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Growth, testis size, spermatogenesis, semen parameters and seminal plasma and sperm membrane protein profile during the reproductive development of male goats supplemented with de-oiled castor cake

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ABSTRACT

The present study was conducted to evaluate the effect of de-oiled castor cake on reproductive traits of crossbreed goats. Fourteen males were grouped into two lots ($n = 7$/group), as described: group without de-oiled castor cake (WCC) and group fed with de-oiled castor cake (CC). Goats received two diets containing a mixture of Bermudagrass hay and concentrates with the same energy (73% total digestive nutrients) and protein content (15% crude protein) during 150 days, corresponding to ages from 40 (puberty) to 60 weeks. Blood plasma concentrations of urea, albumin, lactate dehydrogenase, creatinine, alanine aminotransferase and testosterone were determined. We also evaluated scrotal circumference, sperm parameters, quantitative aspects of spermatogenesis and daily sperm production (DSP), as well as the proteome of seminal plasma and sperm membrane. Seminal fluid and sperm proteins were analyzed by 2D SDS–PAGE and mass spectrometry. After 150 days of castor cake feeding, animals had no changes in the biochemical composition of blood plasma, suggesting the absence of intoxication by ingestion of ricin. There were no alterations in dry mater intake, weight gain, testis size, peripheral concentrations of testosterone, sperm concentration, motility and morphology. Sertoli and germ cell populations in the testis and DSP were not affected either. However, there were significant variations in the expression of five seminal plasma proteins and four sperm membrane proteins. In conclusion, the replacement of soybean meal by castor cake (with ricin concentrations of 50 mg/kg) did not interfere with the growth and core reproductive development of male goats. However, the diet with ricin altered the expression of certain seminal plasma and sperm membrane proteins, which play roles in sperm function and fertilization. Lower expression of these proteins may impair the ricin-fed animals to perform as high-fertility sires.

1. Introduction

Oleaginous seeds such as castor (*Ricinus communis* L.) have been used for biodiesel production worldwide. Consequently, this process generates a by-product known as castor cake, which has high protein concentration [1] and proper ruminal degradability [2]. Despite these desirable nutritional aspects, castor cake contains ricin, an inhibitor of ribosomal activity, ricinine, an alkaloid with low toxicity, and allergenic proteins known as CB-1A [3]. Thus, castor cake has in fact potential use as a feeding source but the precise effects of their toxins on animal reproduction need to be evaluated in detail.

Based on results obtained in previous studies, The European Food Safety Authority [4] reports that cattle fed with castor residues for 14 months did not show fertility problems or clinical signs of intoxication, tolerating 15–20 mg ricin per kg of body weight. Also, Silva et al. [5] provided de-oiled castor cake for sheep and found no

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effect of feeding on folliculogenesis. In contrast, Sandhyakumary et al. [6] and Nithya et al. [7] observed that oral administration of the root extract of *R. communis* for rodents caused reductions in both sperm concentrations and motility and increased morphologic alterations of spermatozoa through suppression of testosterone production. Thus, it is clear that the type of ricin-containing feed stuff may affect the reproduction of males and females in several species but conclusive studies about the subject are still absent. Therefore, the present study was conducted to evaluate the influence of de-oiled castor cake on reproductive parameters and development of crossbred male goats.

2. Material and methods

2.1. Castor cake and ricin quantification

The present study represents an effort to investigate how ricin-containing diets influence animal reproduction, following a primary evaluation focused on effects of castor cake on anatomical components, meat quality and meat proteome of goats [8]. As described therein, castor cake was purchased from BOM Brasil Company (Salvador, Bahia, Brazil; 12°55′S and 38°29′W). To calculate ricin concentration in the de-oiled castor cake [8], samples of castor cake were diluted in 0.15 M NaCl (1:10), homogenized for 30 min and centrifuged at 32,980 × *g* (20 min, 4 °C) for separation and collection of the supernatant. Then, the total protein concentration was quantified in the supernatant samples according to Bradford's method [9], using BSA as a standard. Next, samples were subjected to 1D electrophoresis (12%), under non-denaturing conditions (native PAGE). Gels were run at a constant current (20 mA) and 120 V for 90 min (Mini-gel vertical Bio-Rad PowerPac Basic Supply; Bio Rad, USA). Gels were stained with Coomassie Blue R250, scanned (One Touch scanner, Visioneer, USA) and analyzed with the Image Master Platinum software (GE Healthcare, USA). Protein bands were automatically detected by the software, with manual adjustments when necessary. Bands were evaluated in volume units (area times intensity) after normalization based on the volume of each band as related to the total volume of all bands detected in the gel, according to software's instructions. Ricin has a molecular weight of approximately 65 kDa [10]. Thus, ricin concentration was established by the direct relation between the total volume of each band at 65 kDa and total protein quantified in the samples [11].

2.2. Animals and experimental design

The present investigation was carried out in the Northeast of Brazil (4°01′S and 38°31′W), from June to November. This study was approved by the Ethics Committee for Animal use of the State University of Ceará (protocol # 09503497-8/82), which follows the regulations from the National Council for Control of Animal Experimentation (Law # 11,794; October 8, 2008; Brazil). Starting at 32 weeks of age, 14 cross-bred male goats were evaluated every 15 days to verify the occurrence of penile detachment from the prepuce and seminal parameters. During this period, the animals were kept in individual and shaded pens, with free access to water and salt, and subjected to 30 days of housing adaptation. Penile detachment was evaluated and given a scale from zero to five (0 = completely attached, 5 = completely detached), as described before [12]. Then, puberty of the male goats was defined as the time when the penis was completely detached (score 5) and ejaculates had a minimum of 0.6 ml, sperm concentration $\geq 0.8 \times 10^9$ cells/ml and wave motion ≥2. At this developmental state, two groups of seven animals each were allocated into treatments. Treatments consisted of two diets, both composed of Bermuda grass (*Cynodon dactylon*) hay and concentrates with the same energy (73% total digestive nutrients) and protein content (15% crude protein). By choosing concentrates with such composition, we were able to evaluate the diet effects based only on ricin content, avoiding any potential bias caused by differences in energy and protein content. As described in detail [8], the current study used control (WCC) and de-oiled castor cake diets (CC). The control diet included Bermuda grass hay and concentrate with corn (80%) and soybean meal (15%). In the second group, goats were fed hay and concentrate containing de-oiled castor cake (15%) and corn (80%). Both rations contained 5% of minerals and a mixture of urea/ammonium sulfate (9:1) was used to adjust the crude protein content of the diet. Body weight and age of animals were, respectively, 21.3 ± 1.7 kg and 282.7 ± 2.0 days (WCC group) and 21.3 ± 1.2 kg and 284.5 ± 2.1 days (CC group) at the beginning of the experiment. Diets for WCC and CC groups were formulated according to nutritional requirements for male goats [13] and had identical concentrate:roughage ratios (40:60). Thus, for each kg of feedstuff given to the animals, 40% was concentrate (formulated as described above) and 60% contained hay. The 40:60 concentrate:roughage ratio allows fair conditions for development of the bacteria present in the rumen–reticulum complex, essential for optimal digestion and absorption of nutrients. Animals were fed twice a day (07:00 and 15:00 h) and, after the adaptation period, the experiment lasted 150 days. Chemical compositions of ingredients (organic and mineral matter, crude protein, ether extract, neutral and acid detergent fiber) used in the experimental diets are shown in Table 1.

2.3. *Performance* in vivo *and biometric measurements of male goats*

Intakes of dry matter (g/day; % BW), de-oiled castor cake (g/day) and ricin (mg/kg BW), average daily weight gain (g/day) and total weight gain (kg) were measured every 15 days during the experimental period (150 days). Daily leftovers were collected for determination of animal intake, allowing up minimum leftovers of 10%, with readjustment every 15 days. The following parameters were also evaluated in all animals: body weight (kg), thoracic perimeter (cm), testicular length and width (cm), scrotal circumference (cm) and testicular volume (cm^3). Testicular volume was calculated using cylinder formula [14], in which $VOL = 2[r^2 \times \pi \times h]$, with radius ($r$ = testicular width/2), $\pi = 3.14$ and h is the testicular length.

2.4. Dosage of hormones and metabolites

Monthly, blood samples were collected by puncture of the jugular vein using heparinized Vacutainers (Labor Import®, Wei Hai, China). The tubes were centrifuged at 600 × *g* for 15 min to obtain the plasma and stored at −20 °C. Subsequently, plasma testosterone concentration was determined by Microparticle Enzyme Immunoassay (Abbott Diagnostics AxSYM System, USA), using a commercial kit (Axsym testosterone, Abbott Japan Co., Japan). The sensitivity and inter-assay coefficient of variation were 0.2 ng/mL and ≤20%, respectively. Plasma concentrations of urea, albumin, lactate dehydrogenase (LDH), creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by an automated biochemical analyzer (LabMax Labmax 240, Labtest®, Japan), using commercial kits (Labtest®, Lagoa Santa, MG, Brazil).

2.5. Collection and evaluation of semen

Semen collection was performed monthly by electroejaculation (Torjet 65; Eletrovet, São Paulo, Brazil) with a probe specially adapted for small ruminants. Ejaculate volume was measured in a graduated tube, and a small aliquot was reserved for the evaluation of sperm parameters. Sperm concentration was determined