DOI: 10.5433/1679-0359.2017v38n3p1605

Fermentation kinetics and ruminal parameters of animals fed diets containing Brazil nut cake inclusion levels

Cinética de fermentação e parâmetros ruminais de animais alimentados com dietas contendo níveis de inclusão de torta da amêndoa de castanha-do-pará

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Abstract

The inclusion effect of 0 (control), 15, 30, 45, 60, and 100% dry matter (DM) of Brazil nut (*Bertholletia excelsa* Bonpl.) cake (BNC) aiming at replacing corn silage was assessed on fermentation kinetics and effective degradability (ED) by means of in vitro gas production at 3, 6, 9, 12, 24, 48, 72 and 96 h. A randomized block design was used with six treatments, three blocks, and two replications per block. France's model was fit to the data. An in vivo experiment, conducted in fistulated ovine, assessed the effects of BNC inclusion levels of 0, 15, 30, and 45% DM replacing corn silage on ruminal parameters. Ruminal fluid samples were collected postprandial at 8, 10, 12, 14, 16, and 18 h for determining the concentrations of short-chain fatty acids (SCFA), pH, and N–NH_3. A completely randomized design with repeated measures in time was used, with four treatments and three replications. Results of acetate, propionate, butyrate, acetate to propionate ratio, pH, and NH_3 were submitted to analysis of variance and regression (linear and quadratic) considering treatment, time and interaction of both. In addition, the F test with a 5% ($P < 0.05$) significance level was applied to the data. Fermentation kinetics pattern and ED presented a linear decreasing ($P < 0.05$), which means a decrease in total gas production at each coproduct inclusion level. No interaction effect between treatment and time was observed for total SCFA ($P > 0.40$), acetic acid ($P > 0.41$), propionic acid ($P > 0.85$), butyric acid ($P > 0.62$) and pH ($P > 0.57$). BNC replacements of 0, 15, 30 and 45% did not change ($P > 0.05$) total SCFA concentration, as well as acetic acid concentration in ovine. When including 45% DM of BNC, concentrations (mMol/100 mL) of propionic ($P < 0.001$) and butyric ($P < 0.022$) acids was reduced in the ruminal fluid. The highest concentrations at measurement times were observed 4 hours after feeding. The pH values presented a quadratic effect on both inclusion ($P < 0.001$) and time ($P < 0.001$). An interaction was observed between treatment and time for N–NH_3 concentration (mg/ml) ($P < 0.001$) and acetic to propionic acids ratio ($P < 0.014$). Fermentation kinetics was negatively affected by Brazil nut cake inclusion to corn silage-based diet. Therefore, the use of this coproduct is recommended associated with non-structural carbohydrate sources.

Key words: Amazon. *Bertholletia excelsa*. Coproduct. Ovine. Supplementation.

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Received: Aug. 17, 2016 - Approved: Dec. 22, 2016

Resumo

Avaliou-se o efeito da inclusão da torta da amêndoa de castanha-do-pará (*Bertholletia excelsa* Bonpl.) – (TAC) nas proporções 0 (controle), 15, 30, 45, 60 e 100% da matéria seca (MS) em substituição **à** silagem de milho (SM), sobre a cinética de fermentação e a degradabilidade efetiva (DE), através da produção de gases *in vitro*, nos tempos 3, 6, 9, 12, 24, 48, 72 e 96 horas. Utilizou-se delineamento em blocos casualizado, com seis tratamentos, três blocos e duas repetições por bloco. O modelo de France foi ajustado aos dados. Ensaio *in vivo*, conduzido em ovinos fistulados, avaliou-se a influência da TAC nos níveis 0, 15, 30 e 45% (MS), em substituição à silagem de milho, sobre os parâmetros ruminais desses animais. As coletas de líquido ruminal para determinação da concentração dos Ácidos Graxos de Cadeia Curta (AGCC), pH e N-NH_3 foram realizadas às 08h00, 10h00, 12h00, 14h00, 16h00 e 18h00, pós-prandial. Utilizou-se delineamento inteiramente casualizado, com medidas repetidas no tempo, sendo quatro tratamentos e três repetições. Os resultados das variáveis: acetato, propionato, butirato, relação acetato:propionato, pH e NH_3 foram submetidos à análise de variância e regressão (linear e quadrática) considerando-se tratamento, tempo e a interação de ambos. Aplicou-se teste F, com nível de significância de 5% ($P < 0,05$). O padrão de cinética de fermentação e DE foi linear decrescente ($P < 0,05$), diminuiu a produção de gás total à cada nível de inclusão do coproduto. Não houve efeito da interação tratamento *vs* tempo para as variáveis AGCC total ($P>0,40$), ácido acético ($P > 0,41$), propiônico ($P > 0,85$), butírico ($P > 0,62$) e pH ($P > 0,57$). As substituições de TAC de 0, 15, 30 e 45% não alteraram ($P > 0,05$) a concentração de AGCC total, da mesma maneira, não modificou a concentração do ácido acético dos ovinos. Com inclusão de 45% MS de TAC, a concentração dos ácidos propiônico e butírico mMol/100 mL de líquido ruminal reduziram ($P < 0,001$) e ($P < 0,022$), respectivamente. As maiores concentrações, nos tempos de mensuração foram observadas 4 horas após a alimentação. Os valores de pH apresentaram efeito quadrático tanto na inclusão ($P < 0,001$), quanto no tempo ($P < 0,001$). Houve interação tratamento vs tempo na concentração de N-NH_3 (mg/ml) ($P < 0,001$) e na relação dos ácidos acético:propiônico ($P < 0,014$). A cinética de fermentação foi afetada negativamente pela inclusão de torta da amêndoa da castanho-do-pará, à dieta à base de silagem de milho. Recomenda-se o uso desse coproduto, associado à fontes de carboidrato não estrutural.

Palavras-chave: Amazônia. *Bertholletia excelsa.* Coproduto. Ovinos. Suplementação.

Introduction

The increase of world population generates demand for food such as animal-origin products. This situation generates new possibilities of economic gain for productive sector, but reinforces the need for adjustments in production, making it increasingly sustainable (PAULA et al., 2014). However, in much of Brazil, performance, production cycles, and age of slaughter are compromised by forage seasonality, affecting economic and environmental sustainability (MORAES et al., 2010).

Attractive alternatives have been studied in recent years, including a proposal to supplement the deficiencies caused by forage with low nutritional value. Increasingly, agroindustrial coproducts have characteristics of replacing energy and/or protein concentrates that commonly compete with human nutrition, as well as subsidize the productive development of small and medium farmers, even in periods of low forage supply.

In the Amazon region, almost all Brazil nut (*Bertholletia excelsa* Bonpl.) is derived from extraction of forest peoples as a source of employment and income, aiming at their subsistence and sustainable use of local ecosystems. Oil properties of this nut have attracted attention and generated high demand nationally and internationally. Brazil nut cake (BNC) is a residue from oil extraction carried out by a mechanical press (SOUZA; MENEZES, 2004), being added rice husk in the process to potentiate oil removal, which makes it impossible using this coproduct in the food industry. However, it has a great potential for ruminant feeding use. Ramos et al. (2016) included BNC in ovine diet and observed that 56% inclusion is the maximum limit so that the ingestion behavior of dry matter (DM) of animals was not altered.

Assessments performed by using in vitro techniques are among those that offer good correlations with the results found in vivo, quickness and cost reduction. Food quality can be inferred through fermentation kinetics. The parameters volatile fatty acids (VFA), pH, and ammoniacal nitrogen ($N–NH_3$) are considered as an indicative of reactions that occur from food ingestion. Interactions that occur when oilseed coproducts are included in animal diet have been supported by several studies (SANTOS et al., 2012; CHANJULA et al., 2011; WATANABE et al., 2010) and indicate that changes in patterns are dependent, among other factors, on quantity and quality of coproducts included in the diet.

Studies on BNC use as an alternative to ruminant feeding are scarce but necessary for being a viable option for small regional farmers. Thus, this study aimed to assess the fermentation kinetics and ruminal parameters of ovine fed diets containing Brazil nut cake inclusion levels.

Materials and Methods

Fermentation kinetics

The in vitro gas production experiment was performed at the Nutrition Laboratory of the Federal University of Minas Gerais (UFMG) located in Belo Horizonte, Minas Gerais, Brazil (19°55'15" S and 43°56'16" W), where corn silage was replaced with 0, 15, 30, 45, 60 and 100% BNC, based on dry matter. BNC was obtained by mechanical pressing and by using rice husk in the Amazon Oil agroindustry located in Ananindeua, Pará, Brazil.

The experimental design for gas production (in vitro study) was randomized block design with six treatments, three blocks, and two replications per block; the inocula collected from three bovines configured the blocks. Cumulative profiles of gases produced by in vitro fermentation were performed by a semi-automated technique as Theodorou et al. (1994), modified by Maurício et al. (1999).

Inocula were obtained from rumen-fistulated bovine maintained on pasture and supplemented with standard concentrate from the UFMG laboratory. The inoculum was mixed and filtered with cotton fabric layers and maintained in a water bath at 39 °C with carbon dioxide continuously insufflated for preparing 100 mL of buffered ruminal fluid (9:1 buffer to liquid ratio). The buffer solution was prepared according to Theodorou et al. (1994). Samples of 0.5 g were pre-dried in a forced ventilation oven at 65 °C for 72 hours and ground in a 1-mm sieve mill.

Subsequently, mixture and samples were incubated in individual 160-mL glass bottles previously identified with hard-to-remove paint, sealed with rubber stoppers, and maintained at 39 °C, as Menke et al. (1979). Bottles with only buffered ruminal fluid were used as standards. Pressure readings were obtained by means of a pressure transducer at 3, 6, 9, 12, 24, 48, 72, and 96 h after inoculation. Gas production at each time was corrected by subtracting the production in bottles with samples from the production obtained in bottles with only ruminal fluid (standard). Pressure was converted into gas volume using the equation $V = -0.004$ (s.e. 0.06) + 4.43 P (s.e. 0.043) + 0.051 P^2 (s.e. 0.007), previously obtained when the equipment was calibrated, as in Maurício et al. (2003).

Ruminal parameters

The in vivo experiment was approved by the Ethics Committee on Experimental Animal Research of the Federal University of Pará under the protocol BIO 120–13 and conducted at Embrapa Eastern Amazon, Animal Research Unit "Senador Álvaro Adolpho," Belém, Pará, Brazil (1°27'21" S and 48°30'16" W).

The experimental design was completely randomized design with repeated measures in time with four treatments and three replications. Variables were collected every 2 hours between 0 and 10 h

postprandial. Twelve non-defined-breed rumen-fistulated ovines, with an average weight of 35 kg, were maintained in individual metabolic cages in a closed shed and fed twice a day at 8 and 18 h, allowing 10% leftovers from that was supplied, in addition to water ad libitum.

Ovine underwent adaptation for 21 days to diets with corn silage replacement by Brazil nut cake at levels of 0, 15, 30, 45, 60, and 100% (DM). On the 22nd day, representative portions of ruminal contents were collected and filtered in double cotton fabric to extract the rumen fluid at 0 (before feeding), 2, 4, 6, 8 and 10 h postprandial. The pH admeasurement was performed at collection time by using a portable digital pH meter (MB–10, Marte, Sapucaí, Brazil). Subsequently, this material was stored in identified bottles and maintained in a freezer for other analyses.

Concentrations of short-chain fatty acids (SCFA) were determined by gas chromatography by using aliquots of 8 mL and 1 mL meta-phosphoric acid solution addition (AZEVEDO, 1998). In order to assess the ammoniacal nitrogen ($N–NH_3$) concentration in the ovine ruminal fluid, distillation and titration were performed as described by Fenner (1965), adapted by Vieira (1980). Aliquots of 80 ml were collected from each collection, adding 1 mL sulfuric acid (H_2SO_4 1:1).

Chemical analyses

The chemical composition of diet ingredients was determined at the Nutrition Laboratory of the Federal University of Pará, Castanhal campus, Pará, Brazil (1°17'38" S and 47°55'35" W). In pre-dried samples, contents of dry matter (DM), organic matter (OM), mineral matter (MM) and crude protein (PB) were determined according to AOAC International (AOAC, 2005) procedures. Neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose (CEL), hemicellulose (HEMI) and lignin (LIG) were determined by the sequential method of Van Soest et al. (1991). In addition, ethereal extract (EE) was determined by the method described by Silva and Queiroz (2002). In all analyses, INCT recommendations in Detmann et al. (2012) were followed. Table 1 presents bromatological composition percentage of experimental diets.

Table 1. Bromatological composition of experimental diets.

Variable (DM, %)	BNC[1] inclusion (%)					
	0	15	30	45	60	100
Dry matter	33.07	40.98	48.90	56.82	64.73	85.85
Organic matter	95.21	94.35	93.49	92.63	91.77	89.48
Mineral matter	4.79	5.65	6.50	7.36	8.22	10.52
Neutral detergent insoluble ash	1.05	1.17	1.30	1.43	1.56	1.90
Acid detergent insoluble ash	0.76	0.80	0.84	0.89	0.93	0.10
Crude protein	7.88	11.95	16.02	20.09	24.17	35.03
Neutral detergent insoluble protein	0.71	2.10	3.49	4.88	6.27	9.99
Acid detergent insoluble protein	0.24	0.63	1.02	1.41	1.80	2.85
Ethereal extract	4.38	5.97	7.56	9.15	10.75	15.00
Neutral detergent insoluble fiber ap[2]	38.21	37.06	35.90	34.75	33.60	30.54
Acid detergent insoluble fiber	19.25	18.61	17.97	17.33	16.70	15.00
Lignin	2.18	2.47	2.76	3.05	3.35	4.13
Hemicellulose	18.96	18.44	17.93	17.42	16.90	15.54
Cellulose	17.07	16.14	15.21	14.28	13.35	10.87
Non-fibrous carbohydrates	44.74	39.36	33.99	28.61	23.24	8.91

BNC[1]: Brazil nut cake; ap[2]: corrected for ashes and protein.

Statistical analyses

Data normality was verified by the Shapiro-Wilk test and homogeneity of variances by the Bartlett and Cochran test. In order to estimate the in vitro ruminal fermentation kinetic parameters of each treatment, France and Siddons (1993) model was fitted to the data, as expressed in $Y = A \times \{1 - \exp^{[-b \times (t \times T) - c \times (\sqrt{t} - \sqrt{T})]}\}$, where Y is the accumulated gas production (mL), A is the total gas produced (mL), t is the incubation time (h), T is the colonization time (h), and $b^{(h-1)}$ and $c^{(h-0.5)}$ is the average fractional degradation rate μ (h^{-1}), calculated as $\mu = b + c / 2\sqrt{t}$. Equations generated were compared by parallelism and curve identity tests ($P<0.05$), according to Regazzi and Silva (2004).

Effective degradability was estimated through $ED = S_0 \times Exp^{-kt} \times (1 - k) / (S_0 + U_0)$, where ED is the effective degradability, k = passage rate, calculated for k = 2, 5, and 8% h^{-1}, which means a low, medium, and high intake, respectively, according to the recommendations of the Report… (1984), and S_0 and U_0 are the initially fermentable fractions and non-fermentable fractions, respectively (FRANCE; SIDDONS, 1993).

For the in vivo experiment, results of concentrations of acetate, propionate, butyrate, acetate to propionate ratio, pH, and NH_3 from ovine ruminal fluid were submitted to analysis of variance and regression (linear and quadratic) considering treatment, time and interaction of both. The F test was used with a 5% significance level.

Results and Discussion

Fermentation kinetics

Among the assessed parameters, the maximum potential of gas production (A), fractional degradation rate (μ) and effective degradability of DM (EDDM) were numerically higher at 0% inclusion level (only corn silage), in addition to being found in the same treatment lower colonization time (T). The opposite was observed as BNC was included in the diet (Table 2) since EDDM presented reduced values. Similarly, the higher the passage rates were, the lower the observed EDDM ($2 < 5 < 8\%$ h^{-1}, i.e. low, medium and high intake, respectively).

Table 2. In vitro fermentation kinetics parameters of diets containing BNC inclusion levels, from France model and effective degradability of DM (EDDM) obtained for passage rates of 2, 5, and 8% h^{-1}.

France parameter	BNC[1] inclusion					
	0	15	30	45	60	100
A[2]	194.6047	188.9308	180.7920	182.5716	169.4461	141.1446
T[3]	00:30:47	00:32:21	00:32:43	00:44:33	00:49:15	02:32:52
μ[4]	0.0586	0.0595	0.0615	0.0624	0.0625	0.0636
EDDM* (2%)	52.2302	50.5915	50.5178	49.9181	49.4170	46.9138
EDDM (5%)	49.9791	48.3713	48.3009	47.4489	46.8574	42.2427
EDDM (8%)	47.7845	46.2094	46.1427	45.0643	44.3931	38.0050

BNC[1]: Brazil nut cake; A[2]: total gas produced (mL); T[3]: colonization time (hours: minutes); μ[4]: fractional degradation rate (h^{-1}).

Gas production is directly proportional to food microbial fermentation and allows assessing the way the microbial attack occurs during food degradation in the rumen (NEIVA JÚNIOR et al., 2010). Microorganisms can more or less easily degrade fractions that constitute a food. Non-fibrous carbohydrates (NFC), for example, present little in BNC (8.91% DM), are important chemical constituents since they represent sources of fast energy availability, which implies in the initial growth of ruminal microorganisms (MIZUBUTI et al., 2011).

The highest colonization time with 100% BNC (2 h 32 min) (Table 2) may have been a result of high levels of EE (15% DM), which probably made it difficult to attack the diet fiber, higher amount of indigestible components, especially lignin (4.13% DM), and, especially, NFC reduction (8.91% DM). An identical colonization time was found in a study that assessed fermentation kinetics of dry tamarind cake under 1.35 rpm, presenting 15% EE and 33.7% FNC (WANG et al., 2016). A colonization time of 5 h and 40 min was found in a study in which the babassu mesocarp flour was studied (SOUSA et al., 2014), which gives a higher nutritive value to BNC when compared to babassu flour.

EDDM can be considered as the energy digested in the rumen. For this reason, the highest EDDM values are in accordance with the maximum potential of gas production data. Food particle size and its flow through rumen directly influence the ruminant's digestive processes. Jobim et al. (2011) found EDDM values of corn silage by means of an in situ technique that corresponded to 47.34 (5% h^{-1}) and 41.99 (8% h^{-1}), which are lower than those determined for the same passage rates in this study (49.97 and 47.78). On the contrary, Pôssas et al. (2015) assessed the in vitro silage EDDM from three corn hybrids and found that even the lowest values cited were higher (67.04 and 62.56 for passage rates of 2 and 5% h^{-1}) than those found here. In either case, those results are due to NDF and cellulose contents of silages, which were lower in the first study and higher in the second when compared to this study. Wang et al. (2016) observed EDDM values of 73.8 and 64.1 for passage rates of 2 and 5% in an in situ assessment of tamarind cake.

Equations and curves related to regression analysis of ruminal fermentation kinetic data of diets (Table 3) presented gas production curves parallel to each other, with no statistical difference. However, by the curve identity test, a statistical difference was observed between equations of each inclusion level; all of them were different from each other, except for BNC inclusion levels of 15, 30, and 45%, which did not differ from each other.

Table 3. Cumulative gas production (CGP) equations (mL g^{-1} DM) in diets with Brazil nut cake inclusion.

Inclusion	Equation (France model)	R^2
0	$\hat{Y}=194.6047x\{1-exp[-(0.0777) \times (t \times 0.5123)-(-0.1517) \times (\sqrt{t}-\sqrt{0.5123})]\}$ a A	99.78
15	$\hat{Y}=127.3000x\{1-exp[-(0.0774) \times (t \times 0.5393)-(-0.1391) \times (\sqrt{t}-\sqrt{0.5393})]\}$ a B	99.49
30	$\hat{Y}=124.5000x\{1-exp[-(0.0835) \times (t \times 0.5453)-(-0.1765) \times (\sqrt{t}-\sqrt{0.5453})]\}$ a B	99.42
45	$\hat{Y}=125.4000x\{1-exp[-(0.0858) \times (t \times 0.7427)-(-0.1891) \times (\sqrt{t}-\sqrt{0.7427})]\}$ a B	99.62
60	$\hat{Y}=115.3000x\{1-exp[-(0.0822) \times (t \times 0.8210)-(-0.1523) \times (\sqrt{t}-\sqrt{0.8210})]\}$ a C	99.84
100	$\hat{Y}=96.3458x\{1-exp[-(0.0861) \times (t \times 2.5480)-(-0.1773) \times (\sqrt{t}-\sqrt{2.5480})]\}$ a D	99.56

R^2: coefficient of determination (%). Equations followed by equal lowercase letters in the same column are parallel by the curve parallelism test at 5% probability. Equations followed by equal uppercase letters in the same column are identical by the curve identity test at 5% probability (REGAZZI; SILVA, 2004).

The differences ($P<0.05$) observed in the curve identity test (Table 3), more specifically for inclusion levels from 0 to 15% and from 45 to 60% BNC, can be compared and explained by two events observed in an in vivo experiment conducted by Ramos (2014): fiber digestibility affected by a 16% BNC inclusion, which corresponds to 6% EE in the diet; and DM consumption affected by a 58.4% BNC inclusion.

Ruminal parameters

No interaction effect between treatment and time was observed for total SCFA, acetic acid, propionic acid, butyric acid and pH. No effect was also observed in the concentrations of total SCFA and total acetic acid among treatments. From BNC inclusion to the diet, a decrease in propionic acid concentration was observed; for butyric acid

concentration, on the other hand, a quadratic effect was observed, with higher concentrations for 15 and 30% BNC inclusions and lower in 45% DM. The pH showed a quadratic effect and an inverse tendency to that observed for SCFA; with higher values in the diet with 45% BNC inclusion and lower values when 18%, DM of BNC was added to the diet (Table 4).

Table 4. Short-chain fatty acids concentration (mMol 100 mL^{-1} ruminal fluid) and average pH values of ruminal fluid of ovine fed Brazil nut cake increasing levels in replacement to corn silage.

Item	BNC inclusion (%)				Regression	R^2	P-value	CV
	0	15	30	45				
SCFA[1]	56.66	52.77	50.55	46.66	$\hat{Y}=56.50$	–	1.000	54.20
AA[3]	38.41	33.79	32.38	32.70	$\hat{Y}=38.34$	–	0.141	21.88
PA[4]	14.99	14.43	14.13	12.68	$\hat{Y}=15.14-0.004x$	89.83	<0.001	8.39
BA[5]	3.75	3.98	3.98	3.19	$\hat{Y}=3.72+0.004x-0.0001x^2$	95.97	0.022	24.28
pH	6.56	6.45	6.41	6.65	$\hat{Y}=6.57-0.015x+0.0004x^2$	93.11	0.001	2.67

SCFA[1]: total short-chain fatty acids; R^2: coefficient of determination (%); AA[3]: acetic acid; PA[4]: propionic acid; BA[5]: butyric acid; CV: coefficient of variation (%).

Decreasing NDF digestibility, caused by EE excess in the diet (RAMOS, 2014), was probably the main factor in reducing propionic and butyric acids concentration. Similar conditions were observed when assessing 30% rubber tree cake + 30% palm cake inclusion (CHANJULA et al., 2011). Recent studies have demonstrated unsatisfactory effects due to fiber digestion reduction caused by high oil concentrations in ruminant diets (PATRA, 2014; SANTOS et al., 2016).

Values of pH at all inclusion levels remained within the range considered as appropriate (6.0 to 7.0) to occur fiber and protein digestion (HOOVER, 1986; VAN SOEST, 1994) and may have been higher in the diet with 45% BNC (6.65) due to fermentation decreasing in diet compounds, limited amount of non-fibrous carbohydrates, and lignin and ethereal extract content increasing. Nutritional factors such as fiber and carbohydrate contents in the diet are the main influencers of ruminal pH since rumination time variation and fermentation products, such as volatile fatty acids (ALVES et al., 2012), may influence them.

Total and individual SCFA concentrations and pH values of ruminal fluid at measurement time presented quadratic effects. SCFA concentrations showed peaks at 4 h 25 min (acetic acid), 4 h 83 min (propionic acid) and 5 h 62 min (butyric acid) after feeding, and decrease henceforth. Ruminal fluid pH decreased from diet supply (after 0 h) and increased gradually over time (Table 5).

Table 5. Short-chain fatty acids concentration (mMol 100 mL^{-1} ruminal fluid) and average pH values of ruminal fluid of ovine between 0 and 10 h postprandial.

Item	SCFA concentration between 0[1] and 10 h						Regression	R^2	P-value	CV
	8	10	12	14	16	18				
SCFA	44.16	62.50	63.33	45.83	50.83	43.33	$\hat{Y}=49.01+0.421x-0.050x^2$	47.49	0.001	20.54
AA[3]	30.05	40.28	40.76	31.32	34.43	29.09	$\hat{Y}=32.59+0.255x-0.030x^2$	52.03	0.002	22.44
PA[4]	10.42	17.67	18.25	12.59	13.45	11.97	$\hat{Y}=12.28+0.174x-0.018x^2$	45.56	<0.001	24.6
BA[5]	2.73	4.55	4.71	3.35	3.75	3.26	$\hat{Y}=3.18+0.045x-0.004x^2$	45.36	0.001	29.45
pH	6.79	6.21	6.43	6.5	6.54	6.63	$\hat{Y}=6.66-0.114x+0.011x^2$	43.96	<0.001	2.95

0[1]: SCFA concentration before first feeding; R^2: coefficient of determination (%); SCFA: total short-chain fatty acids; AA[3]: acetic acid; PA[4]: propionic acid; BA[5]: butyric acid; CV: coefficient of variation (%).

When assessed in relation to time after feeding, the lowest pH value (6.21) of ruminal fluid, observed two hours postprandial, may be due to the fermentative effect of food offered, with a tendency to increase up to 10 h of measurements, when fermentation product concentrations are lower. Mesacasa et al. (2015) did not observe a significant difference in pH values of ruminal fluid after feeding ruminants with sunflower cake.

The average values observed from the interaction between treatment and time for ammoniacal nitrogen ($N–NH_3$) of ruminal fluid are shown in Table 6. At all inclusion levels, $N–NH_3$ concentrations in ruminal fluid were higher two hours after feeding and, from this time on, a decrease was observed for this variable.

Table 6. $N–NH_3$ concentration (mg dl^{-1}) in the rumen of ovine fed Brazil nut cake increasing levels (%) between 0 and 10 h postprandial in replacement to corn silage.

Inc.	SCFA concentration between 0[1] and 10 h						Regression	R^2	P-value	CV
	8	10	12	14	16	18				
0[3]	5.92	13.38	6.28	3.58	3.72	3.4	$\hat{Y} = 9.215-0.632x$	38.74	<0.001	22.21
15	8.45	15.88	9.12	6.36	7.71	7.48	$\hat{Y} = 11.464-0.458x$	25.21	0.001	
30	7.10	20.34	17.99	15.35	13.44	12.44	$\hat{Y} = 10.073+3.147x-0.309x^2$	53.73	<0.001	
45	11.62	22.16	10.18	11.30	8.48	8.54	$\hat{Y} = 16.000-0.790x$	33.25	0.001	

Inc.: inclusion; 0[1]: $N–NH_3$ concentration before first feeding; R^2: coefficient of determination (%); CV: coefficient of variation (%).

When evaluating BNC inclusion levels and time after supplementation, the average $N–NH_3$ concentrations (mg mL^{-1}) in rumen fluid were in accordance with the minimum amount established for microbial growth, i.e. 5 mg N100 mL^{-1} of ruminal fluid (SATTER, SLYTER, 1974), except for 0% BNC diet between times 6 and 10. This fact can be explained by the lower crude protein percentage in the silage (7.88) when compared to other treatments. Two hours after supplementation, considering all inclusion levels, a $N–NH_3$ concentration peak occurred in the rumen, especially for 30 and 45% BNC diets, which presented averages closer to those proposed by Mehrez et al. (1977), so that maximum ruminal fermentation activity occurs (19 to 23 mg Dl^{-1}). However, decreases were observed when comparing $N–NH_3$ concentrations in 30 and 45% BNC diets during collection time, indicating that 30% BNC inclusion in the diet would provide, for a longer time, a greater ammonia availability for microbial protein synthesis. Nitrogen supply increases fiber digestion by improving microbial efficiency from substrate addition to ruminal flora (LIMA et al., 2013). Mesacasa et al. (2015) observed lower $N–NH_3$ concentrations in the ruminal fluid by including sunflower cake in the diet, which is due to the highest coproduct indigestible fraction.

The interaction between time and treatment of acetic to propionic acids ratio (P<0.014) (Table 7), showed a quadratic effect in the diets with 0 and 30% BNC, but a decrease was observed in this relation with BNC inclusion. The lowest values were observed for these treatments two and four hours after feeding, respectively. An ethereal extract content higher in the diet containing 30% BNC possibly explains the acetic to propionic acids molar ratio, which decreased with BNC inclusion in the diet. Pimentel et al. (2012) observed that the acetate to propionate ratio was not affected by cashew nuts addition to the diet and Vargas et al. (2002) observed a decrease in acetate to propionate ratio of 3.09:2.93, suggesting that unsaturated lipid supply (ground soybean grain and soybean oil) inhibited gram-positive ruminal bacteria and stimulated those that produce propionate.

Table 7. Acetic to propionic acids mole ratio in the rumen of ovine fed Brazil nut cake increasing levels (% DM) at different time after supplementation.

Inc.	Acetic to propionic acids ratio between 0[1] and 10 h						Regression	R^2	P-value	CV
	8	10	12	14	16	18				
0	3.33	2.33	2.33	2.67	3.00	3.33	$\hat{Y} = 3.142-0.323x+0.035x^2$	79.55	<0.001	10.16
15	2.67	2.33	2.00	2.33	2.67	2.00	$\hat{Y}=2.535$	–	0.469	
30	3.00	2.00	2.00	2.33	2.33	2.33	$\hat{Y} = 2.773-0.251x+0.022x^2$	53.21	0.001	
45	3.00	2.33	2.33	3.00	2.67	2.33	$\hat{Y}=2.750$	–	0.808	

Inc.: inclusion; 0[1]: acetic to propionic acids ratio before first feeding; R^2: coefficient of determination (%); CV: coefficient of variation (%); NS: not significant.

Conclusion

Fermentation kinetics was negatively affected by Brazil nut cake inclusion to corn silage diet. Regarding ruminal parameters, propionic and butyric acids decreased from 30% Brazil nut cake inclusion, but within that considered acceptable to ovine physiology. The use of this coproduct is recommended associated with non-structural carbohydrate sources.

Acknowledgements

To the Project Pecus/Bioma Amazônia for the financial support, to Embrapa Eastern Amazon, Federal University of Minas Gerais (UFMG) and Federal University of Pará (UFPA) for the laboratory support, and to Coordination for the Improvement of Higher Education Personnel (CAPES) for granting the scholarships.

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