Journal of Veterinary Science & Animal Husbandry
Volume 11 | Issue 1
ISSN: 2348-9790

Research Article Open Access

Productive Performance Progenitor-Progeny on Cottonseed Supplementation in Santa Ines Ovines

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Citation: Carolina Rodriguez Jimenez, Débora Botéquio Moretti, Tairon Pannunzio Dias e Silva, Egon Hion Ieda, Natasha Kelly Mantuam, et al. (2023) Productive Performance Progenitor-Progeny on Cottonseed Supplementation in Santa Ines Ovines. J Vet Sci Ani Husb 11(1): 102

Abstract

The use of cottonseed by-products are sources of protein, energy, and fiber, excellent nutrient feed alternatives for animals. However, cottonseed use is limited because of the presence of gossypol, as it causes serious problems to animal health. Thus, was evaluated ewe long-term supplemented of cottonseed during the pre-breeding season, breeding season, pregnancy, and lactation period (10 months). We used Santa Inês breed ewe with 43.2 ± 2.0 kg body weight (BW) and 3.0 ± 0.1 body condition score (BCS). Two treatments were evaluated: ewe fed on soybeans seed (CO, n =18) and ewe fed on cottonseed (CA, n = 21). Have been evaluated of productivity parameters, such as, hematology and biochemical. Body mass parameters, such as, BW, BCS and the tissue composition of the Longissimus dorsi muscle by in vivo ultrasound, and helminth infection control parameters such as, egg per gram count. Both treatments had a satisfactory breeding season, maintained pregnancy, and took their lambs to weaning. The parameters hematological and biochemicals proved to be stable for both treatments. The BW, BCS and ultrasonography measurements in vivo were lower in ewe fed with CA. We observed no differences in the fecal egg counts between treatments. It is concluded that cottonseed intake (360 ppm of gossypol/animal per d) during long periods (10 months) for ewe did not result in intoxication, did not aid in endoparasite control and did not improve productivity because the body dynamics in ewe fed on cottonseed was less efficient both in ewe and in their lambs until weaning.

Keywords: weight; gossypol; ewe; gestation; body mass

Introduction

Cottonseed is a cotton by-product that is an alternative source of protein, fiber, and energy at a low cost for animals. Feed nutritional quality depends on its chemical composition and nutrient availability as well as the presence of a bioactive compound, such as gossypol [1].

From the 1960s to the 1980s, studies of gossypol toxicity in ewe were performed [2,3]. From this, studies in vitro [4,5] and in nutrition [6, 7,8,9] among others) were developed to be able to use cotton by-products more efficiently in ewe farming.

In 2006 the European Union (22002L0032— EN—3 20.10.2006 — 006.001— 1 (established 500 ppm as the maximum concentration of free gossypol for cottonseed in ewe, goats, and adult cattle (FAO 2003; [10, 9]). But, the European Union in 2013 (2002L0032 — EN — 26.02.2013 — 017.001 — 1) decreased to 300 ppm of free gossypol to complete feed for ewe (except lambs). The effect of gossypol depends beyond the dose, of time of intake too. This information in the literature is scarce in ewe females during pregnancy, lactation and their lambs, bringing forth the importance and effect of the analysis of the prolonged use of the cottonseed in this species.

Additionally, gossypol, which is a phenolic substance, has been studied for its action against helminths in ruminants [11]. The use of food that can help control worms in ewe (function food) is of great value and should be investigated. Ewe are more sensitive to endoparasites [12,13]. Mainly in the phenomenon "spring rise", or breaking immunity, in peripartum, which increases nematode infection, allowing a high establishment of new larvae and/or high fecundity of females [14].

Many questions regarding the cottonseed intake, especially for long periods, by ruminants remain unanswered. Research is required for a better understanding of its action in small ruminants to recommend the safe use of cottonseed in diets, without impairing animal welfare. Thus, this study had the objective the supplementation of 500 -600 g/kg of cottonseed in de pre-breeding season, gestation, and lactation (10 months) in ewe females Santa Ines evaluating productivity parameters, such as, hematology and biochemical. Body mass parameters, such as, weigh, body condition and the tissue composition of the Longissimus dorsi muscle by in vivo ultrasound, and helminth infection control parameters such as, egg per gram count.

Material and Methods

Animals and Handling

The experiment was conducted in Piracicaba, SP, Brazil (22°42'30"S, 47°38'01"W; average altitude of 546 m). The study was approved by the Ethics Commission on Animal Use of the Center for Nuclear Energy in Agriculture of the University of São Paulo (CENA/USP/Protocol number 008/2015)

The 41 Santa Inês ewe females breed were used clinically healthy, with average BW 43.2 ± 2.0 kg and BCS 3.0 ± 0.1. The experimental period had adaptation period (1 month) and three experimental phases (i) breeding season (2 months) that only pregnant female continued the experiment; (ii) pregnancy (5 months) and (iii) lactation (2 months). thus, two female control group don't pregnant and the treatments were divided this: ewe fed soybean seed (control group CO, n =18) and ewe fed cottonseed (CA, n = 21), for a total of 39 ewe.

Both experimental groups were kept on pastures of Panicum maximum cv. Aruana supplemented with coast-cross hay, mineral salt, and water ad libitum. The ewe received concentrated (500 to 600 g/kg per animal to both treatments) every day in the late afternoon (16h:00). The diets were elaborated (Moretti et al 2019) to meet the requirements of protein and metabolizable energy for maintenance in the reproductive period (NRC 2007), which can be observed in Table 1.

Chemical Composition (%)	Sheep Diet			Creep Feling	Hay
	Forage	CO	CA	Lambs	Sheep/Lambs
Dry Matter	92.5	93.2 ± 6.36	94.0 ± 2.16	87.7	90.0
Organic Matter	90.0	96.1 ± 2.16	96.4 ± 0.74	96.6	94.0
Crude Protein	17.0	24.5 ± 4.43	24.8 ± 17.48	21.8	6.5
Neutral Detergent Fiber	67.1	47.7 ± 36,51	58.6 ± 25.83	40.2	80.9
Acid Detergent Fiber	38.0	12.9 ± 77.71	42.6 ± 16.58	6.7	44.9
Lignin	6.8	9.4 ± 2.61	15.8 ± 18.62	-	9.0
Ether Extract	1.9	19.3 ± 2.50	21.0 ± 2.01	3.72	1.7
Mineral Matter	10.1	3.9 ± 2.16	3.6 ± 0.74	3.4	5.9
Gossipol Free (mg/kg)	-	-	300 - 360	-	-

Hay and Forage ad libitum, CO and CA treatments Offered (500 to 600 g/kg). CO: soybean seed concentrate; CA: cottonseed concentrate. Free gossypol was determined according to the methodology adapted from Botsoglou (1992).

Table 1: Chemical composition and quantity offered and of forage and concentrates, values expressed in % of dry matter.

The chemical analyses of Dry matter content (DM; ID no. 934.01), crude protein (CP; ID no. 2001.11), ether extract (EE; ID no. 2003.5), and ash fraction (A; ID no. 942.05) were determined according to methods approved by the Official Association of Analytical Chemists (AOAC, 2011). The Lignin (sa)-Lignin determined by solubilization of cellulose with sulphuric acid. Neutral detergent fiber (assayed with a heat stable amylase and expressed exclusive of residual ash - aNDFom-NDF) and acid detergent fiber (expressed exclusive of residual ash-ADFom-ADF) fractions were determined using a fiber analyzer (Tecnal TE-149, Piracicaba, Brazil) according to the methodology described by Van Soest et al. (1991) and adapted by Mertens et al. (2002; Table 1). Free gossypol was determined by a methodology adapted from Botsoglou, (1992). The soybean seed was submitted in a drying oven (FANEM LTDA) to 90º C for 1h30m based on studies by [15, 16]. The lambs remained on breastfeeding until the experiment was finished. The lambs received water, Cynodon ssp. Hay, mineral salt, and creep feeding (crushed corn and soybean meal in the proportion of 70:30 respectively) ad libitum, prepared according to [9] Table 1).

Hematological And Biochemical Parameters

Blood two samples were collected monthly (Jan to Oct) by jugular venipuncture, using needles coupled to a vacutainer tube containing 0.05 mL of ethylenediaminetetraacetic acid (EDTA) to perform hemogram and the other sample without EDTA to perform biochemical parameters. Serum and plasma samples were obtained after centrifugation at 2000 rpm for 10 min and kept frozen at -20 °C until further analysis.

Blood samples with EDTA were used to perform the complete blood count using a hematological analyzer (Davol poch-100 iV, São Paulo, Brazil). The following parameters were determined: red blood cell count (RBC), hemoglobin concentration (Hg), hematocrit (HT), platelet count (PLT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and white blood cell (WBC). The differential leukocyte count was performed utilizing light microscopy with the count of 100 cells in the blood smear stained with rapid dye (Panótico Rápido – LABORCLIN® LTDA, Pinhais, Paraná, Brazil) for hematology to determine lymphocytes, neutrophils, eosinophils, and monocytes.

Blood biochemical determinations were performed using commercial kits (LABTEST® Lagoa Santa, MG, Brazil) by spectrophotometry procedures in automated equipment (Spectrophotometer ThermoFisher Scientific, Waltham, Massachusetts, EUA). The biochemical tests were quantified for glutamic-oxaloacetic transaminase (GOT) (kinetic method), creatinine (colorimetric-creatinine method), urea (colorimetric enzymatic method), total protein (colorimetric-biuret method) and albumin (bromocresol colorimetric-green method). Beta-Hydroxybutyrate (BHB) levels were analyzed using the Optium Xceed equipment with f-Keto strips (FreeStyle/Optium).

Ultrasound Measurements

Ultrasound measurement images were obtained monthly with (CHISON D600VET ultrasound, Jiangsu, China) equipped with a linear transducer probe of 5-MHz frequency. The local trichotomy was performed between the 12th and 13th rib on the left side of the animal. We measured the longissimus dorsi area (LA), longissimus dorsi growth depth (LGD), longissimus dorsi growth width (LGW), and longissimus dorsi fat thickness (LFT).

Helminth Infection (Fecal Egg Counts)

Fecal samples were collected monthly, directly from the animal rectum, for fecal egg count (FEC), according to [39].

Statistical Analysis

The data were analyzed in a completely randomized design with measurements repeated over time (10 months) in the MIXED procedure of SAS v. 9.1® software (SAS Institute Inc; Cary, North Carolina, USA). The fixed effects were dietary treatments (CO or CA), time, and interactions with the random effect of the experimental unit (animal). Three types of covariance structures were used for the repeated analysis: unstructured (UN), compound symmetry (CS), and auto-regressive (AR (1)). That with the lowest BIC was chosen for the analysis. Data for FEC were previously log (x + 10) transformed to stabilize the variance before the analysis, and the results were back-transformed and presented with the original values. Comparisons between the means were performed using Tukey´s test at 5% probability. The graphics were generated with the SIGMAPLOT v.10 software.

Results

The results of HT and Hg are shown in Figure 1 a, c. The percentages showed variation during the experiment in both treatments, yet within the reference values (24 to 50% and 8 to 16g/dL respectively). In the CO and CA treatments, RBC decreased from birth (July) to lactation end (October; $p > 0.05$), with values below those recommended (<8 – 16 106/µL; Figure 1 b). In terms of time, ewe fed on CO presented higher Hg values at birth that CA treatment (July; $p > 0.05$). And the variables, PLT, MCV and MCHC indicated no differences between treatments ($p > 0.05$ Figure 1 d, e, f). In both treatments, ewe had a higher amount of PLT at the lactation end (September/October) differing from early pregnancy (May; $p > 0.05$).

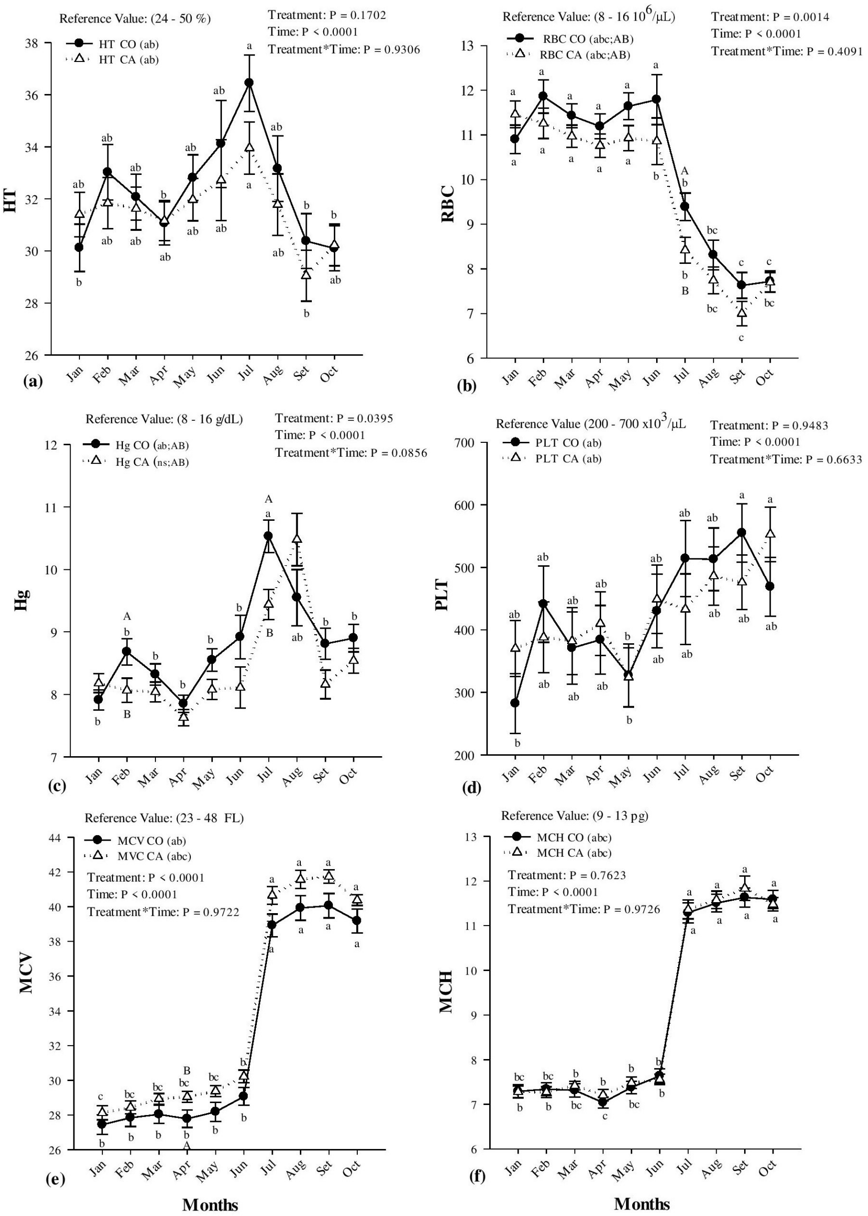

Figure 1: Means ± standard error of (a) hematocrit (HT), (b) the red blood cell (RBC), (c) hemoglobin (Hg), (d) platelet count (PLT), (e) mean corpuscular volume (MCV) and (f) mean corpuscular hemoglobin concentration (MCHC) of ewe (CA and CO). a, b Lowercase letters indicate differences within the treatment in the time (P <0.05). A, B Uppercase letters indicate differences between treatments (P<0.05), Reference values according to Kaneko et al. (2008).

Similarly, in WBC count no differences were found between treatments (p > 0.05). In the analyses during the time, there was a decrease during pregnancy and an increase at birth and lactation in both treatments (p > 0.05; Figure. 2 a, b). The results of the differential leukocyte count (lymphocytes, neutrophils, eosinophils, and monocytes) are shown in Figure 2 c -f, respectively. In lymphocyte counts (Figure 2 c), from the beginning of pregnancy (April to June), the ewe (CO and CA) presented decreased values (< 2.0 x103/µL) but increasing until birth (p < 0.05). The neutrophil count increased in late pregnancy (July and August; p > 0.05; Figure 2 d). During the assessed period, eosinophilia in the ewe was observed in six months for the ewe fed on CO and in three months for ewe fed on CA (Figure 2 e). The monocyte count had little variation in the experimental period (Figure 2 f).

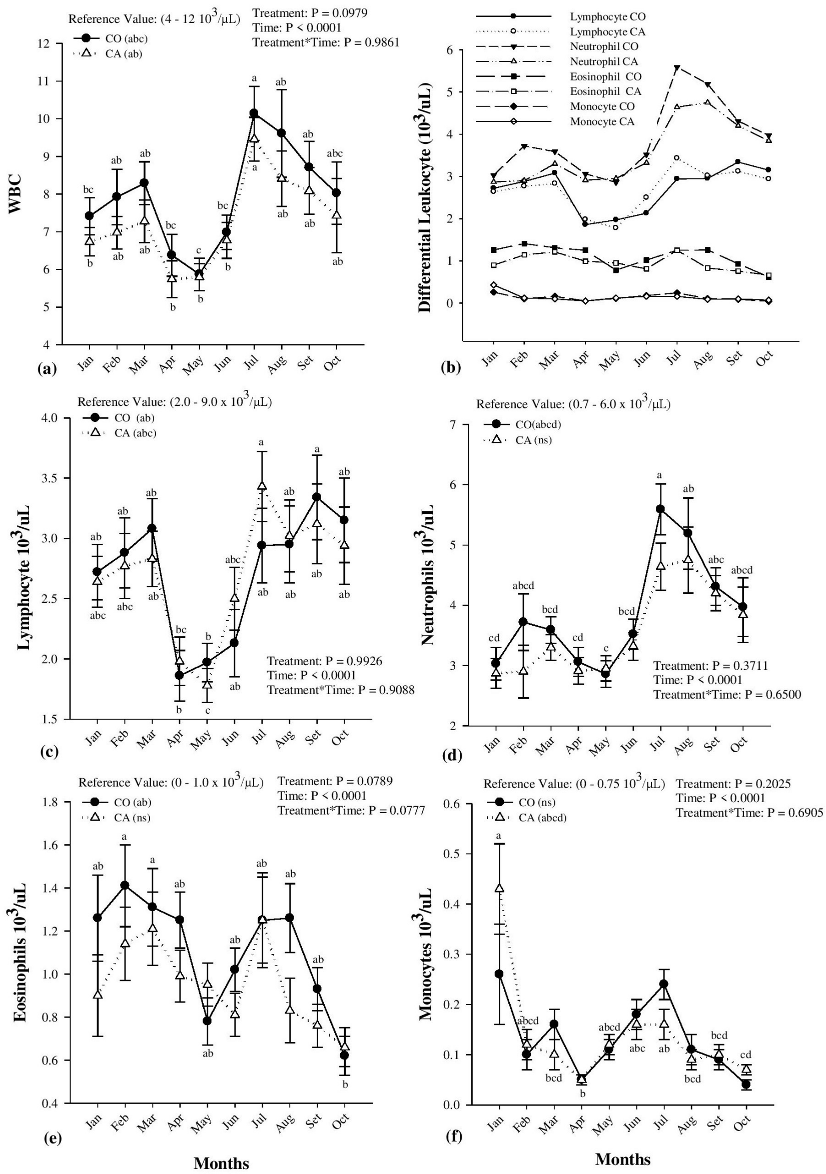

Figure 2: Mean ± standard error of the white blood cell (WBC), (b) differential leucocyte, (c) lymphocytes, (d) neutrophils, (e) eosinophils and (f) monocytes of ewes (CA and CO). a, b Lowercase letters indicate difference (P<0.05) within treatment in the time; Reference values according to Kaneko et al. (2008).

In both treatments (CO/CA), the total protein and globulin levels were above the reference levels (6.0 to 7.9 g/dL and 3.5 to 5.7 g/dL, respectively [17]. The CA treatment presented higher values of total protein and globulin level in the pre-breeding season and 15 days postpartum, differing from CO treatment ($p < 0.05$; Table 2). The albumin levels did not differ between treatments and experimental period ($p > 0.05$). Urea data were within desired levels for both treatments (CO / CA). The levels were higher on the 60th day prepartum in the CO treatment compared to the CA treatment ($p > 0.05$).

	Albumin		Total protein		Globulins		Urea	
	CO	CA	CO	CA	CO	CA	CO	CA
PBS	2.75 ± 0.35	2.46 ± 0.33	7.80 ± 0.49 ^{B}	9.37 ± 0.45 ^{abA}	4.23 ± 0.60 ^{B}	5.96 ± 0.55 ^{abA}	39.4 ± 2.7 ^{de}	40.3 ± 2.5 ^{de}
60 pre	3.16 ± 0.18	3.19 ± 0.17	8.65 ± 0.30	8.56 ± 0.28 ^{ab}	5.46 ± 0.44	5.74 ± 0.41 ^{ab}	66.7 ± 2.5 ^{aA}	60.1 ± 2.3 ^{abB}
45 pre	2.77 ± 0.16	2.51 ± 0.15	7.17 ± 0.82	7.69 ± 0.76 ^{ab}	4.46 ± 0.90	5.02 ± 0.83 ^{ab}	46.3 ± 2.9 ^{cde}	43.2 ± 2.7 ^{cde}
30 pre	2.41 ± 0.20	2.47 ± 0.18	8.94 ± 0.55	9.25 ± 0.51 ^{ab}	6.52 ± 0.53	6.74 ± 0.49 ^{ab}	57.6 ± 2.7 ^{abc}	50.5 ± 2.5 ^{bcd}
15 pre	2.54 ± 0.25	2.42 ± 0.23	8.48 ± 0.53	8.29 ± 0.49 ^{ab}	5.31 ± 0.56	5.87 ± 0.52 ^{ab}	65.6 ± 2.6 ^{a}	56.7 ± 2.4 ^{abc}
Birth	2.71 ± 0.26	2.66 ± 0.24	8.42 ± 0.83	8.96 ± 0.77 ^{ab}	5.79 ± 0.90	6.62 ± 0.83 ^{ab}	50.3 ± 3.9 ^{bcde}	53.0 ± 3.6 ^{abcd}
15 post	2.62 ± 0.33	3.09 ± 0.30	8.33 ± 0.67 ^{B}	10.26 ± 0.62 ^{aA}	5.58 ± 0.81 ^{B}	7.80 ± 0.75 ^{aA}	50.4 ± 3.2 ^{bcd}	48.2 ± 3.0 ^{bcde}
30 post	3.18 ± 0.30	2.81 ± 0.28	7.51 ± 0.31	7.53 ± 0.29 ^{b}	4.73 ± 0.39	5.05 ± 0.36 ^{ab}	36.2 ± 2.9 ^{e}	37.4 ± 2.7 ^{e}
45 post	3.56 ± 0.30	3.40 ± 0.28	9.33 ± 0.94	10.03 ± 0.87 ^{ab}	6.79 ± 0.90	6.84 ± 0.83 ^{ab}	64.1 ± 3.7 ^{ab}	65.1 ± 3.4 ^{a}
60 post	2.62 ± 0.21	2.34 ± 0.20	6.67 ± 0.46	7.43 ± 0.42 ^{b}	4.04 ± 0.58	4.34 ± 0.54 ^{b}	38.7 ± 4.0 ^{de}	43.0 ± 3.7 ^{cde}
Minimum	2.41	2.34	6.67	7.43	4.04	4.34	36.18	37.40
Maximum	3.56	3.40	9.33	10.26	6.79	7.80	66.69	65.12
***P*-value**								
Treatment	0.3900		0.0262		0.018		0.1948	
Time	<.0001		<.0001		<.0001		<.0001	
Treatment*Time	0.9306		0.4437		0.8178		0.2859	
Mean ± SE	2.83 ± 0.08	2.73 ± 0.07	8.13 ± 0.19	8.74 ± 0.18	5.29 ± 0.20	6.0 ± 0.21	51.52 ± 1.0	49.74 ± 0.92
Ref-value	**2.4 - 3.0 g/dL**		**6.0 - 7.9 g/dL**		**3.5 - 5.7 g/dL**		**36 - 92 mg/dL**	

a,b,c,d,e Lowercase letters in columns indicate differences within treatment in the time (P < 0.05); A,B Uppercase letters in rows indicate difference between treatments (P < 0.05). Reference values according to (Kaneko et al. 2008). Pre-breeding season (PBS), days before childbirth (pre), days after childbirth (post), and reference value (Ref-value).

Table 2. Means ± standard error of the concentrations of albumin, total protein, globulin and urea of *Santa Inês* race sheep fed with soybean seed (CO) and cottonseed (CA) treatments.

Creatinine levels increased (p > 0.05) in both treatments at birth compared to other periods. Higher levels of GOT were observed in the CO treatment on the 15th day postpartum concerning CA treatment (p > 0.05; Table 3) and BHB values did not show differences between treatments (CO and CA; p > 0.05). Also, creatinine, GOT and BHB were maintained within the reference levels for the species.

	Creatinine		GOT		BHB	
	CO	CA	CO	CA	CO	CA
PBS	1.50 ± 0.09 ^{ab}	1.52 ± 0.08 ^{bc}	82.58 ± 5.77 ^{bc}	86.46 ± 5.46 ^{abc}	0.42 ± 0.03	0.50 ± 0.03 ^{ab}
60 pre	2.57 ± 0.30 ^{a}	2.53 ± 0.28 ^{ab}	100.94 ± 6.29 ^{abc}	101.22 ± 5.82 ^{abc}	0.38 ± 0.03	0.39 ± 0.04 ^{b}
45 pre	1.49 ± 0.12 ^{abc}	1.52 ± 0.11 ^{bcd}	95.87 ± 5.54 ^{abc}	108.64 ± 5.13 ^{a}	0.39 ± 0.04	0.48 ± 0.04 ^{ab}
30 pre	1.03 ± 0.08 ^{c}	1.01 ± 0.07 ^{e}	112.91 ± 3.62 ^{a}	108.14 ± 3.35 ^{a}	0.46 ± 0.08	0.65 ± 0.08 ^{ab}
15 pre	1.56 ± 0.09 ^{ab}	1.55 ± 0.08 ^{bc}	121.58 ± 9.06 ^{a}	104.36 ± 8.39 ^{abc}	0.63 ± 0.10	0.61 ± 0.09 ^{ab}
Birth	2.47 ± 0.20 ^{a}	2.72 ± 0.18 ^{a}	73.86 ± 5.90 ^{c}	82.17 ± 5.46 ^{bc}	0.65 ± 0.22	0.58 ± 0.20 ^{ab}
15 post	1.28 ± 0.08 ^{abc}	1.34 ± 0.07 ^{cde}	99.02 ± 7.63 ^{abcA}	77.15 ± 7.06 ^{cB}	0.61 ± 0.09	0.73 ± 0.08 ^{a}
30 post	1.30 ± 0.12 ^{abc}	1.39 ± 0.11 ^{cde}	88.17 ± 7.17 ^{abc}	94.33 ± 6.63 ^{abc}	0.47 ± 0.04	0.47 ± 0.03 ^{ab}
45 post	1.28 ± 0.29 ^{abc}	1.41 ± 0.26 ^{bcde}	106.35 ± 6.18 ^{ab}	107.63 ± 5.72 ^{ab}	0.50 ± 0.03	0.48 ± 0.02 ^{ab}
60 post	1.12 ± 0.11 ^{bc}	1.02 ± 0.11 ^{de}	108.68 ± 6.91 ^{a}	108.03 ± 6.40 ^{ab}	0.52 ± 0.03	0.48 ± 0.02 ^{ab}
Minimum	1.03	1.01	73.86	77.15	0.38	0.39
Maximum	2.57	2.72	121.58	108.64	0.65	0.73
***P*-value**						
Treatment	0.6093		0.6756		0.4018	
Time	<.0001		<.0001		0.0001	
Treatment*Time	0.9952		0.2220		0.4963	
Mean ± SE	1.56 ± 0.05	1.60 ± 0.05	98.99 ± 2.0	97.81 ± 1.91	0.50 ± 0.02	0,54 ± 0.02
Ref-value	**1,2 - 1,9 mg/dL**		**0 - 280 U/L**		**<1 mM/L**	

a,b,c,d,e Lowercase letters in columns indicate differences within treatment in the time (P < 0.05); A,B Uppercase letters in rows indicate difference between treatments (P < 0.05). Reference values according to (Kaneko et al. 2008). Pre-breeding season (PBS), days before childbirth (pre), days after childbirth (post), and reference value (Ref-value).

Table 3. Means ± standard error of the concentrations of creatinine, glutamic oxaloacetic transaminase (GOT) and beta hydroxybutyrate (BHB) of *Santa Inês* breed sheep fed with the soybean seed (CO) and cottonseed (CA) treatments.

From early pregnancy (May) until the end of the experiment, the BW variation of ewe in the CA treatment was lower compared to the ewe in the CO treatment (p > 0.05; Figure. 3 a). The BCS was higher in the ewe fed on CO in the first month of pregnancy (April) and near birth (July/August; p > 0.05; Figure. 3 b).

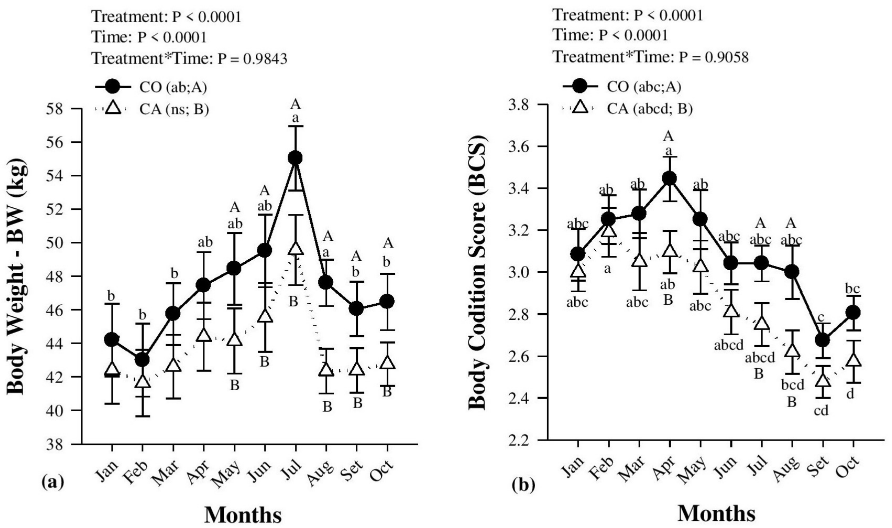

Figure 3. Means ± standard error of (a) body weight (BW) and (b) body condition score (BCS) in Santa Inês ewe fed on CO and CA. a, b Lowercase letters indicate difference (P <0.05) within treatment in the time, A, B Uppercase letters indicate differences between treatments, asterisks indicate no difference within the treatment in the time.

The results of in vivo carcass measurements (LA, LGD, LGW, and LFT) performed through ultrasonography are shown in Table 4. Ewe fed with CO had better measurements of LA and LGD from the early pregnancy to lactation than ewe that were fed with CA, except for LGD, (p > 0.05). For LGW, no differences were observed during the experimental time in the treatments (p > 0.05). However, the CO treatment presented higher LGW in Abril and June in comparison to the CA treatment (p > 0.05). The LFT measurements indicated differences at the end of pregnancy and the start of lactation (Jul and Aug) between CO and CA (p > 0.05; Table 4).

In the evaluation of lambs born from ewe that were fed with CA, has been identified that lambs presented lower body weight at birth and weaning (p < 0.05; Figure. 4). No differences were observed between the sex variable and weight of lambs (female or male) between treatments CO/CA (p > 0.05), except in females to 60d (p < 0.05).

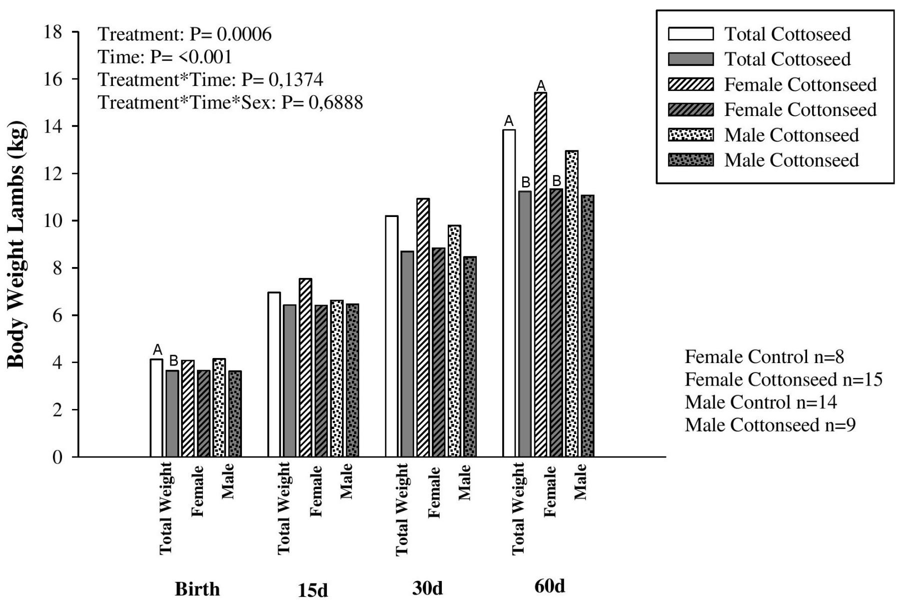

Figure 4. Total body weight and by sex (male and female) a birth, 15, 30 and 60 days of lambs descended from Santa Inês ewe fed with cotton seed and soybeans seed. A, B Uppercase letters indicate differences (P <0.05) between treatments.

The study showed no difference ($p > 0.05$) in endoparasite infection between treatments (CO and CA) during the experimental period. However, there was interaction with time, highlighting a gradual increase in FEC in late pregnancy and lactation ($p > 0.05$; Figure. 5).

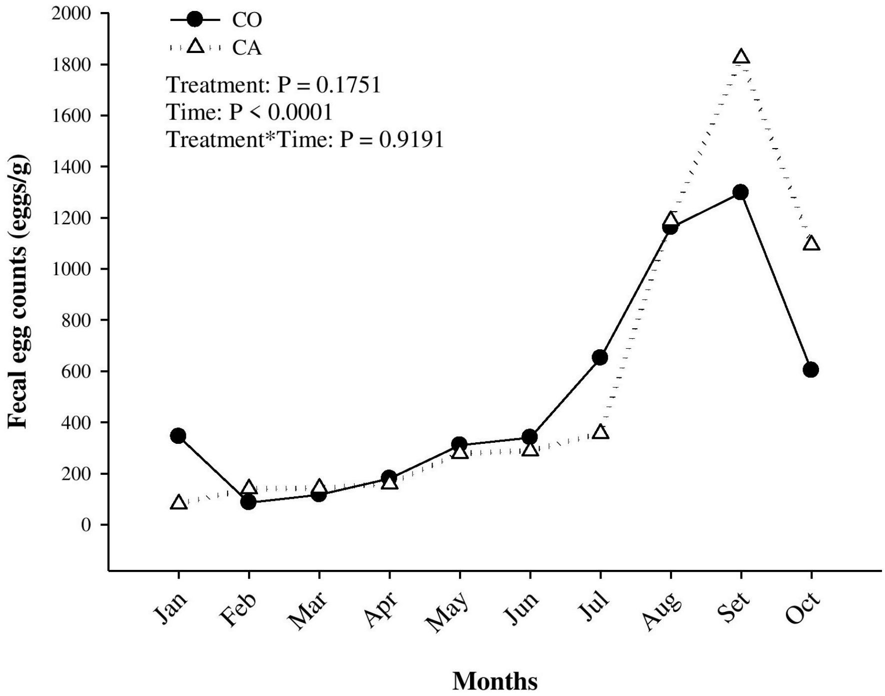

Figure 5: Fecal egg counts in ewe fed with soybeans seed (CO) and cottonseed (CA).

Discussion

The study evaluated the hematological and biochemical parameters, body dynamics, and in vivo ultrasonography measurement and helminth infection of ewe fed with cottonseed during all reproductive periods. Thus, understanding the safe use of this cotton by-product as a feed alternative. During the experimental period (10 months), was intake 360 ppm of free gossypol/animal per day in the cottonseed. In this scenario, no negative cumulative effects were identified related to health and/or toxicity that could cause serious problems in ewe fed with CA treatment in the reproductive period [10].

Blood metabolite variation is observed, mainly in the final third of pregnancy and early lactation, corresponding to the moments of high metabolic requirement. There was no difference in the hematological constituents between pregnant and puerperal ewe, with an evident decrease in HT similar to birth. Similarly, in this study, variations from 29.05% to 36.44% in HT were identified with higher values in early pregnancy decreasing near birth [18].

The WBC counts remained within the reference values, with a lower limit in early pregnancy and upper limit near birth and decreasing in the lactation. Indicating that white blood cells and/or immune system were not affected in both treatments. This happened because, in the early pregnancy, the ewe's immune system is suppressed to keep gestations. On the other hand, in the final third of gestation, blood volume may change in response to placental circulation and fetal development [19]. Thus, we can say that the good levels observed in white blood cells in ewe (CO / CA), contributed to the good health of the flock and lambs, once during lactation the lambs stayed equally healthy. [20] evaluated the effect of maternal cottonseed feed on the immune and antioxidant status of Santa Ines lambs and identified that concentration of alpha-beta globulin, gamma globulin, immunoglobulin G and M, the activity of glutathione peroxidase, catalase, and oxygen radical absorbance capacity were not affected during the lactation period.

Ewe (CO and CA) presented eosinophilia according to the increase in endoparasite load, which is associated to the immune response to helminths [21,22, 23, 18]. The biochemical profile stands out as an excellent tool to evaluate the health of animals, which may be altered by normal physiological metabolic processes, diseases, or nutritional changes or intoxication [24]. Treatment CA showed that cottonseed caused neither hematological nor biochemical disorders in ewe. Similarly, the consumption of 400 g. kg−1 cottonseed cake (3.28 mg. g-1 of free gossypol by ewe for 63 days caused no consistent changes in serum biochemical parameters, indicating no damage to the liver or kidneys [4]. Total protein and globulin levels remained slightly above reference levels throughout the experiment in both treatments (CO and CA), being something more physiological of the race. Protein levels can increase during pregnancy, which is related to kidney function to filter fluid excess in this reproductive stage. The albumin level showed that ewe in both treatments gradually increased their levels from birth to 45 days postpartum, and urea levels remained normal. Albumin and urea are protein indicators that also provide information on animal health. Urea shows protein status in the short term, whereas albumin demonstrates in the long-term [25].

BHB is used to monitor energy balance in ruminants [26]. During the end of pregnancy, BHB concentration between 0.8 mM/L to 1.6 mM/L is suggestive of moderate malnutrition; and between 1.7 mM/L to 3.0 mM/L indicates a worrying lack of dietary energy and, in clinical cases, of pregnancy toxemia [27]. In ewe with satisfactory pregnancy, BHB concentration is below 0.5 mmol/L [28]. Therefore, the results in the experiment indicated that ewe had energy intake according to the use of fiber from the CA treatment.

Creatinine is a waste product from the normal breakdown of muscle tissue. As creatinine is produced and filtered through the kidneys after excreted in the urine. Any changes in levels of creatinine in the blood are related to excretion and therefore reflect kidney function [29]. Thus, cottonseed intake did not cause nephrotoxic problems during the experimental period, showing creatinine levels similar to the control treatment. Besides, cottonseed intake did not cause liver disorders, even though GOT levels were similar between groups. This enzyme participates in the metabolism of the amino acids and is present at high levels when there is liver damage [30]

However, cottonseed did not exceed expectations regarding BCS. Ewe fed on CO presented superior results in almost all body dynamics parameters (BW, BCS, LA, LGW, and LGD) compared to ewe fed on CA. Moreover, lambs developed lower weights in birth and the weaning. We highlighted those lambs did not intake cottonseed, but were indirectly affected by the diet of ewe during gestation and breastfeeding. studies developed by [9] in the male descendants of the ewes of the present study identified that in the ewe milk fed with cottonseed, had lower dry matter, protein, ether extracts, Ca, and K, and consequently lower BW in male lambs compared with the soybean seed group. This fact shows possible breastfeeding with lower milk quality in CA treatment than soybean seed, and that affected lambs' weight gain. Equally, [20] observed that the colostrum of ewe fed cottonseed had a positive effect on the immune quality and antioxidant potential of the colostrum.

Feed requirements increase during pregnancy because the animal needs nutrients for udder development, maintenance, and fetal growth, therefore, during pregnancy and lactation, there is a decline in body reserves. Considering that the diets of both treatments were formulated to have similar energetic and protein values, this difference between ewe fed on CO compared to ewe fed on CA can be related to the degradability and digestibility of grain in the rumen and small intestine, respectively. The ADF and lignin values in the CA treatment were higher than in the CO treatment (Table 1). These characteristics are inherent to each grain used. It is known that the higher the ADF and lignin contents compromises rumen degradability with less energy availability for the animal. Lignin is classified as an indigestible substance due to its physical and chemical resistance and its strong bond with cellulose, making this nutrient have a reduced degradability [31]. Also, [32, 33] stated that lignin has the ability to reduce the hydrolytic action of enzymes and rumen microorganisms on fibrous carbohydrates due to inter-resistant bonds, resulting in less degradability. This situation can explain the less BW and BSC performance ewe from CA against CO.

Despite that, regions with scarcity, problems of acquisition, or cost increase in traditional feed, such as soybean, among others. The ewe farmer can ease and tranquility of offering your animals the cottonseed or plus another carbohydrate source to minimize the effect of lower quality fiber. Representing a great cost-benefit, providing a nutritional value without harm to the health of the ewes during the breeding, gestation, and lactation periods, regardless of the differences in body dynamics observed in the present study, the body dynamics showed stable body maintenance values.

In treatments, In the diet, nutrients as the protein always were balanced in both treatments and the levels of albumin, total protein, globulins and urea in serum are proof of this. In this way, the protein was not a limiting nutrient for cottonseed treatment. We hypotized that the BW and BCS performance were reduced due to the cottonseed lower quality fiber, as previously discussed. But despite the difference in body dynamics between CO and CA treatments, both treatments were efficient for the maintenance of a satisfactory pregnancy, lactation, and weaning (see blood variables). And the cottonseed treatment can be used when the soybean won't economically accessible Therefore, that may have been an important fact which probably interfered with the body condition of the ewe and consequently their lambs.

Endoparasites are one of the most serious health problems in ewe production, especially in the ewe that present the phenomenon widely known in the literature as spring-rise [14]. The increase in the verminosis in the peripartum period may occur because the organism distributes the nutrients for fetal growth and milk production during these phases, which are a priority, reducing nutrient availability for the immune system to fight parasites [34]. After weaning, the immune response of the ewe is restored and there is reduction in the FEC [35].

Several literature reports show many biological effects of gossypol, such as anticancer, antiviral, antifungal, antimicrobial due to its integration to the group of phenols [36][11]. Besides that, among the various drug properties of gossypol is also the parasitic action [37], inhibiting tapeworm larval development and eliminating the tapeworm due to the stimulation of host immunity [38]. However, this hypothesis was not confirmed in our study because, in the treatments tested (CO/CA), no differences were identified in egg count per gram, which showed similar behavior.

The changes observed in the blood variables were due to the reproductive phases of the ewe (gestation and lactation), aggravated by the increase in the endoparasites, and no effect of the diet was identified. There is evidence of a decrease in red cells after birth and, consequently, increase in the parasite population, with levels near to anemia levels in the lactation period. Consequently, PLT remained high in late pregnancy and after birth, due to possible mucosal lesions caused by worms and blood loss at birth and placental expulsion. Besides, the cell size also changed, with increase in MCV and MCHC to compensate possible anemia.

Conclusions

It is concluded that cottonseed intake (360 ppm of gossypol/animal per d) during long periods (10 months) for ewe did not result in intoxication, did not aid in endoparasite control and did not improve productivity because the body dynamics in ewe fed on cottonseed was less efficient both in ewe and in their lambs until weaning.

Conflict of Interest

The authors declare that there are no conflict of interests.

Acknowledgments

The authors thank the Center for Nuclear Energy in Agriculture (CENA), University of São Paulo (USP), Foundation for Research Support of the State of São Paulo (FAPESP) and National Counsel of Technological and Scientific Development (CNPq) for financial support.

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