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Levels of supplementation for grazing pregnant beef cows during the dry season

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ABSTRACT - The objective of this study was to evaluate the effect of different levels of multiple supplement supply on the nutritional characteristics and productive performance of pregnant cows grazing on *Brachiaria decumbens* Stapf. during the dry season. The experimental area was composed of four 3.0 ha paddocks with availability of potentially digestible dry matter of 2,582 kg/ha. Twenty-eight crossbred pregnant cows with predominance of Zebu breed at body weight of 446±12 kg were utilized in a completely randomized experimental design. Treatments consisted of mineral supplement (control treatment) and supply of multiple supplement at 0.5, 1.0 and 1.5 kg/animal/day with 300 g crude protein (CP)/kg of dry matter (DM). There was quadratic effect of multiple supplementation levels on daily weight gain and a linear increase for final body condition score. The intakes of DM, organic matter (OM) and total digestible nutrients presented no effect with levels of multiple supplement. The coefficients of apparent digestibility of DM and OM had cubic effect with multiple supplementation levels. There was no effect of levels of multiple supplementation on the microbial nitrogen flow and efficiency, but the microbial nitrogen flow in relation to nitrogen intake showed decreasing linear profile. The supply of 1.0 kg of multiple supplement optimizes the performance of grazing cows during the dry season.

Key Words: multiple supplement, nutritional parameters, weight gain

Introduction

The production chain of beef cattle in Brazil devotes most of its efforts to animal fattening. The common scenario in many rural properties is the loss of the development of the breeding herd due to growth of males for slaughter, which generally have better access to pastures and forage supply.

The rearing activity, despite having the lowest profitability in the beef cattle livestock, is the one that can withstand the phases of growing and fattening, because its success depends on the quality of animals produced and offered in the first. However, the rearing phase not only involves the calf rearing, but also the breeders (cows and bulls).

The nutrition of the dam throughout its life is responsible for adequate response in terms of number of calves weaned per year, and one of the indexes that allow the evaluation of reproductive efficiency is the calving interval. Cows poorly nourished and with low body condition score are inefficient in the following breeding season, and the moment to establish the body condition of the female occurs in the pre-partum period.

The objective of this study was to evaluate the effect of providing different levels of multiple supplement on the nutritional characteristics, productive performance and body condition score of pregnant cows grazing on *Brachiaria decumbens* Stapf. during the dry season.

Material and Methods

The experiment was conducted in the beef cattle sector of Universidade Federal de Viçosa - UFV, Viçosa, Brazil, in a 12 ha area with four paddocks for grazing with continuous stocking, corresponding to treatments from June to August 2009 in the dry season (Figure 1).

Treatments consisted of mineral supplement (control treatment) and supply of three daily levels of multiple supplement: 0.5, 1.0 and 1.5 kg/animal (Table 1) with 300 g of crude protein (CP)/kg dry matter (DM) composed of soybean meal (200 g/kg), cottonseed meal (200 g/kg), corn (285 g/kg), sorghum (285 g/kg) and urea:ammonium sulfate at a 9:1 ratio (30 g/kg).

Twenty-eight crossbred pregnant cows (3-4.5 months of gestation) with predominance of Zebu breed at initial weight of 446±12 kg, properly vaccinated and wormed, were used.

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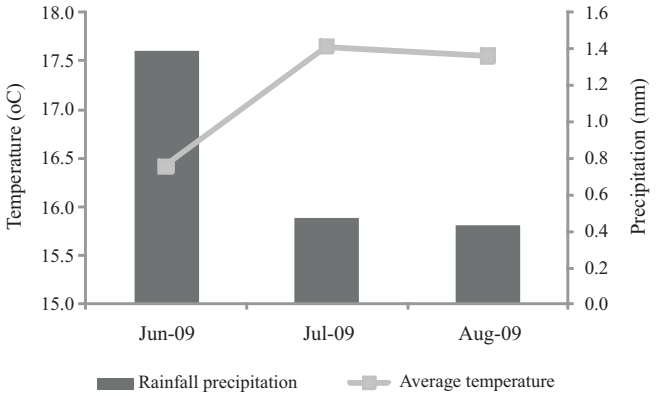


Source: Departamento de Engenharia Agrícola - UFV.

Figure 1 - Precipitation in millimeters (mm) and average temperature in °C during the experimental period.

Water was provided *ad libitum*. Multiple supplement was supplied at 10h00 throughout the experimental period in covered troughs (2.00 × 0.40 m) with access from both sides. The heifers in the treatment with multiple supplement at any levels of supply were fed mineral supplement (composition on the basis of natural matter: dicalcium phosphate, 500.00 g/kg; sodium chloride, 477.75 g/kg; zinc sulfate, 14.00 g/kg, copper sulphate, 7.00 g/kg; cobalt sulphate, 0.50 g/kg; potassium iodide, 0.50 g/kg; and sodium selenite, 0.25 g/kg) at the same amount (80 g/animal/day). The heifers in the control treatment had unrestricted access to mineral supplement.

The experiment was set and conducted following a completely randomized design with four treatments, seven replicates per treatment and three experimental periods of 28 days.

The animals were weighed at the beginning and end of the experiment, without fasting, and after being fasted for liquids and solids for 14 hours, aiming to reduce the possible differences in the filling of the digestive tract, as well as at each grazing cycle of 28 days without fasting, so that their development could be monitored. The total weight gain (TWG) was quantified by the difference between the final weight and initial weight at fast, with daily weight gain (DWG) as the ratio between TWG and the number of experimental days (84). The animals were subjected to paddock rotation every seven days, in order to minimize possible differences concerning forage availability and characteristics of the paddocks (and location of drinkers, troughs, relief and shading). The monitoring of the body condition score (BCS) was performed by three trained individuals, using the scale recommended by the NRC (1996).

On the fourteenth day of each experimental period, a collection was performed to determine DM total mass/ha (Table 2). The area to be sampled was delimited with an iron square (0.5 × 0.5 m) in four random sites in each experimental paddock. The samples were cut at ground level with scissors and then aliquots of each collected sample were taken, and composite samples were prepared for each paddock. An aliquot of the composite sample was separated into green leaf blade, dry leaf blade, green stem and sheath and dry stem and sheath to determine the mass of morphological components (Table 2).

Afterwards, the samples were weighed and dried in a forced circulation oven (60 °C) for 72 hours, processed in a knife mill (1 and 2 mm) and placed in containers previously identified for further analysis. The DM content was quantified (Silva & Queiroz, 2002).

Sampling for qualitative assessment of the pasture consumed by the animals was obtained via manual grazing simulation on the fourteenth day of each experimental period. The samples were dried under forced ventilation (60 ºC), processed in a knife mill (1 and 2 mm mesh sieve) and then packed in containers previously identified for analysis. During the digestibility trial, grazing manual simulation was performed on the eighth day (42nd of the productive performance).

For assessments of the nutritional characteristics, the same heifers and the area of productive performance were

Table 1 - Chemical composition on the dry matter basis

Item (g/kg)	Multiple supplement	*B. decumbens*[1]			
		June	July[2]	August	Average
Dry matter	888.7	313.8	323.6	419.4	352.3±33.7
Crude protein	298.5	71.0	68.0	64.9	68.0±1.8
Ether extract	22.4	16.5	16.9	14.3	15.9±0.8
Neutral detergent fiber corrected for ash and protein	147.6	632.4	632.8	687.8	651.0±18.4
Organic matter	967.2	905.3	896.3	896.5	899.4±3.0
Non-fibrous carbohydrates	498.7	185.4	178.6	129.5	164.5±17.6
Lignin	15.0	39.2	39.0	44.9	41.0±1.9

[1] Samples obtained by manual grazing simulation.
[2] Sample collected during digestibility trial.

Table 2 - Herbage mass and morphological components of pasture during experiment periods

Item	*Brachiaria decumbens* (kg/ha)			
	June	July	August	Average
Total dry matter	4,967.0	4,498.5	4,105.8	4,523.8±248.9
Potentially digestible dry matter	2,636.6	2,647.0	2,461.5	2,581.7±60.2
Green leaf blade	640.3	599.6	582.7	607.5±17.1
Dry leaf blade	865.5	918.6	762.5	848.8±45.8
Green stem and sheath	2,115.0	1,414.4	910.6	1,480±349.2
Dry stem and sheath	1,346.3	1,566.0	1,850.1	1,587.4±145.8

used. The digestibility trial lasted nine days, starting on the 35th day of the productive performance and ending on the 43rd day; the first six days were for adaptation to chromic oxide external marker (to estimate fecal excretion) and titanium dioxide (to estimate supplement intake) and the last three days were for collection of feces at different times, 15h00, 10h00 and 7h00.

Ten grams of chromic oxide marker were given to each animal per day, introduced with the aid of an applicator via the esophagus at 9h00 and 10 grams of titanium dioxide marker were given to each animal per day mixed with multiple supplement.

Feces were collected immediately after animal defecation or directly in the rectum, at quantities of approximately 200 g, individually identified and dried in a forced air circulation oven (60 °C). After this period, the samples were processed in a knife mill (1 and 2 mm mesh sieve) and samples composed of the three days of collection were made.

On the 9th day of the digestibility trial, spot urine sample (10 mL) was collected from animal spontaneous urination four hours after supplement supply (Valadares et al., 1999). After collection, urine samples were diluted in 40 mL of H_2SO_4 (0.036N) and stored at -20 °C for subsequent quantification of the levels of creatinine, urea and purine derivatives.

Samples of forage, feces and ingredients used to produce the supplement, processed in a 1 mm mesh sieve mill, were evaluated for DM, organic matter (OM), CP, ether extract (EE) and lignin (H_2SO_4 72% w/w) according to the techniques described by Silva & Queiroz (2002); neutral detergent fiber (NDF) was evaluated according to techniques described by Mertens (2002), using thermostable α-amylase, but omitting the use of sodium sulfite; corrections for protein and ash in the NDF followed the procedures described by Licitra et al. (1996) and Mertens (2002), respectively.

The levels of non-fibrous carbohydrates (NFC) were obtained according to the equation proposed by Detmann & Valadares Filho (2010):

$$NFC = 100 - [\,MM + EE + NDFap + (CP - CPu + U)]$$

in which: NFC = non-fibrous carbohydrates; MM = mineral matter content; EE = ether extract content; NDFap = content of neutral detergent fiber corrected for ash and protein; CP = crude protein content; CPu = urea crude protein content; and U = urea content. All other items are expressed as DM %.

Fecal samples were analyzed for the levels of titanium dioxide according to the colorimetric technique described by Titgemeyer et al. (2001) and chromic oxide in atomic absorption spectrophotometer as described by Williams et al. (1962). Feces excretion was estimated through the relationship between dose and fecal concentration of chromic oxide.

To estimate the voluntary feed intake, indigestible neutral detergent fiber (iNDF) was used according to Detmann et al. (2001), quantified by *in situ* incubation procedures with Ankon® bags (F57) for 288 hours in samples processed to 2 mm. The estimate was done by the following equation:

$$IIpDM = \frac{[(FE \times iFC) - iS]}{iFoC}$$

in which: IIpDM = individual intake of pasture dry matter (kg/day); FE = feces excretion (kg/day); iFC = iNDF fecal concentration (kg/kg); iS = iNDF intake from the supplement (kg/day) and iFoC = iNDF forage concentration (kg/kg).

The estimation of individual supplement intake was obtained by the following equation:

$$SupII = \frac{(FE \times iFC)}{iFG} \times SupFG$$

in which: SupII = individual intake of supplement (g/day); FE = feces excretion (g/day); iFC = titanium dioxide fecal concentration (g/g); iFG = titanium dioxide in the supplement fed to the group of animals (g/day); SupFG = amount of supplement fed to the animals (g/day).

The total DM intake (kg/day) was estimated by summing IIpDM and SupII.

Forage samples collected for evaluation of moment mass at a given experimental period were evaluated for DM, NDF and iNDF as described above. The percentage of potentially digestible DM (DMpd) in the forage in each experimental period was estimated according to Paulino et al. (2008):

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

in which: DMpd = forage content of potentially digestible DM (DM%); 0.98 = true digestible coefficient of the cell content; and NDF and iNDF = forage content of NDF and iNDF, respectively (DM%).

Urine samples, after thawing, were analyzed for levels of creatinine, according to the modified method of Jaffé (Bioclin® K016-1); uric acid, by enzymatic-colorimetry with clearing factor of lipid (Human® 10687); allantoin, according to the colorimetric method described by Chen & Gomes (1992), and urea, by the method Urease/GLDH.

The total volume of urine was estimated through the relationship between daily excretion of creatinine in function of the body weight and urine creatinine concentration. Creatinine excretion per body weight unit was obtained according to the equation (Chizzotti et al., 2006):

$$CE = 32.27 - 0.01093 \times BW$$

in which: CE = daily excretion of creatine (mg/kg BW); and BW = body weight (kg).

The urea daily urinary excretion was estimated by multiplying its concentration in urine spot samples and the urinary volume estimated. The excretion of purine derivatives was calculated by the sum of allantoin and uric acid excreted in the urine.

The purines absorbed were calculated from the excretion of purine derivatives by the equation (Barbosa et al., 2011):

$$AP = \frac{PD - 0.301 \times WB^{0.75}}{0.80}$$

in which: AP = absorbed purines (mmol/day); PD = excretion of purine derivatives (mmol/day); 0.301 = excretion of endogenous purine derivatives (mmol) in the urine per unit of metabolic size ($BW^{0.75}$); and 0.80 = recovery of absorbed purines as purine derivatives in the urine (mmol/mmol).

The rumen synthesis of microbial nitrogenous compounds was estimated in function of the AP using the equation described by Chen & Gomes (1992):

$$N_{mic} = \frac{70 \times AP}{0.83 \times R \times 1000}$$

in which: N_{mic} = microbial nitrogen compound flow in the small intestine (g/day); R = N_{RNA}:N_{TOTAL} ratio in the microorganisms (mg/mg); 70 = nitrogen content in the purines (mg/mmol); and 0.83 = intestinal digestibility of microbial purine (mg/mg). The N_{RNA}:N_{TOTAL} ratio of 0.134 was used, according to Valadares et al. (1999).

The experiment was analyzed in a completely randomized design with four treatments (mineral supplement and three levels of of multiple supplement supply) and seven replicates. After the analysis of variance, treatments were compared by means of orthogonal decomposition of the sum of squares of the treatments in linear, quadratic and cubic order effects related to the effect of level of supplementation, with subsequent adjustment of the linear regression equations. Calving order (primiparous and multiparous) was considered as criterion for local control.

Statistical procedures were conducted by means of PROC GLM of SAS (Statistical Analysis System, version 9.2), adopting 0.10 as the critical level of probability of type I error and body weight as a covariate.

Results and Discussion

The evaluation of pasture DM production and quality is paramount in a grazing cattle system, because this is the baseline feed for the animal that selects and collects the material to be consumed.

Thus, it is appropriate to discuss the production of potentially digestible DM (DMpd), which corresponds to the fraction potentially convertible into animal product because it integrates quantity and quality regardless of season.

The average masses (Table 2) throughout the DM and DMpd trial period were 4,524 and 2,582 kg/ha, respectively. The mass of DMpd expressed in function of the body weight (BW) was 27.6 g/kg, below the value of 40 to 50 DMpd g/kg BW of animals suggested by Paulino et al. (2004), aiming at associating production per animal and per areas satisfactory.

Due to the recognized seasonality of "qualitative-quantitative" production of tropical forages, to define grazing management strategies based on pasture condition, one should establish management targets for each season, whereas in the dry season, one should minimize the morphological differentiation and live with senescence (Paulino et al., 2008).

In this period, forage production is severely reduced, senescence of leaves and tillers is accelerated and tropical pastures, especially those kept under grazing, usually have low availability of good quality forage (Santos et al., 2004).

As the dry season progresses, the percentage of dry leaf and dry stem and sheath, that is, dead and low nutritional value material, increased (Table 2).

The weight gain of animals is linearly and negatively influenced by dead forage availability (Santos et al., 2004) and linearly and positively influenced by the mass of green forage (Cabral et al., 2011), consisting of green leaf blade and green stem and sheath.

In their feeding procedure, herbivores have the challenge of feeding from a resource that is complex and dynamic in time and space, in which the potentially

grazeable and desirable portion of a forage canopy is the layer represented by the leaf blades.

The average content of 68 g CP/kg DM of forage (Table 1) was below the minimum needed to sustain microbial growth and to promote digestion of fibrous carbohydrates of low quality forage (Lazzarini et al., 2009).

Daily weight gain (DWG) presented a quadratic pattern (P<0.10) for the multiple supplementation levels (Table 3), with a maximum performance at the level of 0.98 kg of multiple supplement supply.

The decrease in gain efficiency as the content of the supplement in the diet increased highlights the effect of catalytic supplementation of essential limiting microbial substrates cited by Paulino et al. (2008). The basic principle in pasture supplementation should be the lack of effect of substitution of forage by the supplement with promotion of intake and digestibility of nutrients.

The final body condition score showed a positive linear pattern (P<0.10), confirming the data of Ruas et al. (2000), working with protein supplementation of Nellore cows. The evaluation of body condition score (BCS) is efficient because it takes into account the accumulation of body reserves, which the female has to mobilize during the phase of lactation (Oliveira et al., 2006).

The initial body condition score of the cows was greater than the minimum body condition score at parturition (5.0) recommended by the NRC (2000) so females have a good reproductive performance in the next breeding season, showing the importance of quantitative and qualitative traits of the basal feeding, because those animals were grazing only with mineral supplementation during the rainy season prior to this experiment.

The females of the control treatment maintained the BCS with a small increase to the level of 0.5 kg of multiple supplement supply and significant difference as the level of supplementation increased (Figure 2).

Ruas et al. (2000) observed no change in the BCS of cows without supplementation during the study period, and there was a linear increase in the BCS in the treatments in which animals were supplemented (1 or 2 kg of concentrate per cow per day).

There was an increasing linear effect (P>0.10) of the levels of multiple supplement supply on intake of CP, EE and non-fibrous carbohydrates (NFC). There was no effect (P>0.10) for intakes of DM (kg/day), pasture DM (PDM), OM, pasture OM, neutral detergent fiber corrected for ash and protein (NDFap), indigestible NDF (iNDF), digested NDF (dNDF) and total digestible nutrients (TDN) (Table 4).

The increasing intake profiles CP, EE and NFC occurred by increasing the supply of multiple supplement in the different treatments, and this was the greater source of these nutrients (Table 1) compared with pasture. In contrast, NDFap and iNDF intake showed the same profile (P>0.10) of PDM intake (Table 4) as the primary source of these fractions was the forage.

Although multiple supplementation increases the CP content of the diet, it did not cause significant increase in the extraction of energy from the NDF, because there was no effect on dNDF intake and the increase in the intakes of CP, EE and NFC was not sufficient to increase TDN intake.

The DM intake (g/kg BW) showed a linear pattern with levels of multiple supplementation, close to that found by

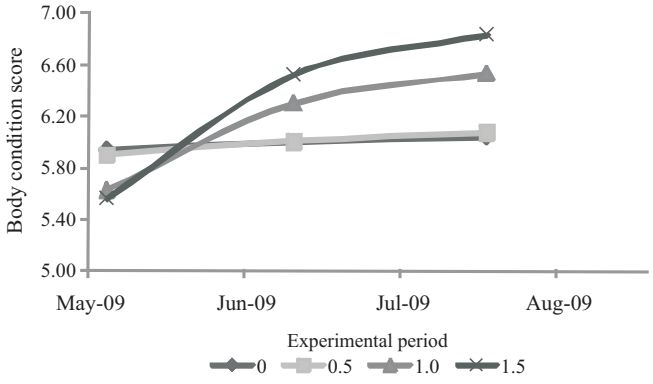


Figure 2 - Body condition score evolution over the experimental period.

Table 3 - Least square means, standard error of the mean and significance of effects for productive performance

	Multiple supplement (kg/day)				Standard error	P value		
	0	0.5	1.0	1.5		Linear	Quadratic	Cubic
Initial body weight (kg)	456.9	447.2	446.7	429.9	2.0			
Final body weight (kg)	490.5	485.4	496.8	470.6	8.3	0.540	0.555	0.541
Daily weight gain (g)[1]	391.2	445.1	582.5	472.7	22,8	0.080	0.095	0.167
Initial body condition score	5.94	5.90	5.63	5.56				
Final body condition score[2]	6.04	6.07	6.53	6.83	0.04	<0.001	0.204	0.217

[1] $\hat{Y} = 374.66 + 321.9300x - 163.7000x^2$ ($R^2 = 0.7190$).
[2] $\hat{Y} = 5.94 + 0.5660x$ ($r^2 = 0.9183$).

Valente (2009) of 20.7 g/kg BW previous year with the same herd of females.

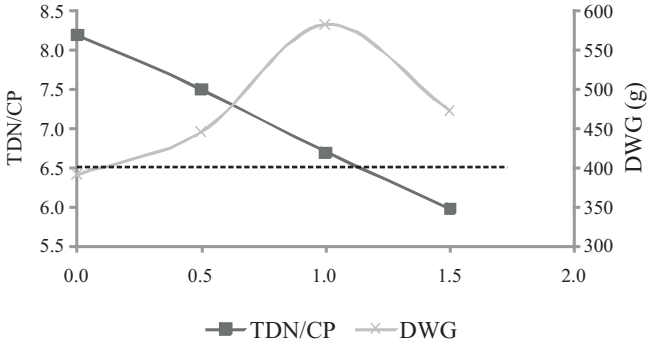
Results obtained in tropical conditions with low-quality forages indicate that direct responses on total intake or digested components are stimulated by supplementation with nitrogen compounds up to levels of 80 to 100 g CP/kg DM in the diet (Lazzarini et al., 2009; Detmann et al., 2010; Figueiras et al., 2010). From this point, the response to this type of supplementation becomes not very evident on the intake and nitrogen compound losses become more prominent (Detmann et al., 2009).

The average levels of CP in the diet, calculated from the ratio of the total CP intake (pasture and supplement) and total DM intake, were 68, 80, 90 and 101 g/kg, for treatments 0, 0.5, 1.0 and 1.5 kg supplement/animal/day, respectively, but there was no exclusive supplementation with nitrogen compounds as quoted by the authors above in this study, and this is probably the reason of the absence of effect on dNDF intake.

The TDN/CP ratios in diets for the different treatments, from control up to 1.5 kg supplement/animal/day, were 8.2, 7.5, 6.7 and 6, with maximum performance occurring at 1.0 kg supplementation, which promoted a TDN/CP ratio closer to the recommended by the NRC (2000), of 6.5 for maintenance and BW gain of 500 g/day (Figure 3).

The ratio between metabolizable protein and metabolizable energy, herein represented by the TDN/CP ratio, is one of the determinants of intake (Illius & Jessop, 1996) and the adjustments made by the animal concerning increase or decrease of fiber utilization (digestibility of NDFap; Table 5).

The total apparent digestibility of DM and OM showed cubic profiles (P<0.10) with maximum points at 0.64 and 0.68 kg of multiple supplement supply, respectively (Table 5). The lowest apparent digestibility of DM and OM occurred at 1.33 and 1.29 kg of supplement/animal/day.



The dashed line represents the TDN/CP ratio dictated by nutritional requirement for maintenance and gain of 0.5 kg/day according to data from the NRC (2000).
TDN - total digestible nutrients; CP - crude protein; DWG - daily weight gain.

Figure 3 - Relationship of levels of total digestible nutrients and crude protein (TDN/CP) and performance (DWG) in g according to the multiple supplement intake in the different treatments.

Table 4 - Least square means, standard error of the mean and significance of effects for voluntary intake

	Multiple supplement (kg/day)				Standard error	P value		
	0	0.5	1.0	1.5		Linear	Quadratic	Cubic
			kg/day					
Dry matter	9.03	9.29	10.20	9.81	0.36	0.354	0.692	0.607
Pasture dry matter	9.03	8.81	9.25	8.45	0.32	0.672	0.681	0.582
Organic matter	8.09	8.36	9.21	8.89	0.32	0.308	0.693	0.609
Pasture organic matter	8.09	7.89	8.29	5.57	0.27	0.672	0.681	0.582
Crude protein[1]	0.62	0.75	0.92	0.99	0.03	0.001	0.739	0.690
Ether extract[2]	0.15	0.16	0.18	0.18	<0.01	0.096	0.701	0.625
Non-fibrous carbohidrates[3]	1.63	1.81	2.12	2.19	0.08	0.012	0.717	0.652
NDFap	5.72	5.66	6.03	5.60	0.21	0.992	0.684	0.589
iNDF	1.77	1.74	1.84	1.70	0.06	0.869	0.682	0.586
dNDF	3.69	3.82	3.99	3.64	0.13	0.999	0.403	0.680
Total digestible nutrients	5.09	5.59	6.15	5.93	0.22	0.144	0.447	0.715
			g/kg of body weight					
Dry matter[4]	18.65	19.45	20.97	21.38	0.55	0.074	0.884	0.746
Pasture dry matter	18.65	18.49	19.05	18.51	0.49	0.977	0.859	0.729
Organic matter	18.10	18.70	20.59	19.89	0.72	0.308	0.693	0.609
Pasture organic matter	18.10	17.66	18.54	16.93	0.65	0.672	0.681	0.582
NDFap	12.79	12.67	13.49	12.53	0.47	0.992	0.684	0.589
iNDF	3.65	3.65	3.79	3.72	0.10	0.704	0.863	0.732

NDFap - neutral detergent fiber correct for ash and protein; iNDF - indigestible neutral detergent fiber; dNDF - digested neutral detergent fiber.
[1] $\hat{Y} = 0.63 + 0.2566x$ ($r^2 = 0.9791$).
[2] $\hat{Y} = 0.15 + 0.0210x$ ($r^2 = 0.8576$).
[3] $\hat{Y} = 1.64 + 0.4000x$ ($r^2 = 0.9498$).
[4] $\hat{Y} = 18.66 + 1.9420x$ ($r^2 = 0.9582$).

In addition to increasing the level of CP in the diet, the multiple supplement also increased the level of the NFC, which could have caused competition between fermenting NFC and fibrolytic microorganisms at higher levels of multiple supplementation, which led to negative effects on the degradation of NDF (Table 5), the so-called "carbohydrate effect". In this context, the low level of supplementation (catalytic supplementation) was sufficient to supply the essential limiting microbial substrates and maximize degradation of NDFap.

The multiple supplementation levels promoted a quadratic effect (P<0.10) on apparent digestibility of CP and EE and on the digestibility of NDFap and TDN. The maximum digestibility for each of the variables described above occurred at 1.52, 0.72, 0.78 and 1.1 kg, respectively. However, the maximum point in the apparent digestibility of CP occurred outside the range of supplementation levels analyzed in this study and should be observed as a linear relationship with the treatments.

The apparent digestibility of NFC showed a positive linear profile (P<0.10) with the levels of supplement supply. The apparent digestibility of CP, EE and NFC were lower for the control treatment by the effect of higher proportion of metabolic fecal fraction in relation to the nutrient ingested (Cabral et al., 2006).

For the calculation of TDN, information from the digestibility of CP, EE, NFC and NDFap is used; therefore, its maximum value occurs at an intermediate level of supplementation among the maximum digestibilities of these fractions.

An increasing linear effect (P<0.10) of the levels of multiple supplements on the urinary urea nitrogen excretion was observed (Table 6), which is a pattern similar to that found by several authors (Pereira et al., 2007; Figueiras et al., 2010).

The urea concentration found in the urine is positively correlated to plasma concentrations of nitrogen and CP intake (Van Soest, 1994). Thus, urinary urea nitrogen excretion is an indicator of the ruminal nitrogen use efficiency and of the balance in the dietary protein/energy.

Gestation reflects in greater efficiency in the use of nitrogen in the tissues in maternal system (Hanks et al., 1993; Scheaffer et al., 2001), which could result in less formation of urea in the liver, lower recycling to the rumen and excretion in the urine.

There was no effect (P>0.10) for flow of microbial nitrogen compounds (FMNC) and microbial synthesis efficiency (MSE) in relation to the levels of multiple supplementation. In relation to nitrogen intake, FMNC showed decreasing linear behavior (P<0.10) in function of the different treatments (Table 6).

Table 5 - Digestibility coefficients and total digestible nutrients according to different treatments

	Multiple supplement (kg/day)				Standard error of the mean	P value		
	0	0.5	1.0	1.5		Linear	Quadratic	Cubic
Dry matter[1]	57.03	61.00	60.69	60.48	0.20	<0.001	<0.001	0.092
Organic matter[2]	60.21	63.92	63.79	63.77	0.18	<0.001	<0.001	0.067
Crude protein[3]	46.01	55.38	56.51	60.18	0.74	<0.001	0.089	0.181
Ether extract[4]	4.28	20.21	13.66	13.74	2.90	0.717	0.020	0.454
Non-fibrous carbohydrates[5]	66.35	69.37	73.22	75.42	0.86	<0.001	0.827	0.785
NDFap[6]	64.61	67.55	66.38	65.34	0.32	0.738	0.009	0.220
Total digestible nutrients[7]	56.05	60.02	60.17	60.40	0.22	<0.001	<0.001	0.104

NDFap - neutral detergent fiber correct for ash and protein.
[1] $\hat{Y} = 59.63 + 12.9400x - 14.9200x^2 + 5.0400x3$ ($R^2 = 1.000$).
[2] $\hat{Y} = 62.41 + 13.1600x - 14.7800x^2 + 5.0000x3$ ($R^2 = 1.0000$).
[3] $\hat{Y} = 46.55 + 17.2780x - 5.7000x^2$ ($R^2 = 0.9468$).
[4] $\hat{Y} = 5.24 + 37.1410x - 25.8500x^2$ ($R^2 = 0.9032$).
[5] $\hat{Y} = 66.43 + 6.2120x$ ($r^2 = 0.9902$).
[6] $\hat{Y} = 64.82 + 6.1740x - 3.9800x2$ ($R^2 = 0.8170$).
[7] $\hat{Y} = 56.25 + 8.2500x - 3.7400x2$ ($R^2 = 0.9414$).

Table 6 - Metabolism of nitrogenous compounds according to the different treatments

	Multiple supplement (kg/day)				Standard error of the mean	P value		
	0	0.5	1.0	1.5		Linear	Quadratic	Cubic
Urinary urea nitrogen excretion (g/day)[1]	38.30	67.80	80.20	108.00	5.05	<0.001	0.934	0.513
Flow of microbial nitrogen compounds (g/day)	93.12	110.96	119.06	110.19	4.29	0.232	0.192	0.877
Microbial synthesis efficiency (g microbial CP/kg TDN)	115.11	125.41	120.15	121.47	2.74	0.654	0.481	0.457
FMNC/NI[2]	0.76	0.80	0.71	0.66	0.02	0.095	0.352	0.371

FMNC/NI - flow of microbial nitrogen compounds in relation to nitrogen intake (g/g nitrogen intake).
[1] $\hat{Y} = 40.4 + 44.3000x$ ($r^2 = 0.9786$).
[2] $\hat{Y} = 79.05 - 7.6020x$ ($r^2 = 0.6683$).

Several authors (Lazzarini et al., 2009; Figueiras et al., 2010; Souza et al., 2010) also found no increase in FMNC with supplementation in tropical conditions, compared with the control treatment. The estimate of average MSE of 120.5 g microbial CP/kg TDN is close to that suggested by Valadares Filho et al. (2010).

Lazzarini et al. (2009) found that nitrogen intake was lower than the FMNC at the lowest level of CP in the diet (52.8 g CP/kg) and evaluation of FMNC/NI by using the regression equation as a function of CP levels in the diet indicated that the estimates of these variables become equivalent to each other at 71.3 g CP.

In this study, there was no equivalence between nitrogen intake and flow of microbial nitrogen compounds, even at the level of 68 g of CP in the diet of animals in the control treatment, probably due to the increased use of nitrogen in the tissues, which resulted in lower formation of urea in the liver and recycling to the rumen.

Conclusions

Multiple supplementation optimizes performance of grazing pregnant cows in the dry season and promotes the increase of body condition score. The level of supply of 1.0 kg of multiple supplement with 300 g CP/kg DM maximizes the performance of cows grazing in the dry season.

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