Dietary protein reduction on microbial protein, amino acids digestibility, and body retention in beef cattle. I. Digestibility sites and ruminal synthesis estimated by purine bases and ^{15}N as markers¹

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ABSTRACT: The objectives of this study were to evaluate the effect of reducing dietary CP contents on 1) total and partial nutrient digestion and nitrogen balance and 2) on microbial crude protein (MCP) synthesis and true MCP digestibility in the small intestine obtained with ^{15}N and purine bases (PB) in beef cattle. Eight bulls (4 Nellore and 4 Crossbred Angus $\times$ Nellore) cannulated in the rumen and ileum were distributed in duplicated 4×4 Latin squares. The diets consisted of increasing CP contents: 100, 120, or 140 g CP/kg DM offered ad libitum, and restricted intake (RI) diet with 120 g CP/kg DM. The experiment lasted four 17-d periods, with 10 d for adaptation to diets and another 7 for data collection. Omasal digesta flow was obtained using Co-EDTA and indigestible NDF (iNDF) as markers, and to estimate ileal digesta flow only iNDF was used. From days 11 to 17 of each experimental period, ruminal infusions of Co-EDTA (5.0 g/d) and ^{15}N (7.03 g of ammonium sulfate enriched with 10% of ^{15}N atoms) were performed. There was no effect of CP contents (linear effect, $P = 0.55$ and quadratic effect, $P = 0.11$) on ruminal OM digestibility. Intake of CP linearly increased ($P < 0.01$) with greater dietary CP. The $\text{NH}_3\text{-N}$ ($P < 0.01$) and urinary

N excretion ($P < 0.01$) increased in response to dietary CP, whereas retained N increased linearly ($P = 0.03$). Liquid-associated bacteria (LAB) in the omasum had greater N content ($P < 0.05$) in relation to the particle-associated bacteria (PAB). There was no difference between LAB and PAB ($P = 0.12$) for $^{15}\text{N}:$ ^{14}N ratio. The $^{15}\text{N}:$ ^{14}N ratio was greater ($P < 0.01$) in RI animals in relation to those fed at voluntary intake. Microbial CP had a quadratic tendency ($P = 0.09$) in response to CP increase. Microbial efficiency (expressed in relation to apparent ruminally degradable OM and true ruminally degradable OM) had a quadratic tendency ($P = 0.07$ and $P = 0.08$, respectively) to CP increasing and was numerically greatest at 120 g CP/kg DM. The adjusted equations for estimating true intestinal digestibility of MCP (Y_1) and total CP (Y_2) were, respectively, as follows: $Y_1 = -16.724_{(\text{SEM} = 40.06)} + 0.86X_{(\text{SEM} = 0.05)}$ and $Y_2 = -43.81_{(\text{SEM} = 49.19)} + 0.75X_{(\text{SEM} = 0.05)}$. It was concluded that diets with 120 g/kg of CP optimize the microbial synthesis and efficiency and ruminal ash and protein NDF digestibility, resulting in a better use of N compounds in the rumen. The PB technique can be used as an alternative to the ^{15}N to estimate microbial synthesis.

Key words: ammonia, Co-EDTA, ileum, nitrogen efficiency, omasum, rumen

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INTRODUCTION

The Brazilian system of nutritional requirements for cattle (BR-CORTE, [Valadares Filho et al., 2016](#)) assumes an 80% intestinal digestibility (ID) of microbial crude protein (MCP), as previously recommended by the [NRC \(1985\)](#). However, there are inconsistent values among different studies, e.g., [Bird \(1972\)](#), using ^{35}S as microbial marker, obtained the value of 74%, whereas [Salter and Smith \(1977\)](#) obtained 85% for MCP ID in sheep cannulated in duodenum and ileum. These last authors, when ^{15}N was used as a microbial marker, obtained 79% MCP ID ([Salter and Smith, 1977](#)). Therefore, more studies with ileum cannulated animals are required to accurately estimate MCP ID.

Purine bases (PB) and ^{15}N have been widely used as markers to assess MCP synthesis ([Broderick et al., 2010](#), [Rotta et al., 2014a](#)). The MCP measurements from PB or ^{15}N are usually similar ([Broderick and Merchen et al., 1992](#); [Rotta et al., 2014a](#)). Even though some authors have found differences between N flow estimated by PB and ^{15}N ([Reynal et al., 2005](#); [Ipharraguerre et al., 2007](#)), [Carro and Miller \(2002\)](#) reported that PB as a microbial marker can be performed by simpler colorimetric or chromatographic techniques, whereas the continuous intraruminal infusion of ^{15}N ammonium salts may be more laborious and expensive under certain conditions.

The MCP synthesis is an important component to calculate MP and to assess protein requirements. According to [Menezes et al. \(2016\)](#), increasing dietary CP levels from 100 to 140 g/kg did not improve retention and performance, but increased N excretion in beef cattle.

Thus, we hypothesized that reducing CP content in beef cattle diets could improve N use efficiency, and that the PB marker would be satisfactory as well as ^{15}N to estimate microbial synthesis and digestibility. Therefore, the objectives were to evaluate the effects of reducing dietary CP contents on 1) total tract, ruminal, small and large intestinal nutrient digestion and N balance, and 2) on MCP synthesis and true MCP digestibility obtained with ^{15}N and PB in beef cattle small intestine.

MATERIALS AND METHODS

Animals, Experimental Design, and Diets

This study was approved by the Institutional Animal Care and Use Committee at the Federal University of Viçosa (CEUAP/UFV), protocol number 06/2013. The surgery for ileum fistulation used in this study was described by [Leão and Coelho da Silva \(1980\)](#), in which a T-shaped latex cannula was inserted approximately 10 cm from the ileocecal junction. The trial was carried out at the Experimental Feedlot and in the Ruminant Nutrition Laboratory of the Animal Science Department at the Federal University of Viçosa, Brazil.

Eight steers, 4 Nellore (BW 241.3 ± 43 kg and 13 mo old), and 4 crossbred Angus $\times$ Nellore (initial BW 263.4 ± 47 kg and 13 mo old), with rumen and ileum cannulas were allocated in duplicated 4×4 Latin squares, simultaneously. The different genetic groups were justified by the limited number of ileum-cannulated cattle because only these 8 animals were available for this trial. Then, the genetic group was not a study object, though it was included in the statistical model.

The experiment lasted four 17-d periods. Each period consisted of 10 d for adaptation of the animals to the experimental diets and 7 d for data collection ([Koenig et al., 2013a](#); [Rotta et al., 2014a](#)). The animals were housed in individual stalls (8 m²) with feeding troughs and free access to water throughout the experiment.

The 4 experimental diets consisted of increasing CP contents: 100, 120, or 140 g of CP/kg of DM offered ad libitum, and a diet containing 120 g/kg CP offered at restricted intake (RI) of 12 g DM/kg BW. The treatment RI allowed us to understand how the intake at maintenance level affects N dynamics. The RI was also used to quantify the true intestinal digestibility of the amino acids in a complementary study.

The diets consisted of 50% corn silage (CS) and 50% concentrate on DM basis. The total CS amount was supplied at 0700 h for all animals. Animals fed ad libitum received half of the daily amount of the concentrate at 0700 h, and the other half at 1500 h ([Pazdiora et al., 2014](#)). Intake was adjusted to maintain theorts near to 5% of the offered amount. The animals at RI were fed once a day, at 0700 h.

Table 1. Chemical composition of the diets and ingredients proportion

Item	Dietary CP (g/kg DM)		
	100	120	140
Ingredient, g/kg DM			
Corn silage	500	500	500
Corn grain	397	396	396
Wheat meal	60	30	0
Soybean meal / U+AS ¹	22	53	83
Sodium chloride	5	5	5
Mineral mixture ²	5	5	5
Sodium bicarbonate	8	8	8
Magnesium oxide	3	3	3
Chemical composition, g/kg DM			
OM	954	949	944
CP	99	121	142
Ether extract	40	40	40
apNDF ³	314	307	299
NFC ⁴	507	494	482
RDP, g/kg CP ⁵	668	696	716

¹U + AS = Urea + ammonia sulfate; 83.3% of soybean meal e 16.7% of urea + ammonia sulfate.

²Mineral mixture = 223 g/kg calcium; 174 g/kg phosphorus; 24 g/kg sulfur; 100 mg/kg cobalt; 1,250.0 mg/kg copper; 1,795.0 mg/kg iron; 90 mg/kg iodine; 2,000.0 mg/kg manganese; 15.00 mg/kg selenium; 5,270.00 mg/kg zinc and 1,740.00 mg/kg fluor.

³apNDF = NDF corrected for ash and protein.

⁴NFC = nonfibrous carbohydrates.

⁵RDP = Calculated by the Brazilian Feed Composition Tables for Ruminants (Valadares et al., 2017), considering kp = 0.05.

The diets were formulated based on the nutritional requirement system for pure and cross-bred Zebu cattle (BR CORTE 2.0) described by Valadares Filho et al. (2010) to allow 1 kg ADG (Table 1).

Nitrogen Balance and Total Digestibility

The CS and leftovers were sampled during the collection period and partially dried in a forced air ventilation oven (55 °C) for 72 h. After drying, the samples were ground in a Wiley mill (TE-648, TECNAL, Piracicaba, Brazil) with 2 mm (for using in indigestible NDF [iNDF] analyses) and subsequently with 1-mm mesh. The samples were composed proportionally to dry weight, per animal for each period. The concentrate ingredients were sampled directly from the grain storage at the feed mill in the days when they were mixed. These samples were stored at -20 °C for later chemical analyses. After these procedures and thawing, these samples were ground similarly as described above for CS and leftovers.

To estimate the nutrient digestibility, total feces collection was done from the 11th to 13th d of each experimental period. Feces were collected directly

from the concrete floor that was washed immediately after defecation to prevent sample contamination. After completed 24 hours of collection, the feces were weighed, homogenized and a daily sample was partially dried and ground in a Wiley mill (TE-648, TECNAL, Piracicaba, Brazil) with 2 (for and 1-mm mesh, respectively). Feces samples were composed for each animal per period, proportional to the dry weight of each collection day.

To assess the nitrogen compound balance, a total collection of urine from each animal was made in each experimental period, during the same 72 h as the total feces collection. Collector funnels were used coupled to hoses, which conducted the urine to plastic containers with 200-mL H₂SO₄ at 20%, to conserve the nitrogenous compounds. At the end of each collection day (24 h), the daily urine volume was quantified and a sample was stored proportional to the daily volume excreted by each animal. Urine samples were composed per period for each animal, proportional to the total daily volume. The composite samples were stored at -20 °C for total N analysis.

Markers Infusion and Digesta Sampling

The double marker system was used (France and Siddons, 1986) to estimate the digesta flow, using the Co-EDTA, to label the liquid and small particles phases, and iNDF to label the large particles phase (Mariz et al., 2013; Rotta et al., 2014b).

Continuous Co-EDTA infusions were made with 5.0-g/d Co-EDTA (0.7-g Co, diluted in 2.7 liter of water) via ruminal cannula using peristaltic pumps (BP-600.4; Milan Scientific Equipment, Inc., Colombo, Paraná, Brazil), from 11th to 16th d of each experimental period. Also, a total of 7.03 g of ammonium sulfate enriched with 10% of ¹⁵N atoms [ammonium sulfate (¹⁵NH₄)₂SO₄] [Sigma Aldrich (Isotec), Miamisburg, OH] was added to the previously described Co-EDTA solution, providing a daily supply of 150 mg of ¹⁵N to each animal. In all periods, the ¹⁵N ammonium sulfate infusion began 60 h prior to the first digesta sampling to allow uniform distribution of ¹⁵N into the ¹⁵NH₃ in the ruminal microbial pool (Broderick and Merchen, 1992). Infusions were performed until the last day (16th) of digesta sampling.

Before starting the ¹⁵N infusion, a sample of the ruminal content was collected from each animal and stored at -20 °C for ¹⁵N background determination in the ruminal digesta. The iNDF was used as a single marker to estimate the ileal DM flow.

A total of 8 samples of omasal (Huhtanen et al., 1997; Leão, 2002) and ileal digesta were obtained per animal from the 14th to 16th d of each period,

with 9-h intervals between them, as follows: on 14th d these samplings were made at 0600 and 1500 h; on the 15th d, 0000, 0900, and 1800 h; and on the 16th d at 0300, 1200, and 2100 h, totaling 8 samples of each digesta (omasal or ileal), which were used to make a composite sample per animal. The ileum digesta was sampled into plastic bags placed at the orifice of ileum fistula, allowing the normal digesta flow, totaling 200 mL of digesta per sampling time.

The individual samples of omasal and ileal digesta were immediately frozen (-20°C) for later processing. At the end of each experimental period, samples were thawed at room temperature and composed per animal, resulting in a composite sample with approximately 5.6 liter of omasal digesta and another with approximately 1.6 liter of ileal digesta. The composite omasal digesta sample was filtered using a 100- μm nylon filter with an area of pores on 44% of the surface (Sefar Nitex 100/44, Sefar, Thal, Switzerland) so that 2 phases were obtained: liquid and small particles phase and large particles phase.

After these procedures, the composite samples of omasal and ileal digesta were immediately frozen at -80°C . Then, these samples were lyophilized, grounded in a Wiley mill at 2 and 1 mm, and stored for later analysis.

From the 14th to 16th d of each experimental period, at the same time as the omasal and ileal digesta, the ruminal fluid was sampled manually to quantify ruminal ammonia nitrogen ($\text{NH}_3\text{-N}$) concentration. The ruminal fluid was obtained at different points of the liquid-solid interface and later filtered through 3 layers of cheesecloth. An aliquot of 40-mL ruminal fluid was mixed with 1-mL H_2SO_4 (50% v/v) and frozen at -20°C for later analysis.

The procedure for bacterial isolation described by Reynal et al. (2005) with modifications suggested by Krizsan et al. (2010) was used. After every 4 samplings taken at 0600, 1500, 0000, 0900 h, and at 1800, 1500, 1200, and 2100 h, a composite sample was pooled, resulting in 1.2 liter of omasal composite sample and 800 mL of ileal composite sample.

The composite samples (omasal and ileum) were filtered through a 100- μm nylon mesh filter with 44% of pore surface area (Sefar Nitex 100/44, Sefar, Thal, Switzerland) and the material that remained on the filter was washed with 800 mL of 0.90% (wt/vol) saline solution (NaCl). The phase remaining on the filter was saved for isolation of particle-associated bacteria (PAB). The filtrate material was centrifuged at $1,000 \times g$ for 10 min at 5°C for isolation of liquid-associated bacteria (LAB). More details about bacterial isolation are described in Rotta et al. (2014a). Pellets resulting

from LAB and PAB isolation were stored at -80°C , freeze dried (Freezemobile 24, Virtis, Gardiner, NY), and then macerated with a plastic pestle and mortar and stored at -20°C in plastic containers with a lid for further chemical DM, OM, CP, PB, and ^{15}N analysis.

Blood was collected on the 17th d of the experimental period by puncturing the jugular vein, using tubes with separator gel. These samples were immediately centrifuged at $3000 \times g$ for 15 min and serum was added to a labeled plastic centrifuge tube and stored at -20°C for later urea analysis.

Chemical Analyses

Samples of feeds, orts, feces, omasal, and ileal digesta were analyzed for DM, ash, and N according to the official methods 934.01, 942.05, and 968.06, respectively (AOAC, 2006). The EE content was analyzed according to method 920.39 (AOAC, 2005).

To analyze the NDF corrected for ash and protein (apNDF) concentration, samples were treated with heat stable α -amylase, without adding sodium sulfite, corrected for ash residue (Mertens, 2002) and N-residual compounds (Licitra et al., 1996).

The iNDF was evaluated with 2-mm samples, ruminally incubated along 288 h (Valente et al., 2015). Cobalt concentration was determined using an atomic absorption spectrophotometer (Spectr AA-800; Varian spectrometer, Harbor City, CA).

Nonfibrous carbohydrates (NFC) were quantified according to Detmann and Valadares Filho (2010): $\text{NFC} = 100 - [(\% \text{CP} - \% \text{CP of urea} + \% \text{urea}) + \% \text{apNDF} + \% \text{EE} + \% \text{ash}]$.

The $\text{NH}_3\text{-N}$ concentration in ruminal fluid was quantified using the colorimetric technique described by Chaney and Marbach (1962). Serum urea-N (SUN) was quantified by the fixed time kinetic method, using an automatic biochemistry analyzer (Mindray brand, model: BS200E).

The composite samples of omasum and ileal digesta, BAL and BAP isolated by omasum and ileum, were analyzed for PB and ^{15}N . The PB analyses were performed according to Zinn and Owens (1980) as modified by Ushida et al. (1985).

The ^{15}N analyses were performed according to Machado et al. (2013). The ^{15}N excess atoms were measured using an isotope ratio mass spectrometer (Delta S; Finnigan MAT, Bremen, Germany). Samples containing approximately 100 μg of N were weighed and placed in 5- to 8-mm capsules for future readings. The stable isotopes' rate of the same chemical element ($^{15}\text{N}:$ ^{14}N) was evaluated in terms of Δ per thousand, according to specific international standard.

CALCULATIONS

Assuming that digesta samples reaching the omasum are unrepresentative, the marker concentrations at the different phases of the omasal digesta were used to calculate the reconstitution factor (France and Siddons, 1986). The ileal digesta flow (g/d) was obtained by dividing the iNDF intake by its concentration in the ileal digesta sample. The nutrient flow (g/d) was obtained by multiplying its concentration in the digesta (g/kg DM) by the DM flow. The apparent ruminal and intestinal nutrient digestibilities were calculated in relation to the total ingested. The amount of absorbed N was obtained by the difference between N-intake and N-fecal, whereas the N-retained was obtained by subtracting the N-urinary and N-fecal from the N-intake.

Statistical Analyses

The variables regarding intake and apparent digestibility in different sampling sites of the gastrointestinal tract were analyzed by the PROC MIXED of SAS software (version 9.1), using the following statistical model:

$$Y_{ijkl} = \mu + q_i + N_j + qN_{ij} + a_k + P_l + \epsilon_{ijkl}$$

where Y_{ijkl} is the dependent variable, μ is the general mean, q_i is the random effect of the Latin square/genetic group i , N_j is the fixed effect of the dietary CP treatment ($j = 1$ to 4), qN_{ij} is the random effect of the interaction between Latin square i and the dietary CP content j , a_k is the random effect of animal k , P_l is the random effect of period l , and ϵ_{ijkl} is the random error taken as normal and independently distributed (NID) ($0; \sigma^2_\epsilon$).

The degrees of freedom were estimated by the Kenward–Roger method. In the case of significant effects for CP content, the following orthogonal contrasts were studied: C1: RI vs. voluntary intake of the 120 g of CP/kg of DM; C2: linear effect of CP dietary contents 100, 120, and 140 g/kg; and C3: quadratic effect for the same increasing contents. All the statistical procedures were carried out using 0.05 as a critical level of probability for type 1 error, and P -values between 0.05 and 0.10 were considered as a trend. The data for NH_3 -N concentration were analyzed as repeated measures. The model tested was similar to that described above, including time as a fixed effect and all its interactions.

The Bacterial chemical composition was compared in a split plot scheme, considering the type of bacterial isolation (LAB or PAB), using the mixed

procedure of SAS software (version 9.1), by the following model:

$$Y_{ijklm} = \mu + q_i + N_j + B_k + BN_{jk} + a_l + p_m + E_{ijklm} + \epsilon_{ijklm}$$

where Y_{ijklm} is the dependent variable, μ is the general mean, q_i is the random effect of the Latin square/genetic group, N_j is the fixed effect of the dietary CP content j , B_k is the fixed effect of the type of bacterial isolation k , BN_{jk} is the fixed effect of the interaction between dietary CP content j and the type of bacterial isolation k , a_l is the random effect of animal l , p_m is the random effect of period m , E_{ijklm} is the random residual error between the types of bacterial isolation within the experimental units, and ϵ_{ijklm} is the unobservable random error, assuming a normal distribution (NID) ($0; \sigma^2_\epsilon$).

The variables MCP synthesis, MCP/apparent ruminally degradable OM (MCP/aRDOM), and MCP/true ruminally degradable OM (MCP/tRDOM) were also compared with split plot scheme, considering the microbial markers (^{15}N or PB), using the mixed procedure of SAS software (version 9.1) by the following model:

$$Y_{ijklm} = \mu + q_i + N_j + M_k + NM_{jk} + a_l + p_m + E_{ijklm} + \epsilon_{ijklm}$$

where Y_{ijklm} is the dependent variable, μ is the general mean, q_i is the random effect of the Latin square/genetic group, N_j is the fixed effect of the dietary CP content j , M_k is the fixed effect of the microbial marker (^{15}N or PB) k , NM_{jk} is the fixed effect of the interaction between dietary CP content j and the microbial marker k , a_l is the random effect of animal l , p_m is the random effect of period m , E_{ijklm} is the random residual error between the microbial markers within the experimental units, and ϵ_{ijklm} is the unobservable random error, assuming a normal distribution (NID) ($0; \sigma^2_\epsilon$).

In the case of significant effects for CP content in both analyses, the following orthogonal contrasts were studied: C1: RI vs. voluntary intake of the 120 g of CP/kg of DM; C2: linear effect of CP dietary contents 100, 120, and 140 g/kg; and C3: quadratic effect for the same increasing contents. All the statistical procedures were carried out using 0.05 as critical level for probability of the type 1 error.

True intestinal digestibility of the total CP and microbial CP was estimated by linear regression model fitted between the total CP or microbial

CP absorbed in small intestine ($\hat{Y}$; g/d) and their respective omasal flow (X ; g/d). The intercept of the equation represented the endogenous losses, and the slope represented the true digestibility of the total CP or microbial CP. The adjusted model for the true digestibility of the microbial CP took into consideration the effect of the microbial markers on the obtained estimates, using this factor as a Dummy variable. In case of similarity between the estimates, a single regression equation was adjusted for the data described above. The data were analyzed using the PROC MIXED of SAS (version 9.2) and 0.05 as a critical level for the probability of type 1 error. Animal and period were considered random variables similarly to the previous statistical models.

RESULTS

Total and Partial Nutrient Digestibility and Balance of Nitrogen

Intake of OM tended to increase linearly ($P = 0.07$) in response to CP content (Table 2). Apparent ruminal digestibility (linear effect, $P = 0.55$ and quadratic effect, $P = 0.11$) and total OM digestibility (linear effect, $P = 0.58$ and quadratic effect, $P = 0.98$), both expressed in percentage, were not affected by CP content, but animals in the RI treatment had greater ($P = 0.01$) ruminal apparent digestibility in relation to voluntary intake. Intake of CP ($P < 0.01$) (kg/d) increased linearly in response to CP content. The CP intake was lower ($P < 0.01$) for animals in RI compared with voluntary intake.

Ruminal apparent CP digestibility, in both units, responded quadratically (linear effect, $P < 0.01$ and quadratic effect, $P = 0.02$ expressed in g/day; linear effect, $P < 0.01$ and quadratic effect, $P = 0.03$ expressed in % of intake) to CP increase and were numerically greatest at 140 CP content. Ruminal CP digestibilities had negative values for RI, 100 and 120 CP treatments. The total apparent CP digestibility (g/d and % of intake) increased linearly ($P < 0.01$) according to CP content.

There was no effect (linear effect, $P = 0.34$ and quadratic effect, $P = 0.48$) of CP on apNDF intake (Table 3). The apNDF ruminal digestibility (%) responded quadratically (linear effect, $P = 0.76$ and quadratic effect, $P < 0.01$) to the CP increase and it was greatest at the 120 CP level. The apNDF apparent digestibility in small intestine (ID) had quadratic behavior (linear effect, $P = 0.82$ and quadratic effect,

$P < 0.01$) in response to increasing CP content and it was numerically smaller at 120 CP treatment. The total apNDF digestibility was not affected (linear effect, $P = 0.49$ and quadratic effect, $P = 0.64$) by CP contents, but RI showed lower ($P < 0.01$) ruminal apparent digestibility (kg/d) in relation to voluntary intake at 120 g of CP/g of DM.

The NFC intake was not affected by dietary CP (linear effect, $P = 0.77$ and quadratic effect, $P = 0.12$). Rumen digestibility of NFC (%) was quadratically (linear effect, $P = 0.52$ and quadratic effect, $P = 0.01$) affected by treatments, where 120 CP had numerically the lowest value. The same behavior was observed for digestibility in small intestine; however, the greatest value was observed for the 120 CP treatment. Total NFC digestibility (%) was not affected (linear effect, $P = 0.49$ and quadratic effect, $P = 0.45$) when CP content increased.

Increase in dietary CP resulted in a linear increase ($P < 0.01$) in urinary N excretion, and also in a trend to linear increase ($P = 0.07$) fecal N excretion (Table 4). The SUN concentration in animals fed the RI diet was similar ($P = 0.13$) compared with voluntary intake. Retained N (g/d) increased linearly ($P = 0.03$) in response to CP contents. Nitrogen retention ($P < 0.01$) was lower for animals in RI compared with the other fed ad libitum.

There was an interaction ($P < 0.01$) between CP contents and sampling time for ruminal $\text{NH}_3\text{-N}$ concentrations (Figure 1). The partitioning of the interaction demonstrated that, with an exception for Time 0, the 140 CP resulted in the greatest ruminal N-NH_3 concentration for all the time points evaluated (Time 3: C2 $P = 0.06$ and over all time points $P < 0.01$).

Chemical Composition of Bacteria and ^{15}N : ^{14}N Ratio

There were no interactions ($P > 0.10$) among the types of isolated bacteria (LAB and PAB) and dietary CP levels on any of the evaluated variables in the bacterial chemical composition neither in omasum nor in ileum digesta (Table 5). The N content (in g/kg OM) was greater ($P < 0.01$) for LAB than for PAB in the omasal digesta. There was a positive linear effect ($P < 0.01$) on N content (g/kg OM) of the isolated bacteria in the omasal and ileal digesta in response to the increasing dietary CP content.

The ^{15}N : ^{14}N ratio observed for LAB and PAB isolated in the omasal digesta was similar ($P = 0.12$). The ^{15}N : ^{14}N ratio in the bacteria isolated in the omasal digesta was greater ($P < 0.01$) for RI animals compared with animals fed ad libitum.

Table 2. Effects of dietary CP on intake and apparent digestibility of OM and CP on the intake and TDN quantity

Item ⁴	CP content (g/kg of DM) ¹				SEM	Contrast ²	Level effect ³	
	RI	100	120	140		RI vs. VI	Linear	Quadratic
OM intake								
kg/day	2.83	4.89	4.89	5.35	0.34	< 0.01	0.07	0.28
Ruminal digestibility								
kg/day	1.35	2.25	2.03	2.43	0.25	< 0.01	0.34	0.07
%	47.7	45.1	40.8	43.6	3.14	0.01	0.55	0.11
Digestibility in the small intestine								
kg/day	0.55	0.76	1.07	1.01	0.10	< 0.01	0.02	0.04
%	19.3	15.5	22.7	19.3	2.43	0.09	0.06	< 0.01
Digestibility in the large intestine								
kg/day	0.33	0.63	0.48	0.62	0.13	< 0.01	0.93	0.06
%	11.6	12.7	10.4	11.5	2.83	0.94	0.55	0.32
Total digestibility								
kg/day	2.25	3.65	3.6	4.07	0.31	< 0.01	0.08	0.21
%	78.7	73.7	73.9	74.4	1.95	0.02	0.58	0.98
CP intake								
g/day	361	533	641	823	50	< 0.01	< 0.01	0.20
Ruminal digestibility								
g/day	−79.8	−82.8	−103	48	0.04	0.56	< 0.01	0.02
%	−23.1	−16.3	−18.2	3	6.56	0.40	< 0.01	0.03
Digestibility in the small intestine								
g/day	305	402	527	541	0.03	< 0.01	< 0.01	0.06
%	86.4	75.5	84.0	66.4	5.96	0.64	0.09	< 0.01
Digestibility in the large intestine								
g/day	38.0	44.3	41.7	54.8	0.02	0.80	0.47	0.53
%	10.5	8.2	7.7	7.1	3.11	0.27	0.67	0.98
Total digestibility								
g/day	263	364	466	644	0.04	< 0.01	< 0.01	0.16
%	73.4	68.8	73.5	77	1.63	0.97	<0.01	0.68
TDN intake								
kg/day	2.25	3.66	3.61	4.09	0.3	< 0.01	0.07	0.20
Contents; %	73.5	68.9	69.2	69.9	1.89	0.03	0.60	0.93

¹Treatments: RI = restricted intake to 1.2% BW containing 120 g CP/kg DM; 100, 120, and 140 = voluntary intake containing 100, 120, and 140 g CP/kg DM, respectively.

²P-value associated with the contrast RI vs. VI = restricted intake vs. voluntary intake (120 g CP/kg DM).

³P-value associated with CP content evaluation (voluntary intake of the diets containing 100, 120, and 140 g CP/kg DM).

⁴Units expressed in % of intake.

There was a negative linear effect ($P < 0.01$) of CP on $^{15}\text{N}:^{14}\text{N}$ ratio in bacteria isolated in the omasal digesta. There was also a negative linear effect ($P < 0.01$) on $^{15}\text{N}:^{14}\text{N}$ ratio in ileal digesta isolated bacteria, with values of 395.55, 349.88, 330.81, and 257.29 (Δ per thousand) for RI, 100, 120, and 140 g CP/kg DM, respectively.

Microbial Markers and Efficiency of Microbial CP Synthesis

There was no interaction ($P > 0.10$) between dietary CP contents and microbial markers for any of the evaluated variables (Table 6). Animals in RI showed lower ($P < 0.01$) MCP synthesis than

others fed ad libitum. There was a linear increase in MCP synthesis ($P < 0.01$) according to dietary CP contents. There was a tendency to increase MCP synthesis using PB ($P = 0.07$) in comparison to ^{15}N .

The MCP efficiency expressed in relation to aRDOM ($P = 0.12$) and tRDOM ($P = 0.43$) was similar for RI in relation to ad libitum fed animals (Table 7). The microbial efficiency expressed in relation to the aRDOM and tRDOM had a tendency quadratic behavior (linear effect $P = 0.07$ and quadratic effect for aRDOM; linear effect $P = 0.04$ and quadratic effect $P = 0.08$ for tRDOM) to the dietary CP content, being numerically greater for animals receiving 120 g CP/kg DM in the diet (Table 6).

Table 3. Effects of dietary CP on NDF corrected for ash and protein (apNDF) and nonfibrous carbohydrates (NFC) intakes and apparent digestibilities

Item ⁴	CP content (g/kg of DM) ¹				SEM	Contrast ²	Level effect ³	
	RI	10	12	14		RI vs VI	Linear	Quadratic
apNDF intake								
kg/day	1	1.67	1.66	1.77	0.13	< 0.01	0.34	0.48
Ruminal digestibility								
kg/day	0.59	0.67	0.83	0.74	0.08	< 0.01	0.33	0.06
%	58.8	40.8	50.5	41.8	4.01	0.02	0.76	< 0.01
Digestibility in the small intestine								
kg/day	0.032	0.18	0.053	0.18	0.072	0.74	0.99	< 0.01
%	2.6	10.9	2.70	10.2	4.55	0.95	0.82	< 0.01
Digestibility in the large intestine								
kg/day	0.081	0.11	0.13	0.15	0.071	0.36	0.51	0.97
%	7.7	6.1	7.7	8.6	4.83	0.99	0.47	0.91
Total digestibility								
kg/day	0.69	0.96	1.00	1.07	0.10	< 0.01	0.19	0.90
%	69.10	58.30	60.90	60.70	3.46	0.02	0.49	0.64
NFC intake								
kg/day	1.38	2.55	2.39	2.59	0.19	0.03	0.77	0.12
Ruminal digestibility								
kg/day	0.86	1.70	1.38	1.69	0.17	<0.01	0.93	< 0.01
%	62.1	65.2	55.3	62.9	4.07	0.73	0.52	0.01
Digestibility in the small intestine								
kg/day	0.11	−0.014	0.26	0.054	0.062	0.02	0.32	< 0.01
%	8.1	−0.301	12.2	2.90	3.69	0.16	0.47	0.02
Digestibility in the large intestine								
kg/day	0.23	0.49	0.32	0.44	0.071	0.16	0.47	0.02
%	16.2	19	14.4	16.5	3.07	0.84	0.41	0.21
Total digestibility								
kg/day	1.2	2.18	1.95	2.18	0.17	< 0.01	0.97	0.05
%	86.3	83.3	81.7	82.2	1.67	< 0.01	0.49	0.45

¹Treatments: RI = restricted intake to 1.2% BW containing 120 g CP/kg DM; 100, 120, and 140 = voluntary intake containing 100, 120, and 140 g CP/kg DM, respectively.

²P-value associated with the contrast RI vs. VI = restricted intake vs. voluntary intake (120 g CP /kg DM).

³P-value associated with CP content evaluation (voluntary intake of the diets containing 100, 120 and 140 g CP/kg DM).

⁴Units expressed in % of intake.

Table 4. Effects of dietary CP on nitrogen balance and serum nitrogen urea

Item	CP content (g/kg of DM) ¹				SEM	Contrast ² RI vs VI	Level effect ³	
	RI	100	120	140			Linear	Quadratic
Nitrogen intake, g/day	56.9	86.1	102	135	8.65	< 0.01	< 0.01	0.13
Nitrogen excretion, g/day								
Nitrogen urinary	29.7	23.9	33.6	63.6	7.16	0.08	< 0.01	0.76
Nitrogen fecal	15.1	26.7	27.3	31.9	2.72	< 0.01	0.07	0.44
Retained nitrogen								
g/day	12.1	35.4	40.9	44.5	5.26	< 0.01	0.03	0.97
% ingested	20.2	40.6	38.4	35.8	3.77	< 0.01	0.19	0.94
% absorbed	30.6	59.1	52.5	45.9	4.89	< 0.01	0.02	0.99
SUN ⁴ , mg/dL	13.7	9.38	11.4	13.1	1.87	0.13	0.04	0.42

¹Treatments: RI = restricted intake to 1.2% BW containing 120 g CP/kg DM; 100, 120, and 140 = voluntary intake containing 100, 120, and 140 g CP/kg DM, respectively.

²P-value associated with the contrast RI vs. VI = restricted intake vs. voluntary intake (120 g CP/kg DM).

³P-value associated with CP content evaluation (voluntary intake of the diets containing 100, 120, and 140 g CP/kg DM).

⁴SUN = serum urea-N.

Figure 1. Effect of dietary CP contents and feeding times on ruminal ammonia nitrogen concentrations. Additional slicing for contrast (C1: restricted intake vs. voluntary intake of the 120 g of CP/kg of DM; C2: linear effect of CP dietary contents 100, 120, and 140 g/kg; and C3: quadratic effect for the same increasing contents) effect in each time points: Time 0: C1 $P = 0.25$, C2 $P = 0.85$, C3 $P = 0.23$; Time 3: C1 $P = 0.34$, C2 $P = 0.06$, C3 $P = 0.92$; Time 6: C1 $P = 0.47$; C2 $P < 0.01$; C3 $P = 0.99$; Time 9: C1 $P = 0.31$, C2 $P < 0.01$, C3 $P = 0.18$; Time 12: C1 $P = 0.66$, C2 $P < 0.01$, C3 $P = 0.23$; Time 15: C1 $P = 0.16$, C2 $P < 0.01$, C3 $P = 0.93$; Time 18: C1 $P = 0.28$, C2 $P < 0.01$, C3 $P = 0.21$; Time 21: C1 $P = 0.54$, C2 $P < 0.01$, C3 $P = 0.51$.

Table 5. Effects of isolated bacteria and microbial markers on nitrogen and OM content, and ^{15}N : ^{14}N ratio

	Bacteria ¹		CP content (g/kg DM) ²					(P-value)		
Item	LAB	PAB	RI	100	120	140	SEM	Bacteria	CPC ³	B × CPC ⁴
Omasum										
Nitrogen, g/kg OM	83.2	79.3	82.6	77.9	81.7	83.1	1.002	< 0.01	< 0.01	0.09
OM, g/kg DM	845	824	831	835	838	833	0.05	< 0.01	0.66	0.71
¹⁵ N: ¹⁴ N, Δ per thousand ⁵	463	454	614	474	437	309	28.7	0.12	< 0.01	0.72
%N-RNA	0.90	0.73	0.76	0.69	0.82	0.99	0.13	0.20	0.45	0.35
N-RNA/N-bacteria	0.13	0.11	0.11	0.12	0.12	0.12	0.009	< 0.01	0.93	0.75
Ileum										
Nitrogen, g/kg OM	72.7	71.6	69.7	69.4	72.8	76.7	1.31	0.30	< 0.01	0.98
OM, g/kg DM	793	747	770	775	762	765	0.10	< 0.01	0.59	0.48

¹LAB = liquid-associated bacteria, PAB = solid-associated bacteria.

²Treatments: RI = restricted intake to 1.2% BW containing 120 g CP/kg DM; 100, 120, and 140 = voluntary intake containing 100, 120, and 140 g CP/kg DM, respectively.

³CPC = CP content (g CP/kg of DM).

⁴P-value associated to the interaction B $\times$ CPC (bacteria and CP content).

⁵ Value obtained after subtracting ^{15}N background value.

Estimates of True Intestinal Digestibility of Microbial Protein

After testing the Dummy variable referent to the effect of the different markers on the regression between the total CP or microbial CP absorbed in small intestine ($\hat{Y}$; g/d) and their respective omasal flow (X ; g/d), it was observed similarly ($P > 0.10$) intercept and slope estimates between the markers. Then, a single equation was fitted to estimate MCP true intestinal digestibility in small intestine (MCPsi), since there was no significant effect ($P > 0.10$) of the microbial markers (PB and ^{15}N) used to determine the microbial synthesis. After adjusting a single regression based on data of the 2 makers, the

regression slope was significant ($P < 0.01$), thus generating the following regression equation (Figure 2):

$$\text{MCPsi} = -16.7_{(\text{SEM}=40.1)} + 0.86 \\ \times \text{MCP omasal flow}_{(\text{SEM}=0.05)}$$

The same fitting was performed for total CP (TCPsi) true digestibility in small intestine. Regression slope was significant ($P < 0.01$), thus generating the following regression equation (Figure 3):

$$\text{TCPsi} = -43.8_{(\text{SEM}=49.2)} + 0.75 \\ \times \text{TCP omasal flow}_{(\text{SEM}=0.05)}$$

Table 6. Effect of dietary CP contents and microbial markers on microbial CP (MCP) synthesis and its efficiency expressed as MCP/apparent ruminally degradable OM (MCP/aRDOM), MCP/true ruminally degradable OM (MCP/tRDOM)

Item	CP content (g/kg of DM) ¹				SEM	(P-value)		
	RI	100	120	140		CPC ²	MM ³	CPC × MM ⁴
		MCP synthesis ⁵ , g/day						
Purine basis	352	509	671	671	24.8	< 0.01	0.07	0.57
¹⁵ N	352	483	582	608				
t-test ⁸	0.98	0.56	0.35	0.31				
		MCP/aRDOM ⁶ , g/kg						
Purine basis	260	237	319	304	24.3	< 0.01	0.12	0.63
¹⁵ N	264	222	304	273				
t-test ⁸	0.83	0.46	0.34	0.23				
		MCP/tRDOM ⁷ , g/kg						
Purine basis	172	156	194	186	9.71	< 0.01	0.14	0.69
¹⁵ N	175	144	183	169				
t-test ⁸	0.84	0.41	0.47	0.24				

¹Treatments: RI = restricted intake to 1.2% BW containing 120 g CP/kg of DM; 100, 120, and 140 = voluntary intake containing 100, 120, and 140 g CP/kg DM, respectively.

²CPC = crude protein content (g CP/kg of DM).

³MM = microbial markers.

⁴P-value associated with the interaction CPC × MM (CP content and microbial marker).

⁵MCP synthesis = microbial crude protein synthesis.

⁶MCP/aRDOM = microbial crude protein/apparent ruminally degradable organic matter.

⁷MCP/tRDOM = microbial crude protein/true ruminally degradable organic matter.

⁸*t*-test = *P*-value from paired *t*-test.

Table 7. *P*-value of the evaluated contrasts for the dietary CP content effects on isolate bacteria, ¹⁵N:¹⁴N ratio, microbial synthesis, and efficiency

Item	Contrast ¹	Level effect ²	
	RI vs. VI	Linear	Quadratic
Omasum			
Nitrogen, g/kg OM	0.13	< 0.01	0.97
¹⁵ N: ¹⁴ N, Δ per thousand	< 0.01	< 0.01	0.12
Ileum			
Nitrogen, g/kg OM	0.81	0.09	0.52
MCP synthesis ³ , g/day	< 0.01	< 0.01	0.45
MCP/aRDOM ⁴ , g/kg	0.12	0.07	0.07
MCP/tRDOM ⁵ , g/kg	0.43	0.04	0.08

¹*P*-value associated with the contrast RI vs. VI = restricted intake vs. voluntary intake (120 g CP/kg DM).

²*P*-value associated with the tested regression level effects.

³MCP synthesis = microbial crude protein synthesis.

⁴MCP/aRDOM = microbial crude protein/apparent ruminally degradable organic matter.

⁵MCP/tRDOM = microbial crude protein/true ruminally degradable organic matter.

DISCUSSION

In the present study, although a tendency to greater OM intake (OMI) for the 140 CP/kg DM, the diets with 100 and 120 CP/kg DM had similar OMI values. In other studies with beef cattle fed with 100 to 140 g CP/kg DM, no effect was

observed of CP content on this variable (Koenig et al., 2013a; Menezes et al., 2016). However, there is no agreement in the literature about the effects of CP on OMI. Imaizumi et al. (2010) reported possible reductions in DM and OMI in diets with low CP concentrations that could be attributed to insufficient ruminal NH₃-N available for ruminal fermentation and microbial synthesis. Similarity OMI results between 100 and 120 dietary CP may indicate that 100 g CP/kg DM is sufficient to meet the N requirements of the ruminal microorganisms without damaging OMI in beef cattle.

Decreasing dietary CP did not affect OM ruminal digestibility (%), demonstrating that 100 CP was sufficient to optimize OM ruminal digestion. These results are in agreement with those reported in other studies, which varied CP contents between 80 and 175 g/kg (Ipharraguerre et al., 2005; Yuangklang et al., 2010; Mutsvangwa et al., 2016) and demonstrated that decreasing dietary CP had no effect on ruminal OM digestibility. Contrary to these studies, increases in OM digestibilities were positively related to CP diet concentration (Allen, 2000; Archibeque et al., 2007). In spite of the low N supply in RI, this treatment promoted adequate levels of N for ruminal digestion, showing that a balance between N and energy is more important

Figure 2. Microbial CP true intestinal digestibility estimated by microbial markers (purine bases [PB] and ^{15}N) in beef steers fed with increasing CP content in the diets. The regression was adjusted regardless of the markers since there was no significant difference ($P > 0.10$) between them.

Figure 3. Total CP true intestinal digestibility in beef steers fed with increasing CP content in the diets.

than the total daily N offered (g/d) (Broderick, 2003; Ipharraguerre et al., 2005).

The ruminal $\text{NH}_3\text{-N}$ concentration has effects on microbial growth and consequently on nutrient digestibility (Faverdin, 1999). In the present study, the means of the ruminal $\text{NH}_3\text{-N}$ concentrations obtained during the feeding cycle were 8.56, 5.45, 8.16, and 14.99 mg/dL for the RI diets, 100, 120, and 140 CP, respectively. Although an increase was observed in the ruminal $\text{NH}_3\text{-N}$ concentrations of approximately 175% and 84% with the 140 CP level compared with the 100 and 120 CP diets, there was no difference in the OM ruminal digestibility in response to increasing CP contents. These results indicated that 5.45-mg/dL ruminal $\text{NH}_3\text{-N}$ concentrations were not limited to microbial growth, and it was therefore possible to optimize OM digestion using 100 CP in the diets of beef cattle.

The large ruminal $\text{NH}_3\text{-N}$ losses resulting from great CP dietary concentrations have been indicated as one of the main factors responsible for the low efficiency of N use by ruminants (Tamminga, 1992).

Increase in CP contents, in addition to providing a significant increase in ruminal $\text{NH}_3\text{-N}$, resulted in important effects on N ruminal metabolism. Increases in N flow to the rumen associated with reductions in N intake were obtained in the RI treatments: 100 and 120 CP, which justifies the negative values for ruminal digestibility ($\text{RI} = -23.1$; 100 g of CP/kg DM = -16.3 and 120 g of CP/kg DM = -18.2) observed for CP in these treatments.

The improvements in the efficiency of N usage can also be attributed to the lower total N intakes associated with lower N urinary excretions. The N excretions obtained with 100 and 120 CP were reduced by 39.7 and 29.9 g/d in relation to 140 CP level, respectively, demonstrating that there are significant reductions in N excretions when dietary CP concentrations are reduced. However, it is important to note that some volatile N may have been lost during fecal drying; therefore, the absolute values for N retention reported herein are likely greater than expected; however, these means still represent treatment differences.

Animals in RI tended to present intermediate values of urinary N excretion when compared with animals fed with 120 g CP/kg DM. The N excretions obtained with RI can be attributed to a possible increase in protein catabolism when a basal condition is established. Considering that the blood pool of free amino acids can be used to synthesize urea (Waterlow, 2006), an increase in protein turnover represents an adjustment to maintain an optimal amount of circulating free amino acids.

Bacteria Chemical Composition and ^{15}N : ^{14}N Ratio

The ruminal bacteria pool contributes to a significant amount of N and OM absorbed in the SI of ruminants (Clark et al., 1992). The present study shows that N content is greater in LAB compared with PAB, suggesting that there is a greater contribution of N originated from LAB to the SI, since similar amounts of LAB and PAB leave the rumen. The differences in bacteria composition are generally attributed to changes in growth rates among species, microenvironment conditions in the liquid and solid phases of the digesta, diversity of bacterial species, dietary factors, and a combination among them (Ipharraguerre et al., 2007). A lower growth rate of PAB compared with LAB was reported by Cecava et al. (1991). Supporting the results found in the present study, Yang et al. (2001) reported greater N values in LAB (9.35%) than in PAB (9.06%), based on a dilution effect on N content in response to the greater polysaccharide concentrations in PAB compared with LAB.

The similarity in the ^{15}N : ^{14}N ratio obtained between the ruminal microbial pool in the different phases agrees with the results reported in other studies (Krizsan et al., 2010; Rotta et al., 2014a). In contrast, a greater ^{15}N enrichment in PAB compared with LAB was reported (Ahvenjarvi et al., 2002; Reynal et al., 2005; Brito et al., 2006). This behavior was attributed to the greater use of ruminal $\text{NH}_3\text{-N}$ as N sources by LAB than by PAB (Ahvenjarvi et al., 2002; Carro and Miller 2002; Reynal et al., 2005). It has been postulated that PAB preferentially incorporates greater amounts of N derived from amino acids and peptides, resulting in higher ^{15}N dilution (Bruto et al., 2006). However, the contribution of different N sources to MCP synthesis is highly variable and depends on the availability of these sources in the ruminal environment (Wallace et al., 2001).

In the present study, the lack of differences in ^{15}N : ^{14}N ratios obtained for LAB and PAB suggests no limitation on the availability of N sources used

by ruminal bacteria. In addition, studies have shown increases in maintenance requirements of ruminal microorganisms in response to possible reductions in the digesta time retention in the rumen of animals receiving lower feed levels (Clark et al., 1992; Valadares Filho et al., 2010), justifying the greater $\text{NH}_3\text{-N}$ incorporation in the MCP of RI animals.

Microbial Markers and Microbial CP Synthesis Efficiency

The highest microbial efficiency obtained in animals fed with 120 g CP/kg DM can be attributed to the N compounds availability and the optimized fiber digestibility at this CP content. This allowed an adequate supply of degradable carbohydrates in the rumen for the microorganism's growth. Animals in RI showed similarity in microbial efficiency compared with others in the voluntary intake. Despite the lower N supply in the ruminal environment, synchronization between energy and N represents an important factor influencing the usage efficiency of ruminal substrates (Broderick, 2003; Ipharraguerre et al., 2005). In addition, increases in the retention time of the digesta in the rumen, associated with the lower DMI of RI animals, probably result in an increase in the extension of ruminal fermentation (Scholljegerdes et al., 2004). These events allow the substrates availability synchronization in the rumen of the RI animals, leading to a similar microbial efficiency compared with animals fed ad libitum.

The use of ^{15}N marker for the microbial synthesis quantification is a well-established and an accurate method (Broderick and Merchen, 1992; Carro and Miller 2002; Reynal et al., 2005). In the present study, microbial markers (^{15}N and PB) represented similar estimates for microbial protein synthesis and efficiency, indicating that both techniques were adequate to estimate microbial synthesis. However, there was a statistical tendency to obtain a greater microbial protein estimate when using PB in contrast with ^{15}N . These results suggest that novel studies comparing microbial markers need to be conducted to improve the accuracy of the estimates.

As PB is almost exclusively localized in the bacterial cytoplasm, the lysis process may result in a reduction of the N purine:N microbial ratio and, consequently, an overestimation of the microbial N flow (Carro and Miller, 2002). However, due to the uniform distribution of ^{15}N in microbial cells, the enrichment of this microbial marker is not affected by the bacterial isolation (Carro and Miller, 2002; Valadares Filho et al., 2016), justifying the accurate results obtained in the literature.

Our results demonstrated that PB isolation procedure is similar to the standard ^{15}N method to quantify the MCP, indicating that the methodology used in this study was efficient for PB recovery. According to [Marshak and Vogel. \(1951\)](#), the errors of extraction and fractionation of the nucleic acids can be avoided by direct hydrolysis of the sampled material with HClO_4 and by using spectrophotometry method to determine PB. Thus, considering the similarity in results between ^{15}N and PB, it can be recommended to use the method described by [Zinn and Owens \(1980\)](#) as modified by [Ushida et al. \(1985\)](#) to quantify the CP microbial synthesis.

Estimation of Intestinal True Digestibility of Microbial Protein

Rumen undegraded CP requirement for ruminants can be calculated by assuming 80% of intestinal digestibility according to [NRC \(2000\)](#); however, based on the constant 86% for true intestinal digestibility of MCP obtained in this study, we may infer that CP requirements for beef cattle are overestimated when using the American System ([NRC, 2000](#)). Data recently published under similar conditions reported that CP intake predicted by the Brazilian Tables of Nutrient Requirements of Zebu Beef Cattle—BR-CORTE ([Valadares Filho et al., 2016](#)) was, on average, 17% greater than the values observed ([Amaral et al., 2014](#); [Menezes et al., 2016](#)), which confirms the excess of CP use in beef cattle diets. Considering the lack of studies reporting the true digestibility of MCP in beef cattle under tropical conditions and the current CP requirement based on data obtained in different climate conditions, we suggested the value of 86% for MCP digestibility. This will allow a reduction of the dietary CP levels in these animals.

CONCLUSIONS

The present study confirms that increasing CP contents in beef cattle diets result in increased ruminal $\text{NH}_3\text{-N}$ concentrations, but also confirms that dietary CP between 100 and 120 g/kg is enough to meet net nitrogen requirements of ruminal microorganisms and to optimize OM ruminal digestion. A total of 120 g CP/kg DM is recommended to improve apNDF ruminal digestibility, to optimize microbial synthesis and efficiency, and to promote efficient N usage. The similarity between microbial markers shows that PB technique can be used as an alternative to ^{15}N to estimate microbial synthesis. The MCP true digestibility obtained for beef cattle under tropical conditions was 86% based on both microbial markers ^{15}N and PB.

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