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Bioactive Compounds of Barbatimão (*Stryphnodendron* sp.) as Dietary Additive in Lamb Diets

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Abstract: This study aimed to evaluate barbatimão bark extracts as a feed additive and substitute for lasalocid sodium (LAS) for feedlot lambs. Lambs were distributed into three treatments: LAS (0.018 g of lasalocid sodium), DBB (1.500 g of dried and milled barbatimão bark), and BHE (0.300 g of barbatimão hydroalcoholic extract). There was no effect ($p = 0.32$) of the inclusion of DBB and BHE extracts on the average daily gain. Inclusion of BHE in lamb diets reduced ($p < 0.05$) the fatness score compared to LAS, which was similar to DBB. The BHE decreased the yellowness intensity and hue angle ($p < 0.05$) of meat compared to the LAS. Animals that consumed DBB and BHE had a reduced ($p = 0.04$) total cholesterol level. Thus, the use of barbatimão bark extracts can replace lasalocid sodium in the diet of feedlot lambs, with no detrimental effects on performance or metabolic parameters.

Keywords: ruminants; tannins; bioactive compounds; saponins; feed additives

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1. Introduction

Lasalocid sodium (LAS) is a synthetic product used as an additive in animal feed, related to its potential to mitigate methane (CH4) emissions, improve animal performance [1], and increase production profitability. However, the market is increasingly demanding, resulting in the rejection of chemicals in animal protein production, due to the evolution of antibiotic-resistant pathogens [2]. In the search for substitutes for synthetic products, the animal feed additive industry has intensified its investments in biocompounds [3]. These biocompounds modify ruminal subtract availability and microbial ecosystem, thus reducing CH4 emissions [4]. The natural additive effects are related to the type of diet (high or low concentrate), concentration and amount of ingested additive, mode of action in the gastrointestinal tract, and physiological state of the animal [3,5]. In addition, the composition of biocompounds in plants can be affected in several ways, from the plant development to the final extraction [6].

Barbatimão (*Stryphnodendron* sp.) is a plant native to the Brazilian savanna and produces several chemical metabolites in its secondary metabolism, such as tannins and saponins [7]. These biocompounds have antimicrobial, healing, anti-inflammatory, and antioxidant activities; thus, it is a plant used by communities in traditional medicine [8,9]. Tannins are phenolic compounds with properties that precipitate proteins [10]. High doses of tannins (>5% in dry matter; DM) in a ruminant diet may lead to a reduction in DM intake, digestibility, and performance [11]. However, at moderate doses, tannins potentiate nutrient use efficiency, due to the greater availability of these nutrients in the small intestine [3]. Saponins are active photochemical components of plants, which are part of the plant defense system, and that have an antimicrobial and antioxidant potential [8,12] that can affect ruminal microorganisms. In the literature, several studies have shown

that the inclusion of sources of tannins and saponins in ruminant diets can reduce enteric methane production, improving feed efficiency and animal performance [11,13,14].

In this context, barbatimão extract has advantages because of its various active components and modes of action, being a natural and safe option as an additive [15]. Thus, the objective of this study was to evaluate the effects of the use of barbatimão bark extracts, as a food additive in place of LAS, on the metabolic parameters, performance, and carcass characteristics of feedlot lambs.

2. Materials and Methods

2.1. Location and Experimental Facilities

The experiment was carried out at the Federal University of Grande Dourados. The Animal Use Ethical Committee of the Federal University of Grande Dourados, Brazil, approved the experimental animal procedures (Protocol 032.2020).

2.2. Collection, Production, and Phytochemical Analysis of Extracts

Barbatimão barks (*Stryphnodendron rotundifolium*) were manually collected from several trees during the summer season in the morning and dried in a forced ventilation oven at 55 °C for 72 h. Then, the material was milled in a Willey mill with a 2 mm sieve, to obtain the dry barbatimão bark (DBB). Barbatimão hydroalcoholic extract (BHE) was obtained by submersion of 250 g of DBB in a water–ethanol solution 50:50 [16] and incubated in a water bath at 60 °C for 60 min. Hot filtration was performed in a funnel with four layers of cheesecloth and dried (40 °C) to a constant weight. Then, the material was macerated until a powder granulometry was obtained.

The extracts were submitted to phytochemical evaluation [17], to confirm the secondary metabolite classes [18]. The presence of triterpenes and steroids was confirmed through hydrolysis of the dry methanolic extract. This procedure was performed with potassium hydroxide (0.5 mol/L) and with reflux for 1 h. Then, the compounds were extracted with ethyl ether and then submitted to a Liebermann–Burchard reaction. To determine the presence of secondary metabolite classes, the reactions of characterization intensities were classified as follows: negative reaction (− = 0%), partial intensity (±/+ = 10%), low intensity (+ = 50%), medium intensity (++ = 75%), and high intensity (+++ = 100%) [18]. The extracts were solubilized at a concentration of 1 mg/mL in methanol, to analyze phenolic compounds and flavonoids [19]. The content of phenolic compounds was determined based on the Folin–Ciocalteau colorimetric method [19]. The tannin content was determined using the Folin–Denis spectrophotometric method, with tannic acid as a reference [20].

2.3. Animals, Diets, and Experimental Design

Twenty-four lambs, non-castrated males, with 150 ± 4.59 days of age and 21.2 ± 3.63 kg of body weight (BW) were used. At the beginning of the experiment, the lambs were weighed, identified, and dewormed (Baycox, 1 mL/3.5 kg BW). The lambs were allocated to individual covered stalls with an area of 2.2 m^2 (1.5 m × 1.5 m), with a cement floor lined with rice husk. They had free access to water and had access to ground oat hay (*A. sativa*) and concentrate ad libitum for 14 days for adaptation.

After the adaptation phase, the experimental period began, consisting of three periods of 14 days, counting 42 days of performance evaluation plus six days up to the slaughter, totaling 48 days for the experiment. The experimental diet (Table 1) was formulated based on soybean meal, ground corn, oat hay, and specific commercial mineral supplement for sheep, according to the National Research Council (NRC) [21], to meet the requirement of lamb weight gain of 300 g/animal/day, with the dry matter intake estimated at 3.5% of BW. The forage:concentrate ratio was 20:80. The total diet was offered at 07:00 and 12:00, adjusting the amount provided every three days, considering 5% of leftovers.

Table 1. Ingredients and chemical composition of the experimental diet.

Ingredients	Quantity (g/kg DM)
Hay	200.0
Ground corn	632.0
Soybean meal	152.0
Salt	8.0
Calcium carbonate	8.0
Composition	
Dry matter (g/kg as feed)	895.0
Organic matter (g/kg of DM)	918.0
Crude protein (g/kg of DM)	210.0
Ether extract (g/kg of DM)	35.0
Neutral detergent fiber (g/kg of DM)	247.0
Acid detergent fiber (g/kg of DM)	138.0

The experimental designed used randomized blocks, with three treatments and eight repetitions. The lambs were blocked based on BW, and the treatments were randomly distributed within each block. The treatments tested were addition of DBB, 1.50 g/animal/day; addition of BHE, 0.300 g/animal/day; and addition of LAS, 0.018 g/animal/day. DBB and BHE were mixed with 30 g of concentrate and supplied as a top-dress at morning meals, to ensure total intake. For LAS, 0.150 g/kg of the ionophore (Taurotec-Zoetis, Campinas, São Paulo, Brazil; 15% of lasalocid sodium) was mixed into the diet.

2.4. Animal Performance

The dry matter intake was determined daily from the difference between the offered feed and the leftovers. The leftovers were weighed before the morning meal. A composite sample of diet ingredients and leftovers was collected per period per animal and stored in a freezer at −20 °C. At the end of the collection period, the samples were pre-dried in a forced ventilation oven at 55 °C for 72 h. Lastly, the samples were milled (1 mm) in a Willey mill for further analysis.

The lambs were weighed on the first day (initial body weight) and at the end of each experimental period, with fasting of solids and liquid in the previous 12 h. The average daily gain was measured from the difference in BW between the beginning and the end of the experimental period, divided by the number of days. The relation between dry matter intake and average daily gain was used to determine the feed conversion ratio. The body condition at slaughter was evaluated by two specialists, based on a scale from 1 to 5, with a 0.5 variation.

2.5. Chemical Composition

Diet and extract samples were analyzed for dry matter content (ID 934.01), ash (ID 930.05), crude protein (CP, ID 981.10), and ether extract (ID 920.39) [22]. Neutral detergent fiber and acid detergent fiber contents were evaluated according to Van Soest [23].

2.6. Slaughter and Carcass Evaluation

All slaughter procedures were performed according to the Regulation of Industrial and Sanitary Inspection of Products of Animal Origin and the rules of Technical Regulation of Methods of Insensitization for the Humanitarian Slaughter of Butcher Animals [24]. The lambs were slaughtered at the end of the experiment, after 62 days in the feedlot (14 of adaptation + 48 of the experiment). The lambs were submitted to solid fasting for 16 h and weighed to determine the body weight at slaughter. The lambs were desensitized by electronarcosis, suspended by the hind legs, and the carotid arteries and jugular vein were sectioned for bleeding, then they were skinned and eviscerated. The full and empty gastrointestinal tract, bladder, and gallbladder were weighed, and the weight of the abiotic components was obtained from the difference between the full and empty weights. The empty body weight was determined from the subtraction of the abiotic components from

body weight at slaughter. Carcass conformation and fatness indexes were determined by two specialists, based on a scale from 1 to 5, with a 0.5 variation, where: 1—Very poor; 1.5—Poor; 2—Acceptable; 2.5—Average; 3—Good; 3.5—Very Good; 4—Superior; 4.5—Very Superior, and 5—Excellent [25].

The carcasses were weighed to obtain the hot carcass weight. The carcasses were suspended by the leg tendons and stored in a cold room at 4 °C for 24 h, and were posteriorly weighed to obtain the cold carcass weight. Then, the yields of the hot carcass and cold carcass and the loss by cooling were determined [26].

The meat color in the Longissimus thoracis et lumborum muscle was determined [27] using a digital colorimeter (Minolta CR-400, Minolta Co., Osaka, Japan), calibrated in the CIELAB system. The luminosity (L*), red intensity color (a*), and yellow intensity color (b*) [28] were measured. The saturation index (chroma; C*) was determined according to the equation:

$$C* = \sqrt{(a*^2) + \left(b*^2\right)} \quad (1)$$

The definition of metric hue angle (HUE) was determined according to the equation:

$$\text{HUE} = \text{arctangent } (b^*/a^*) \quad (2)$$

2.7. Metabolic Parameters

Blood samples were taken by jugular vein puncture, four hours after morning feeding, on the 11th day of each experimental period with a vacutainer with heparin. The samples were centrifuged immediately after collection at 3000 rpm for 15 min, and the plasma was frozen for further analysis. The plasma glucose concentration was determined by the enzymatic-colorimetric method of glucose-oxidase, using a commercial kit (Sigma C.C.). Total cholesterol was evaluated using a Cholesterol Labtest Diagnóstica commercial kit. The concentration of urea, aspartate aminotransferase, and alanine aminotransferase were evaluated using Diagnóstica commercial kits.

Urine was sampled after slaughter. Creatinine and urea were evaluated using Gold Diagnóstica commercial kits. The colorimetric method was used for allantoin determination [29]. Blood and urine tests were performed at the Laboratory of the Veterinary Hospital of the University Center of Grande Dourados. Creatinin was used to estimate the total urine excretion.

2.8. Statistical Analysis

The data were analyzed using MIXED PROC from the Statistical Package of SAS (SAS University Edition), except for the data from carcass conformation and fatness, which were analyzed by proc npar1way (SAS University Edition). The means were compared using the Tukey test. Significance was declared when $p < 0.05$. The statistical model included treatment as a fixed effect and the block as a random effect.

3. Results

The high tannin contents of barbatimão bark extracts (Table 2) did not interfere in acceptability because it did not reduce the lamb dry matter intake ($p = 0.56$; Table 3). Barbatimão bark extract's effects were similar to (LAS), and there was no change in the performance variables average daily gain, feed conversion ratio, initial body weight, body weight at slaughter, empty body weight, and body condition at slaughter ($p > 0.05$) with the addition of DBB and BHE (Table 3). The inclusion of DBB and BHE did not influence the variables related to body weight at slaughter and yield ($p > 0.05$), such as hot carcass weight, cold carcass weight, loss by cooling, hot carcass yield, cold carcass yield, and carcass conformation of lambs. The BHE showed a lower carcass fatness index ($p = 0.04$).

Table 2. Chemical composition and secondary metabolic compounds of dry barbatimão bark (DBB) and barbatimão hydroalcoholic extract (BHE).

Chemical Composition	DBB	BHE
Dry matter (g/kg as feed)	362.0	765.2
Organic matter (g/kg of DM)	980.2	975.1
Crude protein (g/kg of DM)	109.0	29.0
Ether extract (g/kg of DM)	6.0	9.0
Neutral detergent fiber (g/kg of DM)	473.0	9.0
Acid detergent fiber (g/kg of DM)	446.0	6.0
Phenolic compounds (mg/g of DM)	89.8	93.2
Flavonoids (mg/g of DM)	35.0	39.1
Tannins (mg/g of DM)	453.70	479.1
Secondary metabolic compounds *		
Phenolic compounds	++	++
Flavonoids	+	+
Tannins	+++	+++
Naphtoquinone	−	−
Coumarin	+	+
Triterpenes and Steroids	+	+
Cyanogenic heterosides	+	+
Cardioactive heterosides	+	+
Reducing sugars	+	+
Saponins	+	+
Alkaloids	−	−

* The presence of secondary metabolic compounds was classified as follows: negative reaction (− = 0%), partial intensity (±/+ = 10%), low intensity (+ = 50%), medium intensity (++ = 75%), and high intensity (+++ = 100%).

Table 3. Productive performance characteristics and carcass evaluation of lambs fed with diets containing lasalocid sodium (LAS), dried barbatimão bark (DBB), or dry barbatimão hydroalcoholic extract (BHE).

Item	LAS	DBB	BHE	[1] SEM	*p*-Value
Dry matter intake (g/day)	880.2	866.8	828.3	51.20	0.56
Average daily gain (g)	221.8	189.2	198.4	0.02	0.32
Feed conversion ratio	3.9	5.1	4.2	0.55	0.09
Initial body weight (kg)	21.6	20.6	21.1	0.97	0.31
Body weight at slaughter (kg)	31.0	29.5	30.5	1.47	0.61
Empty body weight (kg)	27.6	25.9	26.5	1.20	0.40
Body condition at slaughter	2.7	2.4	2.7	0.19	0.22
Total weight gain (kg)	9.1	8.2	8.6	1.04	0.69
Weight hot carcass (kg)	15.6	14.4	14.7	0.68	0.25
Weight cold carcass (kg)	15.0	14.0	14.2	0.65	0.27
Hot carcass yield (%)	50.2	49.0	48.6	0.94	0.23
Cold carcass yield (%)	48.4	47.4	47.0	0.96	0.31
Loss by cooling (%)	4.2	3.3	3.3	0.57	0.23
Conformation [2]	2.3	2.2	2.1	0.11	0.40
Fatness [3]	3.1 a	2.7 ab	2.5 b	0.17	0.04

[1] Standard error mean; [2] Conformation estimated by a scale of 1 (no fat) to 5 (excess fat); [3] Estimated fatness by a scale of 1 (no fat) to 5 (excess fat); a, b Averages followed by different letters on the same line differ ($p < 0.05$) from each other.

The parameters of luminosity, red intensity, and chroma were not influenced ($p > 0.05$) by the DBB and BHE (Table 4). However, the BHE lambs had a lower intensity of yellow and hue angle compared to the other treatments. The barbatimão bark extracts in the diets did not influence ($p > 0.05$) the levels of glucose and urea in the blood and urea and allantoin in urine of the lambs (Table 5). The barbatimão bark extracts reduced ($p = 0.01$) the total blood cholesterol levels compared to the LAS treatment. The barbatimão bark extracts did not influence ($p > 0.05$) the aspartate aminotransferase and alanine aminotransferase levels.

Table 4. Meat color of lambs fed with diets containing lasalocid sodium (LAS), dried barbatimão bark (DBB), or dry barbatimão hydroalcoholic extract (BHE).

Item	LAS	DBB	BHE	[1] SEM	*p*-Value
Luminosity, L*	39.9	39.8	38.9	0.58	0.38
Intensity of red, a*	16.7	16.7	15.7	0.58	0.17
Intensity of yellow, b*	4.6 a	4.6 a	3.7 b	0.27	0.01
Chroma, C*	17.3	17.4	16.2	0.32	0.27
Hue angle, HUE	15.6 a	15.5 a	13.2 b	0.93	0.04

[1] Standard error mean; a, b Averages followed by different letters on the same line differ ($p < 0.05$) from each other.

Table 5. Blood and urinary parameters of lambs fed with diets containing lasalocid sodium (LAS), dried barbatimão bark (DBB), or dry barbatimão hydroalcoholic extract (BHE).

Item	LAS	DBB	BHE	[1] SEM	*p*-Value
Blood					
Glucose (mg/dL)	75.8	66.4	66.0	5.21	0.33
Total cholesterol (mg/dL)	56.4 a	39.7 b	41.5 b	5.01	0.01
Urea (mg/dL)	55.2	43.4	40.5	5.15	0.11
Aspartate aminotransferase (mg/dL)	119.4	119.7	127.2	15.69	0.91
Alanine aminotransferase (mg/dL)	19.2	20.0	16.8	1.99	0.45
Urine					
Urea (mg/day)	3189.0	2336.2	2612.4	357.85	0.23
Alantoin (mg/day)	835.0	624.5	563.6	108.02	0.15

[1] Standard error mean; a, b Averages followed by different letters on the same line differ ($p < 0.05$) from each other.

4. Discussion

Normally, a high tannin content is associated with harmful effects, such as reduced feed intake [30]. However, if the supply is moderate, there are no harmful impacts of intake [5], as occurred in this study. Barbatimão bark extract's effects were similar to LAS, by reducing ruminal proteolysis and promoting the flow of dietary protein into the duodenum. This was inferred to be because the flow of dietary protein with microbial protein increased the availability of nitrogen compounds for protein synthesis in the animal [31]. In addition, the presence of tannins and saponins in the barbatimão bark extracts was associated with reduced energy expenditure, justified by the ability of these phenolic compounds to reduce the process of methanogenesis [32]. Consequently, there is reduction in energy losses by decreasing ruminal methane production [4]. Studies with sheep fed a diet containing less than 50 g of tannins/kg of dry matter showed no effect on daily dry matter intake, presenting a higher feed efficiency and daily weight gain than a treatment without tannins [30,33]. Furthermore, carcass characteristics and meat quality were not affected by tannins [34].

Regardless of the treatment, body weight at slaughter and carcass yields were similar, and this may indicate a positive relation of body weight at slaughter with carcass yield. All treatments had an average within the appropriate variation range, from 40 to 50% for sheep [35]. Loss by cooling was not influenced by treatments, with established indices for sheep ranging from 1 to 7% [36]. Loss by cooling is related to the carcass fatness classification, because it is related to age, nutritional management, live weight, and carcass conformation [37].

The lower carcass fatness index on BHE may be related to the level of extract used in the diet, resulting in a lower influence of barbatimão biocompounds on lambs. The BHE carcasses had an acceptable fatness index (2 to 2.4), with an average of 2.4, followed by DBB, with a medium fatness index (2.5 to 3.0) and LAS with a fatness index considered good (3.0 to 3.4) [25]. Carcass fatness index is directly connected to adiposity, which predicts the tissue composition of the carcass [38]. In addition, it reduces fluid loss and shortening of muscle fibers and increases meat darkening during the cooling process [39].

The BHE may have influenced the meat color through the activity of barbatimão biocompounds, probably due to the level of extract used, since this additive has an antioxidant

action, which can interfere in the meat color [40]. Meat values of b* (9.44), C* (25.29), and HUE (21.92) of feedlot Pantaneiros lambs reported in the literature [41] differ from the values of this study, which were lower.

To verify the metabolic alteration, the blood and urinary parameters were evaluated. Barbatimão bark extracts reduced the total blood cholesterol levels compared to the LAS treatment. Cholesterol levels are indicative of energy balance [42]. The reduction in blood cholesterol concentration can be explained by a possible decrease in rumen acetate production, since acetate is a precursor to cholesterol synthesis in ruminants [43]. The barbatimão tannins can form complexes with fibers, inhibiting the cellulolytic bacteria action and causing lower acetic acid production [44]. Similarly, the antimicrobial activity of saponins present in barbatimão is more evident for Gram-positive bacteria [45]. Inhibition of Gram-positive bacteria in ruminants reduces the proportion of acetate produced in the rumen [46].

The aspartate aminotransferase and alanine aminotransferase enzymes are markers of liver damage and can be identified in the cytoplasm, liver cell mitochondria, cardiac system, and skeletal muscle [42]. Our study's results corroborate the literature reports [47]. This literature indicates that appropriate doses (<5% in dry matter) do not cause adverse effects, even with extensive biotransformation of liver metabolism in mammals and intestinal microbiota. Thus, the extracts used in the present study did not cause liver injury in the lambs.

5. Conclusions

The use of barbatimão bark extracts may replace lasalocid sodium in the diet of feedlot lambs, with no detrimental effects on performance and metabolic parameters. Therefore, it is suggested that further studies are conducted to evaluate the bioavailability of the biocompounds and the inclusion of higher doses of milled bark and hydroalcoholic extract of dried barbatimão in the diet of feedlot lambs.

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