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Original

Supplementation of grazing heifers with different protein sources

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ABSTRACT

Objective. Evaluate the effects of supplementation with different protein sources (soybean meal and wheat bran with urea) on the productive performance, intake, digestibility, microbial protein synthesis, and metabolic profile of grazing beef heifers. **Materials and methods**. Were used twenty Nellore heifers at 8.5±0.06 months of age, with an initial average body weight of 241.5±4.71 kg. The animals were distributed in a completely randomized deseing with two treatments and ten replicates. Two protein sources in the supplements were evaluated: 1) Soybean meal (SBM), and 2) Soybean meal + Wheat bran + Urea (SBM+WB+U). **Results**. Crude protein (CP) and organic matter intakes were higher ($p<0.05$) for heifers from SBM compared with SBM+WB+U. The CP digestibility was increased ($p<0.05$) with SBM supplementation. Mean blood concentrations of glucose, cholesterol, serum urea nitrogen, and total proteins were not affected ($p>0.10$) by protein sources. In the same way, daily weight gain and final body weight were not influenced ($p>0.10$) by protein sources. **Conclusions.** The supplementation with soybean meal or wheat bran with urea in association with soybean meal in multiple supplements for grazing cattle provides similar productive and nutritional performance and metabolic profile in beef heifers.

Keywords: Nelore; nitrogen compounds; nutrient intake; ruminant nutrition; tropical pasture (*Source: USDA*).

RESUMEN

Objetivo. Evaluar el efecto de la suplementación con diferentes fuentes de proteína (torta de soya y salvado de trigo con urea) sobre el desempeño productivo, consumo, digestibilidad, síntesis de proteína microbiana y perfil metabólico de novillas en pastoreo. **Materiales y métodos.** Fueron utilizadas 20 novillas Nelore de 8.5±0.06 meses edad y peso corporal inicial promedio de 241.5±4.71 kg. Los animales fueron distribuidos en delineamiento completamente al azar, con dos tratamientos

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y diez repeticiones. Fueron evaluadas dos fuentes de proteína en los suplementos: 1) Torta de soya (TS) y 2) Torta de soya + salvado de trigo + Urea (TS+ST+U). **Resultados.** El consumo de proteína bruta (PB) y materia orgánica (MO) fue mayor ($p<0.05$) para novillas del tratamiento TS comparado con novillas TS+ST+U. La digestibilidad de la PB fue incrementada ($p<0.05$) con la suplementación con TS. Las concentraciones medias de glucosa, colesterol, nitrógeno ureico en la sangre y proteínas totales no fueron afectadas ($p>0.10$) por las fuentes de proteína suplementadas. De igual modo, la ganancia de peso diario y peso corporal final no fueron influenciados ($p>0.10$) por las fuentes de proteína. **Conclusiones.** La suplementación con torta de soya o salvado de trigo con urea en asociación a la torta soya en suplementos múltiples para bovinos en pastoreo, proporcionan similar desempeño productivo y nutricional y perfil metabólico de los animales.

Palabras clave: Nelore; compuestos nitrogenados; consumo de nutrientes; nutrición de rumiantes; pasturas tropicales (*Fuente: USDA*).

INTRODUCTION

Tropical grasses represent the primary nutritional resource for beef cattle production in a mountainous region Brazil. However, they can rarely be considered to represent a balanced diet for animal production, since own several nutritional constraints, such as protein, energy, and minerals are observed throughout the year (1). During the dry season, the crude protein (CP) content of tropical grasses under grazing is usually greater than 70g of CP kg^{-1} dry matter (DM). During the rainy season, tropical grass pastures present an imbalanced energy:protein ratio, containing relatively excess energy (2) and thereby requiring protein supplementation to reduce nutritional and metabolic deficiencies in an attempt to improve the performance of grazing cattle and the efficiency of the production system (2,3).

Protein is considered the limiting nutrient (4), mainly for cattle production in the tropics. However, it is also the most expensive nutrient of diets and thus deserves more attention in formulations. In addition, because of the high cost of the main protein sources used in the formulation of supplements for cattle, such as soybean meal (SBM), the use of alternative protein sources can optimize results, be it through a reduction of the production costs without compromising performance, or through better adequacy of the nutrients available to meet the metabolic requirements of the animals (5). Consequently, researchers have had an increasing interest in the use of other feed sources, such as cottonseed meal, castor meal, wheat bran plus urea, and urea in cattle diets (6,7,8,9).

The wheat bran as protein food is used partially or wholly in the formulation of supplements for cattle, especially in regions where there presence of wheat crop since wheat bran is a highly available, competitively priced product in the market and desirable nutritional characteristics: a high percentage of rumen degradable protein, low starch content and mineral input, which benefit low-quality fodder-fed animals (10,11). On the other hand, urea is commonly used in the diet of ruminants as a source of non-protein nitrogen (1).

Thus, the objective of this study was to evaluate the effect of supplementation with different protein sources (soybean meal and wheat bran with urea) on the productive performance, intake, digestibility, microbial protein synthesis and metabolic profile of grazing beef heifers.

MATERIALS AND METHODS

Ethical aspects. All the procedures performed on the animals were approved by the Institutional Animal Care and Use Committee of Universidade Federal de Viçosa (protocol CEUAP-UFV number 10/2016).

Experimental area. This experiment was carried out at the Department of Animal Science of Universidade Federal de Viçosa - Brazil, located in a mountainous region at 20°45' S 42°52' W; 657m altitude, between July and November 2015, which corresponded to the dry season and the beginning of the dry-rainy transition. The experimental period presented a total precipitation of 303 mm and an average temperature of 20.8°C.

Experimental design and diets. In this study were used twenty Nellore heifers with 8.5±0.06 months of age and an initial average body weight of 241.5±4.71 kg. The experimental design was completely randomized, with two treatments and ten replicates. The treatments evaluated were: 1) soybean meal (SBM) and 2) supplements with soybean meal + wheat bran + urea (SBM+WB+U). The urea: ammonium sulfate (9:1) mixture was used to adjust the CP content of the wheat bran supplement, due to differences in CP levels in protein foods used. Supplements were composed in addition to protein sources of ground corn and mineral salt and, formulated to contain 30% CP (Table 1). Supplements were given to animals in the amount of 6 g of supplement kg^{-1} of body weight (BW). The supplement amount of 6 g kg^{-1} BW (465 g CP d^{-1}) corresponded to approximately 70% of the dietary requirements of CP for Zebu heifers with BW of 300 kg and expected gain of 0.5 kg d^{-1} (12).

Table 1. Ingredients and chemical composition of supplements consumed by the heifers during the experimental period.

Item	Supplement[2]	
	SBM	SBM+WB+U
Ingredients % (as-fed basis)		
Soybean meal	54.3	30.2
Ground corn	40.7	31.9
Wheat bran	-	30.0
U/AS (9:1)	-	2.9
Mineral salt[1]	5.0	5.0
Chemical Composition (g kg^{-1} of DM)		
Dry matter	915.0	912.9
Organic matter	916.6	913.5
Crude protein	285.9	273.9
Ether extract	16.3	15.2
NFC[4]	485.83	453.94
NDFap	128.6	213.9
INDP (g kg^{-1} of CP)	132.9	41.9
iNDF	15.2	37.7

[1]Centesimal composition: dicalcium phosphate, 50.00; sodium chloride, 47.15; zinc sulfate, 1.50; copper sulfate, 0.75; cobalt sulfate, 0.05; potassium iodate, 0.05 and manganese sulfate: 0.05; AS: ammonium sulfate; [2]SBM = soybean meal; SBM+WB+U: soybean meal + wheat bran + urea; [3]NFC: non-fibrous carbohydrates = OM - (CP + EE+ NDFap); NDFap: neutral detergent fiber corrected for ash and protein; INDP: insoluble neutral detergent protein; iNDF: indigestible neutral detergent fiber.

Animal handling. Animals were subjected to 14d of adaptation to the diet and to the experimental area. At the beginning of the experiment the animals were weighed after 14h of fasting of solids. Animals were allocated to one of two paddocks of 2.5 has each (one for each treatment), uniformly covered with *Brachiaria decumbens* Stapf., and equipped with drinking and feeders. Supplements were delivered daily at 10 am. Water was provided *ad libitum* during the study.

Throughout the experiment animals were weighed every 30d without fasting and always in the morning, in order to adjust the amount of supplement to be provided to each group and to monitor performance. The animals were in grazing continuous system, however, to minimize the possible effects of the plots on the experimental treatments, animals were rotated within each pasture every seven days, so each group stayed for the same period in each plot.

Forage samples. Forage chemical composition (Table 2) was assessed by hand-plucked samples collected (simulated grazing), every 15 days. Every 30d a second pasture sample was collected to estimate the total availability of dry matter (DM) and potentially digestible dry matter (pdDM). Four subsamples were randomly collected in each plot by cutting it close to the ground using a metal square (0.5 m × 0.5m). Samples were oven-dried at 60°C and ground in a Wiley mill (model 3; Arthur H. Thomas, Philadelphia, PA) to pass through a 2-mm screen. After that, half of each ground sample was ground again to pass through a 1-mm screen. Samples were pooled based on each experimental period.

Table 2. Chemical composition of forage consumed by the heifers during the experimental period.

Item	B. decumbens[2,4]	B. decumbens[3,4]
Chemical Composition (g kg^{-1} of DM)		
Dry matter	887.9 ± 1.36	885.8 ± 0.05
Organic matter	919.24 ± 0.57	799.9 ± 0.44
Crude protein	100.9 ± 7.79	91.3 ± 0.64
Ether extract	19.8 ± 0.70	-
NFC[1]	200.3 ± 1.45	-
NDFap	598.1 ± 3.09	621.6 ± 4.05
INDP (g kg^{-1} of CP)	354.6 ± 0.39	329.6 ± 0.19
iNDF	154.9 ± 0.163	157.7 ± 0.35

[1]NFC non-fibrous carbohydrates = OM – [(CP – CPU + U) + EE + NDFap]; NDFap: neutral detergent fiber corrected for ash and protein; INDP: insoluble neutral detergent protein; iNDF: indigestible neutral detergent fiber. [2]Mean values for samples obtained by hand-plucking in the digestion trial; [3]Mean values for samples obtained by hand-plucking throughout the experimental period; [4]Means ± standard error medium.

Nutritional characteristics. On the 75th day of the experiment, a ten-day trial was performed to evaluate the nutritional characteristics. The first six days of the trial were used for the adaptation of animals to the markers (stabilization of markers excretion). Chromium oxide (Cr_2O_3) was used as external marker to estimate fecal excretion was packaged in paper cartridges in the amount of 10 g animal and delivered via esophagus with a metal probe once daily, at 10 a.m. Titanium dioxide (TiO_2) was used to estimate the individual intake of supplement, mixed in the supplement at the proportion of 10 g kg^{-1} of supplement. To estimate forage DM intake, indigestible neutral detergent fiber (iNDF) was used as internal marker. The last four days of the trial, feces samples were collected immediately after defecation or directly from the rectum of animals (at amounts of approximately 200g), at different times according to the following schedule: Day 6 - 18 h, Day 7 - 14 h, Day 8 - 10 h and Day 9 - 06 h. Samples feces were identified, oven-dried at 60°C and ground as previously described. After that, samples were pooled based on each animal.

To evaluate the microbial protein production and urinary urea nitrogen (UUN) excretion by the animals, on the ninth day of the trial a spot urine sample was collected during spontaneous urination, four hours after the supplementation was given. After the collection, 10mL of urine were diluted in 40 mL H_2SO_4 (0.036 N) and frozen at -20ºC for later analysis.

Blood collections. On days 45, 90 and 135 of the experiment, blood samples were collected to quantify the concentration of glucose, cholesterol, serum urea nitrogen (SUN), total proteins and albumin. Samples were collected at 7h00, via jugular venipuncture in vacuum tubes containing separator gel and clot accelerator (BD Vacutainer® SST II Advance, Phymouth, UK) and vacuum tubes containing sodium fluoride and EDTA (BD Vacutainer® Fluoreto/EDTA, São Paulo, Brazil) as glycolytic inhibitor and anticoagulant, respectively, for glucose analysis. Samples collected with separator gel and clot accelerator were immediately centrifuged (3.600 × g for 20 min) and, samples collected with glycolytic inhibitor were immediately centrifuged (2.600 × g for 10 min), the plasma was frozen at 20°C for later analysis.

Performance productive. To evaluate average daily gain (ADG) and final body weight (FBW), the animals were weighed at the beginning and end of the experiment after 14h of solids fasting.

Analytical procedures. Samples of forage, feces and supplement (processed to pass through 1-mm sieves) were analyzed for DM (dried overnight at 105°C; method INCT-CA number G-003/1), ash (complete combustion in a muffle furnace at 600°C for 4h; method INCT-CA number M-001/1), CP (Kjeldahl procedure; method INCT-CA number N-001/1), ether extract (Randall procedure; method INCT-CA number G-005/1), NDF corrected for ash and protein (using a heat-stable α-amylase, omitting sodium sulfite and correcting for residual ash and protein; method INCT-CA number F-002/1) according to (13). The iNDF content in samples of feces, forage and supplement (processed to pass through 2-mm sieves) was estimated using the *in situ* ruminal incubation procedure for 288 h (method INCT-CA number F-008/1) (14).

Feces samples were also analyzed for chromium concentration using nitroperchloric digestion and atomic absorption spectrophotometry (13) and titanium dioxide by colorimetry (15).

The pdDM was estimated as described by Detmann et al (17), using the following equation:

$$pdDM = 0.98 \times (100 - NDF) + (NDF - iNDF)$$

The fecal DM excretion was estimated using the chromic oxide marker, based on the ratio between the amount of chromium supplied and its concentration in the feces. Individual supplement intake was estimated (SI) by elation of excretion of TiO_2 in feces and marker concentration in the supplement.

Dry matter intake (DMI) was estimated by using iNDF as an internal marker and calculated by the following equation:

$$DMI = [(FE \times iNDFfeces - iNDFsupplement) / iNDFforage] + IS$$

Where FE = fecal excretion (kg per day), iNDFfeces = concentration of iNDF in the feces (kg per kg), iNDFsupplement = concentration of iNDF in the supplement (kg per kg) and iNDFforage = concentration of iNDF in the forage (kg per kg) and IS = intake of supplement.

Daily urinary volume was calculated using the relationship between the daily creatinine excretion (CE), taking as reference the equation proposed by Costa e Silva *et al.* (18), and its concentration in the spot samples:

$$CE(g/d) = 0.0345 \times BW^{0.9491}$$

Where: BW = body weight

Excretion of the purine derivatives in urine was calculated by the sum of the allantoin and uric acid excretions, which were obtained by the product between their concentrations in urine by the daily urinary volume. Absorbed purines were calculated from the excretion of purine derivatives according to Chen & Gomes (19).

$$AP = X - 0.301 \times BW^{0.75} / 0.80$$

Where AP = absorbed purines (mmol/d), X = excretion of purine derivatives (mmol d^{-1}), 0.8 = recovered absorbed purines. The 0.301 × $BW^{0.75}$ value = endogenous excretion of purine derivates.

Ruminal synthesis of nitrogen compounds was calculated as a function of the absorbed purines using the equation described by Barbosa et al (20).

$$MICN = 70 \times AP / 0.93 \times 0.137 \times 1.000$$

where MICN= ruminal synthesis of nitrogen compounds (g d^{-1}), AP = absorbed purines (mmol d^{-1}), 70 = purine N content (mg mol^{-1}), 0.93 = purine digestibility and 0.137 = relation of purine N:total N of microorganisms.

Efficiency of protein microbial synthesis (EMS) was estimated by dividing protein microbial production by the DOM intake.

The blood glucose (Ref. Number K082-2, Bioclin® Quibasa, Belo Horizonte, Brazil) and cholesterol concentrations (Ref. Number K083-2, Bioclin® Quibasa, Belo Horizonte, Brazil) were quantified by an enzymatic-colorimetric test. Blood and urine urea concentration using the enzymatic kinetic test (Ref. Number K056-1, Bioclin® Quibasa, Belo Horizonte, Brazil) and, albumin (Ref. Number K040-1, Bioclin® Quibasa, Belo Horizonte, Brazil) and total protein (Ref. Number K031-1, Bioclin® Quibasa, Belo Horizonte, Brazil) by an colorimetric test. Urinary creatinine with a colorimetric kinetic test (Ref. Número K067-1, Bioclin® Quibasa, Belo Horizonte, Brazil) and, urinary uric acid by an enzymatic-colorimetric test (Ref. Número K139-1, Bioclin® Quibasa, Belo Horizonte, Brazil). Serum urea N (SUN) was estimated as 46.67% of total serum urea. These metabolites were analyzed in accordance with an automatic biochemistry analyzer (Mindray BS200E, Shenzhen, China).

Statistical analyses. The experiment was analyzed according to completely randomized design. All statistical procedures were conducted using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The intake, digestibility, production of microbial protein, average daily gain (ADG) and, final BW (FBW) were submitted to analysis of variance, adopting the initial body weight as covariate. Serum concentrations of glucose, cholesterol, SUN, albumin, and total proteins were analyzed using the procedure for repeated measures. The most appropriate covariance structure was chosen the best (co) variance structure was chosen based on Akaike's information criterium with correction. Statistical significance was considered at $p \leq 0.05$, and tendencies were considered at $0.05 < p \leq 0.10$. In the absence of interaction treatment and collection, main effects are reported.
Thus, the experiment was analyzed according to the model:

$$Y_{ij} = \mu + T_i + e_{(i)j}$$

Where: Y_{ij} = average observation between individuals taken in the experimental unit j subjected to treatment I; μ = general constant; T_i = fixed effect of treatment i; $e_{(i)j}$ = random error, non-observable associated with each experimental unit, assumption NID (0, σ^2).

RESULTS

Forage samples. The mean availability of total forage DM and pdDM during the experiment was 4.7±0.37 and 3.2±0.28 t ha^{-1}, respectively. The concept of pdDM encompasses the quantity and quality of the forage available to be transformed into animal product (1). The forage samples obtained by the hand-plucking method had an average CP content of 91.3 g kg^{-1} DM (Table 2).

Nutritional characteristics. No effect of treatments was detected ($p > 0.10$) on the voluntary intakes (kg day^{-1}) of DM, supplement, NDFap, digestible organic matter (DOM), digestible NDF, and NDFap (Table 3). However, CP and OM intake were higher ($p < 0.01$) for animals from treatment SBM (Table 3). Additionally, an upward trend ($p = 0.087$) was observed in forage DM (FDM) intake for heifers from treatment SBM (Table 3).

Table 3. Voluntary intake of beef heifers under grazing supplemented with different protein sources.

Item	Supplement[1]		SEM	P-Value
	SBM	SBM+WB+U		
	kg day^{-1}			
DM	6.82	6.47	0.185	0.210
FDM	5.30	4.99	0.120	0.087
SDM	1.52	1.47	0.088	0.679
OM	6.27	5.94	0.110	0.049
CP	1.01	0.87	0.012	0.003
NDFap	3.38	3.29	0.071	0.351
iNDF	0.84	0.83	0.019	0.607
DOM	4.12	3.96	0.073	0.147
DNDF	2.03	2.01	0.048	0.769
	g kg^{-1} of BW			
DM	23.6	23.0	0.70	0.547
DMF	18.3	17.7	0.51	0.383
OM	21.7	21.1	0.51	0.439
NDFap	11.7	11.7	0.30	0.896
iNDF	2.9	2.9	0.20	0.818

DM: dry matter; FDM: forage dry matter; SDM: supplement dry matter; OM: organic matter; CP: crude protein; NDFap: neutral detergent fiber corrected for ash and protein; iNDF: indigestible neutral detergent fiber; DOM: digestible organic matter; DNDF: digestible neutral detergent fiber; [1]SBM: soybean meal; SBM+WB+U: soybean meal + wheat bran + urea; SEM: standard error medium.

In relation to intake (g kg^{-1} of BW) of DM, FDM, OM, NDFap, and iNDF were not influenced (p>0.10) by protein sources (Table 3).

No effect of treatments was observed (p<0.10) on the digestibility coefficients of DM, OM, and NDFap (Table 4). However, CP digestibility was higher (p<0.05) for animals from SBM compared with SBM+WB+U (Table 4). Similarly, supplementation with SBM increased (p<0.01) the dietary content of DOM (Table 4).

Table 4. Digestibility coefficients and nitrogen levels of beef heifers under grazing supplemented with different protein sources.

Item	Supmlement[1]		SEM	P-Value
	SBM	SBM+WB+U		
Dry matter (g g^{-1})	0.632	0.634	0.0054	0.806
Organic matter (g g^{-1})	0.658	0.668	0.0045	0.125
Crude protein (g g^{-1})	0.739	0.720	0.0063	0.049
NDFap (g g^{-1})	0.598	0.601	0.0090	0.163
DOM (g kg^{-1} of DM)	777.1	616.2	9.21	<0,001
MICN (g day^{-1})	72.8	66.5	4.80	0.368
MICNR (g g^{-1} N)	0.447	0.491	0.0379	0.420
EMS (g CP kg^{-1} DOM)	110.5	109.7	9.41	0.948
UUN (g day^{-1})	66.4	74.3	4.05	0.182

NDFap: neutral detergent fiber corrected for ash and protein; MICN: production of microbial nitrogen compounds; RMICN: relative microbial nitrogen; EMS: efficiency of microbial protein synthesis; UUN: urea nitrogen excretion in the urine; [1]SBM: soybean meal; SBM+WB+U: soybean meal + wheat bran + urea; SEM: standard error medium.

Microbial nitrogen (MICN) production, microbial nitrogen relative to the ingested nitrogen (MICNR), and efficiency of microbial protein synthesis (EMS) were not affected by the protein sources (p>0.10) (Table 4). Additionally, there was no effect of treatments (p>0.10) on urine urea nitrogen excretion (UUN) (Table 4).

Metabolic profile. Overall, no interaction (p>0.10) was observed between protein sources and collection days on the variables evaluated associates with the metabolic profile (Table 5). An interaction effect was only observed (p<0.01) between protein sources and collection days on SUN (Table 5); the study of this effect indicated that both treatments led to an increase in the concentration of SUN from the first collection, with maximum concentration observed in the second collection (Figure 1).

Table 5. Metabolic profile of beef heifers under grazing supplemented with different protein sources.

Item	Supplement[1]		SEM	Valor-P[2]		
	SBM	SBMWBU		Treat	Col	TreatCol
Glucose (mg dL^{-1})	75.0	76.1	1.37	0.583	<0.001	0.694
Cholesterol (mg dL^{-1})	89.6	88.5	4.40	0.859	0.073	0.642
SUN (mg dL^{-1})	15.2	16.4	0.51	0.122	<0.001	<0.001
Total proteins (g dL^{-1})	6.19	6.10	0.163	0.719	0.026	0.163
Albumin (g dL^{-1})	3.41	3.27	0.050	0.059	0.004	0.175

[1]SBM: soybean meal; SBMWBU: soybean meal + wheat bran + urea; [2]Treat: treatments effects; Col: Collections effects; SEM: standard error medium; TreatCol: interaction between treatment and collection.

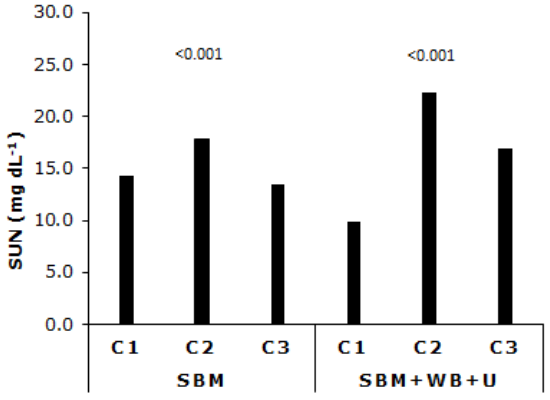

Figure 1. Serum urea nitrogen (SUN) concentration of different treatments according to collection one (C1), collection two (C2) and collection three (C3) during the experimental period.

No effect of treatments was observed (p>0.10) on the plasma concentrations of glucose, cholesterol, SUN, and total proteins (Table 5). However, the albumin concentration showed an upward trend (p=0.059) in heifers from SBM (Table 5).

Regarding to the collection days, an effect was observed (p<0.01) on plasma glucose concentration, whose lowest value was seen in the third collection (Figure 2). Additionally, there was a downward trend (p=0.073) in cholesterol concentration from the first collection onwards (Table 5). The total protein concentration had its maximum value (p<0.01) in the last collection (Figure 3). Lastly, an effect was observed (p<0.01) on albumin concentration (Table 5), whose maximum value was detected in the last collection (Figure 4).

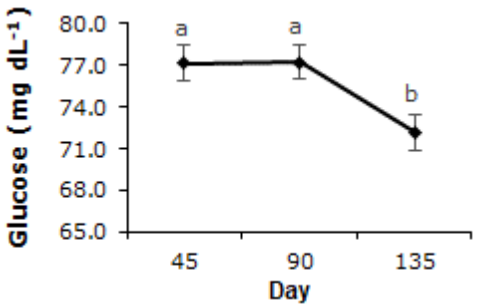

Figure 2. Blood concentration of glucose during the experimental period. [1]Means in the row, followed by different letters, differ (p<0.05).

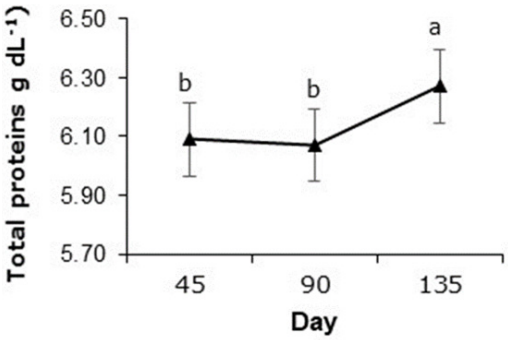

Figure 3. Blood concentration of total proteins during the experimental period. [1]Means in the row, followed by different letters, differ (p<0.05).

Productive performance. The ADG and FBW of the animals were not affected (p>0.10) by protein sources (Table 6).

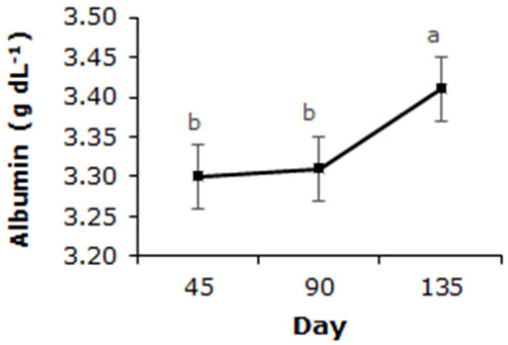

Figure 4. Blood concentration of albumin during the experimental period. [1]Means in the row, followed by different letters, differ (p<0.05).

Table 6. Productive performance of beef heifers under grazing supplemented with different protein sources.

Item[1]	Supplement[2]		SEM	P-Value
	SBM	SBM+WB+U		
FBW (kg)	314.2	308.7	2.95	0.188
ADG (kg day^{-1})	0.486	0.449	0.0190	0.183

[1]FBW: final body weight; ADG: average daily gain. [1]SBM: soybean meal; SBM+WB+U: soybean meal + wheat bran + urea; SEM: standard error medium.

DISCUSSION

Supplementation with nitrogenous compounds for cattle favors the growth of fibrolytic bacteria, increasing the ruminal degradation of fiber and forage intake (2,21). Thus, the upward trend in FDM intake in heifers from treatment SBM (Table 3) can be explained by the higher CP intake these animals in relation to heifers from SBM+WB+U (Table 3), which confirms the positive effect of nitrogen supplementation on forage intake. However, this pattern was not observed in NDFap intake (Table 3).

The higher intake of CP for heifers from treatment SBM compared with SBM+WB+U (Table 3) may be attributed to the higher CP content in the SBM supplement. Although the supplements were formulated to be isoproteic, they had different contents of CP (Table 1). The higher intakes of OM and DOM for animals from SBM compared with SBM+WB+U can only be attributed to their higher CP intake (Table 3), because there was no difference in the intakes of NDFap or DNDF, respectively.

The higher digestibility coefficient of CP for heifers from SBM compared with SBM+WB+U can be justified by their higher protein intake (Table 3). A higher intake of nitrogen compounds leads to lower participation of endogenous protein and reduces the representativeness of the fecal metabolic fraction of nitrogenous components (22). The higher digestibility of CP elevated the dietary content of DOM.

Evaluating the nutritional value of the diet, the CP content was 148.5 and 134.1 g kg^{-1} DM for treatments from SBM and SBM+WB+U, respectively. This shows that there was no deficiency of nitrogen compounds for the growth of fibrolytic microorganisms, which may explain the similar digestibility of NDFap between treatments (Table 4). Similar results were reported by other authors supplementing cattle in tropical conditions with different types and quantities of supplements (9,22,23,24).

The similar estimates of MICN and EMS between treatments (Table 4) evidence that there were no deficiencies of substrate essences (energy and nitrogen compounds) for the growth of ruminal microorganisms from the diets. The mean value of EMS in the treatments were 110.1g CP kg^{-1} DOM, this value being slightly lower than the 120g CP kg^{-1} DOM recommended by Valadares Filho et al. (12) for cattle managed in tropical conditions.

Although CP intake was greater for heifers from treatment SBM compared with SBM+WB+U (Table 3), this was not sufficient to affect RMICN between the treatments. These results indicate that there were no deficiencies of nitrogen compounds in the rumen.

In spite of the higher CP intake for SBM compared with SBM+WB+U heifers (Table 3), SUN and UUN was similar between treatments, which was expected, given the presence of urea in the supplement (Table 1). Urea increases the rumen-degradable protein content (RDP), which results in increased ruminal ammonia, consequently, higher blood nitrogen, in addition, increasing in ruminal ammonia decreased utilization efficiency.

The SUN concentration is positively associated with CP intake, RDP, and ruminal ammonia concentration (25). Optimal SUN concentrations in growing beef heifers range from 11 to 15 mg^{-1} (26), suggesting that the animals in this study did not present deficiencies or excess protein (Table 5). On the other hand, the higher SUN concentration in the second collection may be attributed to the increase in CP content of the forage consumed by the animals. In this sense, higher protein intake leads to increases the ruminal ammonia concentration and consequently the nitrogen transport by diffusion to the blood flow, leading to an increase in the SUN concentration (27).

The glucose concentration in the blood is positively associated with the intake of DM (28). Similar DM intakes between treatments (Table 3) may explain the lack of difference in glucose concentrations of the animals (Table 5). In ruminants, glucose requirements are met primarily via hepatic gluconeogenesis (28). Thus, the decreasing in glucose concentration from the second collection time (Figure 2) can be attributed possibly to a reduction in the rate of gluconeogenesis caused by the increase in insulin concentrations. Supporting these results, the uptake of glucose precursors as well as the release of hepatic glucose are reduced by insulin (28). Da Silva et al. (29) y Almeida et al. (30), also reported no difference in glucose concentrations of supplemented heifers in tropical conditions.

Plasma cholesterol concentration is an indicator of energy metabolism and nutritional status of animals. Thus, the similar plasma cholesterol concentrations between treatments (Table 5) indicate that both supplements provided similar energy statuses in animals.

Plasma proteins are mainly composed of albumin, globulins, and fibrinogen. In the liver, its synthesis is directly related to the nutritional status and availability of amino acids in the animal (31). The lack of differences in total protein concentration across treatments (Table 5) indicates that there was no protein deficiency in the diet. By contrast, the trend of increase in plasma albumin concentration for SBM heifers (Table 5) may be attributed to the higher CP intake by these animals (Table 3). Supporting this rationale, albumin is a more sensitive indicator to evaluate the protein nutritional status than are total proteins (31). This may explain of increase in albumin concentrations and a lack of effect on total protein plasma concentrations across treatments (Table 5). The higher plasma concentrations of total proteins and albumin in the third collection (Figure 2 and Figure 3, respectively) may be associated with the higher CP content of the forage consumed by the animals, as mentioned above.

The similar ADG and FBW between treatments may be justified by the sufficient level of CP in the consumed forage (8,27) and lack of difference in DM intake of animals (Table 3). In addition, it can also be inferred that the difference in CP and OM intake between animals was minimal and not enough to impact ADG and, consequently, FBW.

The results obtained in this study indicate that, the supplementation with soybean meal or wheat bran with urea in association with soybean meal in multiple supplements for cattle grazing provides similar productive and nutritional performance and metabolic profile in the animals. Thus, the use of wheat bran with urea associated with soybean meal in multiple supplements for grazing cattle is recommended.

Conflict of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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