Rev. Bras. Saúde Prod. Anim., Salvador, v.19, n.2, p.178-192 abr./jun., 2018 **ISSN 1519 9940**
http://dx.doi.org/10.1590/S1519-99402018000200004

Inclusion of copaiba oil (*Copaifera sp.*) as additive in supplements for cattle on pasture

Inclusão de óleo de copaíba ("Copaifera sp.") como aditivo em suplementos para bovinos em pastagens

LIMA, Fabiola Espindola Ortega de[1]; GOES, Rafael Henrique de Tonissi e Buschinelli de[2]*; GANDRA, Jefferson Rodrigues[2]; PENHA, Diego dos Santos[1]; OLIVEIRA, Raquel Tenório de[2]; GRESSLER, Maria Gizelma de Menezes[2]; SILVA, Thaiano Iranildo de Sousa[1]; SILVA, Nayara Gonçalves[1]

[1]Universidade Federal da Grande Dourados, Programa de Pós-Graduação em Zootecnia, Dourados, Mato Grosso do Sul, Brasil.
[2]Universidade Federal da Grande Dourados, Faculdade de Ciências Agrárias, Dourados, Mato Grosso do Sul, Brasil.
*Endereço para correspondência: rafaelgoes@ufgd.edu.br

SUMMARY

This study analyzed the effect of including copaiba oil as an additive for cattle supplemented on pasture, during the dry season. Four crossbred steers, castrated, with 245 ± 25 kg, aged about 18 months, fitted with permanent rumen cannula; were randomly assigned to a 4x4 Latin square. All animals were housed in individual paddocks (0.3 ha), uniformly covered with *Brachiaria brizantha* cv Marandu, with through and drinking fountain; and were given a concentrate at 500g/100 kg BW containing 380 g/kg crude protein (%DM). The copaiba oil was added to the supplement as a spray, in the proportions of 0, 0.5; 1.0 and 1.5 g/kg DM intake. Spraying supplementation was performed daily at the time of supply of the supplement. Data of intake and digestibility of nutrients, ruminal pH; ruminal ammonia nitrogen, and microbial protein synthesis were tested by analysis of variance and polynomial regression, adopting a significance level of 5%. The inclusion of copaiba oil quadratically affected total dry matter intake (P=0.030) and CP digestibility (P=0.043), without altering ruminal metabolism (P>0.05) and microbial protein synthesis (P>0.05) of the animals kept on pasture.

Keywords: cattle, copaiba resin, ruminal additives, supplementation

RESUMO

Objetivou-se avaliar o efeito da inclusão do óleo de copaíba, como aditivo, para bovinos suplementados a pasto, durante a época da seca do ano. Foram utilizados quatro novilhos, castrados, de 245 ± 25 kg, com idade de aproximadamente 18 meses, providos de cânula ruminal, dispostos aleatoriamente em delineamento quadrado latino 4x4. Os animais foram mantidos em piquetes individuais de *Brachiaria brizantha* cv Marandu, de 0,3 hectares providos de cocho e bebedouro e alimentados com suplemento contendo 380 g/kg de PB (%MS) na proporção de 500g/100 kg de PC. O óleo de copaíba foi acrescido ao suplemento na forma de spray nas proporções de 0, 0,5; 1,0 e 1,5 g/kg de MS ingerida. A pulverização do suplemento foi realizada diariamente no momento do fornecimento do suplemento. Os dados de consumo e digestibilidade dos nutrientes, pH ruminal, nitrogênio amoniacal ruminal e a síntese de proteína microbiana dos animais foram submetidos à análise de variância e regressão polinomial, adotando-se nível de significância de 0,05. A adição do óleo de copaíba afetou quadraticamente o consumo de matéria seca total (P=0,030) e o coeficiente de digestibilidade da PB (P=0,043); sem alterar os parâmetros de fermentação ruminal (P>0,05) e a síntese de proteína microbiana (P>0,05) dos animais mantidos a pasto.

Palavras-chave: aditivos ruminais, bovinos, copaíba-resina, suplementação

Rev. Bras. Saúde Prod. Anim., Salvador, v.19, n.2, p.178-192 abr./jun., 2018 ISSN 1519 9940
http://dx.doi.org/10.1590/S1519-99402018000200004

INTRODUCTION

The use of feeding strategies such as the supplementation during different periods of the year, especially during the dry season, are solutions that guarantee the supply of animals and the profitability of production systems. In many cases, supplementation may provide improvement in animal performance, but the response is not always satisfactory; the variation between the observed and the expected can be explained by the associative effect (interaction between the diet components) of the supplement on the forage intake and available energy of the diet (GOES, et al., 2004).

According to Goes et al. (2008), when using the pasture supplementation technique, the limiting nutrient, which may be mineral, vitamin, protein or energy, must be taken into account. Generally, supplementation of grazing animals is carried out mainly during the dry season and during periods of low availability and quality of the forage. In the mentioned conditions, the supplement is almost always used for maintenance, and nitrogen is the most limiting nutrient, which under a situation of deficiency, does not allow the proper development of ruminal microorganisms that act in the digestion of ingested foods, thus compromising the use of nutrients.

In order to alter the ruminal fermentative characteristics, Van Der Merwe et al. (2001) cited the use of non-ionophore antibiotic and growth promoters. The main additives used in Brazil are ionophores, however, problems with toxicity and bacterial resistance, prevent the use of these products as food additives for ruminants in many countries (BARTON, 2000). As a consequence, the scientific community actively seeks alternative food additives without leaving residues in animal products and, at the same time, reducing the release of polluting gases to the environment (SILVA et al., 2012). Among the options, copaiba oil-resin (*Copaifera* sp.) may be an alternative because it has antibiotic properties (PIERI et al., 2011).

Pieri et al. (2012) showed that Gram negative and positive bacteria were inhibited by the concentration of solutions of 10% oil. Mendonça et al. (2009) showed that *Copaifera multijuga hayne* oil resin has the ability to inhibit bacterial.

Based on the above, the goal of this study was to evaluate the effects of the inclusion of copaiba oil in supplements for grazing cattle on nutrient intake and digestibility, microbial protein synthesis, pH and ruminal ammonia nitrogen concentration.

MATERIAL AND METHODS

The experiment was developed according to principles established by the Ethics Committee on Animal Experimentation of the Federal University of Grande Dourados (Protocol approved 023/2015); and conducted in the Ruminant Nutrition Sector of the Faculty of Agricultural Sciences, Dourados, State of Mato Grosso do Sul, 22°14'S latitude, 54°49'W longitude and 450 m altitude, between June and August, totaling 60 experimental days (4 periods of 15 days). The experimental period (Table 1) presented low rainfall, allied to extreme cold with occurrence of frosts.

The study animals were four Jersey steers weighing 245 ± 25 kg, castrated, with approximately 18 months of age, fitted with ruminal cannula, dewormed with Ivermectin (1%). The animals were kept in individual paddocks (0.3 ha) of *B. brizantha* cv Marandu, provided with trough and drinking fountain; and arranged in a 4 x 4 Latin square design.

Table 1. Maximum (Tmax) and minimum (Tmin) temperature, maximum (URmax) and minimum (URmin) relative humidity and rainfall (Prec) in Dourados, State of Mato Grosso do Sul, from June to August 2013

Month	Tmax (T°C)	Tmin (T°C)	URmax (%)	URmin (%)	Prec(mm)
June	26.5	11.6	92.0	48.9	14.3
July	22.6	6.5	95.0	43.2	8.9
August	23.7	6.4	80.5	24.14	1.4

The animals received daily a supplement with 380 g/kg CP at 500g/100 kg body weight (BW) (Table 2 and 3), by the morning until 10h00min to reduce influence on forage intake. Due to the low availability of forage presented, the animals received daily 2.59 kg oat hay (Table 3).

Table 2. Percentage composition of the supplements used

Percentage composition (%DM)	
Corn grain	40.00
Soybean meal	9.00
Urea	11.00
Mineral mix(1)	40.00

Guarantee levels: Crude protein (min) 38.0%; Protein equivalente NPN (max) 32.3%; Phosphorus 20.0g; Calcium 50g; Sulfur 13.0g; Sodium 74.0g, Cobalt 7.5mg; Manganese 147.2; Selenium 1.8mg; Zinc 525.0mg; Fluorine (max) 200mg.

Table 3. Composition of oat hay and supplement provided during the experimental period

Item	Chemical composition (g/kg)				
	DM	CP	NDF	ADF	MM
Hay	764.5	18.4	596.0	318.0	73.2
Supplement	873.3	383.0	317.8	219.5	366.9

DM = dry matter, CP = crude protein, NDF = neutral detergent fiber, FDA = acid detergent fiber, MM = mineral matter.

The treatments consisted of the inclusion of copaiba oil (OC) in the proportions of 0; 0.5; 1.0 and 1.5 g/kg DM ingested (0.0, 2.9, 5.8 and 8.7 g copaiba oil). Copaiba oil was added to the supplement as a spray; due to its high density (0.5g OC), it was diluted with isopropyl alcohol (7 mL); and spraying was performed daily at the time of supply. The composition of copaiba oil (Table 4) was performed according to methodology described by Adams (2017).

On the first day of each experimental period, the total availability of dry matter was determined by cutting close to the ground of 10 delimited areas (0.25m^2), randomly within each paddock. Subsequently, two samples were taken, one for the evaluation of the dry matter and chemical composition and another for the quantification of the components: green leaf, green stem and senescent material.

Table 4. Chemical characterization of copaiba oil used

Sesquiterpenes	%
β-caryophyllene	9.78
β- bisabolene	8.15
α- humulene	8.08
β- selinene	7.76
α-bisabolol	7.14
β-elemene	6.19
γ- cadinene	5.98
α-cadinol	5.67
Diterpenes	
Hardwickiic acid	5.78
Colavenol	3.03
Copaifera acid	2.99
Copaiferolic acid	2.65
Calavenic acid	2.34
Patagonic acid	2.22
Copalic acid	2.03
Fatty acids	
14:0	1.67
16:0	3.67
18:0	2.98

The forage ingested by the animals (extrusa) was collected on the 15th day, through rumen emptying, after a 12 hour fast. At 08h, the rumen was emptied, dried with cotton cloths and cleaned. After rumen emptying, the animals were returned to their respective paddocks and grazed for approximately 30 minutes. An average of 400 g extrusa was collected, which was stored in identified plastic bags and transported inside a Styrofoam box to the Animal Nutrition Laboratory/FCA/UFGD.

After collecting the extrusa, the ruminal content was changed between the animals according to the diet change, with the purpose of reducing the adaptation period (KIM et al., 2014; OSMARI et al., 2017)

Forage and supplement samples were analyzed for dry matter (DM: method 930.15), crude protein (PB: Nx6.25, method 984.13), ether extract (EE: method 920.39) and mineral matter (MM: 942.05) according to AOAC methodologies (2016). The contents of acid detergent fiber (ADF) were determined as described by Van Soest and Robertson (1999); lignin content (LIG) was obtained by oxidation with potassium permanganate (Van Soest and Wine, 1968). For neutral detergent fiber (NDF) analysis, the samples were treated with heat stable alpha amylase without the addition of sodium sulfite and corrected for ash (MERTENS, 2002).

Voluntary intake was determined by the ratio between the amount of fecal dry matter excreted using the external indicator (TiO_2) and the internal indicator (ADFi). The animals received titanium dioxide (TiO_2) for ten consecutive days, with adaptation to the external indicator for five days (1^{st}-5^{th} day) and five days of collection (6^{th}-10^{th} day). Titanium dioxide (10 g/day) was packed in paper cartridges and introduced directly into the rumen of the fistulated animals at 08 and 17h; as described by Ferreira et al. (2009a).

Fecal samples were collected for five days directly from the rectum of the animals once a day, at different times (6, 8, 10, 12 and 14h); in approximate amounts of 200 g, as described by Ferreira et al. (2009b). Samples were conditioned in plastic bags and frozen at -10ºC for future analysis, according to the methodology described by Myers et al. (2004).

For the determination of fecal dry matter production, the following formula was used: fecal DM excreted per day = (100 x TiO_2 supplied)/(% TiO_2 in fecal DM). The indigestible ADF (ADFi) was used to estimate forage intake and determined according to a procedure described by Detmann et. al. (2012) based on in situ degradability for 288 hours.

The dry matter intake was determined according to:

CMS = {[(EF x CIFZ) - IS]/CIFO} + CMSS;

Where, CMS = dry matter intake (kg/day); EF = fecal output (kg/day); CIFZ = concentration of the indicator in feces (kg/kg); IS = indicator present in the supplement (kg/day); CIFO = concentration of the indicator in the forage (kg/kg), CMSS = supplement dry matter intake (kg/day).

On the 13rd experimental day, the amount of concentrate of 500g/100 kg BW was introduced directly into the rumen at 08h00min. The ruminal fluid collection for the determination of pH and ruminal ammonia nitrogen (NAR) were carried out at the liquid/solid interface of the ruminal environment and filtered through a triple layer of cheesecloth; prior to the supply of the concentrate (0h) and 2, 4, 6 and 8 hours after supply.

Determination of pH was performed immediately in 40 mL ruminal fluid collected from each animal. The determination of NAR was performed in 40 mL of ruminal fluid, which was preserved with 1 mL of 1: 1 HCl and packed in a glass container with polyethylene cap, identified and frozen at -20°C. The quantification of the ammonia nitrogen contents was performed by the Micro-Kjeldahl method using the apparatus TE-0364 - Tecnal®, without acid digestion with distillation with 2N potassium hydroxide (KOH) according to the INCTCA method N-006/1, described by Detmann et al. (2012).

For the determination of the microbial synthesis efficiency, spot urine samples were collected on days 11 and 12 of each experimental period, 3 to 4 h after supplying the supplements through spontaneous urination of animals. Immediately after collection, the urine was homogenized and filtered through cloth filters; and a 10 mL aliquot was diluted in 40 mL H_2SO_4 (0.036 N), in order to avoid destruction of the purine derivatives and precipitation of uric acid. A second aliquot of 50mL was stored in 1 mL H_2SO_4 (36 N) for the determination of urea and creatinine concentrations.

Allantoin analyses were performed using the colorimetric method, according to Fujihara et al. (1987) described by Chen and Gomes (1992). Commercial kits (Gold Analisa®) were used to determine the concentration of creatinine and uric acid.

The total excretion of purine derivatives (DP) calculated by the sum of the amounts of allantoin and uric acid excreted in urine, expressed as mmol day^{-1}. The absorbed microbial purines (Pabs, mmol day^{-1}) calculated from the excretion of purine derivatives in urine (DP, mmol day^{-1}), by the equation: $DP = 0.85*Pabs + 0.385*BW^{0.75}$. Where, 0.85 corresponds to the recovery of purines absorbed as urinary derivatives of purines and 0.385 $BW^{0.75}$ is the endogenous contribution to purine excretion (VERBIC et al., 1990).

The total urinary volume was estimated by the ratio between creatinine concentration in urine and its excretion per unit of body weight, adopting as standard the value of 27.36 mg kg^{-1} BW (RENNÓ et al., 2000). The daily excretions of urea-N and creatinine-N were obtained by means of the product of urea and creatinine concentrations by urinary volume within 24 hours, multiplied by 0.466 or 0.3715, corresponding to the levels of N in urea and creatinine, respectively.

The microbial nitrogen synthesis (Nmic, g N d^{-1}) was calculated by means of absorbed purine bases (Pabs, mmol d^{-1}), according to the equation described by Chen & Gomes (1992): Nmic = (70*Pabs)/(0.83*0.134*1000); where 70

is the amount of N present in purines (mg N mol^{-1}); 0.134 is the N of the Purine: total bacterial N ratio (VALADARES et al., 1999); and 0.83 is the intestinal digestibility of microbial purines.

On days 0, 3, 6, 9 and 12, four hours after supplementation, blood samples were collected, totaling five samples, for subsequent serum collection. The collection was performed by puncture of the jugular vein into Vacutainer® tubes containing heparin, which were centrifuged at 3000 rpm for 15 minutes, to remove the plasma. The resulting plasma was poured into Eppendorf tubes and frozen at -20°C for analysis of urea, by colorimetric using a commercial kit (Gold Analisa®).

The ingestive behavior of the supplement was determined on the 14th day by weighing the supplement leftovers in the troughs 20, 40, 60, 90, 120, 180, 300, 420, 540 and 1440 minutes after supplying the concentrate (GOES et al., 2015).

Data were analyzed in a 4x4 Latin square using PROC MIXED SAS 9.2, according to the following model:

$$Y_{ijk} = \mu + A_i + P_j + D_k + e_{ijk},$$

where: Y_{ijyk} = observation of animal i, in period j, submitted to dose k; μ = overall mean, A_i = random effect of animal i ($_i$ = 1 to 4), P_j = random effect of period j ($_j$ = 1 to 4), D_k = fixed effect of dose used ($_k$ = 1 to 4), and e_{ijk} = random effect of the error, associated with each observation, assuming that NID (0; σ^2).

Ruminal fermentation data were analyzed using the REPEAT command of PROC MIXED, using the covariance matrices: compound symmetric (CS); heterogeneous compound symmetry (CSH); first-order autoregressive (AR1); first-order heterogeneous autoregressive (ARH1); toeplitz (TOEP); heterogeneous toeplitz (TOEPH); analytical factor (FA); Huynh-Felt (HF); unstructured (UN) and components of variance (CV). The variance and variance matrices were evaluated by means of SBC criteria (Schwarz's Bayesian Criterion).

The mathematical model adopted was:

$$Y_{ijk} = \mu + A_i + P_j + D_k + T_y + T_y(D_k)\ e_{ijk}$$

where: Y_{ijyk} = observation of animal i, in period j, submitted to dose k; in time y; μ = overall mean; A_i = random effect of the animal (i= 1 to 4), Pj = random effect of the period (j = 1 to 4), D_k = fixed effect of the dose used (k = 1 to 4), Ty = fixed effect of collection time (k = 1 to 5), $T_y(D_k)$ = interaction between doses used and collection time, and e_{ijk} = random effect of error, associated with each observation, assuming that NID (0; σ^2).

Data were tested by analysis of variance and polynomial regression, adopting a level of significance of 5%.

For the ingestive behavior of supplement for each treatment, we fit a model of the type:

$Y = a^* (1 - e^{(-k*X)})$,

where "a" and "k" are the parameters of the model, "e" is the Napierian number, and Y and X are the variables, in the same way as Goes et al. (2015).

This model was fit to describe the intake pattern of the concentrate supplement (Y) as a function of time (X) for 24 hours. Supplement intake patterns were compared considering the 95% confidence interval of the estimated model parameters.

RESULTS AND DISCUSSION

The amount of dry matter available during the experimental period ranged from 1512.78 to 1578.69 kg DM/ha and 896.4 kg green DM/ha (Table 5). Silva

et. al. (2009) recommended that in order to obtain animal selectivity, average values of 4,500 kg DM/ha and 1,200 kg green DM/ha should be obtained. However, in the present research, due to the climatic conditions that affected the forage quality, the supply of pasture was below the values mentioned by the authors, impeding the selective grazing by the animals. For this reason, approximately 2.59 kg oat hay was given to the animals daily.

In addition to the availability of green forage, it is necessary to take into account the amount of chemical compounds present in the feed offered (Tables 3 and 5), since they influence the animal CMST. CP values of pasture and hay remained around 4.0%, below the minimum limit of 7.0%, thus becoming a limiting factor for adequate microbial activity and growth, impairing forage digestibility due to the high levels of NDF and ADF of the forage offered.

Table 5. Availability of green dry matter (green DM kg/ha), leaf (%), stem (%) and senescent material (%), and chemical composition of Marandu palisade grass

Item	Levels of inclusion of copaiba oil (g/kg ingested DM)			
	0	0.5	1.0	1.5
Availability of greenDM (kg/ha)	870.52	812.24	798.66	908.88
Leaf (%)	27.17	18.40	20.12	23.18
Stem (%)	29.18	35.05	30.47	36.90
Senescent Material (%)	43.29	46.35	49.21	39.72
Chemical composition (g/kg)				
DM	571.9			
CP	43.5			
NDF	789.1			
ADF	698.1			
LIG	76.4			
MM	40.6			
TDB*	351.5			
TDN:CP	8.08			

*%TDN = 74.49 – 0.5635*ADF (r^2=0.82) Capelle et al (2001).

The inclusion of copaiba oil affected quadratically the forage dry matter intake (CMSF) (P≤0.004), supplement dry matter intake (CMSS) (P≤0.005), and total dry matter intake (CMST) (P ≤0.03), stimulated by higher values for DM digestibility (P≤0.038) and CP digestibility (P≤0.043) (Table 6). During the experiment, the daily intake of supplements ranged from 56 to 495g (Table 7). The parameter "a" represents the estimate of daily intake of the supplement with 95% reliability. The parameter "k" for the inclusion of 1.0 g/kg DM had the lowest velocity estimate but presented a higher estimate for the parameter "a" of intake. The inclusion level of 0.5 g/kg DM presented for the parameter "a" the lowest intake estimate, however, it presented a higher estimate for the parameter "k" of ingestion rate. The estimates (quantity found by the mathematical model) showed different values for the parameters "a" of intake (g) and parameter "k" of ingestion rate in the different levels of inclusion of copaiba oil in the supplement.

Rev. Bras. Saúde Prod. Anim., Salvador, v.19, n.2, p.178-192 abr./jun., 2018 **ISSN 1519 9940**
http://dx.doi.org/10.1590/S1519-99402018000200004

Table 6. Mean values of forage dry matter intake (CMSF), supplement dry matter intake (CMSS), hay intake (CHay) and total dry matter intake (CMST)

Item	Levels of inclusion of copaiba (g/kg DM)				SEM[1]	P-value[2]	
	0	0.5	1.0	1.5		Linear	Quad
CMSF(1)	1.68	2.54	1.95	1.37	0.16	0.804	0.004
CMSS(2)	0.298	0.494	0.420	0.321	0.75	0.486	0.005
CHay	2.59	2.59	2.59	2.59	-	-	-
CMST(3)	4.57	5.62	4.87	4.38	0.16	0.156	0.030
Digestibility Coefficients							
Dry matter(4)	0,52	0,59	0,54	0,51	3.59	0.641	0.038
Crude protein (5)	0,56	0,78	0,72	0,56	6.44	0.535	0.043
NDF	0,46	0,47	0,43	0,43	2.43	0.640	0.949

[1]Standard error of the mean.[2] Linear and quadratic effect.
Y(1)= 1.75 + 1.85X – 1.44X^2 r^2= 0.56; Y(2)= 0.31 + 0.44X – 0.29X^2 r^2 = 0.63; Y(3)= 4.67 + 2.04 – 1.54X2 r^2= 0.28; Y(4)=0.199+0.466x – 0.094X^2 r^2= 0.96; Y(5)= 0.436 +0.114x – 0.024X^2 r^2= 0.75.

Table 7. Intake estimates, asymptotic confidence intervals and asymptotic standard error for Brody model parameters of the concentrate supplement intake with different copaiba inclusion

Levels of inclusion of copaiba (g/kg DM)	Estimate	Asymptotic confidence interval		Asymptotic standard error
		Lower limit	Upper limit	
			Parameter "a"	
0	144.0	56.9378	259.60	56.93
0.5	131.4	63.7535	199.00	33.25
1.0	182.8	100.6000	265.00	40.39
1.5	367.4	239.7000	495.10	62.91
			Parameter "k"	
0	0.1307	-0.1363	0.3976	0.1315
0.5	0.2237	0.0650	0.3825	0.0780
1.0	0.1437	-0.00056	0.2880	0.0709
1.5	0.0985	-0.3903	-0.0532	0.0830

Parameter "a": intake intervals; Parameter "k": ingestion rate.

The pH and ruminal ammonia concentration (NAR) were not influenced by the inclusion levels of copaiba oil in the animal diet (Table 8). However, there was a time effect for both the pH values and the NH3-N values caused by the determination schedules (Figures 1 and 2). Apparently, the functional oils do not alter ruminal pH characteristics (OSMARI et al., 2017).

The highest mean pH value observed was at 2 hours, differing from the other values according to the hours of determination. The lowest pH value was observed at 6 hours for the diet with inclusion of 1.0 g/kg/DM, however, all treatments influenced the drop in pH values between 2 and 6 hours of collection. According to Hiltner & Dehority (1983). these values are between 6.6 and 7.0 and a pH less than 6.2 entails in a significant reduction of the degradation process and with values smaller than 6.0, there is practically no digestion of the fiber.

Rev. Bras. Saúde Prod. Anim., Salvador, v.19, n.2, p.178-192 abr./jun., 2018 **ISSN 1519 9940**
http://dx.doi.org/10.1590/S1519-99402018000200004

Table 8. Mean values of pH and the ammonia concentration (mg/dL) of ruminal fluid of cattle supplemented on pasture receiving copaiba oil

Item	Inclusion levels of copaiba oil (g/kg DM)				SEM[1]	P-value[2]	
	0	0.5	1.0	1.5		Linear	Quad
pH	6.9	6.8	6.8	6.8	0.02	0.32	0.94
NH_3-N (mg/dL)	13.9	12.2	12.3	13.4	0.75	0.88	0.56

[1]Standard error of the mean.[2] Linear and quadratic effect.

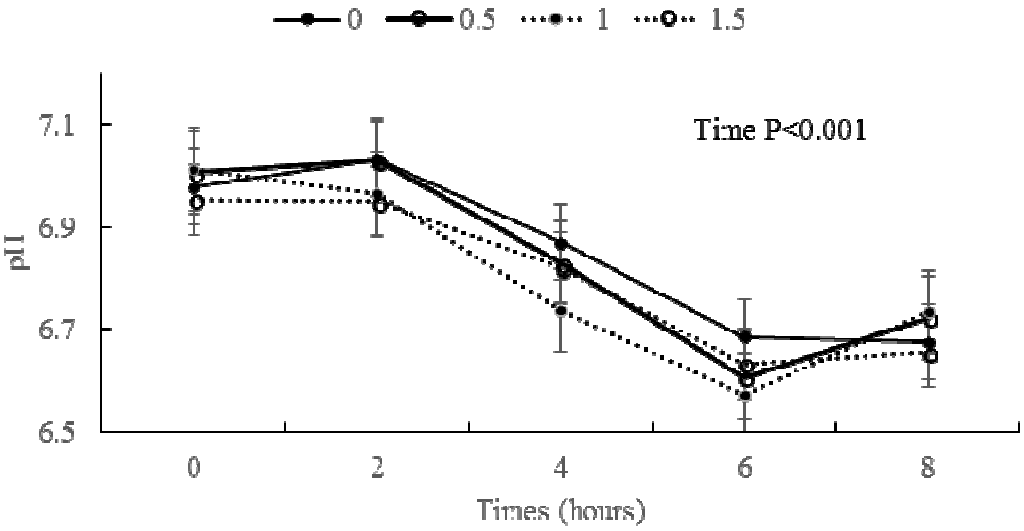

Figure 1. Mean ruminal pH after supplementation with different inclusion levels of copaiba oil

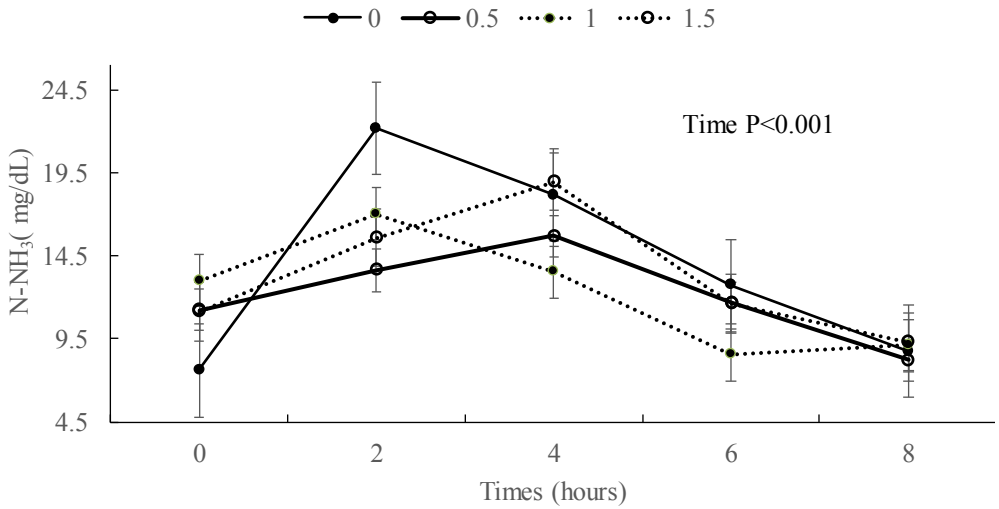

Figure 2. Ruminal Ammonia Nitrogen Concentration (mg/dL) after supplementation with different levels of inclusion of copaiba oil

The pH values in this study varied little between inclusion levels tested, presenting a mean of 6.82. This value of pH found reinforces reports that diets with predominance of forage should have pH close to neutrality. The values found are above the limit of 6.2, proposed by Hiltner & Dehority (1983), as the minimum limit so that there is no reduction of the microbial synthesis and inhibition of the NDF degradation.

For NAR, there was a difference between the supplement levels that were influenced by time, according to Figure 2. The mean value for NAR was 12.92 mg/dL; close to the minimum values required for maximum microbial growth and ruminal digestion, which is 10 mg/dL, as described by Detmann et al. (2007), in order to increase the suitability of the growth medium to the availability of nitrogen compounds for microbial anabolism.

The collection times where the highest NAR production rates were observed, per level of inclusion of copaiba oil to the supplement consumed, were in the collections of 2 hours for all treatments. The values obtained, after the supplementation, can be explained by the solubility of the supplements used, mainly by the use of urea in the composition.

Diets with levels of 0.5 and 1.5 g/kg/DM continued with high levels of NAR production up to 4 hours of collection, and diets with inclusion of 0 and 1.0 g/kg/DM had a decrease in production in the same time interval. However, all levels of inclusion of copaiba oil in the diet provided a decline in production between 4 and 6 hours, decreasing to the 8-hour interval, except for diet 3 (1.0 g/kg/DM) that maintained production with a slight increase.

The mean concentrations of NAR obtained in the ruminal fluid of the diets were close to the minimum recommended by Detmann et al. (2007) for the maximum microbial growth and ruminal digestion, of 10 mg/dL, thus occurring the suitability of the growth medium to the availability of nitrogen compounds for microbial anabolism.

According to Mehrez et al. (1977), in order to reach the maximum microbial synthesis and potentiate the dry matter intake, the concentration of 23 mg NH_3-N/100 mL is recommended. Rumen ammonia levels are important for the synthesis of microbial protein and nitrogen deficiency limits microbial growth, reducing cell wall digestibility, intake and, consequently, animal performance.

Creatinine formed in the muscle is a metabolic residue that is constantly and largely excreted by the kidneys. The daily production and excretion of creatinine depend on the muscle mass and is proportional to the weight of the animal.

According to NRC (NASEM, 2016), in the animal fed energetic diets, the percentage of protein decreases and that of fat increases as its weight approaches weight at maturity. Thus, in growing animals, the percentage of muscle tissue varies according to animal weight and, consequently, creatinine excretion can be altered. Adult animals present less variation in body composition and, therefore, creatinine excretion at live weight becomes less variable (LEAL et al., 2007).

Although the animals in this experiment were in the growth phase, creatinine levels did not show significant differences for the different levels of inclusion of copaiba oil (Table 9). The mean total creatinine value was 1.63 mg/ dL, and the reference intervals recommended by Kaneko et al. (1997) for cattle are 1 to 2 mg/dL.

Rev. Bras. Saúde Prod. Anim., Salvador, v.19, n.2, p.178-192 abr./jun., 2018 **ISSN 1519 9940**
http://dx.doi.org/10.1590/S1519-99402018000200004

There was no effect on the metabolism of urea and creatinine and on the microbial protein synthesis of the animals (Tables 9 and 10). Diets with inclusion of 0.5 and 1.0 g/kg/DM, respectively, presented higher values for plasma urea, however, the total mean value obtained was 48.73 mg/dL, a result close to that reported by Kaneko et al. (1997), who cited reference values between 17 and 45 mg/dL.

Table 9. Metabolism of urea and creatinine from steers on pasture and supplemented with copaiba oil

Item	Levels of inclusion of copaiba oil (g/kg DM)				SEM[1]	P-value[2]	
	0	0.5	1.0	1.5		Linear	Quad
Urine (mg/dL)							
Urea	57.50	47.50	67.50	46.75	3.62	0.695	0.444
Creatinine	6.12	5.25	8.75	10.25	1.02	0.089	0.560
Urea-N	19.28	23.93	24.77	22.85	1.35	0.356	0.242
Creatinine-N	2.27	1.95	3.25	3.80	0.38	0.089	0.560
Blood (mg/dL)							
Urea	41.38	51.36	53.15	49.04	4.19	0.550	0.449
Creatinine	1.72	0.85	2.00	1.97	0.35	0.580	0.580
Urea-N	26.79	22.13	31.45	21.78	1.68	0.695	0.444
Creatinine-N	0.64	0.31	0.74	0.73	0.09	0.393	0.392
Excretion (mg/kg BW)							
Urea	35.71	35.46	54.79	40.40	4.15	0.371	0.397
Creatinine	28.80	28.84	28.88	28.78	0.02	0.903	0.131
Clearance (24 hours)							
Urea	0.84	0.71	1.01	0.68	0.05	0.731	0.355
Creatinine	26.63	35.23	21.45	23.47	2.23	0.232	0.446
Fractional Excretion (%)							
Urea	5.07	2.21	7.47	3.68	0.79	0.871	0.759

[1]Standard error of the mean;[2] Linear and quadratic effect.

Table 10. Microbial protein synthesis of steers on pasture and supplemented with copaiba oil

Item	Levels of inclusion of copaiba oil (g/kg DM)				SEM[1]	P-value[2]	
	0	0.5	1.0	1.5		Linear	Quad
mmol/L							
Allantoin	7.84	7.85	8.84	8.34	0.56	0.657	0.842
Uric acid	0.81	1.19	1.11	1.26	0.16	0.397	0.730
mmol/day							
Allantoin	85.96	77.41	115.50	109.47	8.19	0.286	0.955
Uric acid	9.62	13.22	13.68	18.66	2.54	0.274	0.899
Total purine	95.58	90.63	129.18	128.13	1.68	0.695	0.444
Abs purine	78.42	72.93	118.53	116.58	11.09	0.267	0.955
(g/day)							
Nitrogen	71.67	67.50	100.94	99.80	4.15	0.265	0.947
Protein	447.99	421.90	630.90	623.76	7.02	0.265	0.947

[1]Standard error of the mean;[2] Linear and quadratic effect.

Urinary excretion may fluctuate between days, causing some bias in the excretion of purine derivatives, which could influence the data obtained herein. Plasma concentrations of urea at baseline levels are important to avoid energy losses through nitrogen excretion. Control of urea excretion by the kidneys may influence the concentration of urea in the blood, depending on the animal's dietary conditions (HARMEYER & MERTENS, 1980). Broderick et al. (1993) concluded that concentrations of plasma urea in cattle lower than 11 mg/dL indicated CP deficiency in the diets provided, which probably did not occur in this study, since the values obtained were higher than reported.

The inclusion of copaiba oil in supplements altered the total dry matter intake and the digestibility coefficients, without altering the pH and the concentrations of ruminal ammonia nitrogen, and the microbial protein synthesis of the animals kept on pasture. It is recommended to use the dose of 0.66 g copaiba oil/kg ingested DM.

ACKNOWLEDGEMENTS

To Coordination for the Improvement of Higher Education Personnel - CAPES; Federal University of Grande Dourados, for financial support and scholarships. To Foundation to Support the Development of Education, Science and Technology of the State of Mato Grosso do Sul (FUNDECT) and the National Council for Scientific and Technological Development (CNPq); for financing part of this work. In addition, the authors express thanks to Euclides Reuter de Oliveira for the donation of copaiba oil; and Claudia Andrea Lima Cardoso, for the characterization of the copaiba oil used.

REFERENCES

ADAMS, R.P. **Identification of essential oil components by Gas Chromatography/Mass Spectroscopy**. 5th Ed. Gruver, TX UDA: Texensis Publishing, 2017. 698p.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS - AOAC. **Official methods of analysis**. 20th Ed. Arlington, VA: AOAC International, 2016. 3172p.

BARTON, M.D. Antibiotic use in animal feed and its impact on human health. **Nutrition Research Reviews**, v.13, n.2, p.279-299, 2000.

BRODERICK, G.A.; CRAIG, W.M.; RICKER, D.B. Urea versus true protein as supplement for lactating dairy cows fed grains plus mixtures of alfafa and corn silages. **Journal of Dairy Science**, v.76, p.2266-2274, 1993.

CHEN, X.B.; GOMES, M.J. **Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives – an overview of technical details**. Bucksburnd: Rowett Research Institute, 1992. 21p. (Occasional publication).

DETMANN. E.; SOUZA, M.A.; VALADARES FILHO. S.C.; QUEIROZ, A.C.; BERCHIELLI, T.T.; SALIBA, E.O.S.; CABRAL, L.S.; PINA, D.S.; LADEIRA, M.M.; AZEVEDO, J.A.G. **Métodos de Análise de Alimentos**. Instituto Nacional de Ciência e Tecnologia de Ciência Animal. Visconde do Rio Branco, MG: Suprema, 2012. 214p.

DETMANN, E.; SOUZA, R.; GARCIA, S.C., VALADARES FILHO, S.C.; CABRAL. L.S.; ZERVOUDAKIS. J.T. Avaliação do vício de "tempo longo" de indicadores internos em ensaio de digestão com ruminantes. **Arquivo Brasileiro de Medicina Veterinária Zootecnia**, v.59, n.1, p.182-188, 2007.

FERREIRA, M.A.; VALADARES FILHO, S.C.; MARCONDES, M.I.; PAIXÃO, M.L.; PAULINO, M.F.; VALADARES, R.F.D. Avaliação de indicadores em estudos com ruminantes: digestibilidade. **Revista Brasileira de Zootecnia**, v.38, n.8, p.1568-1573, 2009a.

FERREIRA, M.A.; VALADARES FILHO, S.C.; COSTA E SILVA, L.F.; NASCIMENTO, F.B.; DETMANN, E.; VALADARES, R.F.D. Avaliação de indicadores em estudos com ruminantes: estimativa de consumos de concentrado e de silagem de milho por vacas em lactação. **Revista Brasileira de Zootecnia**, v.38, n.8, p.1574-1580, 2009b.

FUJIHARA, T.; ORSKOV, E.R.; REEDS, P.J.; KYLE, D.J. The effect of protein infusion on urinary excretion of purine derivatives in ruminants nourished by intragastric nutrition. **Journal of Agriculture Science**, v.109, n.1, p.7-12, 1987.

GOES, R.H.T.B.; ALVES, D.D.; MANCIO, A.B.; ZERVOUDAKIS, J.T. Efeito associativo na suplementação de bovinos a pasto. **Arquivos de Ciências Veterinárias e Zoologia da UNIPAR**, v.7, n.2, p.163-169, 2004.

GOES, R.H.T.B.; GANDRA, J.R.; MARQUEZ, A.F.; OLIVEIRA, E.R. DE; FERNANDES, H.J.; CARDOSO, T.J. DE L.; BRABES, K.C. DA S.; YOSHIHARA, M.M. Metabolismo nitrogenado em bovinos suplementados a pasto durante a transição águas-seca. **Archivos de Zootecnia**, v.64, n.247, p.281-290, 2015.

GOES, R.H.T.B.; LAMBERTUCCI, D.M.; BRABES, K.C.S.; ALVES, D.D. Suplementação proteica e energética para bovinos de corte em pastagens tropicais. **Arquivos de Ciências Veterinárias e Zoologia da UNIPAR**, v.11, n.2, p.129-197, 2008.

HARMEYER, J.; MERTENS, H. Aspects of urea metabolism with references to the goat. **Journal of Dairy Science**, v.63, n.10, p.1707–1728, 1980.

HILTNER, P.; DEHORITY, B.A. Effects of soluble carbohydrates on digestion of cellulose by pure cultures of rumen bacteria. **Applied and Environmental Microbiology**, v.46, n.5, p.642-648, 1983.

KANEKO, J.J.; HARVEY, J.W.; BRUSS, M.L. (Eds.) **Clinical Biochemistry of Domestic Animals**. San Diego: Academic Press, 1997. 932p.

KIM, D.H.; MCLEOD, K.R.; KOONTZ, A.F.; FOOTE, A.P.; KLOTZ, J.L.; HARMON, D.L. Effect of intake on fasting heat production, respiratory quotient and plasma metabolites measured using the washed rumen technique. **Animal**, v.9, p.1-9, 2014.

LEAL, T.L.; VALADARES, R.F.D.; VALADARES FILHO, S.C.; CAMPOS, J.M.S.; DETMANN, E.; BARBOSA, A.M.; TEIXEIRA, R.M.A.; MARCONDES, M.I. Variações diárias nas excreções de creatinina e derivados de purinas em novilhas. **Revista Brasileira de Zootecnia**, v.36, n.4, p.905-911, 2007.

MEHREZ, A.Z.; ØRSKOV, E.R.; MCDONALD, I. Rates of rumen fermentation in relation to ammonia concentration. **British Journal of Nutrition**, v.38, n.3, p.437-443, 1977.

MENDONÇA, D.E.; ONOFRE, S.B. Atividade antimicrobiana do óleo-resina produzido pela copaiba – Copaifera multijuga Hayne (Leguminosae). **Revista Brasileira de Farmacognosia**, v.19, n.2, p.577-581, 2009.

MERTENS, D.R. Gravimetric determination of amylase treated neutral detergent fiber in feeds with refluxing in beakers or crucibles. Collaborative study. **Journal of AOAC International**, v.85, p.1212-1240, 2002.

MYERS, W. D.; LUDDEN, P.A.; NAYIHIGIHUGU, V.; HESS, B. Technical Note: a procedure for the preparation and quantitative analysis of samples for titanium dioxide. **Journal of Animal Science**, v.82, p.179-183, 2004.

NATIONAL ACADEMIES OF SCIENCES, ENGINEERING, AND MEDICINE - NASEM. 2016. **Nutrient Requirements of Beef Cattle**. Eighth Revised Edition. Washington, DC: The National Academies Press, 2016.

OSMARI, M.P.; BRANCO, A.F.; GOES, R.H.T.B.; DIAZ, T.G.; MATOS, L.F. Increasing dietary doses of cashew nut shell liquid on rumen and intestinal digestibility of nutrient in steers fed a high-grain diet. **Archivos de Zootecnia**, v.66, n.255, p.375-381, 2017.

PIERI, F.A.; SILVA, V.O.; SOUZA, C.F.; COSTA, J.C.M.; SANTOS, L.F.; MOREIRA, M.A.S. Antimicrobial profile screening of two oils of Copaifera genus. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.64, n.1, p.241- 244, 2012.

PIERI, F.A.; SOUZA, C.F.; COSTA, J.C.M.; BARRERO, M.A.O.; ESPESCHIT, I.F.; SILVA, V.O.; MOREIRA, M.A.S. Inhibition of *Escherichia coli* from mastitic milk by copaiba oil. **Semina: Ciências Agrárias**, v.32, n.1, p. 1929-1934, 2011.

RENNÓ, L.N.; VALADARES, R.F.D.; VALADARES FILHO, S.C.; LEÃO, M.I.; COELHO DA SILVA, J.F.; CECON, P.R.; GONÇALVES, L.C.; DIAS, H.L.C.; LINHARES, R.S. Concentração plasmática de uréia e excreções de uréia e creatinina em novilhos. **Revista Brasileira de Zootecnia**, v.29, n.4, p.1235-1243, 2000.

SILVA, F.F.; SÁ, J.F.; SCHIO, A.R.; ITAVO. L.C.V.; SILVA, R.R. e.; MATEUS, R.G. Suplementação a pasto: disponibilidade e qualidade x níveis de suplementação x desempenho. **Revista Brasileira de Zootecnia**, v.38, p.371-389, 2009.

SILVA, J.T.; BITTAR, M.C.; FERREIRA, L.S. Evaluation of mannan-oligosaccharides offered in milk replacers or calf starters and their effect on performance and rumen development of dairy calves. **Revista Brasileira de Zootecnia**, v.41, n.3, p.746-752, 2012.

VALADARES, R.F.D.; BRODERICK, G.A.; VALADARES FILHO, S.C.; CLAYTON, M.K. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. **Journal of Dairy Science**, v.82, n.11, p.2686-2696, 1999.

VAN DER MERWE, B.J.; DUGMORE, T.J.; WALSH, K.P. The effect of flavophospholipol (Flavomycin) on milk production and milk urea nitrogen concentration of

grazing dairy cows. **South Africa Journal of Animal Science**, v.31, n.2, p.101-105, 2001.

VAN SOEST, P.J.; ROBERTSON, J.B. **Analysis of forages and fibrous foods**. A Laboratory Manual. Ithaca, NY: Cornell University, 1999.

VAN SOEST, P.J.; WINE, R.H. Determination of Lignin and Cellulose in Acid-Detergent Fiber with Permanganate. **Journal of AOAC International**, v.51, p.780-785, 1968.

VERBIC, J.; CHEN, X.B.; MACLEOD, N.A.; ØRSKOV, E.R. Excretion of purine derivatives by ruminants. Effect of microbial nucleic acid infusion on purine derivative excretion by steers**. Journal of Agricultural Science**, v.114, n.3, p.243-248, 1990.

Receipt date: 30/11/2017
Approval date: 23/03/2018