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Comparative expression profiles of genes related to oocyte development in goats after long-term feeding with biodiesel castor industry residues

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ABSTRACT

The aim of this study was to determine whether the consumption of detoxified castor meal (DCM) by goats over a long period of time affects mRNA levels in oocytes, and in mural granulosa and cumulus cells. A total of 41 adult does were supplemented (DCM group, $n = 21$) or not (control group, $n = 20$) with detoxified castor meal (DCM) for a period of 500 days. Then, 13 and 12 does were randomly selected for slaughter from the DCM and control treatments groups, respectively, for the determination of the number of visible ovarian follicles, retrieved cumulus-oocyte complexes (COCs), and viable and non-viable oocytes. The relative expression levels for distinct genes were determined by quantitative PCR in viable immature oocytes prior to *in vitro* maturation (IVM), in oocytes attaining or not the metaphase stage after IVM, as well as in granulosa cells obtained upon oocyte collection, and in cumulus cells obtained after IVM. The number of follicles ≥4 mm did not differ between treatments (overall mean 23.3 ± 2.0) and no significant differences were observed in the recovery of viable, non-viable, or total mean numbers of oocytes (control group: 44.7 ± 4.6, DCM group: 54.9 ± 5.9, respectively) between control and DCM fed goats. The maturation rate was significantly higher for control than DCM oocytes (58.0% *vs.* 45.3%; $P < 0.05$). The mRNA levels in immature COC for controls were significantly higher for GLUT1 and lower for HSP70 ($P < 0.05$) than for DCM. Following maturation, MII oocytes from both treatments had mRNA levels that were significantly higher for GDF9 and lower for BMP15 than for NC oocytes ($P < 0.05$). In cumulus cells, the mRNA levels were significantly higher for LHR, FSHR, LeptinR, and IGF1, and lower for MnSOD in the control group compared with the DCM group ($P < 0.05$). In conclusion, the inclusion of DCM in goat feed for long periods of time changed gene expression in immature oocytes and in cumulus cells. This was reflected by a decrease in the *in vitro* oocyte maturation rate.

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1. Introduction

For biodiesel production, the castor seed plant (*Ricinus communis* L.) has been highlighted as a potential candidate for oil extraction in developing regions of the world, including Brazil (Food and Agriculture Organization of the United Nations, 2008). Therefore, the animal industry is strategically positioned to take advantage of biodiesel byproducts. Castor meal can be highlighted among these byproducts because of its high nutritional value (34.5% protein) and its low cost (Moreira et al., 2003). However, its direct use in animal feed is not recommended due to the presence of ricin, which is considered one of the most toxic compounds in nature (European Food Safety Authority, 2008). Ricin toxicity is related to its ability to inhibit protein synthesis, and it can cause multiple biological effects such as apoptosis, cell membrane injury and increased inflammatory mediators (Audi et al., 2005). Previous studies have shown that ricin is extremely toxic to pre-implantation embryos in mice (Storeng and Jonsen, 1983), decreases fertility in female rabbits (Salhab et al., 1999), and reduces rat semen quality (Sandhyakumary et al., 2003; Ekwere et al., 2011).

Despite the presence of ricin in castor meal, it is currently possible to efficiently detoxify this byproduct through different methods. The use of calcium oxide by Oliveira et al. (2010) was successful in the detoxification of castor meal, producing a safe product for ruminant feed. Silva et al. (2013) demonstrated that detoxified castor meal could be used for a long period as an alternative source of protein without affecting preantral or antral folliculogenesis in sheep. In another study, Arruda et al. (2013) found that the supplementation of goat feed with detoxified castor meal showed results similar to the control group; however, there were changes in IgG plasma levels and in HSP70 the mRNA levels, as well as a lower rate of transferable embryos in animals fed non-detoxified castor meal. However, there have been no studies on gene expression associated with meiotic competence of oocytes in goats fed with detoxified castor meal for a long period of time.

The aim of this study was to determine whether detoxified castor meal intake for long periods of time affects mRNA levels in oocytes and granulosa and cumulus cells. The expression of genes related to the acquisition of meiotic competence, including genes involved in oocyte quality (growth differentiation factor, GDF9 and bone morphogenetic protein 15, BMP15), metabolism (insulin-like growth factor-1, IGF1, IGF1 receptor, IGF1R, and glucose transporters 1, GLUT1), oxidative stress (heat shock protein, HSP70), and apoptosis (BAX/BCL2L10), were investigated in oocytes. The endocrine activity (luteinizing hormone receptor, LHR, and follicle-stimulating hormone receptor, FSHR), metabolism (leptin receptor, LeptinR, and IGF1), and oxidative stress (HSP70 and manganese superoxide dismutase, MnSOD) of genes related to the granulosa and cumulus cells were analyzed.

2. Material and methods

This study was submitted and approved by the Ethics Committee for Experimental Use of Animals of the State University of Ceará (CEUA-UECE) with the protocol number 09230950-0/74.

Table 1
Composition of the concentrates incorporating soybean meal or detoxified castor meal used to supplement the diets of goat does.

Constituent (% DM)	Diet	
	Control	DCM
Ground corn	81.8	79.6
Soybean meal	12.1	–
Detoxified castor meal	–	14.5
Urea	1.0	1.0
Vitamin mineralized premix	4.3	4.1
White salt	0.8	0.8

2.1. Detoxification of castor bean and quantification of ricin

The detoxification procedure was carried out according to Oliveira et al. (2010), with some modifications. Briefly, castor cake was added to a calcium oxide solution (1 kg CaO per 9 L of water) at a proportion of 60 g of CaO per kg of castor meal, and after standing overnight, the treated material was dried for storage and utilization. The efficiency of the detoxification process was verified by the absence of ricin as demonstrated by 12% polyacrylamide gel electrophoresis under non-denaturing conditions (native-PAGE) followed by staining the gels with Coomassie Blue R-250. Ricin levels were quantified by assaying the total proteins in samples of detoxified and non-detoxified castor cake, following the method of Bradford (1976) and using bovine serum albumin as the protein standard. The digitized image of the polyacrylamide gel was analyzed using the program Image Master Platinum (GE Healthcare Biosciences, Little Chalfont, UK). The protein bands were quantified in volume units (area × intensity) following the method described by Meunier et al. (2005).

2.2. Animals and experimental design

The experiment was conducted in the Padre João Piamarta Farm, in Itaitinga-CE, located at 4°01′S and 38°31′W, in the period from April to September. The area, characterized by a constant photoperiod regimen, has a warm, tropical, sub-humid climate with a mean annual rainfall and temperature of 1416.4 mm and 26–28 °C, respectively, with two distinct seasons: rainy, from January to May, and dry, from June to December.

Forty one mixed-breed, adult, and cyclic goats, generally homogeneous (mean ± SEM) in weight (33.34 ± 1.05 kg) and body condition score (2.34 ± 0.09), were divided into two treatment groups: a diet with soybean meal as the nitrogenous source in the concentrate (control, $n = 20$), and a diet with detoxified castor meal as a replacement for the soybean meal (DCM, $n = 21$) in the concentrate (Tables 1 and 2). The diets were composed of a mixture of guinea grass hay (*Panicum maximum cv*. Mombasa) and were isoenergetic (74% of TDN) and isonitrogenous (14% CP on DM basis). The diet formulations were based on the nutritional requirements for adult non-dairy does (NRC, 2007). The feed was provided twice a day (07:00 and 15:00)