradable protein (RDP) was not affected (P>0.05), but the intake of rumen-undegradable protein (RUP) reduced from 0.67 kg/kg of replacement (Table 4). The absence of effect on RDP intake is because of the increase in the RDP in the diet (Table 2), which compensated the reduction in DM intake. As previously discussed, although the rumen degradability of CMT is

Table 3 - Daily intake of ricin and plasma levels of gamma glutamyl transpeptidase, alanine aminotransaminase and aspartate aminotransaminase in dairy cows receiving diets with different levels of replacement of soybean meal by castor seed meal treated with 60 g calcium oxide/kg

Item	Replacement of soybean meal by castor seed meal (kg/kg)				CV (%)
	0.00	0.33	0.67	1.00	
Ricin, mg/day	0.00	63.25*	114.40	168.87	21.67
Ricin, mg/body weight/day	0.00	0.115*	0.209	0.311	19.58
Gamma glutamyl transpeptidase, UI/L	23.75	21.50	24.22	24.09	14.83
Alanine aminotransaminase, UI/L	24.81	25.44	25.88	27.31	13.29
Aspartate aminotransaminase, UI/L	68.53	65.44	68.19	71.28	17.77

Means followed by (*) indicate the level of inclusion from which there is difference in relation to the control diet (level zero) by the test of William (P<0.05).
CV - coefficient of variation.

lower than that of SBM (Oliveira et al., 2010b), the increase in the RDP content in the diet with replacement of SBM by CMT happened because of the increase in the inclusion of the mixture of urea and ammonium sulfate (Table 2).

Although the digestibility of all the components of the diet (DM, OM, CP, NDFap and NFC), except for EE and the TDN content were reduced (P<0.05) from 0.33 kg/kg replacement of SBM by CMT, the intake of DM, OM, CP, NDFap, digestible NFC and TDN were not affected (P>0.05) up to this level (Table 5).

The decrease in CP digestibility was probably due to increase in the fractions of NDIN and ADIN of the diet, as well as due to decrease in CP intake, which increase the participation of the fecal metabolic fraction. The reduction in the digestibility of NDFap occurred due to the enlargement of the lignin content and especially cutin, with replacement

of SBM by CMT (Oliveira et al., 2010b). The decrease in the NFC digestibility, in turn, happened due to the decrease in NFC intake, which increased the participation of the fecal metabolic fraction (Van Soest., 1994).

The urinary excretion of purine derivatives, the synthesis of microbial N and the utilization efficiency of the RDP for the synthesis of microbial N in the rumen were not affected (P>0.05) up to 0.67 kg/kg of replacement, but reduced (P<0.05) with the total replacement of SBM by CMT (Table 6).

The reduction in the synthesis of microbial N as well as in the utilization efficiency of RDP occurred because of the lower availability of digestible carbohydrates (NFC and NDF) with the replacement of SBM by CMT, which are essential to make energy and carbon skeleton available for the microbial growth (NRC, 2001; Bach et al., 2005).

Table 4 - Daily intake of lactating dairy cows fed diets containing different levels of replacement of soybean meal by castor seed meal treated with 60 g calcium oxide/kg

Item	Replacement of soybean meal by castor seed meal (kg/kg)				CV (%)
	0.00	0.33	0.67	1.00	
Total dry matter (kg/day)	16.41	17.20	15.73*	15.20	6.32
Organic matter (kg/day)	15.27	15.88	15.40	13.79*	6.27
Ether extract (kg/day)	0.40	0.42	0.41	0.40	5.77
Crude protein (kg/day)	2.67	2.75	2.47*	2.43	7.77
Rumen-degradable protein (kg/day)	1.59	1.71	1.61	1.61	7.11
Rumen-undegradable protein (kg/day)	1.08	1.04	0.86*	0.82	11.33
NDFap (kg/day)	6.40	7.01*	6.66	6.52	6.90
Non-fibrous carbohydrates (kg/day)	5.79	5.70	4.87*	4.45	7.21
Dry matter (g/kg body weight)	30.3	31.7	29.1*	28.3	6.36
NDFap (g/kg body weight)	11.9	12.9*	12.3	12.2	7.18

Means followed by (*) indicate the level of inclusion from which there is difference in relation to the control diet (level zero) by the test of William (P<0.05).
NDFap - neutral detergent fiber corrected for ash and protein; CV - coefficient of variation.

Table 5 - Total digestibility of the diet components, total digestible nutrients of the diet and on the daily intake of digestible components of lactating dairy cows fed diets containing different levels of replacement of soybean meal by castor seed meal treated with 60 g calcium oxide/kg

Item	Replacement of soybean meal by castor seed meal (kg/kg DM)				CV (%)
	0.00	0.33	0.67	1.00	
Digestibility, g/g					
Dry matter	0.673	0.645*	0.623	0.592	5.15
Organic matter	0.691	0.665*	0.641	0.609	4.78
Ether extract	0.873	0.895*	0.942	0.938	6.46
Crude protein	0.711	0.683*	0.689	0.671	5.57
NDF corrected for ash and protein (NDFap)	0.576	0.548*	0.517	0.475	8.54
Non-fibrous carbohydrates	0.798	0.780*	0.756	0.736	2.87
Total digestible nutrients, g/g DM	0.671	0.643*	0.619	0.584	4.74
Intake, kg					
Digestible dry matter	11.04	11.09	9.80*	9.00	7.15
Digestible organic matter	10.56	10.56	9.88*	8.40	6.81
Digestible ether extract	0.35	0.38	0.39	0.38	10.50
Digestible crude protein	1.90	1.88	1.70*	1.63	9.96
Digestible NDFap	3.69	3.84	3.44*	3.09	9.14
Digestible non-fibrous carbohydrates	4.61	4.44	3.70*	3.27	7.41
Total digestible nutrients	10.99	11.01	9.71*	8.84	6.78

Means followed by (*) indicate the level of inclusion from which there is difference in relation to the control diet (level zero) by the test of William (P<0.05).
DM - dry matter; CV - coefficient of variation.

It was verified that the fixed value of utilization efficiency of RDP for the synthesis of rumen microbial N utilized by the NRC (2001) and suggested by Bach et al. (2005), of 0.85 (disregarding the fraction of endogenous N), was not within (P<0.05) the confidence interval (CI) obtained from the observed values of CI $(\mu)_{95\%} = 0.77\pm0.05$. On the other hand, considering only the diets with 0 and 0.33 kg/kg of replacement of SBM by CMT, the mean value was 0.85, similar to the adopted by the NRC (2001), demonstrating that for diets with adequate supply of digestible carbohydrates, the fixed value utilized by the NRC (2001) seems to be adequate.

The utilization efficiency of energy available for the synthesis of rumen microbial N was not affected (P>0.05) by the replacement of SBM by CMT (Table 6). Since the availability of rumen N (RDP intake) was kept constant, the absence of effect on the utilization efficiency of energy was expected, because, as demonstrated by the NRC (2001), the utilization efficiency of the energy available for the rumen

microbial growth is not very affected by the intake of available energy, when the availability of rumen N is kept constant. It is worth stressing that the fixed values of 130 and 120 g of microbial CP/kg of TDN ingested suggested by the NRC (2001) and by Valadares Filho et al. (2006) for tropical conditions are within (P<0.05) the confidence interval (CI) obtained from the observed values of CI $(\mu)_{95\%} = 126.39\pm7.46$, which indicates that both values can be utilized for prediction of the synthesis of rumen microbial CP.

Milk yield, MY corrected for 35 g fat/kg of milk, contents of CP and nonfat solids, as well as the daily production of lactose, CP, fat, total solids and nonfat solids in the milk were not affected (P>0.05) up to 0.33 kg/kg of replacement, but decreased (P<0.05) from the replacement of 0.67 kg/kg of SBM by CMT (Table 7). However, feeding efficiency was not affected (P>0.05), since the reduction of MYC was followed by decrease in DM intake. The variation in body weight reduced (P<0.05) from 0.67 kg/kg of replacement, presenting mean values of 0.65, 0.67, 0.20

Table 6 - Effect of the level of replacement of soybean meal by castor seed meal treated with 60 g calcium oxide/kg on the daily excretion of purine derivatives, synthesis and efficiency of the synthesis of microbial nitrogenous compounds (N) in the rumen of lactating dairy cows

| Item | Replacement of soybean meal by castor seed meal (kg/kg) | | | | CV (%) |
	0.00	0.33	0.67	1.00	
Urinary excretion					
Allantoin, mmol/d	286.75	286.60	265.30	229.39*	17.09
Uric acid, mmol/d	30.70	31.05	27.46*	24.10	16.07
Allantoin in the milk, mmol/d	2.14	2.12	1.64	2.36	40.42
Total excreted purine derivatives, mmol/d	319.59	319.67	294.40	255.85*	16.06
Absorbed purines, mmol/d	308.43	308.25	278.98	233.98*	19.93
Microbial N flow, g/d	224.24	224.11	202.83	170.12*	19.93
Microbial crude protein (CP) flow, g/d	1401.50	1400.69	1267.69	1063.25*	19.93
g micCP/kg TDN intake	127.53	127.22	130.56	120.28	17.39
g micCP/g RDP intake	0.881	0.819	0.792*	0.656	18.06

Means followed by (*) indicate the level of inclusion from which there is difference in relation to the control diet (level zero) by the test of William (P<0.05).
CV - coefficient of variation.

Table 7 - Production and composition of the milk from dairy cows fed diets containing different levels of replacement of soybean meal by castor seed meal treated with 60 g calcium oxide/kg

| Item | Replacement of soybean meal by castor seed meal (kg/kg) | | | | CV (%) |
	0.00	0.33	0.67	1.00	
Milk, kg/d	20.82	21.22	19.15*	18.81	7.60
Milk with 3.5% fat (MYC), kg/d	21.11	21.71	19.28*	18.78	7.73
Lactose, g/kg	45.0	45.6	45.6	45.1	1.71
Crude protein, g/kg	31.4	30.9	29.6*	28.8	2.71
Fat, g/kg	37.1	37.0	36.1	35.8	6.70
Protein:fat	0.85	0.84	0.82	0.80*	7.30
Total solids, g/kg	12.41	12.42	12.10	11.94	2.73
Nonfat total solids, g/kg	8.66	8.65	8.50*	8.36	1.38
Lactose, kg/d	0.94	0.97	0.87*	0.85	7.91
Protein, kg/d	0.65	0.66	0.57*	0.54	7.61
Fat, kg/d	0.77	0.79	0.69*	0.67	9.31
Total solids, kg/d	2.58	2.64	2.32*	2.25	7.31
Nonfat total solids, kg/d	1.80	1.84	1.63*	1.57	7.49
MYC/DM intake	0.79	0.81	0.82	0.81	9.08

Means followed by (*) indicate the level of inclusion from which there is difference in relation to the control diet (level zero) by the test of William (P<0.05).
CV - coefficient of variation.

and -0.23 kg/day for the levels 0; 0.33; 0.67 and 1.0 kg/kg of replacement of SBM by CMT.

The reduction in MY occurred mostly due to the decrease in the production of lactose, which represents the main osmotic component of milk. The decrease in the production of lactose was a result of the lower intake of digestible carbohydrates (NFC and NDF), which reduced the flow of blood glucose in the mammary gland. Furthermore, reduction in the synthesis of rumen microbial N might also have contributed to decrease in the production of lactose, because of the diminution in the flow of amino acids to the mammary gland. The protein alpha-lactalbumin, synthesized in the mammary gland from free amino acids absorbed in the blood circulation, plays an essential role in the catalytic action of the enzyme complex lactose synthase (Fonseca, 1995). In this sense, decrease in the flow of amino acids of microbial origin possibly reduced the alpha-lactalbumin synthesis in the mammary gland, compromising the synthesis of lactose as well (Gennadij et al., 2000).

The decrease in the content and production of CP of the milk was caused by the decrease in the synthesis of rumen microbial N, which led to reduction of the flow of amino acids towards the mammary gland. Besides, the decrease in the flow of glucose in the mammary gland from the decrease in the digestible carbohydrate intake might also have contributed to the depression in the CP content of the milk, since the secreting cells of the mammary gland utilize principally the catabolism of glucose to provide the energy necessary for the processes of capture of amino acids, synthesis and transport of milk proteins (Fonseca, 1995; Mackle et al., 2000).

The reduction in the production of fat of the milk might have occurred as a result of the decrease in the availability of acetate and glucose necessary for the synthesis of triglycerides in the mammary gland. The synthesis of triglycerides in the mammary gland depends on the blood supply of long-chain fatty acids, on the *de novo* synthesis of short- and medium-chain fatty acids (SMCFA) and on the concentration of reduction cofactors (NADPH$_2$), necessary for the supply of electrons and for the enzyme complex in charge of the MCFA and of glycerol. The acetate and *B*-OH-butyrate originated from the ruminal degradation of carbohydrates, notably those of the cell wall, are the main sources of carbon for the *de novo* synthesis of SMCFA in the mammary gland in ruminants (Grummer, 1991). Thus, the decrease in digestible NDFap intake from 0.67 kg/kg of replacement of SBM by CMT reduced the availability of carbon sources for the *de novo* synthesis of SMCFA.

At the same time, the diminution in digestible NDF intake might also have affected the synthesis of fat in the mammary gland, through reduction of the flow of glucose in the mammary gland. In *in vitro* studies, utilizing the adipose tissue of cattle and sheep at different ages and physiological stages, it was demonstrated that the addition of glucose to the acetate marked with C^{14} increased the synthesis of fatty acids at more than four times in relation to the treatment without glucose (Ingle et al., 1972). Although glucose represents minimal contribution to the supply of carbon to the *de novo* synthesis of MCFA in the mammary gland of ruminants, it plays a fundamental role in the supply of reduction cofactors and glycerol (Ingle et al., 1972; Van Soest, 1994; Grummer, 2001).

The concentration of plasma urea nitrogen (PUN) increased ($P<0.05$) from 0.33 kg/kg of replacement (Table 8) due to the enlargement of the NPN fraction in

Table 8 - Concentration of plasma urea nitrogen and milk urea nitrogen, urinary excretion of nitrogenous compounds (N), N balance and utilization efficiency of N in lactating dairy cows fed diets containing different levels of replacement of soybean meal (SBM) by castor seed meal treated with 60 g calcium oxide/kg

Item	Replacement of soybean meal by castor seed meal (kg/kg)				CV (%)
	0.00	0.33	0.67	1.00	
PUN, mg/dL	13.15	16.38*	15.37	17.40	17.24
Urinary excretion					
N, g/d	129.20	131.46	123.07	126.02	14.28
N-urea, mg/d	91.68	92.84	89.24	100.48	15.95
N-urea/N, g/g N	0709	0.706	0.725	0.797*	8.63
N ingested, g/d	427.20	440.0	395.20*	388.80	7.55
N in the milk, g/d	104.60	104.91	90.69*	86.68	7.56
N in the feces, g/d	121.60	136.00	121.60	129.60	12.55
N balance, g/d	71.80	67.63	59.84	46.50*	43.35
N in the milk, g/g N ingested	244.8	237.2	229.5*	222.9	7.56
N in the feces, g/g N ingested	284.6	309.1	307.7	333.3*	12.54
N in the urine, g/g N ingested	302.4	298.8	311.4	324.1	14.28
N balance, g/g N ingested	168.1	153.7	151.4	119.6	39.69

Means followed by (*) indicate the level of inclusion from which there is difference in relation to the control diet (level zero) by the test of William ($P<0.05$).
CV - coefficient of variation.

the diet (Table 2). However, the PUN values are within the range considered adequate for the balance of energy and protein, from 10 to 17 mg/dL (Broderick, 1995; Moore & Varga, 1996; Jonker et al., 1998; Ferguson, 2010). The urinary excretion of urea N was not affected (P>0.05) up to 0.67 kg/kg of replacement, but increased (P<0.05) with the total replacement of SBM by CMT (Table 8), due to the increase of the NPN in the diet (Table 2).

The utilization efficiency of the N ingested for the production of N in the milk was not affected (P>0.05) up to 0.33 kg/kg of replacement, but reduced (P<0.05) from the replacement of 0.67 kg/kg SBM by CMT (Table 8), due to the lower efficiency of capture of the N available in the rumen (RDP) for the synthesis of microbial N (Table 6). However, it must be emphasized that there was no negative mean value for NB at any of the levels of replacement of SBM by CMT, indicating that the N intake met the N requirement of cows (Table 8).

Conclusions

Castor seed meal treated with 60 g calcium oxide/kg (natural matter basis) can replace up to one third of the soybean meal in diets (inclusion of 50 g/kg DM) for dairy cows with production of 20 kg milk/day, without affecting the consumption of the digestible components of the diet, animal productive performance or the utilization efficiency of the nitrogenous compounds of the diet.

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