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Moreira Silva, Liliane; de Almeida Oliveira, Cláudio Henrique; Manoel Gomes da Silva, Cleidson; Silva, Aline Maia; Carneiro Linhares Fernandes, César; Ferro Furtado, Roselayne; Sousa Nunes-Pinheiro, Diana Célia; Florindo Guedes, Maria Izabel; Rondina, Davide

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# Use of castor meal (*Ricinus communis* L.) as a source of dietary protein in goats during the mating period: impact on reproductive and metabolic responses

# Uso de farelo de mamona (*Ricinus communis* L.) como fonte de proteína na dieta de caprinos durante o período de monta: impacto sobre a resposta reprodutiva e metabólica

Liliane Moreira Silva[1*]; Cláudio Henrique de Almeida Oliveira[2]; Cleidson Manoel Gomes da Silva[3]; Aline Maia Silva[4]; César Carneiro Linhares Fernandes[1]; Roselayne Ferro Furtado[5]; Diana Célia Sousa Nunes-Pinheiro[6]; Maria Izabel Florindo Guedes[7]; Davide Rondina[6]

## Abstract

The aim of the present study was to evaluate the effects of the total substitution of soybean meal with castor meal, detoxified or non-detoxified, on the response to estrous synchronization, conception rate, early fetal development, presence of IgG, and metabolic-hormonal response. Sixty mixed goats were fed diets without castor meal (WCM), with detoxified castor meal (DCM), and with castor meal (CM) during early pregnancy. The goats had their estrous synchronized and were then submitted to the mating season. The number of fetuses was determined by ultrasonography after 25 days of mating and their development was followed until 60 days of gestation. Plasma levels of progesterone ($P_4$), liver enzymes, and urea were determined along with the evaluation of the immunological response. After 15 days of experimental feeding, immunoglobulin G (IgG) was detected by western blotting only in goats that received non-detoxified castor meal. There was no effect ($p > 0.05$) of type of diet on response to estrous synchronization, plasma $P_4$ levels, conception rate, or embryonic/fetal development. In pregnant goats, there was an effect of diet ($p < 0.001$) on plasma urea levels in multiple-birth pregnancy, gamma-glutamyltransferase (GGT) in single-birth pregnancy, and lactate dehydrogenase (LDH) in both types of pregnancy. In non-pregnant goats, there were increased urea levels in all types of diets and in LDH in WCM goats, but GGT levels decreased in the WCM and CM goats when compared with pregnant goats ($p > 0.05$). In addition, plasma levels of LDH in WCM goats and of urea in all types of diet were higher in non-pregnant goats than pregnant goats. In conclusion, it can be inferred that the inclusion of 15% castor meal, whether or not it is detoxified, to the diet of goats does not affect the reproductive performance, embryonic and early fetal development, or blood metabolites.

**Key words:** Caprine, *Ricinus communis* L., pregnancy, fetal development, metabolites

[1] Discentes do Curso de Doutorado em Ciências Veterinária da Universidade Estadual do Ceará, UECE, Fortaleza, CE, Brasil. E-mail: liligomesvet@hotmail.com; caancesar@gmail.com

[2] Discente do Curso de Doutorado em Biotecnologia da Rede Nordeste de Biotecnologia, RENORBIO, Fortaleza, CE, Brasil. E-mail: claudiohao@hotmail.com

[3] Médico Veterinário, Dr. em Ciências Veterinárias, Pesquisador, UECE, Fortaleza, CE, Brasil. E-mail: gomesvet@hotmail.com

[4] Discente do Curso de Mestrado em Ciências Veterinárias, UECE, Fortaleza, CE, Brasil. E-mail: nine.maia@yahoo.com.br

[5] Bióloga, Drª em Biotecnologia, Pesquisadora da Embrapa Agroindústria Tropical, Fortaleza, CE, Brasil. E-mail: roselayne@cnpat.embrapa.br

[6] Profs., Adjuntos, UECE, Fortaleza, CE, Brasil. E-mail: diana@uece.br; davide.rondina@uece.br

[7] Profª Adjunta, UECE, Fortaleza, CE, Brasil. E-mail: izabel.guedes@uece.br

[*] Author for correspondence

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## Resumo

O objetivo do presente estudo foi avaliar o efeito da substituição total do farelo de soja por farelo de mamona detoxificado ou não sobre a resposta à sincronização do estro, taxa de concepção, desenvolvimento fetal inicial, presença de IgG e resposta metabólica-hormonal. Sessenta cabras mestiças foram alimentadas sem farelo de mamona (SFM), com farelo de mamona detoxificado (FMD) e com farelo de mamona (FM) durante a gestação inicial. Todos os animais tiveram o estro sincronizado e depois foram acasaladas por monta natural. A partir do 25º dia após a monta, foi determinado o número de fetos e realizado o acompanhado do desenvolvimento dos mesmos por ultrassonografia até os 60 dias de gestação. Foi avaliado o perfil de progesterona ($P_4$), os níveis de metabólitos e a resposta imunológica. A partir do 15º dia de alimentação a imunoglobulina G (IgG) foi marcada através da técnica de Western Blotting, apenas em animais que receberam farelo de mamona não detoxificado. Não houve efeito ($p>0,05$) do tipo de dieta sobre a resposta à sincronização do estro, níveis plasmáticos de $P_4$, taxa de gestação e desenvolvimento embrionário/fetal. Em cabras gestantes, observou-se um efeito da dieta ($p<0,001$) sobre os níveis plasmáticos de uréia em animais de gestação múltipla, de gama-glutamil transferase (GGT) em gestação simples e de lactato desidrogenase (LDH) em ambos os tipos de gestação. Verificou-se entre cabras não gestantes e gestantes um aumento significativo nos níveis de ureia em todos os tipos de dietas e de LDH em cabras do grupo SFM, porém os níveis de GGT diminuíram nos grupos SFM e FM em cabras não gestantes ($p>0,05$). Além disso, os níveis plasmáticos de LDH em cabras do grupo SFM e de ureia em todos os tipos de dieta foram maiores nas cabras não gestantes em comparação com cabras gestantes. Em conclusão, pode-se inferir que a inclusão de 15% de farelo de mamona detoxificado ou não na dieta de cabras não afeta o desempenho reprodutivo, bem como o desenvolvimento embrionário e fetal inicial e metabólitos no sangue.

**Palavras-chave:** Caprino, *Ricinus communis* L., gestação, desenvolvimento fetal, metabólitos

## Introduction

Small farmers in Northeast Brazil have received strong incentives for social inclusion in the production of biodiesel from oilseeds, mainly in the semi-arid region. Therefore, in recent years, considerable efforts have been made at extending the use of the castor bean (*Ricinus communis* L.), due to its great edaphoclimatic adaptability. However, its use has generated several environmental concerns with respect to the fate of residues produced, due to the presence of potentially toxic compounds (EFSA, 2008). Ricin is a toxin capable of inactivating protein synthesis in eukaryotic cells (AUDI et al., 2005). In sheep, Aslani et al. (2007) found that feed containing castor bean residues caused cardiovascular, hepatic, and gastrointestinal alterations, followed by death. Despite the presence of ricin in castor meal, studies have demonstrated that this bioproduct has a high protein value (41%) and good ruminal degradability (MOREIRA et al., 2003), which stimulates its use as an alternative dietary source for ruminants (DINIZ et al., 2011). Therefore, it is necessary to adopt methods that promote the inactivation of ricin. Alkaline treatment is the most recommended method for this (OLIVEIRA et al., 2010).

To date, majority of studies have evaluated the effect of castor meal as an alternative protein source for ruminants only with regard to nutritional and production performance aspects (OLIVEIRA et al., 2010; DINIZ et al., 2010), but no study has investigated the effects of a long-term diet with castor meal on reproductive performance in goats. Therefore, the aim of the present study was to evaluate the effect of the total substitution of soybean meal with castor meal, detoxified or non-detoxified, on the response to estrous synchronization, conception rate, early fetal development, presence of IgG, and metabolic-hormonal response in goats during early pregnancy.

## Material and Methods

*Detoxification of castor bean and quantification of ricin*

Castor meal was obtained from the factory BOM Brasil, located in Salvador, Bahia. Detoxification was carried out according to Oliveira et al. (2010), with some modifications. Castor meal was added to a solution of calcium oxide (CaO) (1 kg per 9 L of water) at a proportion of 60 g CaO/kg castor meal. After standing overnight (12-18 h), so that the detoxification process occurred, the treated material was dried for storage and later utilization. This technique was chosen because it was effective, simple, and easy to reproduce.

The absence of ricin after the detoxification process was verified by polyacrylamide gel electrophoresis in a 12% gel under non-denaturing conditions (native-PAGE), performed in a vertical mini-gel system with a Bio-Rad PowerPac Basic unit, where gels were stained with Coomassie Blue R250. Ricin was quantified by assaying the total proteins of samples of detoxified and non-detoxified castor meal following the method of Bradford (1976) with albumin serum bovine as the protein standard. Later, the image of the polyacrylamide gel was digitized in ImageScanner™ of GE Healthcare (compatible with ImageMaster software) and then analyzed by the program Image Master Platinum. The protein bands were quantified in volume units (area *vs*. intensity) following the method described by Meunier et al. (2005).

*Animals and experimental design*

The experiment was conducted on Padre João Piamarta Farm, in Itaitinga-CE, located at 4° 01′ S and 38° 31′ W, during April-July. The area, characterized by a constant photoperiod regimen, has a warm, tropical, sub-humid climate with a mean annual rainfall of 1,416.4 mm and temperature of 26-28°C, with two distinct seasons: rainy from January to May and dry from June to December

Sixty mixed-breed, cyclical, pluriparous goats were assigned into three homogeneous lots (n = 20) (mean ± SEM) considering weight (33.34 ± 1.05 kg), body condition (2.34 ± 0.09) and age (27.88 ± 0.95 months). Goats were kept in two pens separated by a central feed alley where they received mineral salt and water *ad libitum*. Each pen measured 40 × 50 m and contained a 40 × 3 m open front shelter. The feed alley and the front shelter were clay with concrete and faced in an east-west direction. Goats were submitted to 30 days of housing adaptation. During this period, internal and external parasite treatment and control of ovary function by ultrasonography was performed.

The female goats received three different diets composed of mixtures of guinea grass hay (*Panicum maximum* cv. *Mombasa*) and isoenergetic and isonitrogenous concentrates with different sources of nitrogen (Table 1 and 2): soybean meal (WCM), detoxified castor meal (DCM), or castor meal (CM). The formulation of diets was based on the nutritional requirements of mating and early pregnancy (NRC, 2007) and had the same concentrate:roughage ratio (30:70). The diets were provided twice a day (07:00 and 15:00) from 9 days before estrous synchronization up to the 60th day of pregnancy (Figure 1).

**Table 1.** Proportion of the diet ingredients in dry matter basis.

| Constituent (% DM) | Diet | | |
|---|---|---|---|
| | WCM | DCM | CM |
| Ground corn | 81.8 | 79.6 | 81,2 |
| Soybean meal | 12.1 | - | - |
| Detoxified castor meal | - | 14.5 | - |
| Castor meal | - | - | 12.9 |
| Urea | 1.0 | 1.0 | 1.0 |
| Vitamin mineralized premix | 4.3 | 4.1 | 4.1 |
| White salt | 0.8 | 0.8 | 0.8 |

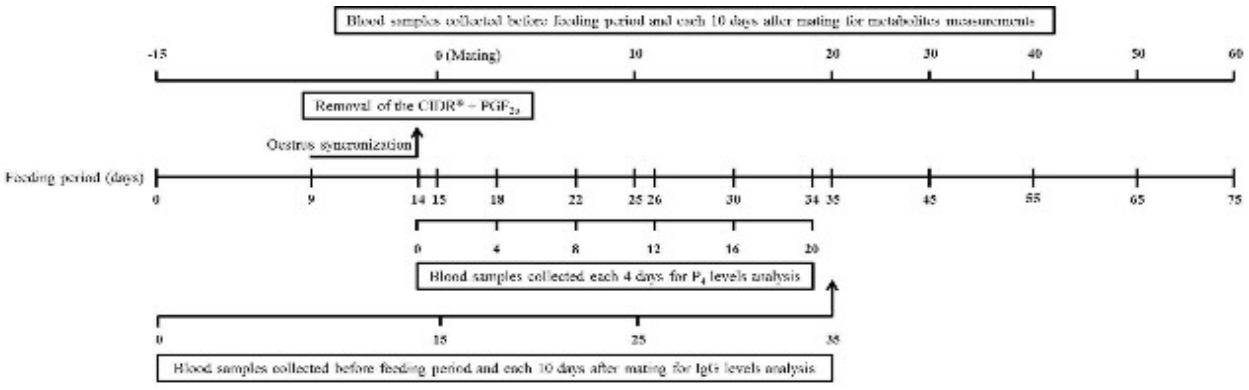
WCM – without castor meal; DCM – detoxified castor meal; CM – castor meal.
**Source:** Elaboration of the authors.

**Table 2.** Chemical composition of diets.

| Ingredients | Composition (% DM) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Organic Matter | Crude Protein | Ether Extract | Ash | Neutral Detergent Fiber | Acid Detergent Fiber | Total Digestible Nutrients |
| Guinea grass hay | 92.8 | 6.8 | 1.2 | 7.2 | 69.9 | 40.5 | 46.0 |
| Detoxified castor meal | 82.4 | 36.1 | 2.9 | 17.6 | 38.4 | 30.6 | 71.2 |
| Castor meal | 89.3 | 41.3 | 2.0 | 10.6 | 40.1 | 33.8 | 71.7 |
| *Concentrate – based supplements* | | | | | | | |
| WCM – diet | 97.2 | 15.0 | 3.1 | 2.8 | - | - | 73.5 |
| DCM – diet | 97.1 | 15.0 | 3.5 | 2.9 | - | - | 73.1 |
| CM – diet | 97.1 | 14.9 | 3.5 | 2.9 | - | - | 73.3 |

WCM – without castor meal; DCM – detoxified castor meal; CM – castor meal.
**Source:** Elaboration of the authors.

**Figure 1.** Protocol for oestrus synchronization and blood sample collection to measure $P_4$, IgG and metabolites levels in goats fed without castor cake, with castor cake and detoxified castor cake. $PGF_{2\alpha}$ : prostaglandin; $P_4$: progesterone.




**Source:** Elaboration of the authors.

Estrous was induced in all females using an intravaginal device (Controlled Internal Drug Release device, CIDR®) impregnated with 0.33 g $P_4$ (Eazi-Breed CIDR®, InterAg, Hamilton, New Zealand), which was put in place for 5 days. Upon removal of the CIDR, the goats received an application of 1 mL of prostaglandin F2α (Lutalyse®, Pfizer, Kalamazoo, USA), as described by Silva et al. (2011a). Next, males of proven fertility and marked in the sternal region were placed together with the females for a period of 72 h, allowing the mated females to be identified by the presence of the marker paint in the region of the croup.

*Western blotting*

The presence of IgG was assayed by western blotting according to Furtado et al. (2011), with some modifications. It utilized 12% polyacrylamide gel run under non-denaturing conditions with samples of crude extracts of detoxified and non-detoxified castor meal. Proteins in the gel were transferred to a nitrocellulose membrane using the semi-dry transfer system, utilizing transfer buffer and the application of 38 mA and 38 V for 2 h. After transfer, the membrane was blocked with 5% Molico® (Nestlé) in PBS and the primary antibody (goat plasma of females fed with detoxified or non-detoxified castor meal) was added. The membrane was then incubated in a humid chamber for 2 h at 37 °C and washed 4 times for 15 min with Molico® solution. Then, a secondary antibody (anti-goat IgG peroxidase conjugated, diluted 1:5000) was added, and the membrane was again incubated under the same conditions. After the incubation period, the membrane was washed twice with Molico® solution, once with PBS, and finally with water. Color development was performed with 30% hydrogen peroxide, nickel chloride, and Tris-HCl buffer, pH 7.2. The blood samples were collected before (Day 0) and on days 15, 25, and 35 after the feeding period (Figure 1).

*Blood sampling, liver enzymes, and urea and progesterone assays*

Blood samples were collected on days −15, 0, 10, 20, 30, 40, 50, and 60 after mating for the determination of metabolites in heparinized tubes by jugular venipuncture (Figure 1). Blood was centrifuged at 3000 rpm for 15 min, and the plasma obtained was frozen at −20 °C until analysis. Plasma concentrations of urea, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and gamma glutamyltransferase (GGT) were determined by spectrophotometric assays in an automated biochemical analyzer (Konelab, Wiener®), utilizing commercial kits (Wiener Laboratorios, Rosario, Argentina). In addition, upon removal of the CIDR® (day 0) and on days 4, 8 12, 16, and 20 after removal of the device, blood samples were collected for $P_4$ determination (Figure 1) by microparticle enzyme immunoassay (MEIA) (Abbott Diagnostics AxSYM® SYSTEM) using a commercial kit (Axsym $P_4$, Abbott Japan Co., Ltd, Tokyo 106-8535 Japan). The sensitivity of the test was 0.2 ng/mL, and the intra- and inter-assay coefficients of variation were 7.9% and 3.3%, respectively.

*Embryonic and fetal measurements*

Diagnosis of pregnancy and the determination of the number of fetuses and of embryonic/fetal development was carried out by real-time ultrasonography (Chisson D600 VET, Chisson Medical Imaging Co. Ltd., China) utilizing a linear transrectal transducer of 3.5-5.0 MHz. The fetal measurements in goats fed WCM (n = 18), DCM (n = 20) and CM (n = 20) were performed every five days. The following parameters were evaluated according to methods proposed by Santos et al. (2004): vesicle diameter (VD), from the 25th to 45th day of pregnancy; crown-rump length (CRL), from the 30th to 50th day of pregnancy; and biparietal diameter (BPD) and abdominal diameter (AD), from the 40th to 60th day of pregnancy. Thoracic

diameter (TD) was determined from the 45th to 60th day of pregnancy according to Lee et al. (2005).

For the measurement of structures of interest, ultrasonography examinations were recorded in the form of videos, followed by the capture and measure of at least three images of each structure and measurement using the previously calibrated Image J program (Image J, National Institutes of Health, Millersville, USA). In twin pregnancy, the mean of the two embryos/fetuses was considered, following the method described by Bulnes, Moreno and Sebastián (1998). The measurements were used to calculate the daily rates of embryonic/fetal growth (mm/day).

*Statistical analysis*

The data were analyzed utilizing PROC GLM in the statistical program SAS (SAS, Inc., Cary, NC, USA). For the liver and kidney parameters, $P_4$, and embryonic/fetal growth, the factors tested were diet (WCM, DCM, and CM), type of pregnancy (single or multiple births), time (time of measurement/sampling), and interactions of diet *vs.* pregnancy and diet *vs*. time. For metabolites in non-pregnant goats, the factors tested were diet and type of pregnancy diagnosis (pregnant or non-pregnant) and interaction. For progesterone concentration measured at CIDR removal, the effect used in the model was diet. The comparison between the means was analyzed by the Duncan test or Student *t*-test according to the number of treatments. For the variables with non-parametric distribution, the effect of diet was determined using PROC NPAR1WAY of SAS, and the frequencies using the chi-squared test. The values were expressed as the mean ± standard error of the mean and the numeric variables as the percentage or frequency.

## Results

Electrophoresis showed the absence of ricin in castor meal after detoxification. Non-detoxified castor meal was found to contain ricin at 50 mg/kg residue. At the beginning of the experimental period (Day −15), IgG was not detected in any group. However, after 15 days of experimental feeding, a positive immunological response was observed only in goats fed CM. In this group, the quantity of ricin supplied was approximately 34.01 g/day/animal, equivalent to 0.043 mg/kg live weight.

There was no effect of type of diet ($p > 0.05$) on the plasma concentration of $P_4$ on the day of CIDR removal, number of goats in estrous, rate of multiple-birth pregnancy, or total conception rate (Table 3). Plasma $P_4$ levels increased ($p < 0.001$) at the measurement time evaluated (Figure 2), and were higher than 3 ng/mL in all groups evaluated eight days after the intravaginal device removal. In goats with multiple-birth pregnancy, the plasma level of this hormone was higher than in goats with single-birth pregnancy (6.34 ± 0.38 ng/mL *vs*. 4.53 ± 0.25 ng/mL; $p < 0.001$). Besides, there was observed an effect of type of diet ($p < 0.01$) on plasma $P_4$ levels in goats with multiple-birth pregnancy, where the DCM group had higher levels than the WCM group (7.18 ± 0.77 ng/mL *vs*. 5.87 ± 0.53 ng/mL; $p < 0.05$).

The data relative to the parameters of embryonic/fetal development (Table 4) demonstrated, in all diets and in both types of pregnancy, a significant increase in fetal growth ($p < 0.001$) during the time interval recorded. However, there were no differences between the types of diets ($p > 0.05$).

With regard to the main liver and kidney function parameters in pregnant goats (Figure 3), an effect of diet was observed ($p < 0.001$) on plasma urea levels in multiple-birth pregnancy, GGT in single-birth pregnancy, and LDH in both types of pregnancy. The WCM group showed higher mean urea levels (28.70 ± 1.07 mg/dL) compared to the other diets (DCM 22.67 ± 1.31; CM 22.92 ± 0.99; $p < 0.001$). For GGT, goats fed CM showed higher mean concentrations (50.82 ± 1.41 IU/L) (WCM 43.48 ± 1.25; DCM 39.50 ± 0.80; $p < 0.001$). Moreover,

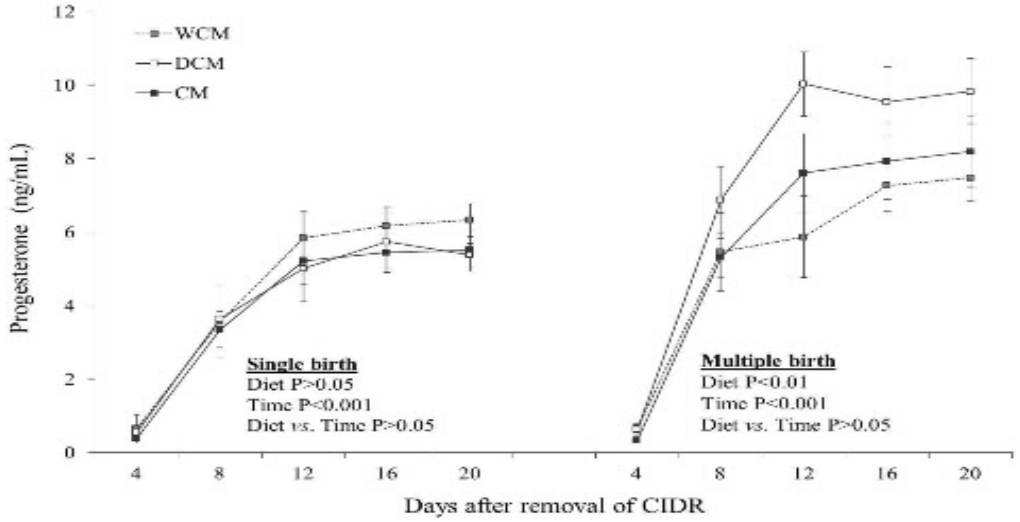
LDH (IU/L) in goats with single-birth pregnancy increased in the WCM group (WCM 736.88 ± 36.03 *vs*. DCM 625.50 ± 24.01 *vs*. CM 600.73 ± 21.32; p < 0.001), whereas in multiple-birth pregnancy, the CM group displayed lower levels of this parameter (CM 471.07 ± 23.36 *vs*. WCM 616.67 ± 28.45 *vs*. DCM 631.00 ± 23.03; p < 0.001).

**Table 3.** Concentration of progesterone ($P_4$) on day of CIDR removal and reproductive performance of goats fed diets without castor meal (WCM), with detoxified castor meal (DCM) and with castor meal (CM).

| Parameters | Groups | | | SEM | p |
|---|---|---|---|---|---|
| | WCM | DCM | CM | | |
| Concentration of $P_4$ on day of CIDR removal (ng/mL) | 7.9 | 6.8 | 6.6 | 0.43 | ns |
| Response to estrus (%) | 75.0 (15/20) | 80.0 (16/20) | 85.0 (17/20) | | ns |
| Total pregnancy rate (%) | 80.0 (12/15) | 87.5 (14/16) | 88.2 (15/17) | | ns |
| Rate of multiple gestation (%) | 50.0 (6/12) | 42.8 (6/14) | 33.3 (5/15) | | ns |

SEM = standard error of the mean.
ns = not significant (p>0.05).
**Source:** Elaboration of the authors.

**Figure 2.** Plasma $P_4$ concentrations in pregnant goats that had single and multiple births, up to day 20 after CIDR removal. Values are means ± SE (standard error). ANOVA results for the effects of diet, time and the interaction diet *vs.* time are represented in the figure. WCM – without castor meal; DCM – detoxified castor meal; CM – castor meal.




**Source:** Elaboration of the authors.

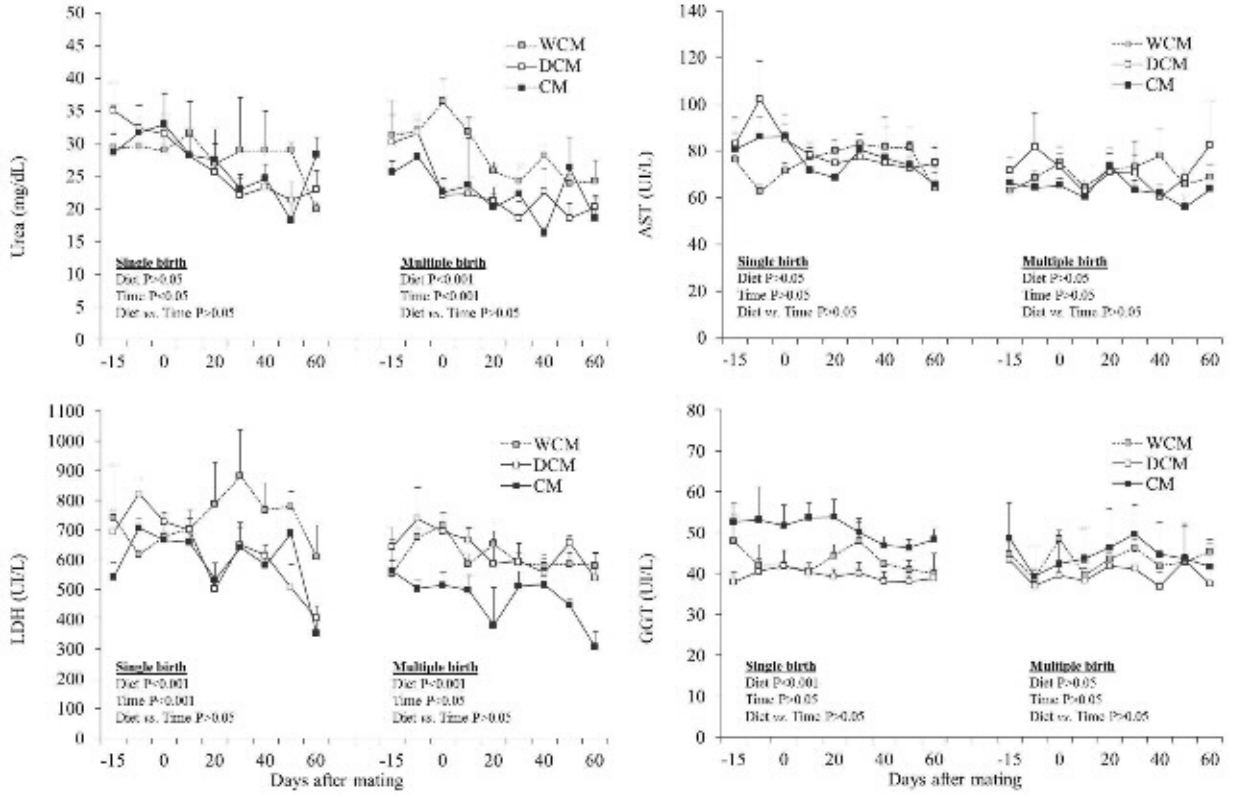
**Table 4.** Embryonic/fetal growth rate (mm/day) in goats fed diets without castor meal (WCM, n=18), with detoxified castor meal (DCM, n=20) and with castor meal (CM, n=20), that showed single and multiple births.

| Parameters | Single birth | | | Multiple birth | | | | |
|---|---|---|---|---|---|---|---|---|
| | WCM | DCM | CM | WCM | DCM | CM | SEM | p |
| Embryonic structure | | | | | | | | |
| Vesicle diameter | | | | | | | | |
| Initial | 17.25$^{A}$ | 16.34$^{A}$ | 17.02$^{A}$ | 16.55$^{A}$ | 16.50 $^{A}$ | 16.11$^{A}$ | 0.09 | ns |
| Final | 38.73$^{B}$ | 39.12$^{B}$ | 38.55$^{B}$ | 39.35$^{B}$ | 38.97$^{B}$ | 40.30$^{B}$ | 0.17 | ns |
| Growth rate | 1.07 | 1.14 | 1.08 | 1.14 | 1.12 | 1.21 | 0.04 | ns |
| Fetal structure | | | | | | | | |
| Crown-rump length | | | | | | | | |
| Initial | 8.51$^{A}$ | 8.79$^{A}$ | 8.79$^{A}$ | 9.01$^{A}$ | 8.95$^{A}$ | 8.72$^{A}$ | 0.05 | ns |
| Final | 38.35$^{B}$ | 36.40$^{B}$ | 36.63$^{B}$ | 35.32$^{B}$ | 36.44$^{B}$ | 36.85$^{B}$ | 0.22 | ns |
| Growth rate | 0.55 | 0.57 | 0.57 | 0.60 | 0.55 | 0.54 | 0.05 | ns |
| Thoracic diameter | | | | | | | | |
| Initial | 8.58$^{A}$ | 8.62$^{A}$ | 8.70$^{A}$ | 8.51$^{A}$ | 8.57$^{A}$ | 8.81$^{A}$ | 0.06 | ns |
| Final | 16.82$^{B}$ | 17.19$^{B}$ | 17.26$^{B}$ | 17.56$^{B}$ | 16.83$^{B}$ | 16.90$^{B}$ | 0.07 | ns |
| Growth rate | 1.49 | 1.38 | 1.38 | 1.32 | 1.37 | 1.41 | 0.01 | ns |
| Abdominal diameter | | | | | | | | |
| Initial | 8.86$^{A}$ | 8.71$^{A}$ | 8.93$^{A}$ | 8.88$^{A}$ | 8.79$^{A}$ | 8.59$^{A}$ | 0.04 | ns |
| Final | 19.15$^{B}$ | 9.89$^{B}$ | 19.45$^{B}$ | 20.00$^{B}$ | 19.37$^{B}$ | 19.33$^{B}$ | 0.08 | ns |
| Growth rate | 0.51 | 0.56 | 0.53 | 0.56 | 0.53 | 0.54 | 0.02 | ns |
| Biparietal diameter | | | | | | | | |
| Initial | 8.25$^{A}$ | 8.21$^{A}$ | 8.05$^{A}$ | 8.21$^{A}$ | 8.10$^{A}$ | 7.92$^{A}$ | 0.04 | ns |
| Final | 14.61$^{B}$ | 14.94$^{B}$ | 14.57$^{B}$ | 14.17$^{B}$ | 14.16$^{B}$ | 14.63$^{B}$ | 0.07 | ns |
| Growth rate | 0.32 | 0.34 | 0.33 | 0.30 | 0.30 | 0.34 | 0.01 | ns |

SEM = standard error of the mean.
$^{A, B}$ = Mean values in the same column with different superscripts differ significantly ($p < 0.05$).
ns = not significant ($p>0.05$).
**Source:** Elaboration of the authors.

A decrease with time was observed ($p < 0.05$) for the plasma levels of urea and LDH in both types of pregnancy (Figure 3). In the goats with a negative pregnancy diagnosis, no significant differences were found for liver and kidney function parameters (GGT, AST, LDH, and urea) between the types of diets (Table 5). The levels of these parameters were also compared between non-pregnant and pregnant goats within each type of diet (Table 5). There was an increase in the urea levels in all types of diets and LDH in the WCM group, but GGT levels decreased in the WCM and CM groups in non-pregnant goats ($p > 0.05$).

**Figure 3.** Plasma concentrations of urea, LDH, AST and GGT in pregnant goats with single and multiple births. ANOVA results for the effects of diet, time and the interaction diet *vs.* time are represented in the figure. WCM – without castor meal; DCM – detoxified castor meal; CM – castor meal.




**Source:** Elaboration of the authors.

**Table 5.** Plasma concentrations of different metabolites in animals negative for diagnosis of pregnancy fed diets without castor meal (WCM), with detoxified castor meal (DCM) and with castor meal (CM).

| Parameters | WCM | NP *vs*. P* | DCM | NP *vs*. P | CM | NP *vs*. P | SEM |
|---|---|---|---|---|---|---|---|
| GGT (UI/L) | 39.70 | < | 42.50 | ns | 44.46 | < | 1.00 |
| AST (UI/L) | 80.10 | ns | 85.85 | ns | 71.80 | ns | 1.88 |
| LDH (UI/L) | 592.70 | > | 609.90 | ns | 640.71 | ns | 16.41 |
| Urea (mg/dL) | 35.67 | > | 35.20 | > | 35.82 | > | 1.04 |

*NP = non-pregnant. P = pregnant.
SEM = standard error of the mean.
< = low P < 0.05. comparison of non-pregnant *vs*. pregnant animals in the same diet group.
> = high P < 0.05. comparison of non-pregnant *vs*. pregnant animals in the same diet group.
ns = not significant. comparison of non-pregnant *vs*. pregnant animals in the same diet group.
**Source:** Elaboration of the authors.

## Discussion

Many studies have demonstrated the efficacy of different physical and chemical methods for the detoxification of castor bean meal (ANANDAN et al., 2005). Recently, the EMBRAPA (2010) was successful in the detoxification of castor bean meal after autoclaving or treatment with sodium hydroxide for feeding small ruminants. In another study, Oliveira et al. (2010) showed that it is possible efficiently detoxify castor seed meal with calcium oxide, obtaining a safe product for feeding to ruminants. In addition, the Department of Science and Food Technology, in partnership with Department of Agricultural Economics of the Federal University of Viçosa (LUANA, 2009), after a careful cost analysis, found that the detoxification of the by-products of castor oil using calcium oxide is effective and economically viable for small producers. Therefore, because of the simplicity, practicability, and economic viability of the use of calcium to detoxify castor meal, we believe that this method is more suitable for farm application.

The immunological response resulting from the ingestion of castor meal was determined by the detection of ricin-specific IgG by Western blotting. After Day 15 of experimental feeding, the specific IgG protein was detected only in goats that received non-detoxified castor meal. These results demonstrated that the ingestion of non-detoxified castor meal induced a humoral response, probably through the production of antibodies against ricin and/or ricinin. Thus, it can be inferred that the presence of IgG is a good indicator of the magnitude of the immunological response to non-detoxified castor meal. Besides, IgG normally appears after a prolonged exposure to the antigen (ZANIN; MARCHINI; CARVALHO, 2002).

In the present study, no effect of diet was observed on the response to estrous synchronization, levels of $P_4$ at the time of CIDR removal, conception rate, or twinning rate in adult goats. These findings demonstrate that the total substitution of soybean meal with detoxified or non-detoxified castor meal does not reduce the reproductive performance of goats. In previous studies by our group, we observed that the inclusion of 50% cashew bagasse in the diet of sheep during the post-partum period also did not influence the response to estrous synchronization (RODRIGUES et al., 2011). Thus, it is believed that the expression of estrous is directly related to the levels of the animal's body reserves (SILVA et al., 2011a), but not to the intake of alternative dietary sources. Besides, studies with goats (OLIVEIRA; GUIDO; LIMA, 2001) and cows (FLORES et al., 2006) demonstrated that the utilization of CIDR in estrous synchronization protocols is a highly efficient treatment.

During the monitoring period (4-20 days after mating), independent of the type of diet, plasma $P_4$ levels increased in pregnant goats with single or multiple births. These results demonstrated that the corpus luteum was functional, producing adequate concentrations of $P_4$ during the period of embryonic implantation. These observations are in accordance with the findings of Kerbler et al. (1997), who reported that the luteal secretion of $P_4$ is essential for the production of oocytes of good quality, as well as for embryonic survival in sheep. In addition, $P_4$ levels have been successfully utilized for the evaluation of ovarian function in goats (SILVA et al., 2011a) and sheep (RODRIGUES et al., 2011).

In the present study, type of diet (WCM, DCM, or CM) had no effect on the embryonic/early fetal growth of goats. These results can be explained by the capacity of ruminal microbiota to partially or totally neutralize the toxic compounds (ricin and/or ricinin) present in castor meal (OLIVEIRA et al., 2010; DINIZ et al., 2010). In agreement with EFSA (2008), well-adapted ruminants can tolerate moderate levels of ricin over long periods of exposure without substantial impact on reproductive performance. In another study, Barros et al. (2011) reported that the substitution of soybean meal with castor meal treated with calcium oxide did not affect production performance in Nelore heifers. Thus,

it can be inferred that the addition of castor meal, detoxified or not, to the diet of goats does not affect early fetal development.

The plasma levels of urea with all types of diets and of LDH in the WCM group were higher in non-pregnant goats than pregnant goats. However, urea and LDH levels in both pregnant and non-pregnant goats tended to decrease over time regardless of the type of diet. In pregnant goats, there was a significant decrease in urea and LDH levels in goats fed DCM and CM, while GGT levels increased only in females fed with CM when compared to goats fed WCM. These results are not in agreement with Silva et al. (2010), who reported that plasma urea levels in sheep with detoxified castor meal were unaltered by the end of the supplementation. In another study, Oliveira et al. (2010) also did not observe alterations in plasma levels of ALT and AST in sheep fed or castor meal detoxified for 21 days. In the present study, despite variation in the levels of urea, LDH, and GGT in goats fed CM, the levels of these physiologic parameters are in agreement with the literature for goats (SMITH; SHERMAN, 2009).

The information currently available does not allow conclusive inferences about the possible interference of detoxified castor bean meal on nutritional metabolism. Some studies have shown that detoxified castor meal can totally replace (100%) soybean meal without causing changes in carcass characteristics in bovine (DINIZ et al., 2011) and ovine (VIEIRA; CÂNDIDO; BOMFIM, 2010), liver function (OLIVEIRA et al., 2010), or plasma levels of urea, hemoglobin, total protein, glucose, AST, ALT, and calcium (MENEZES et al., 2012). However, the use of detoxified castor bean meal in place of soybean meal can negatively affect some productivity parameters. Cobianchi et al. (2012) observed that the substitution of 0.67 kg/kg of soybean meal by detoxified castor bean meal with calcium oxide reduced production and milk composition in cows with daily production of 20 kg. In another study, Pompeu et al. (2012) found that every 1% replacement of soybean meal by castor bean meal was estimated to decrease average daily weight gain by 0.62 g/day. Additionally, Silva et al. (2011b) reported that replacing more than 67% of soybean meal by detoxified castor bean meal reduced the digestibility of dry matter, organic matter, crude protein, and total carbohydrates in finishing animals. On the basis of these results, it is plausible to hypothesize that the discrepancy between the studies found in the literature may be related to differences in species, experimental conditions, and different periods evaluated, making it difficult to compare results obtained by different research groups. Thus, additional studies using more robust techniques are needed to accurately assess the effect of the use of detoxified castor bean meal on ruminant metabolism.

## Conclusions

The supplementation with castor meal before and after detoxification process did not affect the reproductive performance of goats, as well as the embryonic and early fetal development, and the blood metabolites. Therefore, detoxification process is not necessary when castor meal is included up to 15% in goat feeding during early pregnancy.

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## Ethics and Biosafety Committee

The study was approved by the Ethics Commission for the Use of Animals of the State University of Ceará (CEUA-UECE), under Protocol nº. 09503497-8/82.

## References


ANANDAN, S.; ANIL KUMAR, G. K.; GHOSH, J.; RAMACHANDRA, K. S. Effect of different physical and chemical treatments on detoxification of ricin in castor cake. *Animal Feed Science and Technology*, Madri, v. 120, n. 1-2, p. 159-168, 2005.

ASLANI, M. R.; MALEKI, M.; MOHRI, M.; SHARIFI, K.; NAJJAR-NEZHAD, V.; AFSHARI, E. Castor bean (*Ricinus communis*) toxicosis in a sheep flock. *Toxicology,* Hamburg*,* v. 49, n. 3, p. 400-406, 2007.

AUDI, J.; BELSON, M.; PATEL, M.; SCHIER, J.; OSTERLOH, J. Ricin poisoning: a comprehensive review. *The Journal of the American Medical Association*, Boston, v. 294, n. 18, p. 2342-2351, 2005.

BARROS, L. V.; PAULINO, M. F.; DETMANN, E.; VALADARES FILHO, S. C.; LO1PES, S. A.; ROCHA, A. A.; VALENTE, É. E. L.; ALMEIDA, D. M. Replacement of soybean meal by treated castor meal in supplements for grazing heifer during the dry-rainy season period. *Revista Brasileira de Zootecnia,* Visoça, MG, v. 40, n. 4. p. 843-851, 2011.

BRADFORD, M. M. A rapid sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry*, Bethesda, v. 72, n. 7, p. 248-254, 1976.

BULNES, A. G.; MORENO, J. S.; SEBASTIÁN, A. L. Estimation of fetal development in Manchega dairy ewes by transrectal ultrasonographic measurements. *Small Ruminant Research*, Bloemfontein, v. 27, n. 3, p. 243-250, 1998.

COBIANCHI, J. V.; OLIVEIRA, A. S.; CAMPOS, J. M. S.; GUIMARÃES, A. V.; VALADARES FILHO, S. C.; COBIANCHI, F. P.; OLIVEIRA, T. E. S. Productive performance and efficiency of utilization of the diet components in dairy cows fed castor meal treated with calcium oxide. *Revista Brasileira de Zootecnia*, Visoça, MG, v. 41, n. 10, p. 2238-2248, 2012.

DINIZ, L. L.; VALADARES FILHO, S. C.; CAMPOS, J. M. S.; VALADARES, R. F. D.; SILVA, L. D.; MONNERAT, J. P. I. S.; BENEDETI, P. B.; OLIVEIRA, A. S.; PINA, D. S. Effects of castor meal on the growth performance and carcass characteristics of beef cattle. *Asian-Australasian Journal of Animal Sciences*, Korea, v. 23, n. 10, p. 1308-1318, 2010.

DINIZ, L. L.; VALADARES FILHO, S. C.; OLIVEIRA, A. S.; PINA, D. S.; SILVA, L. D.; BENEDETI, P. B.; BAIÃO, G. F.; CAMPOS, J. M. S.; VALADARES, R. F. D. Castor bean meal for cattle finishing: 1 – nutritional parameters. *Livestock Science*, Foulum, v. 135, n. 2-3, p. 153-167, 2011.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA – EMBRAPA. Destoxificação da torta de mamona para uso em ração animal. Brasília, 2010. Disponível em: http://www.hotsites.sct.embrapa.br/diacampo/programacao/2010/destoxicacao-da-torta-de-mamona-para-uso-em-racao-animal>. Acesso em: 15 ago. 2014.

EUROPEAN FOOD SAFETY AUTHORITY – EFSA. Scientific opinion of the panel on contaminants in the food chain on a request from the European Commission on ricin as undesirable substances in animal feed. Parma: The EFSA Journal, 2008. v. 726, p. 1-38.

FLORES, R.; LOOPER, M. L.; KREIDER, D. L.; POST, N. M.; ROSENKRANS JÚNIOR, C. F. Estrous behavior and initiation of estrous cycles in postpartum Brahman-influenced cows after treatment with progesterone and prostaglandin F2{alpha}. *Journal of Animal Science*, Storrs, v. 84, n. 7, p. 1916-1925, 2006.

FURTADO, R. F.; GUEDES, M. I. F.; ALVES, C. R.; MOREIRA, A. C. O. M.; FELIX, W. P.; DUTRA, R. A. F. Produção de anticorpos policlonais anti-ricina. *Ciência e Agrotecnologia*, Lavras, v. 35, n. 1, p. 124-130, 2011.

KERBLER, T. L.; BUHR, M. M.; JORDAN, L. T.; LESLIE, K. E.; WALTON, J. S. Relationship between maternal plasma progesterone concentration and interferon-tau synthesis by the conceptus in cattle. *Theriogenology*, Milan, v. 47, n. 3, p. 703-714, 1997.

LEE, Y.; LEE, O.; CHO, J.; SHIN, H.; CHOI, Y.; SHIM, Y.; CHOI, W.; SHIN, H.; LEE, D.; LEE, G.; SHIN, S. Ultrasonic measurement of fetal parameters for estimation of gestational age in Korean Black Goats. *Journal of Veterinary Medicine Science*, Tokyo, v. 67, n. 5, p. 497-502, 2005.

LUANA, C. A. S. *Análise de viabilidade econômica do processo de pequeno porte para extração de óleo de mamona com solvente e da produção de farelo de mamona detoxicado*. 2009. Dissertação (Mestrado em Ciência e Tecnologia de Alimentos) – Universidade Federal de Viçosa, Viçosa, MG.

MENEZES, D. R.; COSTA, R. G.; ARAÚJO, G. G. L.; PEREIRA, L. G. R.; OLIVEIRA, P. T. L.; SILVA, A. E. V. N.; VOLTOLINI, T. V.; MORAES, S. A. Parâmetros sanguíneos, hepáticos e ruminais de ovinos alimentados com dietas com farelo de mamona destoxificado. *Pesquisa Agropecuária Brasileira*, Brasília, v. 47, n. 1, p. 103-110, 2012.

MEUNIER, B.; BOULEY, J.; PIEC, I.; BERNARD, C.; PICARD, B.; HOCQUETTE, J. F. Data analysis methods for detection of differential protein expression in two-dimensional gel electrophoresis. *Analytical Biochemistry*, Bethesda, v. 340, n. 2, p. 226-230, 2005.

MOREIRA, J. F. C.; RODRÍGUEZ, N. M.; FERNANDES, P. C. C.; VELOSO, C. M.; SALIBA, E. O. S.; GONÇALVES, L. C.; BORGES, I.; BORGES, A. L. C. C. Concentrados protéicos para bovinos. 1. Digestibilidade *in situ* da matéria seca e da proteína bruta. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 55, n. 3, p. 315-323, 2003.

NATIONAL RESEARCH COUNCIL – NRC. Nutrient requirements of small ruminants: sheep, goats, cervids and new world camelids. Washington: The National Academies Press, 2007. 384 p.

OLIVEIRA, A. S.; CAMPOS, J. M. S.; OLIVEIRA, M. R. C.; BRITO, A. F.; VALADARES FILHO, S. C.; DETMANN, E.; VALADARES, R. F. D.; SOUZA, S. M.; MACHADO, O. L. T. Nutrient digestibility, nitrogen metabolism and hepatic function of sheep fed diets containing solvent or expeller castorseed meal treated with calcium hydroxide. *Animal Feed Science and Technology*, Madrid, v. 158, n. 2-3, p. 15-28, 2010.

OLIVEIRA, M. A. L.; GUIDO, S. I.; LIMA, P. F. Comparison of different protocols used to induced and synchronize estrus cycle of Saanen goats. *Small Ruminant Research*, Bloemfontein, v. 40, n. 2, p. 149-153, 2001.

POMPEU, R. C. F. F.; CÂNDIDO, M. J. D.; PEREIRA, E. S.; BOMFIM, M. A. D.; CARNEIRO, M. S. S.; ROGÉRIO, C. P.; SOMBRA, W. A.; LOPES, M. N. Desempenho produtivo e características de carcaça de ovinos em confinamento alimentados com rações contendo torta de mamona destoxificada em substituição ao farelo de soja. *Revista Brasileira de Zootecnia*, Visoça, MG, v. 41, n. 3, p. 726-733, 2012.

RODRIGUES, M. R. C.; RONDINA, D.; ARAÚJO, A. A.; SOUZA, A. L.; NUNES-PINHEIRO, D. C.; FERNANDES, A. A. O.; IBIAPINA, F. L. Respostas reprodutivas e metabólicas de ovelhas alimentadas com bagaço de caju desidratado, durante o pós-parto. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 63, n. 1, p. 171-179, 2011.

SANTOS, M. H. B.; LIMA, P. F.; MESSIAS, J. B.; OLIVEIRA, M. A. L. Medidas do concepto utilizadas na prática ultra-sonográfica de pequenos ruminantes. In: SANTOS, M. H. B.; OLIVEIRA, M. A. L.; LIMA, P. F. *Diagnóstico de gestação na cabra e na ovelha.* São Paulo: Varela, 2004. p. 137-148.

SILVA, D. C.; ALVES, A. A.; OLIVEIRA, M. E.; MOREIRA FILHO, M. A.; RODRIGUES, M. M.; VALE, G. E. S.; NASCIMENTO, H. T. S. Consumo e digestibilidade de dietas contendo farelo de mamona destoxificado para ovinos em terminação. *Revista Brasileira de Saúde e Produção Animal*, Salvador, v. 12, n. 1, p. 96-106, 2011b.

SILVA, D. C.; ALVES, A. A.; VASCONCELOS, V. R.; NASCIMENTO, H. T. S.; MOREIRA FILHO, M. A.; OLIVEIRA, M. E. Metabolismo dos compostos nitrogenados em ovinos alimentados com dietas contendo farelo de mamona destoxificado. *Acta Scientiarum. Animal Sciences*, Maringá, v. 32, n. 2, p. 219-224, 2010.

SILVA, L. M.; RONDINA, D.; ARAÚJO, A. A.; SARGENTINI, C.; LIMA, I. M. T.; RODRIGUES, M. R. C.; SOUZA, A. L.; GIORGETTI, A.; OLIVEIRA, C. H. A.; RODRIGUES, F. V. Reproductive responses and progesterone levels of post-partum oestrus synchronization in goats with different body reserves. *Italian Journal of Animal Science*, Pavia, v. 10, n. 4, p. 220-224, 2011a.

SMITH, M. C.; SHERMAN, D. M. *Goat medicine*. 2th ed. Ames: Wiley-Blackwell, 2009. 871 p.

VIEIRA, M. M. M.; CÂNDIDO, M. J. D.; BOMFIM, M. A. D. Características da carcaça e dos componentes não-carcaça em ovinos alimentados com rações à base de farelo de mamona. *Revista Brasileira de Saúde e Produção Animal*, Salvador, v. 11, n. 1, p. 140-149, 2010.

ZANIN, C. M.; MARCHINI, J. S.; CARVALHO, I. F. Reações adversas a alimentos e imunidade humoral: subclasses de IgG a antígenos alimentares. *Nutrire: Journal of the Brazilian Society of Food and Nutrition*, São Paulo, v. 24, n. 1, p. 125-134, 2002.