DOI: 10.5433/1679-0359.2016v37n1p461

Quantities of supplements for grazing beef heifers in the dry-rainy transition season

Quantidades de suplementos para novilhas de corte sob pastejo durante o período de transição seca - águas

Román Maza Ortega[1*]; Mário Fonseca Paulino[2]; Edenio Detmann[2]; Luciana Navajas Rennó[2]; Aline Gomes da Silva[1]; David Contreras Marquez[1]; Deilen Sotelo Moreno[1]; Felipe Henrique Moura[3]; Jéssika Almeida Bitencourt[3]

Abstract

The objective of this study was to evaluate the effect of supplying different quantities of multiple supplements on the nutritional characteristics and productive performance of heifers during the post-weaning phase on *Urochloa decumbens* pastures during the dry-rainy transition season. Twenty-four heifers (average initial age and weight of 11 mo and 243±6 kg, respectively) were used. The experimental design was completely randomized, with four treatments and six replicates. A supplement containing 25% CP was used, and treatments consisted of the following four levels of supplements: 0, 0.5, 1.0 and 1.5 kg. The animals in the different treatments received a mineral mixture *ad libitum*. A positive linear effect (P<0.10) was observed on ADG and final body weight with the quantities of supplements. An increasing linear effect (P<0.10) was found on the intakes of DM, OM, CP, EE, NFC and TDN with the elevation in the amount of the supplement provided, which was not observed for NDFap intake. A cubic effect was observed (P<0.10) on the apparent digestibility of OM, CP, NDFap, NFC and concentration of TDN among the quantities of multiple supplements. Supplement levels increased (P<0.10) the digestibility coefficient of EE. An increasing linear response was detected (P<0.10) in urine urea nitrogen excretion, serum urea nitrogen (SUN) and flow of microbial nitrogen (MICN) compounds with the quantities of supplement. No difference was observed (P<0.10) in the efficiency of microbial synthesis between the supplementation levels. The quantities of supplement had a decreasing linear effect (P<0.10) on the relative MICN. In conclusion, supplying higher amounts of multiple supplements during the dry-rainy transition season improves the productive performance and nutritional characteristics of beef heifers reared on pasture.

Key words: Digestibility, intake, supplementation, post-weaning, *Urochloa decumbens*

Resumo

O objetivo do trabalho foi avaliar o efeito do fornecimento de diferentes quantidades de suplementos múltiplos sobre as características nutricionais e desempenho produtivo de novilhas durante a fase de recria em pastagens com *Urochloa decumbens* no período de transição seca-águas. Foram utilizadas 24 novilhas com idade e peso médio inicial de 11 meses e 243±3 kg, respectivamente. O delineamento foi inteiramente casualizado com quatro tratamentos e seis repetições. Utilizou-se um suplemento com 25% de PB. Os tratamentos consistiam em quatro quantidades de suplementos: 0; 0,5; 1,0 e 1,5

[1] Discentes do Curso de Doutorado do Programa de Pós-Graduação em Zootecnia, Universidade Federal de Viçosa, UFV, Viçosa, MG, Brasil. E-mail: rmaza777@hotmail.es; alinezooufv@yahoo.com; davidestebas@hotmail.com; mvzsotelo@hotmail.com

[2] Profs., UFV, Viçosa, MG, Brasil. E-mail: mpaulino@ufv.com.br; detmann@ufv.br; lucianarenno@ufv.br

[3] Discentes do Curso de Graduação em Zootecnia, UFV, Viçosa, MG, Brasil. E-mail: felipe.moura@ufv.br; jessika.bitencourt@ufv.br

[*] Author for correspondence

Recebido para publicação 14/03/15 Aprovado em 25/09/15

kg respectivamente. Os animais dos diferentes tratamentos receberam mistura mineral *ad libitum*. Observou-se efeito linear positivo (P<0,10) no GMD e peso corporal final (PCF) com as quantidades de suplementos. Foi verificado efeito linear crescente (P<0,10) no consumo de MS, MO, PB, EE, CNF e NDT com o fornecimento de maiores quantidades de suplemento; efeito não apresentado no consumo de FDNcp. Evidenciou-se efeito cúbico (P<0,10) sobre a digestibilidade aparente da MO, PB, FDNcp, CNF e concentração de NDT entre as quantidades de suplementos múltiplos. As quantidades de suplementos aumentaram (P<0,10) o coeficiente de digestibilidade do EE. Verificou-se comportamento linear crescente (P<0,10) sobre a excreção urinária de nitrogênio uréico (NUU), nitrogênio uréico no soro (NUS) e fluxo de compostos nitrogenados microbianos (NMIC) com as quantidades de suplementos. Não foi observada diferença (P<0,10) das quantidades de suplementação sobre a eficiência de síntese microbiana. As quantidades de suplementos afetaram de forma linear decrescente (P<0,10) o NMICR. Conclui-se que o fornecimento de maiores quantidades de suplementos múltiplos durante a época de transição seca-águas melhora o desempenho produtivo e características nutricionais de novilhas de corte criadas em pastagens.
Palavras-chave: Consumo, digestibilidade, recria, suplementação, *Urochloa decumbens*

Introduction

The pasture model, characterized by the collection *in situ*, represents over 90% of the cattle diet and stands out among the feeding methods due to its low production cost and high practicality (PAULINO et al., 2012). Tropical pastures are characterized by having a high neutral detergent fiber (NDF) content, which can account for more than 60% of the dry matter (DM) (PAULINO et al., 2012), and are a low-cost source of digestible energy for cattle-farming in the tropics (DETMANN et al., 2008). Therefore, actions should be promoted so as to increase the production and use of forage for conversion into animal product.

Supplementing grazing cattle has been one of the main strategies to intensify production systems, and it has become essential for the competitiveness and sustainability of the animal husbandry sector (VALADARES FILHO et al., 2006). Consequently, the use of multiple supplementation is a practice that can be adopted in the management of grazing cattle aiming to increase the carrying capacity of the forage and promote balance in the tropical-pasture-based diet, allowing an increase in the weight gain rates and continuous growth of animals (PAULINO et al., 2004), which leads to an increase in the weight-gain rates and consequently better reproductive responses at younger ages (VIEIRA et al., 2006).

The nutritional management of heifers influences the variability of the age and weight at puberty. Thus, negative correlations have been reported between feed intake and pubertal ages, and positive responses between feed intake and body weight (SÁ FILHO et al., 2008; EMERICK et al., 2009). Therefore, the growth rate at which animals approach adult weight is highly sensitive to variations in the nutritional aspect.

The objective of the present study was thus to evaluate the effect of supplying different amounts of multiple supplements on nutritional characteristics, efficiency of microbial synthesis, and productive performance of beef heifers in their post-weaning phase on *Urochloa decumbens* pastures during the dry-rainy transition season.

Material and Methods

All procedures performed on the animals were approved by the Brazilian Committee of Ethics in Animal Use and Experimentation, under filing no. 28⁄2013.

Location, animals, experimental design and diets.

The experiment was conducted at Universidade Federal de Viçosa, located in the city of Viçosa, MG, Brazil (20°45’45” S latitude and 42°52’04” W longitude; 657 m de altitude), between October and January 2013, which corresponded to the dry-rainy transition season, divided into three 28-day periods. Average temperature and precipitation values of 22.4 ºC and 144.9 mm were observed during the experimental period.

Twenty-four Nellore beef heifers with an initial average age and weight of 11 mo and 243±6 kg, respectively, were used. The animals were tested on an 8-ha experimental area, resulting in a stocking rate o 1.6 AU/ha. The area consisted of four 2-ha paddocks uniformly covered with *Urochloa decumbens* grass, provided with drinkers ad troughs, which were covered and had access from both sides.

The experimental design completely was randomized, with four treatments and six replicates. A supplement was provided to four treatments, at different quantities: 0, 0.5, 1.0 and 1.5 kg, containing 25% CP (as is) (centesimal composition of the supplement, as is: ground corn, 25.60; sorghum grain, 25.40; soybean meal, 22.20; and cottonseed meal, 26.80). Animals received a mineral mixture *ad libitum* (centesimal composition of the mineral mix: 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).

All animals were subjected to the control of ecto- and endoparasites at the beginning of the experiment and over its course.

Animals were weighed at the beginning of the experiment after a water- and feed-deprivation period of 14 hours aiming to reduce the difference in the filling of the digestive tract. Next, treatments were distributed at random to the experimental units (animals). Four lots were formed, by grouping the animals that received the same treatment.

The supplement was given daily at 10h00 during the experimental period. Water was supplied *ad libitum* throughout the experiment. Animals were rotated across the paddocks every seven days to eliminate possible effects of the paddocks on the treatments.

Measurements and sampling

To evaluate performance, the animals were weighed at the beginning and end of the experiment, both after a water- and feed-deprivation period of 14 h, and the resulting value was divided by the number of days in the experiment (112 days).

The pasture was collected on the fourteenth day of each experimental period to quantify the total availability of dry matter (DM) and potentially digestible dry matter (pdDM). The area to be sampled was delimited by a 0.5 × 0.5 m metal frame in four sites chosen randomly in each experimental paddock, and cut at approximately 1 cm above the soil. After collection, each sample was weighed and homogenized, and a composite sample was made from the samples of each paddock. The samples for qualitative evaluation of the forage consumed by the animals were obtained via hand-plucking (grazing simulation) on the fourteenth day of each experimental period. All samples were weighed, dried in a forced air-circulation oven at 60 ºC for 72 h, and ground in a 1- and 2-mm-sieve knife mill. Dry matter (DM) contents were quantified according to INCT-Detmann et al. (2012); neutral detergent fiber (NDF), as recommended by Mertens (2002), using thermostable α-amylase and without using sodium sulfide; and indigestible neutral detergent fiber (iNDF), after incubation in F57 bags (Ankom®) *in situ* for 288 h, according to Valente et al. (2011).

The pdDM was estimated according to the equation described by Paulino et al. (2008):

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

A nine-day trial was conducted from the 43rd day of the experimental period to evaluate the intake and digestibility of the animals, with six of these days used for the animals to adapt to the markers. To estimate fecal output, 10 g of chromium oxide (Cr_2O_3) were supplied daily per animal, conditioned in paper cartridges, and applied using a metal probe via the esophagus, always at 10h00. Titanium dioxide (TiO_2) was given to estimate the individual supplement intake, mixed in the supplement at the proportion of 10 g/kg of supplement. To estimate pasture intake, indigestible neutral detergent fiber (iNDF) was used as internal marker.

On the last three days of the trial, feces were collected at different times - 15h00, 11h00 and 06h00 - aiming to obtain representative fecal samples of each animal. These samples were identified, dried in a forced air-air oven (60 ºC/72 h), and ground in a knife mill with 1- and 2-mm sieves.

On the fifth day of the trial, the grazing simulation was performed in each paddock separately, and these samples were used to estimate the intake and digestibility coefficients.

To evaluate the microbial protein production, spot urine samples were collected on the last day of the trial, during spontaneous urination, approximately four hours after the supplement was given. After the collection, 10 mL of urine were diluted in 40 mL H_2SO_4 (0.036 N) and frozen at -20 ºC to later quantify the contents of creatinine, urea and purine derivatives. After collecting the urine, blood samples were drawn by jugular venipuncture in vacuum tubes containing separator gel and clot accelerator (BD Vacutainer® SST II Advance). The blood was centrifuged immediately at 3600 × *g* for 20 min, and the serum was stored (-20 ºC).

Chemical analyses

The dry matter (DM) (INCT-CA G-003/1), mineral matter (MM) (INCT-CA N-001/1), crude protein (CP) (INCT-CA M-001/1) ether extract (EE) (INCT-CA G-005/1), neutral detergent fiber (NDF) (INCT-CA F-002/1) with corrections for ash (NDIA) (INCT-CA M-002/1) and protein (NDIP) (INCT-CA N-004/1), and indigestible neutral detergent fiber (iNDF) contents were quantified in the samples of forage, feces and supplement according to Detmann et al. (2012). The DM, NDFap, and iNDF contents in the forage samples intended for the calculation of the availability of DM and pdDM were determined as described previously.

Samples of feces were analyzed for the concentration of chromium and titanium dioxide using nitric-perchloric digestion and atomic absorption spectrometry (SOUZA et al., 2013) and colorimetry, respectively (DETMANN et al., 2012).

Fecal DM excretion was estimated using the chromic oxide marker, based on the ratio between the amount of marker supplied and its concentration in the feces:

$$\textit{Fecal dry matter (g/day)} = \frac{\textit{Amount of Marker Supplied (g)}}{\textit{Concentration of Marker in Feces (\%)}} \times 100$$

The individual supplement intake was estimated as the ratio between excretion of TiO_2 in the feces and concentration of marker in the supplement:

$$ISI = ((FE \times CMFe)/MSG) \times SSG,$$

where: ISI = individual supplement intake (kg/day); CMFe = concentration of marker in the feces (kg/kg); MSG = marker present in the supplement supplied to the group (kg/day); and SSG = supplement supplied to the group (kg/day).

Forage dry matter intake (DMI) was determined by employing iNDF as an internal marker, by the following equation:

$$DMI\ (kg/day) = \{[(FE \times CMF)-MS]/CMFo\} + SDMI,$$

where: FE = fecal excretion (kg/day); CMF = concentration of marker in the feces (kg/kg); MS = intake of marker from supplement (kg); CMFo = concentration of marker in the forage (kg/kg); and SDMI = supplement dry matter intake (kg/day).

Creatinine, uric acid and urea were quantified by the kinetic colorimetric, enzymatic colorimetric and fixed-time kinetic methods, respectively, using an automatic device for biochemistry (Mindray® model BS200E) and kits. The daily urinary volume was calculated by employing the ratio between the day excretion of creatinine (EC), adopting the equation proposed by Silva et al. (2012) and its concentration in the spot samples as reference:

$$ECU\ (g/day) = 0.0345 \times BW^{0.9491}$$

where: BW= body weight.

Allantoin in the urine was analyzed as described by George et al. (2006). The total excretion of purine derivatives was calculated as the sum of the amounts of allantoin and uric acid excreted in the urine.

Absorbed purines (Y, mmol/day) were calculated from the excretion of purine derivatives (X, mmol/day), by the following equation:

$$Y = (X - 0.385\ LW^{0.75}) / 0.85$$

where 0.85 is the recovery of absorbed purines as purine derivatives and $0.385LW^{0.75}$ is the endogenous contribution to the excretion of purines (BARBOSA et al., 2011).

The ruminal synthesis of nitrogen compounds (Y, g MICN/day) was calculated as a function of the absorbed purines (X, mmol/day), using the equation described by Chen and Gomes (1992), except for the purine N:total bacterial N ratio of 0.134, according to Valadares et al. (1999):

$$Y = 70X/0.83x0.134x1000$$

where 70 is the N content in the purines (mgN/mol); 0.134 is the purine N:total bacterial N ratio; and 0.83 is the digestibility of the bacterial purines.

Microbial efficiency was expressed as g microbial CP/kg of total digested organic matter (g micCP/kg DOM).

Statistical analysis

Results were subjected to analysis of variance, adopting initial body weight as co-variable. The linear, quadratic and cubic effects of the amount of multiple supplements were evaluated by decomposing the sum of squares of treatments using orthogonal contrasts (STEEL et al., 1997). All procedures were conducted using the PROC MIXED procedure of SAS (version 9.2) software. For all statistical procedures, 0.10 was adopted as critical level of type-I error probability.

Results and Discussion

Mean values of DM and pdDM were 5,275 and 3,766 kg/ha, respectively, with a proportion of potential forage digestibility of 71.17%, representing a supply of 32 g pdDM/kg body weight, which is below the 40 to 60 g pdDM/kg body weight recommended by Paulino et al. (2004) for satisfactory performance of grazing animals.

The average CP content in the forage obtained by the hand-plucking method was 9.86 g/kg (dry matter basis) (Table 1), which is above the minimum necessary to stimulate microbial growth and promote an appropriate degradation of the fibrous substrates of the forage (LAZZARINI et al., 2009; SAMPAIO et al., 2010).

Table 1. Chemical composition of supplement and *Urochloa decumbens*.

Item[1]	Multiple supplement	*U. Decumbens*[4]
DM (g/kg)	88.82	25.46±0.52
OM[2]	96.28	92.07 ±0.46
CP[2]	24.77	10.71 ±0.43
NPN[3]	5.58	19.21 ±2.54
EE[2]	3.07	2.01 ±010
NDFap[2]	21.36	54.80 ±2.13
NIDN[3]	25.33	44.7±1.42
NCF[2]	47.07	24.46 ±1.83
iNDFi[2]	5.10	10.25 ±0.62

[1]/DM - dry matter; OM - organic matter; CP - crude protein; EE - ether extract; NDFap - neutral detergent fiber corrected for ash and protein; NDIN - neutral detergent insoluble nitrogen; NFC - non-fibrous carbohydrates; iNDF - indigestible neutral detergent fiber. [2]/In g/kg DM. [3]/In g/kg total nitrogen. [4]/Mean values for samples obtained by hand-plucking.

However, although the forage obtained by hand-plucking had a higher CP value than the minimum for the rumen microorganisms to enable the proper use of the neutral detergent fiber (NDF) from the basal resource, 44.7% and 19.21% of the CP were available as neutral detergent insoluble nitrogen (NDIN) and non-protein nitrogen (NPN), respectively (Table 2). Neutral detergent insoluble nitrogen is considered to have a slow and incomplete degradation (SNIFFEN et al., 1992). This situation might lead to a metabolic imbalance regarding the MP/ME ratio, which would prevent the maximization of the use of the basal substrate, requiring the application of supplementation with nitrogen compounds (DETMANN et al., 2010), which may explain the animal performance responses.

In this study a positive linear effect (P<0.10) was detected on average daily gain (ADG) as the amount of supplement was raised, which could be verified by the final body weight, which also increased linearly (P<0.10; Table 2). The increasing linear response in the performance of the animals may stem from the increase in the amount of (digestible) multiple supplements. Maximum performance was observed in the animals that received the amount of supplement of 1.5 kg/animal/day, which were also the ones that showed the highest CP intake. This reinforces the idea of prioritizing protein supplementation even under conditions in which forage does not show an apparent deficiency. These results are similar to those reported by Cabral et al. (2014).

Table 2. Adjusted means, standard error (SEM) and indicators of significance for performance of heifers fed the different treatments.

Item	Supplement (kg/day)				SEM	P-value[1]		
	0.0	0.5	1.0	1.5		L	Q	C
ADG (kg)	0.166	0.281	0.349	0.435	0.0398	<0.001	0.720	0.723
FBW (kg)	261.4	274.2	281.9	291.5	4.45	<0.001	0.722	0.719

ADG - average daily gain; FBW - final body weight.
[1]/ L, Q and C - linear-, quadratic- and cubic-order effects referring to supplement quantities.

A positive linear effect (P<0.10) of the amounts of multiple supplement was observed on the intakes of DM, OM, CP, EE, NFC and DOM (kg/day) in the different treatments; however, there was no effect on the intakes of pasture DM (PDM) or DNDF (kg/day) (Table 3).

Table 3. Adjusted means, standard error (SEM) and indicators of significance for nutrient intake with the different treatments.

Item	Supplement (kg/day)				SEM	P-value1		
	0.0	0.5	1.0	1.5		L	Q	C
				kg/day				
DM	5.194	4.818	6.477	6.969	0.4815	0.004	0.379	0.154
FDM	5.194	4.374	5.589	5.636	0.4815	0.252	0.379	0.154
SDM	-	0.444	0.888	1.333	-	-	-	-
OM	4.784	4.478	6.004	6.438	0.4443	0.004	0.415	0.158
CP	0.523	0.577	0.835	0.953	0.0516	<0.001	0.526	0.151
EE	0.108	0.107	0.135	0.149	0.0099	0.003	0.439	0.349
NDFap	3.008	2.474	3.197	3.279	0.2623	0.207	0.255	0.122
NFC	1.142	1.321	1.837	2.057	0.1207	<0.001	0.866	0.255
iNDF	0.561	0.485	0.618	0.597	0.0498	0.295	0.590	0.122
DOM	3.038	2.977	3.864	4.778	0.2956	<0.001	0.115	0.494
DNDF	2.103	2.114	2.126	2.371	0.1821	0.152	0.198	0.250
TDN	2.987	2.964	3.878	4.83	0.2049	<0.001	0.115	0.503
				g/kg body weight				
DM	19.9	18.0	24.4	25.3	1.88	0.016	0.451	0.111
OM	18.4	16.7	22.6	23.4	1.74	0.015	0.490	0.114
NDFap	11.6	9.2	12.1	11.9	1.00	0.405	0.284	0.083
iNDF	2.2	1.8	2.3	2.2	0.19	0.527	0.631	0.082

DM dry matter, FDM forage drymatter, SDM supplement dry matter, OM organic matter, CP crude protein, EE ether extract, NDFap neutral detergent fibre corrected for ash and protein, NFC non-fibrous carbohydrates, iNDF indigestible neutral detergent fibre, DOM digerible organic matter, DFDN digerible neutral detergent fibre, TDN total digestible nutrients.
1/ L, Q and C - linear-, quadratic- and cubic-order effects referring to supplement quantities.

The positive linear effect on the intakes of DM and OM and the absence of effect on pasture DM intake demonstrate that there was no substitution or additive effect on forage intake with any of the supplement levels evaluated (Table 3), which shows that the association of energy sources of rapid ruminal degradation and supplemental nitrogen compounds, at levels at which there are no significant restrictions in the voluntary forage intake, can increase animal performance by promoting a higher amount of metabolizable protein resulting from the higher assimilation of nitrogen in the rumen (SOUZA et al., 2010).

As a complete diet, during the dry-rainy transition and rainy seasons, the pasture has a relatively excessive energy in relation to protein; thus, energy supplementation would further force an imbalance in the DOM/CP ratio by the metabolizable energy surplus (DETMANN et al., 2010). On this basis, supplementation should be focused on protein, so as to maintain the energy:protein balance in the forage aiming at to maintain pasture intake and digestibility (COSTA et al., 2009), with an expected consequent increase in animal performance.

The linear increase in the intakes of CP, EE and NFC was a result of the higher content of these components in the supplement (Table 1) and the increased supply of multiple supplements. The increase in CP, EE and NFC intakes (Table 3) provided a greater intake of DOM, and consequently of TDN.

In this study, it was found that the average CP content of the forage consumed by the animals was above the minimum level for proper use o the fibrous carbohydrates from the basal substrate (Table 1) (LAZZARINI et al., 2009; SAMPAIO et al., 2009), which can explain the lack of a significant effect (P>0.10) on the intakes of PDM and NDFap expressed in kg/day with supplementation (Table 3).

A cubic effect (P<0.10) of the levels of supplement was observed on the intakes of NDF and iNDF in g/kg BW (Table 3). The increased intake of iNDF is often associated with an increase in the rates of passage and digestion of the fibrous particles, with acceleration in the removal of the rumen NDF indigestible components, resulting in a greater rumen turnover (PAULINO et al., 2008; DETMANN et al., 2009). The cubic effect of the quantities of supplement on the intakes of NDFap and iNDF seems to be related to the physiological mechanisms that regulate intake, since the NDFap and iNDF intake values expressed in g/kg BW were low, indicating a low rumen fill.

One of the aspects involved in the better adequacy of the growth medium to the production of microbial enzymes is the availability of ammonia nitrogen (DETMANN et al., 2009), which is used preferably as a precursor for the synthesis of protein by the fibrolytic microorganisms (RUSSELL et al., 1992).

A cubic effect was detected (P<0.10; Table 4) on the digestibility of OM, CP and NFC with the supplement levels, which is associated with the higher inclusion of concentrate (easy digestion) supplements in the diet. The cubic effect of the levels of supplement on CP digestibility may be due to the greater content of nitrogen compounds and lower participation of endogenous protein, which reduces the representativeness of the fecal metabolic fraction of the nitrogen compounds. The digestibility coefficient of NDFap was higher (P<0.10; Table 4) because of the greater consumption of supplement and consequently of CP (Table 3), which reinforces the benefits of supplementation with nitrogen compounds on the fiber degradation by the rumen microorganisms.

A positive linear effect of the amounts of multiple supplement supplied was found on the apparent digestibility coefficient of EE (P<0.10; Table 4), which may be associated with the greater intake of this component with the increasing quantities of supplement; this intake is above the fecal metabolic contribution.

The higher dietary TDN level (P<0.10; Table 4) with the increased supplementation is explained by the greater concentrations of CP, EE and NFC, which leads to a higher intake of these components by the animals. These components are characterized by having better and faster degradation as compared with the forage components. On the other hand, it should be stressed that the range of interference of the fecal metabolic fractions of CP, EE and NFC as the intake of these components is elevated may also reflect in the estimate of the dietary TDN (VAN SOEST, 1994).

Table 4. Adjusted means, standard error (SEM) and indicators of significance for nutrient digestibility with the different treatments.

Item	Supplement (kg/day)				SEM	P-value1		
	0.0	0.5	1.0	1.5		L	Q	C
				g/g				
DOM	0.633	0.668	0.646	0.743	0.0098	<0.001	0.005	<0.001
DCP	0.453	0.543	0.518	0.688	0.0226	<0.001	0.091	0.006
DEE	-0.385	-0.075	0.089	0.280	0.0724	<0.001	0.419	0.594
DNDFap	0.697	0.697	0.667	0.723	0.0072	0.149	<0.001	0.002
DNFC	0.645	0.731	0.708	0.833	0.0128	<0.001	0.134	<0.001
				g/kg dry matter				
TDN	573	619	600	694	11.0	<0.001	0.043	0.002

OM organic matter, CP crude protein, EE ether extract, NDFap neutral detergent fibre corrected for ash and protein, NFC non-fibrous carbohydrates, TDN total digestible nutrients

1/ L, Q and C - linear-, quadratic- and cubic-order effects referring to supplement quantities.

Urine urea nitrogen excretion (UUN), serum urea nitrogen (SUN) and microbial nitrogen (MICN) increased linearly (P<0.10; Table 5). The relative microbial nitrogen (RMICN) decreased linearly as the multiple supplement was increased (P<0.10; Table 5). Supplement levels did not have a significant effect (P>0.10; Table 5) on the efficiency of microbial protein synthesis (EMPS) with the different treatments, averaging 124.7 g CP/kg DOM.

Table 5. Adjusted means, standard error (SEM) and indicators of significance for nitrogen levels with the different treatments.

Item	Suplemento (kg/day)				SEM	P-value1		
	0.0	0.5	1.0	1.5		L	Q	C
MICN (g/day)	63.0	56.2	74.9	84.2	7.71	0.032	0.317	0.317
RMICN (g/g)	0.752	0.654	0.559	0.556	0.0857	0.097	0.585	0.815
EMPS (g/kg DOM)	134.3	134.3	119.9	110.6	16.86	0.315	0.920	0.982
SUN (mg/dL)	9.89	12.22	14.80	17.47	0.976	<0.001	0.859	0.974
UUN (g/day)	39.2	47.0	54.4	60.6	2.65	<0.001	0.780	0.945

MICN production of microbial nitrogen compounds grams per day, RMICN, relative microbial nitrogen grms per grams, EMPS efficiency of microbial protein synthesis (microbial CP synthesis/DOM intake grams per kilogram), SUN serum urea nitrogen in milligrams per deciliter, UUN urea nitrogen excretion in the urine grams per day.

1/ L, Q and C - linear-, quadratic- and cubic-order effects referring to supplement quantities.

The concentration of urine urea nitrogen (UUN) is positively related to the serum urea nitrogen (SUN) and the CP intake (VAN SOEST, 1994).

The linear increase in urine urea nitrogen (UUN), serum urea nitrogen (SUN) and microbial nitrogen (MICN) with the increase in the quantities of multiple supplements may be due to a higher ruminal availability of nitrogen compounds caused by the increasing amounts of supplement that possibly reduced the use efficiency of the ruminal ammonia, indicating that a large amount of nitrogen is not utilized efficiently by the animal. These results are similar to those reported by Figueiras et al. (2010) and Cabral (2014).

The linear decrease shown by the relative microbial nitrogen (RMICN) as the supply of multiple supplements was increased can be attributed to the greater nitrogen intake with elevation in the levels of multiple supplements. These results agree with the values reported by Lazzarini et al. (2009) and Cabral et al. (2014).

Conclusions

Providing multiple supplements in increasing amounts increases the productive performance and improves the nutritional characteristics of grazing beef heifers during the dry-rainy transition season. The supply of 1.5 kg of multiple supplements with 250 g CP/kg DM provides maximum performance.

Acknowledgments

The authors thank Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) for the financial support.

References

BARBOSA, A. M.; VALADARES, R. F. D.; VALADARES FILHO, S. C.; PINA, D. S.; DETMANN, E.; LEÃO, M. I. Endogenous fraction and urinary recovery of purine derivatives obtained by different methods in Nellore cattle. *Journal of Animal Science,* Champaign, v. 89, n. 2, p. 510-519, 2011.

CABRAL, C. H. A.; PAULINO, M. P.; DETMANN, E.; VALENTE, E. E. L.; BARROS, L. V.; CABRAL, C. E. A. Levels of supplementation for grazing pregnant beef Heifers. *Bioscience Journal*, Uberlândia, v. 30, n. 1, p. 226-234, 2014.

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.* International Feed Research Unit. Rowett Research Institute: Aberdeen, 1992. 21 p. (Occasional Publication).

COSTA, V. A. C.; DETMANN, E.; VALADARES FILHO, S. C.; PAULINO, M. F.; HENRIQUES, L. T.; MANTOVANI, H. C. Degradação in vitro da fibra en detergente neutron de forragem tropical de alta qualidade en função da suplementação com proteina e/ ou carboidratos. *Revista Brasileira de Zootecnia*, Viçosa, MG, v. 38, n. 9, p. 1803-1811, 2009.

DETMANN, E.; PAULINO, M. F.; VALADARES FILHO, S. C. Avaliação nutricional de alimentos ou de dietas? Uma abordagem conceitual. In: SYMPOSIUM INTERNATIONAL ON BEEF CATTLE PRODUCTION, 2., 2008, Viçosa, MG. *Proceedings...* Viçosa, MG: UFV, 2008. p. 21-52.

DETMANN, E.; PAULINO, M. P.; MANTOVANI, H. C.; SAMPAIO, C. B.; SOUZA, M. A.; LAZZARINI, I.; DETMANN, K. S. C. Parameterization of ruminal fibre degradation in low-quality tropical forage using Michaelis-Menten kinetics. *Livestock Science*, Amsterdam, v. 126, n. 1-3, p. 136-146, 2009.

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 para análise de alimentos*. Visconde do Rio Branco: Instituto Nacional de Ciência e Tecnologia de Ciência Animal-INCT, Suprema, 2012. 214 p.

DETMANN, E.; VALADARES FILHO, S. C. On the estimation of non-fibrous carbohydrates in feeds and diets. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 62, n. 4, p. 980-984, 2010.

EMERICK, L. L.; DIAS, J. C.; GONÇALVES, P. E. M.; MARTINS, J. A. M.; LEITE, T. G.; ANDRADE, V. J.; VALE FILHO, V. R. Aspectos relevantes sobre a puberdade em fêmeas. *Revista Brasileira de Reprodução Animal,* Belo Horizonte, v. 33, n. 1, p. 11-19, 2009.

FIGUEIRAS, J. F.; DETMANN, E.; PAULINO, M. F.; VALENTE, T. N. P.; VALADARES FILHO, S. C.; LAZZARINI, I. Intake and digestibility in cattle under grazing during dry season supplemented with nitrogenous compounds. *Revista Brasileira de Zootecnia,* Viçosa, MG, v. 39, n. 6, p. 1303-1312, 2010.

GEORGE, S. K.; DIPU, M. T.; MEHRA, U. R.; SINGH, P.; VERMA, A. K.; RAMGAOKAR, J. S. Improved HPLC method for the simultaneous determination of allantoin, uric acid and creatinine in cattle urine. *Journal of Chromatography B,* Izatnagar, v. 832, n. 1, p. 134-137, 2006.

LAZZARINI, I.; DETMANN, E.; SAMPAIO, C. B.; PAULINO, M. P.; VALADARES FILHO, S. C.; SOUZA, M. A.; OLIVEIRA, F. A. Intake and digestibility in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. *Revista Brasileira de Zootecnia*, Viçosa, MG, v. 38, n. 10, p. 2021-2030, 2009.

PAULINO, M. F.; DETMANN, E.; SILVA, A. G.; ALMEIDA, D. M.; VALENTE, E. E. L.; MACIEL, I. F. S.; MARTINS, L. S.; BARROS, L. V.; PAULA, N. F. de; LOPES, S. A.; CARVALHO, V. V. Bovinocultura de alto desempenho com sustentabilidade. In: SIMPÓSIO DE PRODUÇÃO DE GADO DE CORTE, 8., 2012, Viçosa, MG. *Anais...* Viçosa, MG: UFV, 2012. p. 184-196.

PAULINO, M. F.; DETMANN, E.; VALENTE, E. E. L. Nutrição de bovinos em pastejo. In: SIMPÓSIO SOBRE MANEJO ESTRATÉGICO DA PASTAGEM, 4., 2008, Viçosa, MG. *Anais...* Viçosa, MG: UFV, 2008. p. 131-169.

PAULINO, M. F.; FIGUEIREDO, D. M.; MORAES, E. H. B. K.; PORTO, M. O.; SALES, M. F. L.; ACEDO, T. S.; VILLELA, S. D. J.; VALADARES FILHO, S. C. Suplementação de bovinos em pastagens: uma visão sistêmica. In: SIMPÓSIO DE PRODUÇÃO DE GADO DE CORTE, 4., 2004, Viçosa, MG. *Anais...* Viçosa, MG: UFV, 2004. p. 93-139.

RUSSELL, J. B.; O´CONNOR, J. D.; FOX, D. G.; VAN SOEST, P. J.; SNIFFEN, C. J. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *Journal of Animal Science*, Champaign, v. 70, n. 12, p. 3551-3561, 1992.

SÁ FILHO, M. F.; GIMENES, L. U.; SALES, J. N. S.; CREPALDI, G. A.; MEDALHA, A. G.; BARUSSELLI, P. S. Biotecnologia da reprodução em bovinos. In: SIMPÓSIO INTERNACIONAL DE REPRODUÇÃO ANIMAL APLICADA, 3., 2008, Londrina. *Anais...* Londrina: [s.n.], 2008. p. 54-67.

SAMPAIO, C. B.; DETMANN, E.; LAZZARINI, I.; SOUZA, M. A.; PAULINO, M. F.; VALADARES FILHO, S. C. Rumen dynamics of neutral detergent fiber in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. *Revista Brasileira de Zootecnia,* Viçosa, MG, v. 38, n. 3, p. 560-569, 2009.

SAMPAIO, C. B.; DETMANN, E.; PAULINO, M. F.; VALADARES FILHO, S. C.; SOUZA, M. A.; LAZZARINI, I.; PAULINO, P. V. R.; QUEIROZ, A. C. Intake and digestibility in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. *Tropical Animal Health and Production,* Edinburgh, v. 42, n. 7, p. 1471-1479, 2010.

SILVA, L. F. C.; VALADARES FILHO, S. C.; CHIZZOTTI, M. L.; ROTTA, P. P.; PRADOS, L. F.; VALADARES, R. F. D.; ZANETTI, D.; BRAGA, J. M. S. Creatinine excretion and relationship with body weight of Nellore cattle. *Revista Brasileira de Zootecnia,* Viçosa, MG, v. 41, n. 3, p. 807-810, 2012.

SNIFFEN, C. J.; O´CONNOR, J. D.; VAN SOEST, P. J.; FOX, D. G.; RUSSELL, J. B. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *Journal of Animal Science*, Champaign, v. 70, n. 11, p. 3562-3577, 1992.

SOUZA, M. A.; DETMANN, E.; PAULINO, M. F.; SAMPAIO, C. B.; LAZZARINI, I.; VALADARES FILHO, S. C. Intake, digestibility, and rumen dynamics of neutral detergent fibre in cattle fed low-quality tropical forage and supplemented with nitrogen and/or starch. *Tropical Animal Health and Production,* Edinburgh, v. 42, n. 6, p. 1299-1310, 2010.

SOUZA, N. K. P.; DETMANN, E.; PINA, P. S.; VALADARES FILHO, S. C.; SAMPAIO, C. B.; QUEIROZ, A. C.; VELOSO, C. M. Evaluation of chromium concentration in cattle feces using different acid digestion and spectrophotometric quantification techniques. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, Belo Horizonte, v. 65, n. 5, p. 1472-1482, 2013.

STEEL, R. G. D.; TORRIE, J. H.; DICKEY, D. A. *Principles and procedures of statistics*. *A biometrical approach.* 3th ed. New York: McGraw Hill Co, 1997. 666 p.

VALADARES FILHO, S. C.; MORAES, E. H. B. K.; DETMANN, E.; PAULINO, M. P.; VALADARES, R. F. D.; MORAES, K. A. K.; MARCONDES, M. I. Perspectiva do uso de indicadores para estimar o consumo individual de bovinos alimentados em grupo. In: REUNIÃO ANUAL DA SOCIEDADE BRASILEIRA DE ZOOTECNIA, 2006, João Pessoa. *Anais…* João Pessoa: [s.n.], 2006. v. 43, p. 238-262.

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*, Madison, v. 82, n. 11, p. 2686-2696, 1999.

VALENTE, T. N. P.; DETMANN, E.; QUEIROZ, A. C.; VALADARES FILHO, S. C.; GOMES, D. I.; FIGUEIRAS, J. F. Evaluation of rumen degradation profiles of forages using bags made from different textiles. *Revista Brasileira de Zootecnia,* Viçosa, MG, v. 40, n. 11, p. 2565-2573, 2011.

VAN SOEST, P. J. *Nutritional ecology of the ruminant*. 2th ed. Ithaca: Cornell University, 1994. 476 p.

VIEIRA, A.; LOBATO, J. F. P.; CORRÊA, E. S.; TORRES JUNIOR, R. A. A.; COSTA, F. P. Desenvolvimento e desempenho reprodutivo de novilhas Nelore criadas a pasto nos cerrados do Centro-Oeste brasileiro. *Revista Brasileira de Zootecnia,* Viçosa, MG, v. 35, n. 1, p. 186-192, 2006.