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Effect of maternal cottonseed feed on the immune and antioxidant status of Santa Ines lambs

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ABSTRACT

Cottonseed has been used as a nutritional alternative in animal production. However, consequences of this nutrient in the progeny is not well characterized. Thus, this work evaluated the effect on the immune and antioxidant status of the progeny of feeding Santa Ines ewe with or without cottonseed. Twenty-four Santa Ines ewes were distributed in two feeding regimes: cottonseed (CS) concentrate (n = 12) and soybean (SB) concentrate (n = 12). After birth, lambs remained with their mothers and blood samples were collected at 1st, 3rd, 7th, 15th, 30th and 60th day of life of 24 lambs born from mothers fed with (CS, n = 12) or without (SB, n = 12) cottonseed. Serum total protein, albumin, alpha beta globulin, gamma globulin, immunoglobulin G and M, activity of glutathione peroxidase (GPx), catalase (CAT), oxygen radical absorbance capacity (ORAC) and variables related to iron metabolism were affected only by sampling times ($P < 0.05$). The concentration of serum total protein, alpha beta globulin, gamma globulin and immunoglobulin G and M, GPx activity and ORAC values decreased as lamb age increased. Serum albumin concentration and CAT activity, in turn, increased as lamb age increased. In this work, maternal feeding with cottonseed did not affect the serum protein profile and antioxidant status of progeny during the lactation period, indicating no transfer of gossypol effects by milk secretions. Thus, the alternative in ruminants feeding with cottonseed can be used without maternal-descendant effects to immunity and oxidative stress in lambs.

1. Introduction

Cottonseed in animal feed includes an antinutritional factor in the diet, gossypol, which presents negative effects on reproduction [1–3]. Male infertility is an effect of gossypol on decreased sperm concentration and motility, in addition to possible hormonal changes [4]. However, the effects of this toxin on spermatogenesis are slowly reversed with the end of their consumption [5]. In females, gossypol also affects gametogenesis, in addition to determining lesions in embryos [4]. Although cottonseed effects on fertility are extensively studied, investigations are scarce on the consequences of consuming this nutrient on formation and transfer of passive immunity, that is, mobilization of maternal antibodies to the mammary gland and intestinal absorption by the newborn.

Gossypol can decrease serum levels of albumins and immunoglobulins [2,3]. Holstein calves fed with increasing gossypol concentrations in free form showed decreased levels of serum albumin, globulins and total proteins in the first four months of life [1]. In rodents, in turn, oral administration of gossypol four times at 24-hour intervals resulted in a decrease in the total number of lymphocytes in the thymus and lymph nodes of mesentery [3]. In this same study, rodents that were immunized against ovine erythrocytes showed reduced immunoglobulin G production when gossypol was administered prior to immunization and reduced immunoglobulin G and M when applied after immunization. The authors suggest that the immune system is much more sensitive to the gossypol effects than the reproductive system. Thus, discussions are opened on gossypol effects on maternal immunity, as well as the results of these effects on colostrum formation. Changes in serum levels of maternal immunoglobulins during gestation may therefore interfere with colostrum immune potential and acquisition of passive immunity by progeny.

In addition to the effect on the immune system, as it is a phenolic compound, gossypol possibly acts on the oxidative stress. According to Laughton et al. [6], gossypol unavailable iron inhibits lipid peroxidation. However, the authors emphasize that under certain conditions, this phenolic compound can have a pro-oxidant effect and cannot be

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classified in a simplistic way as "antioxidants". Wang et al. [7], in turn, observed that in addition to the ability to sequester free radicals, reduce iron ions and DNA damage prevention induced by ultraviolet radiation, gossypol inhibits cervical cancer, mammary cells and colon. In mammals, birth is associated with oxidative stress, mainly due to the induction of lung breathing. This process results in the generation of reactive oxygen species and antioxidant factors in colostrum, which may be enzymatic such as lactoperoxidase, catalase, superoxide dismutase and glutathione peroxidase, or non-enzymatic, such as vitamins A, E and C, selenium and lactoferrin, are important in this perinatal period to eliminate free radicals [8,9]. In addition to the possible interference in the immune system, maternal nutrition with cottonseed can significantly affect the antioxidant potential in maternal blood and consequently colostrum, affecting offspring health and hygiene. Thus, this study evaluated the maternal-descendent effects of feeding ewes with cottonseed on the immune and antioxidant status of Santa Ines lambs.

2. Materials and methods

2.1. Animals, feeding and experimental procedures

The present experiment was conducted at the Center for Nuclear Energy in Agriculture (CENA), University of São Paulo, Brazil. Thirty-nine Santa Ines ewes non-pregnant, in good sanitary and general clinical condition, weighing 40 ± 15 kg and body score of 3.1 ± 0.5 were used. The experiment was conducted for ten months, divided into: pre-season mating (one month), mating season (two months), gestation (five months) and lactation (two months). The ewes were distributed into two feeding regimes: fed with cottonseed concentrate (CS, n = 12, 6 primiparous and 6 multiparous ewes) or fed with ground corn and soybean concentrate (SB, n = 12, 6 primiparous and 6 multiparous ewes). The diets were elaborated according to NRC (2007) to meet the requirements of protein and metabolizable energy for maintenance in gestation and lactation. Hay was supplied once a day in the afternoon (Table 1). In the morning, ewes were kept in pastures of *Panicum maximo* cv *aruana* and in the afternoon in collective stall containing water, bulky, concentrated and salt. Concentrate consumption was individual (canzil prey) and increased from 500 up to 600 g of concentrate per animal once daily.

After birth, lambs remained with their mothers and blood samples with and without anticoagulant (EDTA) were collected at the 1st (24–36 hours of life), 3rd, 7th, 15th, 30th and 60th day of life from 24 lambs born from ewes fed with (CS) or without (SB) cottonseed (n = 12). Samples collected without anticoagulants were centrifuged at 3000 x *g* for 15 min. and the resulting serum stored at −80 °C. Blood samples collected with anticoagulant were centrifuged at 3000 x *g* for 15 min, the resulting plasma was frozen at −80 °C and 300 μL of the erythrocyte in the pellet was diluted (1:6) with triton-100x (5 g L^{-1}) and frozen at −80 °C for further analysis.

Table 1
Feed provided to Santa Ines ewes during reproductive period.

Chemical composition	Hay	Forage	SB	CS
Offered (g / kg)	Ad libitum	Ad libitum	0.500-0.600	0.500-0.600
Organic matter	940	899	961	964
Dry matter	900	925	932	940
Lignin	90	68	94	158
ADF	449	380	129	426
NDF	809	671	477	586
Crude protein	65	170	245	249
Ethereal extract	16	19	193	210
Mineral matter	59	101	39	36
Free Gossypol * (ppm)	–	–	–	600

ADF ad NDF acid and neutral; SB - soybean concentrate; CS - cottonseed concentrate; *Free gossypol was determined according to the methodology adapted from Botsoglou [34].

2.2. Laboratorial analyses

Electrophoretic fractionation of serum proteins was performed on cellulose acetate film (Cellogel) using a 25 μL sample. The procedure was performed in a horizontal cuba (CELM) containing Tris-glycine buffer pH 9.4. After 20 min of running at 200 V, the film was stained in 200 mL of Ponceau S solution for 10 min. The membrane was then bleached in 200 mL of 5% acetic acid solution and fractional bands were revealed. After drying, membranes with separated serum protein fractions were read in CELM SDS60 densitometer and determined in terms of total serum protein concentration. Total protein was determined by the biuret method [10] and concentrations are expressed in g dL^{-1}.

Immunoglobulin G (IgG) and M (IgM) was determined by radial immunodiffusion [11]. Five uL of samples were applied in duplicate to 1.2% agarose gel containing anti-IgG or anti-IgM (Sigma-Aldrich, Co). After 24 h at 4 °C in a humid chamber, the precipitation arc was measured and compared with the standard curves. Concentrations of immunoglobulin are expressed in mg mL^{-1}.

Glutathione peroxidase activity was evaluated according to Wendel et al. [12]. Samples were incubated with 300 μL of a reaction solution (48 mM buffer phosphate, pH 7.7, 0.38 mM EDTA, 0.95 mM azide (to inhibit catalase), 1 mM glutathione, 0.12 mM nicotinamide adenine dinucleotide phosphate, 3.2 U of glutathione reductase, 0.02 mM DL-dithiothreitol and 0.0007% hydrogen peroxide). A decrease in absorbance was recorded for 5 min in a spectrophotometer with wavelength at 340 nm. The glutathione peroxidase activity values are expressed as unit U mL^{-1}. One unit catalyzes oxidation by H_2O_2 of one mol of reduced glutathione to oxidized glutathione per minute at 37 °C, pH 7.0. Catalase activity was determined according to Iwase et al. [13]. Samples were incubated with Triton-X (1%) and hydrogen peroxide (30%). The height of O_2 foam formed was measured with a digital caliper and compared to a calibration curve. The catalase activity values are expressed as U mL^{-1}. One unit of catalase is responsible for the consumption of one mol of H_2O_2 per minute. The superoxide dismutase activity was measured using a tetrazolium salt to detect superoxide radicals generated by xanthine oxidase and hypoxanthine. The assay measures three types of SOD (Cu/Zn, Mn and FeSOD). The values of SOD activity are expressed as unit U mL^{-1}. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical.

Samples were also evaluated regarding the antioxidant status by the Oxygen Radical Absorbance Capacity (ORAC) protocol, according to Melo et al. [14]. Sample (30 μL) was homogenized in duplicate with 60 μL of fluorescein solution (487 nM) and 110 μL of AAPH (2,2′-Azobis (2-amidinopropane) dihydrochloride, 76 mM) solution. The analyses were performed with a kinetic methodology using emission absorbance of 528 nm and excitation absorbance of 485 nm for 2 h at 37 °C. Values were compared to calibration curve with trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and are expressed as mM of equivalent Trolox per mL.

Serum iron (ug dL^{-1}), latent iron binding capacity (LIBC, ug dL^{-1}), total iron binding capacity (TIBC, ug dL^{-1}), transferrin (mg mL^{-1}) and transferrin saturation index (TSI, %) were determined by commercial kits (Labtest ® Kit).

2.3. Statistical analyses

The experiment was conducted in a completely randomized design. The statistical analysis was performed using SAS software (SAS Institute Inc., 2008) and variables analyzed as a repeated measure-over-time design, considering lambs born from ewes fed with or without cottonseed diet (group) and sampling time as main effects. The lamb effect was considered random.

The data were submitted to the analysis of variance using general linear mixed models (MIXED procedure). The means were compared

Table 2
Serum total protein, albumin, alpha beta globulin, gamma globulin, immunoglobulin G and M in lambs born from ewe fed with or without cottonseed.

		Days of life						General mean	P value		
		1	3	7	15	30	60		*Gr*	*ST*	*GrXST*
Total protein, g dL^{-1}	*CA*	5.33 ± 0.18	5.16 ± 0.19	4.95 ± 0.21	4.51 ± 0.11	4.39 ± 0.10	4.13 ± 0.08	4.72 ± 0.08	n.s.	**	n.s
	CO	5.48 ± 0.18	5.37 ± 0.23	4.99 ± 0.17	4.53 ± 0.14	4.49 ± 0.12	4.26 ± 0.11	4.84 ± 0.08			
	General mean	*5.41 ± 0.12a*	*5.27 ± 0.15a*	*4.97 ± 0.13b*	*4.52 ± 0.09c*	*4.44 ± 0.08 cd*	*4.19 ± 0.07d*				
Albumin, g dL^{-1}	*CT*	1.71 ± 0.04	1.79 ± 0.05	1.91 ± 0.09	1.95 ± 0.10	2.06 ± 0.10	1.82 ± 0.10	1.87 ± 0.04	n.s.	*	n.s.
	CO	1.78 ± 0.11	1.88 ± 0.04	1.81 ± 0.06	1.91 ± 0.10	2.02 ± 0.11	1.94 ± 0.09	1.89 ± 0.04			
	General mean	*1.74 ± 0.05b*	*1.84 ± 0.03ab*	*1.85 ± 0.05ab*	*1.93 ± 0.07ab*	*2.05 ± 0.07a*	*1.88 ± 0.07ab*				
Alpha beta globulin, g dL^{-1}	*CT*	1.71 ± 0.07	1.79 ± 0.06	1.82 ± 0.07	1.63 ± 0.10	1.54 ± 0.9	1.46 ± 0.04	1.65 ± 0.03	n.s.	*	n.s.
	CO	1.67 ± 0.08	1.80 ± 0.07	1.84 ± 0.06	1.65 ± 0.08	1.69 ± 0.09	1.56 ± 0.06	1.70 ± 0.03			
	General mean	*1.69 ± 0.05ab*	*1.79 ± 0.05a*	*1.83 ± 0.05a*	*1.64 ± 0.06ab*	*1.60 ± 0.06ab*	*1.51 ± 0.04b*				
Gamma globulin, g dL^{-1}	*CT*	1.93 ± 0.22	1.58 ± 0.16	1.42 ± 0.14	0.97 ± 0.11	0.74 ± 0.09	0.85 ± 0.06	1.24 ± 0.08	n.s.	*	n.s.
	CO	2.22 ± 0.25	1.64 ± 0.22	1.28 ± 0.18	0.95 ± 0.15	0.80 ± 0.13	0.76 ± 0.05	1.26 ± 0.09			
	General mean	*2.07 ± 0.16a*	*1.61 ± 0.13b*	*1.35 ± 0.11c*	*0.96 ± 0.10d*	*0.77 ± 0.07d*	*0.81 ± 0.0d*				
Immunoglobulin G, mg mL^{-1}	*CT*	37 ± 2	34 ± 2	30 ± 3	24 ± 3	22 ± 2	18 ± 1	27 ± 1	n.s.	**	n.s.
	CO	37 ± 3	33 ± 3	28 ± 4	23 ± 3	19 ± 2	13 ± 1	26 ± 1			
	General mean	*37 ± 2a*	*34 ± 2b*	*29 ± 2c*	*23 ± 2d*	*21 ± 1d*	*15 ± 1e*				
Immunoglobulin M, mg mL^{-1}	*CT*	1.14 ± 0.14	1.09 ± 0.10	0.81 ± 0.14	0.72 ± 0.08	0.85 ± 0.08	0.88 ± 0.05	0.92 ± 0.05	n.s.	**	n.s.
	CO	1.09 ± 0.11	098 ± 0.15	0.78 ± 0.11	0.69 ± 0.04	0.87 ± 0.04	0.81 ± 0.06	0.87 ± 0.04			
	General mean	1.11 ± 0.08a	1.03 ± 0.09a	0.80 ± 0.08b	0.70 ± 0.04b	0.86 ± 0.04ab	0.84 ± 0.04ab				

Lambs born from ewes fed with (CS) or without (SB) cottonseed; Gr – effect of group; ST – effect of sampling time; GrXST interaction between Gr and ST; n.s. – $P > 0.05$; *$P < 0.05$; **$P < 0.01$; [AB]Letter in the same column differ by Tukey´s test, $P < 0.05$; [ab]Letters in the same row differ by Tukey´s test, $P < 0.05$.

based on differences in least-square means, with P values adjusted for multiple comparisons using the Tukey option in the MIXED procedure ($\alpha = 0.05$). The data are presented as means and standard errors. The Pearson and Spearman correlation analysis, through PROC CORR program from SAS software (SAS Institute Inc., 2008), were taken to verify associations between serum variables of interest.

3. Results

Serum total protein, albumin, alpha beta globulin, gamma globulin, immunoglobulin G and M differed only between sampling times ($P < 0.05$) (Table 2). The concentration of serum total protein, alpha beta globulin, gamma globulin and immunoglobulin G and M values decreased as the lamb age increased. Serum albumin concentration, in turn, increased as the lamb age increased.

The activity of the antioxidant enzymes GPx and CAT was affected only by sampling time (Table 3) ($P < 0.05$). The GPx activity was higher at one day of the lamb life compared to 60 days of lamb life. CAT activity, in turn, was higher at 30 and 60 days of lamb life than at one, three, seven and 15 days of lamb life. SOD activity was not influenced by diet and sampling time ($P > 0.05$). The oxygen radical absorbance capacity was also affected by sampling time ($P < 0.05$), with a higher value at the 1st day of life compared to the 7th day of life. Considering iron metabolism, effect of sampling time was observed for all variables evaluated ($P < 0.05$), with higher values at 60 d of life, Table 4.

Correlation between the variables are presented in Table 5. High correlation (above 70%) was observed, as expected, between the variables total protein, gamma globulin, IgG and IgM ($P < 0.05$).

4. Discussion

Maternal-descendent effects were not detected in lambs born from ewes fed with cottonseed. According to Calhoun et al. [15], 5 mg/mL is the safe limit of gossypol in bovine plasma. In this study, ewes consumed around 300 to 360 ppp of gossypol, values under the threshold of the United Nations Food and Agriculture Organization and World Health Organization of 0.6 g/mg (600 ppm) free gossypol in edible cottonseed products [16]. Wang et al. [17] observed that cows fed whole cottonseed with gossypol levels of 385.43 or 611.13 mg/Kg of dry matter, similar to the present work, showed higher gossypol concentrations in plasma and milk compared to cows fed with soybean meal or cottonseed meal with 91.15 or 117.31 mg/Kg of dry matter. However, the authors state that gossypol was not detected at harmful levels 0.153 and 0.223 μg/mL in plasma, respectively, and 0.133 and 0.221 μg/mL in milk, respectively. Thus, possibly the ingestion of low levels of gossypol through lacteal secretions by the newborn and effects still need to be established.

Newborn lambs, like other ruminants, are agammaglobulinemic because immunoglobulin passes through placental barrier during the gestational period and the inexperience of their immune system. Thus, colostrum intake immediately after birth is critical for its survival, and this milk secretion is the sole immunoglobulin source for the newborn and therefore immune protection [18,19]. The colostrum quality is deeply dependent of maternal nutrition [20,21]. According to Banchero et al. [22], in underfed ewes, there are insufficient nutrients for adequate lactation and the hormone regime is inappropriate for good udder development and colostrum synthesis. Cottonseed effects on the mobilization of maternal antibodies to the mammary gland and intestinal absorption by newborns are scarce; however, gossypol in cottonseed has wide range of effects in animal physiology [1–5]. According to Benhaim et al. [23], gossypol has an anti-inflammatory activity due to exhaustion of neutrophil activity. Xu et al. [24] also observed an immunosuppressive effect of gossypol in mice, probably by inhibition of lymphocyte proliferation and by induction of cell apoptosis. Thus, gossypol effects on maternal immunity may change the quality of colostrum. In this study, the maternal ingestion of cottonseed did not

Table 3
Activity of antioxidant enzymes and oxygen radical absorbance capacity (ORAC) in blood of lambs born from ewes fed with or without cottonseed.

		Days of life						General mean	*P value*		
		1	3	7	15	30	60		*Gr*	*ST*	*GrXST*
Glutathione peroxidase, mU mg^{-1} Hb	CT	2.98 ± 0.36	2.77 ± 0.19	2.78 ± 0.23	2.2 ± 0.18	2.52 ± 0.22	2.33 ± 0.29	2.59 ± 0.10	n.s.	*	n.s
	CO	3.11 ± 0.20	2.61 ± 0.18	3.04 ± 0.25	3.07 ± 0.16	2.47 ± 0.22	2.31 ± 0.24	2.78 ± 0.09			
	General mean	*3.05 ± 0.20a*	*2.70 ± 0.13ab*	*2.92 ± 0.17ab*	*2.63 ± 0.15ab*	*2.50 ± 0.15ab*	*2.32 ± 0.19b*				
Catalase, U mg^{-1} HB	*CT*	4.6 ± 0.8	3.4 ± 0.2	4.3 ± 0.6	3.8 ± 0.3	5.9 ± 0.5	8.1 ± 0.7	5.0 ± 0.3	n.s.	**	n.s.
	CO	4.5 ± 0.6	4.5 ± 1.0	4.5 ± 0.7	3.9 ± 0.3	7.6 ± 0.7	8.1 ± 0.6	5.6 ± 0.3			
	General mean	*4.5 ± 0.5b*	*3.9 ± 0.5b*	*4.4 ± 0.4b*	*3.9 ± 0.2b*	*6.7 ± 0.5a*	*8.1 ± 0.4a*				
Superoxide dismutase, U mg^{-1} Hb	*CT*	42 ± 3	42 ± 6	40 ± 2	40 ± 2	43 ± 5	39 ± 3	41 ± 1	n.s	n.s.	n.s.
	CO	40 ± 7	37 ± 6	39 ± 2	43 ± 7	56 ± 2	42 ± 4	42 ± 2			
	General mean	*41 ± 2*	*39 ± 4*	*40 ± 1*	*41 ± 3*	*49 ± 3*	*41 ± 2*				
ORAC, mmol mL^{-1}	*CT*	134 ± 11	128 ± 13	97 ± 7	113 ± 9	101 ± 5	129 ± 10	117 ± 4	n.s.	*	n.s.
	CO	130 ± 10	118 ± 8	106 ± 10	101 ± 7	120 ± 6	109 ± 8	114 ± 3			
	General mean	*132 ± 7a*	*123 ± 7ab*	*101 ± 6b*	*107 ± 6ab*	*110 ± 4ab*	*119 ± 7ab*				

Lambs born from ewes fed with (CS) or without (SB) cottonseed; Gr – effect of group; ST – effect of sampling time; GrXST interaction between Gr and ST; n.s. – $P > 0.05$; *$P < 0.05$; **$P < 0.01$; [ab]Different letters in the row differ by Tukey´s test, $P < 0.05$. Antioxidant enzymes were determined in erythrocytes and are expressed as U (or mU) per g or mg of hemoglobin (Hb); One unit of superoxide dismutase is define as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical; One unit of glutathione peroxidase catalyzes the oxidation by H_2O_2 of one µmol of reduced glutathione to oxidized glutathione per minute at 25 °C, pH 7.0; One unit of catalase is responsible for the consumption of one mol of H_2O_2 per minute; ORAC: oxygen radical absorbance capacity was determined in plasma and is expressed as µmol of equivalent Trolox per mL^{-1}.

influence the acquisition of passive immunity by newborn lamb. The serum protein profile was affected only by sampling time. Serum albumin, produced in the liver is responsible for the transport of substances and regulation of blood oncotic pressure [25]. Thus, the lower values observed in the first days of lamb life may be related to a compensation of high levels of total globulins to keep blood oncotic pressure. The values of total protein, gamma globulin, IgG and IgM, in turn, were higher in the first life days, indicating acquisition of antibodies from maternal colostrum through intestinal epithelium, without differences between the lambs born from ewes fed with or without cottonseed. After 24 h of birth, lambs showed 37 ± 2 mg/mL of IgG, values much higher than necessary to avoid failure of passive immunity acquisition, which occurs when neonates are unable to absorb sufficient amounts of immunoglobulin and is characterized by serum concentrations below 12 mg / mL in the first 12 to 48 h [26]. Moretti et al. [27] supplied 66 or 25 gr of bovine or ovine IgG, respectively, in two colostrum meals of 250 mL to Santa Inês lambs in the first six hours of life, and observed lower serum IgG concentration at 24 h of lamb life (31 and 22 mg/mL, respectively) than the values found in this work. Lambs born from ewes fed with or without cottonseed showed a decrease in serum total protein up to 60 d of life. The high correlation of serum total protein and immunoglobulin reflects the remarkable presence of exogenous IgG (maternal) in the fluctuation of serum total protein, as confirmed by the high correlation between the variables (r = 0.84).

While IgM is the first immunoglobulin class produced in a primary response to an antigen, immunoglobulin G is the most abundant class of serum antibodies. The adaptive immune response depends on antigen recognition and presentation to specific T- and B-cells, activated and proliferate, resulting in antibody synthesis by plasma cells [28]. Xu et al. [24] observed that gossypol acetic acid can significantly inhibit

Table 4
Serum iron, latent iron binding capacity (LIBC), total iron binding capacity (TIBC), transferrin and transferrin saturation index (TSI) in lambs born from ewe fed with or without cottonseed.

		Days of life						General mean	*P value*		
		1	3	7	15	30	60		*Gr*	*ST*	*GrXST*
Iron, ug dL^{-1}	*CT*	118 ± 28	142 ± 36	87 ± 23	161 ± 28	314 ± 35	278 ± 21	183 ± 15	n.s.	**	n.s.
	CO	123 ± 21	214 ± 31	135 ± 27	180 ± 29	231 ± 29	244 ± 26	188 ± 12			
	General mean	*120 ± 17b*	*178 ± 25b*	*111 ± 18b*	*171 ± 20b*	*272 ± 24a*	*261 ± 16a*				
LIBC, ug dL^{-1}	*CT*	189 ± 13	178 ± 15	164 ± 18	145 ± 21	228 ± 31	273 ± 12	196 ± 9	n.s.	**	n.s.
	CO	202 ± 14	210 ± 14	174 ± 14	166 ± 20	205 ± 28	249 ± 17	201 ± 8			
	General mean	*195 ± 9bc*	*194 ± 10bc*	*169 ± 11bc*	*156 ± 14c*	*217 ± 20b*	*261 ± 10a*				
TIBC, ug dL^{-1}	*CT*	307 ± 30	320 ± 48	252 ± 40	306 ± 47	542 ± 59	551 ± 20	380 ± 22	n.s.	**	n.s.
	CO	325 ± 29	424 ± 42	310 ± 38	347 ± 44	436 ± 47	494 ± 37	389 ± 18			
	General mean	*316 ± 21c*	*372 ± 33bc*	*281 ± 28c*	*326 ± 32c*	*489 ± 38ab*	*522 ± 22a*				
Transferrin saturation index (%)	*CT*	33 ± 6	38 ± 4	29 ± 5	49 ± 3	60 ± 4	50 ± 2	43 ± 2	n.s.	**	n.s.
	CO	36 ± 3	48 ± 3	37 ± 5	50 ± 3	54 ± 4	48 ± 3	45 ± 2			
	General mean	*34 ± 3c*	*43 ± 3abc*	*33 ± 3c*	*50 ± 2a*	*57 ± 3a*	*49 ± 2ab*				
Transferrin, mg mL^{-1}	*CT*	215 ± 21	224 ± 34	176 ± 28	214 ± 33	380 ± 41	386 ± 14	266 ± 15	n.s.	**	n.s.
	CO	227 ± 20	297 ± 29	217 ± 27	243 ± 31	305 ± 33	345 ± 26	272 ± 12			
	General mean	*221 ± 14b*	*260 ± 23b*	*197 ± 19b*	*228 ± 22b*	*342 ± 27a*	*366 ± 15a*				

Lambs born from ewes fed with (CS) or without (SB) cottonseed; Gr – effect of group; ST – effect of sampling time; GrXST interaction between Gr and ST; n.s. – $P > 0.05$; **$P < 0.01$; [abc]Different letters in the row differ by Tukey´s test, $P < 0.05$.

Table 5
Pearson and Spearman correlation analysis between variables in the serum of Santa Inês lambs.

	Total protein	Albumin	αβ-globulin	γ-globulin	IgG	IgM	GPx	CAT	SOD	ORAC	Iron	LIBC	TIBC	TSI	Transferrin
Total protein	–	–	0.47	0.90	0.84	0.70	–	−0.36	–	0.35	−0.34	−0.19	−0.32	−0.35	−0.32
Albumin		–	−0.30	−0.29	–	–	–	–	0.30	–	0.22	–	–	0.34	–
αβ-globulin			–	0.27	0.28	–	–	−0.22	–	–	−0.20	−0.33	−0.28	–	−0.28
γ-globulin				–	0.88	0.74	–	−0.29	–	0.40	−0.37	–	−0.28	−0.45	−0.28
IgG					–	0.65	–	−0.38	–	0.32	−0.36	–	−0.31	−0.38	−0.31
IgM						–	–	–	–	0.53	−0.31	–	−0.28	−0.36	−0.28
GPx							–	–	–	–	−0.19	–	−0.18	−0.16	−0.18
CAT								–	–	–	0.23	0.24	0.26	–	0.26
SOD									–	–	–	–	–	–	–
ORAC										–	–	–	–	–	–
Iron											–	0.58	0.94	0.76	0.94
LIBC												–	0.83	–	0.83
TIBC													–	0.55	1.00
TSI														–	0.55

GPx- glutathione peroxidase; CAT – catalase; SOD – superoxide dismutase; LIBC – latent iron binding capacity; TIBC – total iron binding capacity; TSI – transferrin saturation index.

proliferation of murine lymphocytes induced by phorbol dibutyrate (PDB) and ionomycin. Sijun et al. [3] also reported that gossypol administered after priming decreases IgM and IgG production. In Holstein calves fed with increasing concentrations of gossypol in free form results in decreased levels of serum albumin, globulins and total proteins in the first four months of life [1]. Nikokyris et al. [29], in turn, did not detect differences in total protein, globulin and albumin of Karagouniko male lambs fed free gossypol in diets containing up to 30% whole cottonseed for 54 d. Câmara et al. [30] also did not find changes in serum biochemical parameters of sheep consumption of 400 g kg^{-1} of cottonseed cake for 63 d. In our work, maternal feeding with cottonseed also did not affect serum protein concentration of the progeny during the lactation period, indicating no transfer of gossypol effects by milk secretions.

Additionally to the immune function, colostrum ingestion in the first hours of life is related to overcoming of oxidative stress resulted mainly from lung breathing induction [8,9,31]. The antioxidant factors in colostrum, such as lactoperoxidase and vitamins E and C, are also important in this perinatal period to eliminate reactive oxygen species [8,9]. Gossypol has been related to an antioxidant activity due to its capacity to inhibit the induction of lipid peroxidation, forming a stable complex with iron [7]. In our work, the activity of antioxidant enzymes, oxygen radical absorbance capacity and iron metabolism were not influenced by maternal consumption of cottonseed. In ruminants, microbial fermentation in the rumen binds the free gossypol in the diet with proteins, inactivating this compound [4], a condition that may have occurred in ewes fed with cottonseed. Anikumar et al. [32], feeding 1-year-old Deccani sheep on basal diet (green fodder + concentrate), conventional (non-Bt (*Bacillus thuringiensis*)) cotton plants (1.5 kg + green fodder + concentrate), Bt cotton plants (1.5 kg + green fodder + concentrate) and Bt cotton plants (ad libitum + concentrate) did not show differences in the mean concentration of reduced glutathione, thiobarbituric acid reactive substances and in the superoxide dismutase activity and catalase activities in the liver, kidney and heart. The authors concluded that Bt cotton plants do not induce oxidative stress in sheep. Tang et al. [33], in turn, feeding dairy cows with 1000 mg of gossypol per kilogram of dry matter for 28 d showed that gossypol induced oxidative stress and hepatotoxicity, reduced peripheral lipid metabolism, and enhanced hepatic lipid accumulation, decreased amino acid bioavailability and milk protein synthesis, and decreased gluconeogenesis and milk lactose in dairy cows.

In our study, a higher antioxidant defense was observed in the first days of life, when lambs are more susceptible to oxidative stress. However, the ORAC value, unexpectedly, was not correlated with the activity of antioxidant enzymes nor with variables related to iron. Differently, this variable showed a positive correlation with the presence of gammaglobulins, IgG and IgM (r = 0.40, 0.32, 0.53, respectively). While the glutathione peroxidase decreased its activity in the first 60 d of lamb life, catalase activity increased, revealing the importance of each enzyme in distinct phases of animal development.

5. Conclusion

Maternal feeding with cottonseed did not affect the serum protein profile and antioxidant status of progeny during the lactation period, indicating no transfer of gossypol effects by milk secretions. Thus, the alternative in the feeding of ruminants with cottonseed can be used without maternal-descendant effects to immunity and oxidative stress in lambs.

Conflict of interest

No conflict of interest is declared.

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