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RUMINANTS NUTRITION

Babassu mesocarp meal for ewe lambs feeding: *In vitro* ruminal fermentation and *in vivo* apparent digestibility

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ABSTRACT. The purpose of the current study was to evaluate the *in vitro* fermentation (IVRF) and apparent *in vivo* digestibility (AIVD) of diets formulated with four inclusion levels of babassu mesocarp meal (BMM) (0.0; 7.5; 15.0 and 22.5%) in ewe lambs. The IVRF test was performed through the "*Hohenheim Gas Test*" technique, following a randomized complete block design, with four blocks (rumen fluids from four different bovines). Gas production was measured at 3, 6, 9, 12, 24, 48, 72, and 96 hours after incubation. For the AIVD trial, 20 ewe lambs were used in a completely randomized design with five replicates. The animals were housed in metabolic cages during the digestibility test. Diets with BMM showed lower fermentation levels than those without BMM. The BMM reduced the gas production per incubation time and dry matter effective degradability (DMED), possibly due to its chemical characteristics, or even to the influence of other factors, such as physical properties. The BMM negatively influenced the AIVD of DM. Thus, it can be stated that, despite being a starch source, BMM reduces ruminal fermentation parameters when included in ruminant diets, negatively interfering with diet digestibility. Therefore, its use should be cautious.

Keywords: byproducts; feeding; rumination; sheep.

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Introduction

Utilization of alternative feeds, such as agriculture byproducts, is intended to reduce production costs and environmental impacts (Abdalla, Silva Filho, Godoi, Carmo, & Eduardo, 2008).

There are several aspects related to the utilization of byproducts in animal diets. Among them, we can highlight geographic location, economy, and factors related to intake, digestibility, and so on; These latter factors can directly affect nutrition, thereby affecting animal performance, which could possibly result in increased production costs. (Chaves, Stefanello, Burin, Ritt, & Nornberg, 2014). Thus, studies that focus on byproducts characterization should involve ruminal fermentation kinetics as well as apparent *in vivo* digestibility (AIVD).

Babassu is a palm tree typically found in large areas of the Brazilian states of Tocantins, Maranhão and Pará. It produces an oleaginous fruit popularly known as babassu coconut, which can structurally be divided into epicarp, mesocarp, endocarp, and almond. As Babassu oil is extracted, several byproducts are produced. One of them is babassu mesocarp meal (BMM), also called babassu starch meal, used, without scientific support, by local ranchers for feeding ruminants due to its high availability compared to other regionally available by-products (Porro, 2019). Babassu mesocarp meal is rich in starch, which makes it highly energetic and a source of fast-fermenting carbohydrates for ruminants (Sousa, Macedo Júnior, Santos, Silva & Borges, 2014).

Thus, this study aimed to evaluate ruminal fermentation kinetics, apparent digestibility and energy balance in ewe lambs fed diets containing increasing levels of babassu mesocarp meal.

Material and methods

All procedures were conducted between September 29th and November 10th, 2013 at the Federal University of Tocantins in accordance with the guidelines set out by the Brazilian College of Animal Experimentation in the Code of Practice for the Care and Use of Animals for Experimental Purposes, and approved by the Ethics Committee on Use of Animals for Research (CEUA) of the Federal University of Tocantins under the license number 13101.000732/2013-04.

Fermentation and gas production trial

This experiment followed a randomized complete block design, with four treatments (diets containing 0.0, 7.5, 15.0, and 22.5% of babassu mesocarp meal - BMM) and five blocks (rumen fluids from different bovines [*Bos* spp.]).

The diets were composed of cornmeal, soybean meal, mineral mix, Napier grass silage, and BMM. The chemical composition of the BMM is presented in the Table 1.

Table 1. Chemical composition of babassu mesocarp meal (BMM)*.

Item			BMM
Dry matter (DM) (%)			87.83
Ash (% of DM)			4.36
Crude protein (% of DM)			6.09
Ether extract (% of DM)			1.29
Neutral detergent fiber (% of DM)			60.20
Acid detergent fiber (% of DM)			44.02
Hemicellulose (% of DM)			16.17
Cellulose (% of DM)			26.07
Lignin (% of DM)			21.16
Neutral Detergent Insoluble Nitrogen (N) (% of total N)			0.25
Acid Detergent Insoluble Nitrogen (N) (% of total N)			0.31
Carbohydrate fractionation			
TOCH (% of DM)	A + B1 (% of TOCH)	B2 (% of TOCH)	C (% of TOCH)
88.25	36.42	6.05	57.53

TOCH: Total Carbohydrates; *Information provided by the BMM provider

Gas production and degradability were determined using an adaptation of the '*Hohenheim Gas Test*' technique, developed by Menke et al. (1979) using 100-mL graduated glass syringes. Feed samples of 0.2 g were incubated in the syringes with 10 mL of ruminal fluid and 20 mL of culture medium, as described by the authors cited in the previous sentence.

Ruminal fluids were collected from five rumen-fistulated steers fed diets formulated to meet their maintenance requirements. The ruminal fluids were collected before the animals were fed on collection days.

After inoculation, the volume of gas produced was measured at 3, 6, 9, 12, 24, 48, 72, and 96 hours. The data obtained was adjusted with blank syringes (i.e., without samples), as well as for yield per gram of DM. Following this procedure, the model by France et al. (1993) was fitted for the gas yield data through the nonlinear equation (1), where: Y is the cumulative volume of gas produced (mL), t is time (hours), A is total gas production (mL), L is colonization time (hours) and μ is the fractional rate (h^{-1})

$$Y = A\{1 - \exp^{[-b(t-L) - c\times(\sqrt{t} - \sqrt{L})]}\} \quad (1)$$

The equations were contrasted by the model identity test according to Regazzi and Silva (2010), with a 5% probability of error ($p < 0.05$). Equations that had all equal parameters were considered identical.

Average fractional rate (h^{-1}) of gas production (μ) was calculated by the equation:

$$\mu = \frac{b+c}{2\sqrt{t}} \quad (2)$$

Effective degradability was calculated by the equation:

$$ED = So\exp^{-kT}(1 - kI)/(So + Uo) \quad (3)$$

where: ED is effective degradability; k is passage rate; ED is calculated for k equal to 0.05, 0.06, 0.07 and 0.08; S_0 and U_0 are initially fermentable and non-fermentable fractions, respectively, and $I = \exp^{[-(b+k)(t-T) + c(\sqrt{t} - \sqrt{T})]} dt$.

In vivo digestibility trial

For this experiment, 20 crossbred ewe lambs averaging 23.0 ± 3.2 kg of body weight (BW) were housed in individual metabolic cages equipped with feeders, water fountains, and salt shakers, as well as appropriate urine and feces collection devices.

The diets were formulated according to the Nutrient Requirements of Small Ruminants (National Research Council [NRC], 2007) for females of late maturity with intended gains of 150 g BW. day^{-1}. Diets included four levels of BMM: 0.0, 7.5, 15.0, and 22.5%. Diets were isonitrogenous, isoenergetic and similar in NDF levels. The experimental period lasted 20 days, composed of a 15 days adaptation period and a 5 days collection period.

Silage and concentrate were weighted, mixed, and provided to animals twice a day (8 am and 4 pm). The first meal of the day represented 40% of the total daily diet. Water was offered *ad libitum* and the daily water consumption per animal was recorded. Samples of feed, leftovers, and feces were taken daily and 20% of each was separated to make representing the five days of collection and stored in plastic bags at −20 °C. Urine was collected daily and filtered through a gauze, then, a 10 mL aliquot was immediately diluted into 40 mL of 0.2 N sulfuric acid and stored at −20°C.

The collected samples of feed, orts and feces were dried in an oven at 55°C for 72h and ground to pass through a 1 mm mesh screen prior to being analyzed for dry matter concentration (method 967.03), crude protein concentration (method 981.10) and ether extract concentration (Method 920.29) according to the Association Official Analytical Chemists (AOAC, 2016). The concentrations of neutral detergent fiber, acid detergent fiber and lignin were determined with an "Ankon" equipment following the sequential method described in Van Soest, Robertson, and Lewis (1991). Ingredients and chemical composition of diets are showed in Table 2.

Table 2. Ingredients and chemical composition of diets with increasing inclusion levels of babassu mesocarp meal for lambs.

Item (%)	BMM (%)			
	0.0	7.5	15.0	22.5
Napier grass silage	53.34	47.13	40.96	34.78
BMM	0.00	7.50	15.00	22.50
Cornmeal	25.63	24.32	22.98	21.66
Soybean meal	16.03	15.91	15.79	15.69
Phosphate	0.54	0.69	0.84	0.96
Mineral mix	3.00	3.00	3.00	3.00
Dolomitic limestone	1.46	1.45	1.43	1.41
Chemical composition*				
Dry matter	59.16	62.62	66.05	69.44
Crude protein	12.15	11.93	12.26	12.07
Total digestible nutrients	59.70	59.95	60.34	60.86
Neutral detergent fiber	41.07	41.13	41.19	41.25

BMM: babassu mesocarp meal. *Analyzed chemical composition

Dry matter, crude protein, ether extract, neutral detergent fiber, acid detergent fiber and gross energy digestibility were estimated by the difference between their concentration's intake and feces (both measured by the total collection method) divided by intake.

Gross energy (GE) was obtained by using an adiabatic calorimeter pump model PARR 1281 at the Veterinary School's Animal Nutrition Laboratory of UFMG. Urine samples of 10 ml were placed in plastic cups and taken to a forced-air oven at 55°C for 72 hours (pre-drying period) prior to GE measurements in the calorimetric pump. Six plastic cups without samples were used for heat production referencing as blank controls. Digestible energy (DE) values of urine, feces, feed and orts were calculated by a direct energy determination technique using a calorimetric pump.

Metabolizable energy levels (ME) were calculated according to Blaxter and Clapperton (1965), where: DE is equal to the GE intake minus the excreted GE; ME is equal to DE minus GE in urine and gas. Methane production was estimated by the equation: $\mathrm{Cm} = 0.67 + 0.062\mathrm{D}$, where Cm stands for methane production (kcal 100 kcal^{-1} of energy intake) and D stands for apparent digestibility of gross energy.

The experiment followed a completely randomized design with four treatments and five replicates. Data were analyzed using the GLM (General Linear Models) of Statistical Analysis System (SAS, 2015) and the following mathematical model:

$$Y_{ij} = \mu + H_j + e_{ij}$$

where: Y_{ij} represents the observation ij; μ represents the general mean; H_j represents the treatment fixed effect and e_{ij}, the random error.

The data was tested for normality (Shapiro & Wilk, 1965) and homoscedasticity (Cochran, 1941). After the above assumptions were confirmed, the data was subjected to analyses of variance, then, comparisons of levels were performed by regression analyses evaluating linear and quadratic effects through contrasts with a 5% significance level ($p < 0.05$).

Results and discussion

The analysis of ruminal fermentation kinetics, based on the France model, revealed a maximum gas production potential (A) and a shorter colonization time associated with the diet containing 0% of BMM in comparison to the other treatments (Table 3). The control diet was composed of only corn and soybean, as the main staples, which have high fermentability and, therefore, promote fast colonization by ruminal microorganisms (Cabral et al., 2002; Homem Júnior et al, 2019, Liu et al., 2019).

The shorter colonization time (CT) for the control diet, as shown in table 3, indicates the shorter period elapsed prior to the initiation of substrate fermentation, indicating that this diet is more fermentable than the others. Another important aspect was that the CT of diets with BMM were longer, which indicates a reduction in the initial fermentation of these diets. The highest values of effective degradability were in accordance with the data for maximum potential of gas production, which is in accordance with the previous findings, taking place in a more fermentable diet.

Table 3. Parameters of ruminal fermentation kinetics of diets with babassu mesocarp meal (BMM) determined using the France model and effective degradability.

Item	BMM (%)			
	0.0	7.5	15.0	22.5
A*	265.91 A	258.1 B	257.99 B	250.66 C
CT* (hours:minutes)	01:50 B	02:40 A	02:41 A	02:59 A
µ*	0.05993	0.05678	0.05631	0.05324
DMED (5%)	70.99	66.57	64.29	65.98
DMED (6%)	69.00	64.21	61.83	63.85
DMED (7%)	67.05	61.92	59.46	61.76
DMED (8%)	65.16	59.7	57.17	59.74

A: Total gas (mL); CT: colonization time (hours); µ: fractional degradation rate (h^{-1}); DMED: Dry matter effective degradability; * Parameters estimated by France et al. (1993). Values accompanied by the same letters in the lines, are identical by the 5% curve identity test (Regazzi & Silva, 2010).

Gas production per incubation time was reduced proportionally to the level of BMM inclusion in the diet (Table 4), indicating that BMM has a low nutritive value, partially explained by its high lignin concentration (Table 1).

Table 4. Cumulative gas production (CGP) equations expressed as mL. g^{-1} DM of diets including babassu mesocarp meal.

BMM (%)	Equation of France Model	R² (%)
0.0	$Y = 265.9173 \times \{1 - \exp^{[-(0.0743) \times (t - 1.8699) - (-0.1069) \times (\sqrt{t} - \sqrt{1.8699})]}\}$ A	98.80
7.5	$Y = 258.1030 \times \{1 - \exp^{[-(0.0771) \times (t - 2.6481) - (-0.1333) \times (\sqrt{t} - \sqrt{2.6481})]}\}$ B	96.70
15.0	$Y = 257.9985 \times \{1 - \exp^{[-(0.0728) \times (t - 2.6275) - (-0.1297) \times (\sqrt{t} - \sqrt{2.6275})]}\}$ B	98.20
22.5	$Y = 250.6609 \times \{1 - \exp^{[-(0.0697) \times (t - 2.9206) - (0.1119) \times (\sqrt{t} - \sqrt{2.9206})]}\}$ C	97.20

Equations accompanied by equal letters in columns, are identical by the 5% curve identity test (Regazzi & Silva, 2010).

Its use can be economically viable due to its low cost compared to other more expensive fodder (i.e., corn). However, it is necessary to consider the requirement of nutrients for the animals, because such cost reduction may come with a significant decrease in productivity (Seo, Jung, & Seo, 2015). Nevertheless, the use of BMM at the lowest level applied in this work might be considered a feeding option.

As the BMM inclusion increased in the diet, dry matter digestibility decreased and ether extract digestibility increase ($p < 0.05$). There were no significant differences ($p > 0.05$) among treatments for protein and neutral detergent fiber digestibility levels (Table 5).

According to Van Soest (1994), lignin is inversely related to digestibility, leading to a negative effect on dry matter digestibility. The increased digestibility of the ether extract was not expected, as the BMM EE fatty acid content and profile are low (Table 1) and close to the EE of other feeds (Machado, Chaves, & Antoniassi, 2006), respectively.

The high concentrations of lignin in the BMM (Table 1) can increase the colonization time and, consequently, reduce the DM digestibility, because, in addition to being more indigestible, lignin can reduce the digestibility of other dietary components (Reeves, 1985; Jung & Allen, 1995). Thus, it seems that increasing the inclusion of BMM concomitantly reduces ADF and DM digestibility levels.

Table 5. Digestibility coefficients in ewe lambs fed diets with increasing levels babassu mesocarp meal (BMM).

Item	BMM (%)				p-value		
	0.0	7.5	15.0	22.5	L	Q	LD
[1]DMD (%)	71.88	71.71	69.81	66.70	<0.021	0.234	0.432
CPD (%)	66.36	72.76	72.16	68.43	0.342	0.246	0.176
[2]EED (%)	59.39	77.81	75.53	82.19	0.012	0.482	0.441
NDFD (%)	59.95	49.85	53.27	51.06	0.543	0.379	0.105
[3]ADFD (%)	48.75	49.72	41.78	40.06	0.021	0.263	0.519

L: linear effect; Q: quadratic effect; DL: linearity deviation; DDM: dry matter digestibility; CPD: crude protein digestibility; EED: ethereal extract digestibility; NDFD: neutral detergent fiber digestibility; NDAD: acid detergent fiber digestibility. [1]$\hat{Y} = 0.726495 - 0.002328X$ ($R^2 = 87.51\%$); [2]$\hat{Y} = 63.820214 + 0.881422X$ ($R^2 = 76.53\%$); [3]$\hat{Y} = 0.501843 - 0.004537X$ ($R^2 = 81.51\%$).

The average value for crude protein digestibility (CPD) (69.92%) was within the range recommended by Food and Agriculture Organization (FAO, 1992). Ether extract digestibility (EED) (73.72%), on the other hand, was below the recommended (86.6%). Nonetheless, because the diet had more EE, the amount of digestible EE consumed was probably the same or similar among the tested diets. Zinn (1989) showed a linear reduction of organic matter digestibility when oil was included as a supplement with inclusion levels ranging from 0 to 8%, particularly decreasing fiber digestibility.

Sá et al. (2016) reported that babassu endocarp meal (BEM), which has worse nutritional value than BMM, had similar impacts on digestibility to the obtained in this study. In the aforementioned work, BEM negatively influenced almost all digestibility coefficients. When increasing inclusion levels of BMM and BEM, both rich in lignified material, there is a concomitant reduction in the dietary proportion of non-fibrous carbohydrates. As consequences, there are limitations to the utilization of dietary nitrogen, low energy concentrations, and low rates of carbohydrate digestion.

The gross energy intake (GEI) and energy balance (EB) increase linearly and gross energy digestibility (GED) decreases linearly with the inclusion of BMM in the diets of the ewe lambs ($p < 0.05$) (Table 6). In a study with sheep fed increasing levels of pineapple residue (0; 11; 16; and 27%), which is also fibrous but has higher levels of soluble carbohydrates and non-fibrous carbohydrates than BMM, Rogério et al. (2007) did not find differences in gross energy digestibility and energy balance. Sá et al. (2016), working with BEM reported quadratic effects with decreasing onset, contradicting the findings of the present study, which can be explained by the chemical differences between the tested materials, although they originate from the fruit of the same plant.

Table 6. Intake, digestibility and balance of gross energy in ewe lambs fed increasing amounts of babassu mesocarp meal (BMM).

Item	BMM (%)				p-value		
	0.0	7.5	15.0	22.5	L	Q	LD
[1]GEI*	2636.29	3846.71	4407.74	4495.69	>0.001	0.387	0.210
[2]GED (%)	73.29	71.96	69.03	66.57	>0.001	0.521	0.334
[3]EB*	1012.18	1279.69	1718.9	1751.51	0.004	0.387	0.405

*Kcal; L: linear effect; Q: quadratic effect; DL: linearity deviation; GEI: gross energy intake; EGD: gross energy digestibility; EB: energy balance. [1]$\hat{Y} = 2925.71 + 81.856X$ ($R^2 = 75.54\%$); [2]$\hat{Y} = 72.976 + 0.2679X$ ($R^2 = 82.33\%$); [3]$\hat{Y} = 1042.021 + 35.429X$ ($R^2 = 82.04\%$).

It can be emphasized that the decreasing linear pattern of GED is related to the BMM chemical composition (Table 1). However, this reduction influences EB less than GEI (Table 6). It is important to show that lignin, which has a higher latent heat than the other components of the diet (Reeves, 1985), may have increased the calorific value of diets with greater inclusion levels of BMM. This is evident in the gross energy data, as those are only measures of heat. Therefore, it is implied that the higher GEI of diets with BMM leads to animals being provided the highest EB levels, consequently improving their performance and reducing GED.

To prove BMM effective as an alternative feedstuff, it is necessary to consider economic and environmental factors, seasonality and price of BMM, as well as the supply and demand of traditional dietary ingredients, which may influence their use in sheep production systems.

Conclusion

Babassu mesocarp meal reduces *in vitro* rumen fermentation and *in vivo* apparent digestibility of nutrients when included in ewe lamb diets. Its use at low levels may constitute a valid feeding strategy, as long as economic and market aspects are observed.

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