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Supplementation of Banana By-products Hay does not Alter Serum Biochemistry of Lambs

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ABSTRACT

In this study, we evaluated the serum profile of sheep fed with banana residue hays dried using two methods. Thirty crossbred Santa Inês × Dorper, male, non-castrated sheep (average body weight= 26.5 kg) were distributed in a randomized block design with five treatments: control, two types of residues (banana pseudo stem and banana leaf hays), and two drying methods (sun and shade drying). After feeding the experimental diets for 20 days, the animals were fasted for 12 h before collecting venous blood (6 mL) for plasma and serum at 0, 3, and 6 h after feeding. Variance analysis was performed in subdivided plots, and the means were compared using Dunnett's test at 5% probability. No differences were observed in the dry matter intake of animals fed with banana by-product hay; however, the *in vitro* dry matter digestibility was high for pseudo stem hay. The differences in the nutritional value of the hays were not sufficient to alter the serum biochemistry of the animals. No differences were observed in the total protein, serum albumin and globulins, albumin/globulin ratio, and plasma glucose among time points and treatments (5.83 g/dL, 3.43 g/dL, 2.40 g/dL, 1.82 g/dL, and 64.6 mg/dL). Urea and triglyceride levels were the highest (p<0.05) at 3 h after feeding (30.1 and 37.0 mg/dL, respectively) and creatinine at 0 h (0.94 mg/dL). Serum cholesterol levels were the lowest (p<0.05) at 3 h after feeding (38.5 mg/dL). No effect of treatment on these parameters was observed. Thus, sheep can be fed with pseudo stem or banana leaves dehydrated in the sun or shade without compromising blood parameters.

Keywords: blood parameters; crop residues; Musa spp.; small ruminant

INTRODUCTION

Better control of diet and feeding and accurate production planning are the main advantages of finishing lambs in feedlots. Although feed represents most of the production costs of these systems (Greenwood, 2021), extensive research has been conducted to find alternative ingredients, such as agro-industrial by-products, to substitute cereals in ruminant diets (Vastolo *et al.*, 2022).

Bananas are one of the most consumed fruits globally and play an important role in international agribusiness, accounting for 18% of the total fruit produced in 2019 (FAO, 2022). With the pruning and thinning of banana trees, green mass tones are generated, which can be used in animal diets (Roque *et al.*, 2014; Rusdy, 2019). However, these by-products must be stored properly, for which haying is the most suitable method.

The haying method influences the dry matter (DM) content and chemical composition of the feed. Every type of feed has an ideal drying method; however, the information on hay produced from banana tree residues is scarce. Thus, determining the most appropriate drying method is essential for balancing the nutritional value of the feed and optimizing microbial growth, rumen fermentation, and fiber digestion (Collins *et al.*, 2017).

A recent review reported satisfactory performance results for animals fed with banana by-products in combination with fermentable energy and crude protein sources (Rusdy, 2019). However, a better understanding of its effects on animal metabolism, especially in small ruminants (Rahman *et al.*, 2018), is still needed. Several studies used blood parameters to measure the physiological responses of animal metabolism to changes in diet (Buccioni *et al.*, 2017; Jiang *et al.*, 2019; Razmkhah *et al.*, 2017).

Total protein is an essential biomarker that reflects serum protein content. Albumin and globulin are two key components of total protein vital for maintaining blood pressure and transporting essential nutrients throughout the body (Tothova *et al.*, 2016). Urea and creatinine are waste products of protein metabolism, and serum urea and creatinine levels indicate kidney function (Hokamp & Nabity, 2016). Glucose is the primary energy source for cells, and its serum levels can reflect an animal's nutritional status and glucose metabolism. Triglycerides and cholesterol are lipids

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that play essential roles in energy storage and hormone synthesis, respectively (Carlos *et al.*, 2015).

Understanding the serum biochemistry of sheep is particularly important for farmers, as it can help optimize the nutritional management of sheep and ensure that they receive appropriate dietary requirements. In this study, we aimed to evaluate the serum biochemistry (total proteins, serum albumin and globulins, albumin: globulin ratio, serum urea and creatinine, plasma glucose, total cholesterol, and serum triglycerides) of sheep fed with banana by-products processed using different dehydration methods.

MATERIALS AND METHODS

Local, Animals, and Treatments

The experiment was conducted in the sheep sector at the Institute of Agricultural Sciences of the Federal University of Minas Gerais between March and July 2018. According to the Köppen climate classification, this subhumid tropical region has two defined seasons: a rainy summer and a prolonged dry season (Alvares *et al.*, 2013). All animal procedures were approved by the Animal Ethics Committee of the Federal of Minas Gerais of the University under protocol number 270/2016.

Thirty crossbred ½ Santa Inês × ½ Dorper, male, non-castrated sheep, 4–6 months old, with an average initial body weight of 26.5 kg, were used. All the animals were weighed and dewormed before the beginning of the experiment, placed in metabolic cages, and fed twice daily. The animals were blocked according to their initial weights. The experimental diets were calculated according to the recommendations of the National Research Council (2007) for lambs, with a gain of 150 g/day in relation to a forage concentrate of 70:30.

The forages were composed of five treatments: control with Tifton hay (TH), banana leaf hay dehydrated in the sun (LH Sun), shade-dehydrated banana leaf hay (LH Shade), banana pseudo stem hay dehydrated in the sun (PH Sun), and banana pseudo stem hay dehydrated in the shade (PH Shade) (Table 1).

The by-products of dwarf silver banana trees were collected from a property located 70 km from Montes Claros. Leaves and pseudo stems were collected manually soon after the fruit was harvested. The material was processed in a stationary chopper (model EN6500) set to cut particles between 2 and 3 cm. The dehydration process lasted on average 72 hours for LH Sun, 120 hours for LH Shade, 120 hours for PH Sun, and 168 hours for PH Shade.

The experimental period lasted 20 days, and diets were provided twice daily, at 07:00 h and 16:00 h, in an *ad libitum* system, where leftovers consisted of 100 g kg^{-1} of the amount offered in natural matter. The intake was adjusted every morning before the first meal using the weights of food and leftovers. During the experimental period, samples of the diets and leftovers were collected daily.

Samples from the offered food and leftovers were pre-dried in a fan-forced oven at 55 ^oC and ground in a Willey grinder with 1 mm diameter sieves. All samples were analyzed for DM, crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), and ash content according to INCT-CA (Detmann *et al.*, 2012).

DM intake was determined from the relationship between the DM offered and DM in leftovers. Nutrient ingestion was calculated based on its relationship with

Table 1. Ingredients composition and nutritional value of experimental diets

Items	Treatments				
	TH control	LH sun	LH shade	PH sun	PH shade
Ingredients (%)					
Soya meal	16.19	6.59	6.34	6.59	17.00
Ground corn	12.95	22.46	22.68	22.46	12.09
Limestone	0.38	0.36	0.34	0.36	0.32
Mineral and vitamins premix[1]	0.48	0.59	0.64	0.59	0.59
Tifton hay	70.00				
LH Sun		70.00			
LH Shade			70.00		
PH Sun				70.00	
PH Shade					70.00
Nutrients					
DM (%)	90.81	88.92	91.32	88.92	89.28
CP (% of DM)	12.77	12.84	12.73	12.84	13.08
NDF (% of DM)	74.00	66.75	68.27	66.75	66.00
ADF (% of DM)	36.21	37.81	41.26	37.81	44.15
EE (% of DM)	1.50	7.22	5.90	2.29	2.06

Note: TH= Tifton hay, LH Sun= banana leaf hay dehydrated in the sun, LH Shade= banana leaf hay dehydrated in the shade, PH Sun= pseudo stem hay dehydrated in the sun, PH Shade= pseudo stem hay dehydrated in the shade, DM= dry matter, CP= crude protein, NDF= neutral detergent fiber, ADF= acid detergent fiber, EE= ether extract.

(1)Composition of the vitamin mineral premix: calcium – 150 g, phosphorus – 65 g, sodium – 130 g, fluorine - 50 mg, sulfur – 12 g, magnesium – 10 g, iron - 1000 mg, manganese – 3000 mg, cobalt – 80 mg, zinc – 5000 mg, Iodine - 60 mg, selenium - 10 mg, Vitamin A - 50000 I.U., Vitamin E - 312 I.U.

DM, the content of each nutrient in the diet provided, and the leftovers.

Blood Sample Collection and Serum Biochemistry Analysis

At the end of the experimental period, blood samples were collected at three time points: T0h= zero hour, T3h= 3 h after feeding, and T6h= 6 h after feeding. Blood (6 mL) was collected from the right external jugular vein of each animal and divided into two tubes, one with sodium fluoride and the other with a clot activator.

Serum biochemistry samples were centrifuged at 1500 rpm for 10 min to obtain plasma and serum, which were placed in polypropylene bottles (Eppendorf®) and frozen at -20 ^{o}C for further analysis. Plasma glucose concentration was determined by a veterinary laboratory in Montes Claros, MG. Glucose was evaluated via a colorimetric enzymatic test using a Bio 200 VET Alere® spectrophotometer (reagent K082).

Total protein and albumin concentration in the serum was determined using the colorimetric method with biuret reagent, bromocresol green (Katsoulos *et al.*, 2017), and commercial Bioclin®. Globulin content was calculated based on the difference between the total protein and albumin concentrations.

For the serum concentrations of urea, creatinine, triglycerides, and total cholesterol, a colorimetric method was used with a commercial Bioclin kit. All concentrations were analyzed using a semi-automatic spectrophotometer (Bel 1105), with a specific wavelength and calibration factor for each variable.

In Vitro Digestibility

In vitro DM digestibility of the hays (TH, LH Sun, LH Shade, PH Sun, and PH Shade) was carried out according to the methodology described by Silva & Queiroz (2004), Tilley & Terry (1963), and Holden (1999) and modified for the use of the Tecnal® (TE-150) *in vitro* incubator.

Statistical Analysis

Data were tested for normality of residuals and homogeneity of variance. Nutrient intake data were analyzed in a randomized block design using computational software for statistical and genetic analyses (SAEG, 2007). Tukey's test (5% probability) was used to test for differences in the means.

Blood parameters were analyzed in a split-plot arrangement, and data were transformed into log (x+10) to allow for the analysis of variance. Dunnett's test (5% probability) was used to test for differences in the means.

RESULTS

Intake and Digestibility

Animals fed with banana by-product hay presented similar DM intakes of 773.7 g of DM/da, 756.2 g of DM/da, 722.7 g of DM/da, and 666.4 g of DM/day for LH Sun, LH, Shade, PH Sun, and PH shade, respectively (Table 2). The PH shade animals had a lower DM and protein intake than the TH Control animals (888.6 g of DM/day and 115.3 g of CP/day).

The DM *in vitro* digestibility of LH Sun (40%) and LH Shade (42%) was lower than TH Control (52%), whereas the DM *in vitro* digestibility of PH Sun (63%) and PH Shade (66%) was higher than that for the TH Control.

Blood Protein Profile

No differences among treatments and time were observed in average total serum protein concentration, with values ranging between 5.61 g/dL and 6.19 g/dL. Albumin content was within the normal range (3.04 g/dL to 3.73 g/dL) (Table 3). Globulin content ranged from 1.99 g/dL to 2.85 g/dL but was not affected by treatments and time. Moreover, treatments and time did not affect the albumin/globulin ratio (1.21–2.02). Despite not being affected by diet, the average serum urea and creatinine concentrations were high 3 h after feeding ($p<0.05$).

Blood Energy Profile

Plasma glucose levels were similar between time points and treatments, ranging from 60.5 mg/dL to 69.0 mg/dL (Table 4). Triglyceride serum concentrations ranged from 19 mg/dL to 42.3 mg/dL, which were similar between treatments, although the average value at 3 h after feeding was higher than that at 0 h and 6 h

Table 2. Intake of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and ether extract (EE), and *in vitro* dry matter digestibility (IVDMD) of different banana by-products dehydrated by two methods or with Tifton hay

Variables	Treatments					
	TH control	LH sun	LH shade	PH sun	PH shade	CV (%)
DM (g day^{-1})	888.6^{a}	773.7^{ab}	756.2^{ab}	722.7^{ab}	666.4^{b}	12.3
CP (g day^{-1})	115.3^{a}	98.9^{ab}	99.2^{ab}	92.5^{ab}	87.8^{b}	14.6
NDF (g day^{-1})	648.7^{a}	502.6^{b}	516.1^{b}	475.1^{b}	435.7^{b}	16.4
EE (g day^{-1})	13.3^{d}	55.2^{a}	42.3^{b}	23.0^{c}	13.6^{d}	10.7
IVDMD (%)	52.0^{b}	40.0^{c}	42.0^{c}	63.0^{a}	65.0^{a}	13.4

Note: Means in the same row with different superscripts differ significantly by Tukey test ($p<0.05$). CV= Coefficient of variation, TH= Tifton hay, LH Sun= banana leaf hay dehydrated in the sun, LH Shade= banana leaf hay dehydrated in the shade, PH Sun= pseudo stem hay dehydrated in the sun, PH Shade= pseudo stem hay dehydrated in the shade.

Table 3. Mean values for total protein, albumin, globulins, albumin globulin ratio, urea, and creatinine serum in sheep fed with different banana by-products dehydrated by two methods or with Tifton hay measured at three different times

Variables	Treatments					Average	Reference*	CV (%)
	TH control	LH sun	LH Shade	PH sun	PH shade			
Total protein (g/dL)								
T0h	5.94	5.62	5.62	5.88	5.71		6.00-7.90	2.39
T3h	6.19	6.05	6.01	5.7	5.88			
T6h	5.74	5.79	6.11	5.72	5.61			
Albumin (g/dL)								
T0h	3.50	3.67	3.20	3.47	3.06		2.60-4.2	5.25
T3h	3.60	3.34	3.50	3.60	3.04			
T6h	3.60	3.47	3.70	3.73	3.39			
Globulins (g/dL)								
T0h	2.44	2.25	2.43	2.42	2.65		3.50-5.70	13.3
T3h	2.59	2.72	2.52	2.10	2.85			
T6h	2.15	2.31	2.41	1.99	2.20			
Albumin: Globulin (A:G)								
T0h	1.90	1.67	1.41	1.59	1.26		0.42-0.76	20.6
T3h	1.60	1.37	1.90	1.86	1.21			
T6h	1.70	1.52	1.78	2.02	1.67			
Urea (mg/dL)								
T0h	27.0	23.2	24.7	29.8	24.5	25.7^{B}	17.0-43.0	16.9
T3h	26.2	22.7	32.0	40.8	28.8	30.1^{A}		
T6h	25.0	23.0	25.2	33.3	23.5	26.0^{B}		
Creatinine (mg/dL)								
T0h	0.66	0.90	1.02	1.12	1.00	0.94^{A}	1.20-1.90	3.68
T3h	0.62	0.55	0.54	0.50	0.55	0.55^{B}		
T6h	0.55	0.60	0.53	0.51	0.53	0.54^{B}		

Note: Means in the same columns with different uppercases differ significantly by Dunnet's test ($p<0.05$), respectively, *Reference values for sheep (Kaneko *et al*., 2008), CV= Coefficient of variation, TH= Tifton hay, LH Sun= banana leaf hay dehydrated in the sun, LH Shade= banana leaf hay dehydrated in the shade, PH Sun= pseudo stem hay dehydrated in the sun, PH Shade= pseudo stem hay dehydrated in the shade.

Table 4. Mean values for plasma glucose, triglycerides, and serum cholesterol in sheep fed with different banana by-products dehydrated by two methods or with Tifton hay measured at three different times

Variables	Treatments					Average	Reference*	CV (%)
	TH control	LH sun	LH shade	PH sun	PH shade			
Glucose (mg/dL)								
T0h	65.3	64.6	66.9	69.0	66.2		50.0-80.0	2.36
T3h	62.3	60.5	60.7	66.2	66.1			
T6h	65.2	58.6	63.4	67.7	65.7			
Triglycerides (mg/dL)								
T0h	28.5	28.0	25.0	24.7	25.2	26.3^{B}	-	10.74
T3h	31.5	42.0	42.3	38.8	30.3	37.0^{A}		
T6h	30.5	26.5	20.2	25.0	19.0	24.2^{B}		
Total cholesterol (mg/dL)								
T0h	60.8	74.2	64.8	54.7	48.2	60.5^{A}	52.0-76.0	6.06
T3h	37.5	54.2	29.8	38.3	32.8	38.5^{B}		
T6h	63.2	78.8	61.8	54.3	44.8	60.6^{A}		

Note: Means in the same columns with different uppercases differ significantly by Dunnet's test ($p<0.05$), respectively, *Reference values for sheep (Kaneko *et al*., 2008), CV= Coefficient of variation, TH= Tifton hay, LH Sun= banana leaf hay dehydrated in the sun, LH Shade= banana leaf hay dehydrated in the shade, PH Sun= pseudo stem hay dehydrated in the sun, PH Shade= pseudo stem hay dehydrated in the shade.

after feeding. Cholesterol serum values were similar among treatments, ranging from 29.8 mg/dL to 78.8 mg/dL (Table 4). However, the average cholesterol serum concentration at 3 h after feeding was lower ($p<0.05$) than that at other times.

DISCUSSION

DM intake ranged from 3.20% to 2.63% of BW, which was close to that recommended by the NRC (2007). The banana by-product hay presented a similar

DM intake as the TH Control, except for the PH shade. According to Silveira Júnior *et al.* (2020), the presence of tannins in banana by-products, especially in the pseudo stem, is considered an anti-nutritional factor. Shade drying of pseudo stems, which have a high moisture content, may have contributed to the preservation of tannin levels, which increased astringency and reduced the palatability of the food (Frutos *et al.,* 2004). Carmo *et al.* (2016) evaluated the addition of 200 g kg^{-1} or 400 g kg^{-1} leaf or pseudo stem hay dried in the sun and observed superior results for DM intake in relation to control hay (1265 g and 1342 g of DM/day).

Banana leaf hay presented low digestibility. According to Carmo *et al.* (2018), the high lignin concentration (88.1 g kg^{-1}) in leaf hay can reduce digestibility. Despite the change in DM digestibility of the leaf hays, the difference was insufficient to alter animal intake.

Banana pseudo stem hay dehydrated in the shade resulted in a lower protein intake than that with Tifton hay; however, this by-product showed greater IVDMD and resulted in no changes in the blood protein profile of animals. Carmo *et al.* (2016) reported no differences in the CP digestibility coefficients of sheep fed leaf hay and banana pseudo stem hay, indicating no changes in food supply with the use of by-products.

In our study, the protein concentration was close to the lower boundary refereed by Kaneko *et al.* (2008), which could be explained by low values of serum globulin and creatinine concentrations. According to Vivian *et al.* (2017), younger animals present lower creatinine serum concentrations because these animals have lower muscle development than adult animals.

Due to their partially developed immune systems, younger animals present lower concentrations of immunoglobulins (Carlos *et al.,* 2015). In contrast, older animals (Sarda ewes at 4–5 years of age) present an average total protein ranging from 6.10 g/dL to 10.17 g/dL (Pasciu *et al.,* 2019).

The concentration of blood albumin can be influenced by diet (Buccioni *et al.,* 2017; Jiang *et al.,* 2019; Razmkhah *et al.,* 2017), temperature (Dalcin *et al.,* 2016), dehydration, and losses caused by diseases (e.g., gastrointestinal parasitism) as a result of the loss of proteins in the intestine (Atiba *et al.,* 2020). This characteristic makes it a reliable indicator of protein metabolism, as blood albumin concentration is directly related to the food supply and more specifically, to protein expression in the diet (Paula *et al.,* 2013). In the current study, the similarity in blood albumin concentration indicated a lack of change in protein supply by using by-products. The protein quality of the diets was probably similar, and according to Carmo *et al.* (2018), the protein digestibility of diets with the inclusion of banana hay leaf or pseudo stem was similar to that of diets with Tifton hay.

Globulin levels were lower than the normal range in sheep (Kaneko *et al.,* 2008), which could be associated with other variables such as age (Carlos *et al.,* 2015). Similarly, low values were observed for crossbred growing lambs fed a 40:60 forage concentrate diet, with an average of 23 kg LW (Lobo *et al.,* 2020). The similarity in globulin concentration among times and treatments indicates homeostasis of the animals by this serum fraction, which represents several transport proteins of metals, lipids, and bilirubin, in addition to acting on immunity, and is an important indicator of inflammatory processes (Gonzalez & Scheffer, 2003). As albumin concentration showed similar results, using dehydrated banana by-products by different methods presumably does not interfere with globulin concentration, thus not compromising humoral immunity or causing acute inflammatory reactions. In cases of malnutrition or low protein intake, globulins, and other plasma proteins may be reduced (Gonzalez & Scheffer, 2003). However, this hypothesis was rejected in our study because total protein levels were within the normal range (Table 3).

According to Silva *et al.* (2023), the serum urea concentration is the result of synchronization between nitrogen and energy in the rumen environment and protein metabolism in animal tissues (Gonzalez & Scheffer, 2003). The experimental diets had similar protein concentrations, and the low DM *in vitro* digestibility of LH hays was probably insufficient to alter the synchronism between nitrogen and energy, resulting in similar serum concentrations of urea. The highest urea concentration observed at 3 h after feeding may indicate a greater absorption and metabolism of ruminal nitrogenous compounds resulting from greater protein availability.

Creatinine levels in this study were lower than those reported by Kaneko *et al.* (2008). According to these authors, the concentration of circulating creatinine is related to a protein deficit in the diet, which is a late indicator in renal diagnosis, and its detection can be related to the synthesis and deposition of muscle tissue in sheep. Despite being lower than the reference reported, lambs fed with banana hay by-products had values similar to those of the control. This could be associated with other factors, such as the age of the growing animals (4–6 months), resulting in a high metabolic rate of muscle deposition and, consequently, a reduction in the concentration of this non-protein nitrogenous metabolite. The high value of average creatinine at 0 h after feeding, compared to that at other times, is explained by the change from fasting to the feed state.

Although plasma glucose shows little variation in ruminants and is slightly influenced by diet (Lima *et al.,* 2015), this metabolite helps define the energy profile of the animal. The inclusion of by-products in the diet could reduce energy metabolism, as differences in the *in vitro* digestibility of DM existed; however, no changes were observed in the biochemical parameters related to energy metabolism (glucose, triglycerides, and cholesterol). Song *et al.* (2018) subjected lambs to diets with reduced energy intake and observed low plasma glucose and triglyceride levels.

Carmo *et al.* (2016) observed that the inclusion of 20% banana leaf hay increased EE consumption compared to that of other treatments because of the high EE concentration in the banana leaves. However, the EE content in banana leaf hay also originates from pigments unrelated to the lipid content. This could explain the similarity in triglyceride levels found in our experiment, despite the change in EE intake (Table 2). The increase in the average triglyceride level at 3 h after feeding could be explained by the presence of chylomicrons transporting the absorbed lipids from the diet into the blood. In contrast, cholesterol concentration decreased simultaneously.

Cholesterol is a precursor to the synthesis of steroid hormones, vitamin D, and bile salts and participates in the formation of cell membranes (Ribeiro *et al.,* 2018); therefore, in young sheep, such as those in the current study, it may be involved in the growth metabolism of animals (Carlos *et al.,* 2015). The decrease in average serum cholesterol after 3 h of feeding could be related to the lower need for fat mobilization from tissues in the recent feeding state. The cholesterol concentration returned to the fasting state at 6 h.

No differences in cholesterol levels were observed among the treatments; however, some values were below those reported by Kaneko *et al.* (2008). In other studies, lambs fed isoenergetic diets supplemented with glycerol (Saleem & Singer, 2018) or diets with reduced energy intake (Song *et al.,* 2018) showed no differences in serum cholesterol. Thus, intrinsic variables other than diet may significantly affect this parameter. For example, Pasciu *et al.* (2019) observed higher levels (150-250 mg/dL) in older Sarda ewes.

CONCLUSION

Lambs fed 70% banana pseudo stems and leaves dehydrated in the sun and shade showed biochemical variables of glucose, total protein, albumin, globulin, creatinine, urea, cholesterol, and triglycerides with few variations, which indicated that these by-products could be used in the composition of the diets of these animals.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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