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REGULAR ARTICLES

Supplementation of suckling beef calves with different levels of crude protein on tropical pasture

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Abstract The effects of supplementation with different levels of crude protein on performance, intake and nutrient digestibility and efficiency of microbial protein synthesis in suckling beef calves on pasture were assessed. Fifty-five calves, with an average age of 100 days and an initial average body weight of 110±7.5 kg and their respective dams, were used. The experimental design was completely randomised with five treatments and 11 replications. The experimental treatments for calves were as follows: control = calves received only mineral mixture; supplementation levels = calves received supplement containing 8, 19, 30 or 41 % of crude protein (CP, at a rate of 0.5 % of body weight (BW)). The cows received only mineral mixture *ad libitum*. Supplemented calves had higher ($P<0.1$) average daily gain (ADG). Protein levels showed a quadratic effect ($P<0.1$) on average daily gain (ADG) of calves. There was no difference in total dry matter (DM) intake ($P>0.1$). However, intake of dry matter forage (DMF) presented cubic profiles ($P<0.1$), with CP levels in the supplements. Supplementation increased ($P<0.1$) the digestibility of nutrients, except for the digestibility of neutral detergent fibre. Supplementation increased ($P<0.1$) the production of microbial nitrogen and N losses in urine. It can be concluded that multiple supplementations optimise the performance of beef calves on creep feeding. The intake of supplements with CP levels between 8 and 30 % partially replaces of the pasture ingested by calves and increases the digestibility of the diet.

Keywords Calves · Creep feeding · Digestibility · Intake · Nellore · Supplementation

Introduction

In the production of young beef cattle on pastures, continuous weight gain from initial suckling until slaughter is critical to the success of the production system. In beef cattle, milk is not sufficient to supply the calves' requirements when they are about 3 months of age (Henriques et al. 2011). From this age, the calf becomes increasingly dependent on pasture; thus, strategic supplementation can increase animal performance (Valente et al. 2012). Strategic supplementary feeding of suckling calves using the creep-feeding system assumes great importance. The creep-feeding system refers to the supply of concentrated feeds in a place to which access is restricted to calves (Paulino et al. 2012).

The lack of information about the feasibility and the nutritional basis involved in the response to supplementation at every stage of life of animals has hindered the adoption of the practice of supplementation on production systems.

Thus, the objective of this study was to evaluate the effect of supplementation with different levels of crude protein on performance, intake and nutrient digestibility and efficiency of microbial protein synthesis in suckling beef calves on *Urochloa decumbens* pasture.

Material and methods

All procedures involving animals were approved by the Brazilian Committee for Animal Care and Experimentation.

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Animals, experimental design and diets

The experiment was conducted at the Universidade Federal de Viçosa, Viçosa, MG, Brazil, between February and June 2011. The experimental area is located in a hilly region at an altitude of 670 m. Throughout the days of the measurement, the average minimum and maximum temperatures were 18.2 and 30.9 °C in February, 18.9 and 27.5 °C in March, 17.4 and 26.5 °C in April, 13.6 and 24.7 °C in May and 11.4 and 22.9 °C in June. The rainfall was 120 mm in February, 249 mm in March, 43 mm in April, 3 mm in May and 22 mm in June.

Fifty-five beef calves in the suckling phase with an average age of 100 days, and an initial average body weight of 110±7.5 kg and their respective dams (30 Nellore and 25 ¾ Nellore × ¼ Holstein) with an average body weight of 450±15.6 kg were used in this experiment. Only Nellore sires were used in this study. The cow-calf was randomly assigned to one of the five following experimental treatments: control = calves received only mineral mixture; supplementation levels = calves received supplement containing 8, 19, 30 or 41 % of crude protein (CP) (Table 1).

Animals were submitted to 14 days of adaptation to the diet and to the experimental area and 140 days (five periods of 28 days) for experimental evaluation. For performance evaluation, the animals were weighed at the beginning and end of the experiment after 14 h of fasting. At the beginning of the experiment and every 28 days thereafter, they were weighed without fasting and always in the morning, in order to adjust the amount of supplement to be provided to each group (at a rate of 0.5 % of the body weight (BW) of the calves). Cow-calf pairs were placed on a 10-ha plot with *U. decumbens* pastures. There were private feeders for the each group of calves (0.5 m per calf), and the cows had no access. Calves were fed once a day at 11 a.m. Cows received a mineral mixture ad libitum.

In order to minimise the possible effects of the plots on the experimental treatments, animals were rotated among the five pastures every 7 days, so each group stayed for the same period of time on each plot.

Table 1 Supplement composition (%) on the natural matter basis

Ingredient %	Level of crude protein			
	8	19	30	41
Ground corn grain	48	33	18	3
Ground sorghum grain	48	33	18	3
Soybean meal	0	30	60	90
Mineral mixture	4	4	4	4

Percentage composition: dicalcium phosphate, 50.0; sodium chloride, 47.2; zinc sulfate, 1.5; copper sulfate, 0.7; cobalt sulfate, 0.05; potassium iodate, 0.05; and manganese sulfate, 0.5

Experimental procedures and sampling

Forage samples were collected on the 14th day of each experimental period to evaluation of forage mass. In each plot, four forage samples were randomly selected using a metal square (0.5×0.5 m) and cut at approximately 1 cm above the ground.

Sampling for the qualitative assessment of forage consumed by the animals was obtained every 14 day by the hand-plucked method. All the samples were dried (60 °C/72 h) and ground (1 and 2 mm).

Seventy days after the beginning of the experiment, a 9-day trial to evaluate the intake and digestion of the calves was undertaken. It took 6 days for the adaptation of animals to titanium dioxide (TiO_2) and to chromium oxide (Cr_2O_3) and 3 days for faeces collection at different times: 3:00 p.m., 10:00 a.m. and 6:00 a.m. Chromium oxide (Cr_2O_3), used to estimate faecal excretion, was packaged in paper cartridges in the amount of 10 g per animal/day for calves and was directly introduced into the oesophagus by using a rubber tube, always at 10:00 a.m.; while titanium dioxide (TiO_2), used to estimate supplement individual intake, was mixed with the supplement distributed to the calves in an amount equal to 10 g per animal/day. The faecal samples were dried at 60 °C for 72 h, then ground to pass through a 1-mm screen, and proportionally subsampled into a composite sample.

To evaluate the microbial protein production of calves, spot urine samples (10 ml) were collected from spontaneous urination 4 h after supplement intake (in the 80th experimental day). Urine samples were diluted in 40 ml of H_2SO_4 (0.036 N) and frozen at −20 °C. After urine collection, blood samples were collected by jugular vein puncture, using vacuum tubes with separator gel (BD Vacutainer® SSTIIAdvance), centrifuged at 3500× for 10 min, and the plasma was then frozen at −20 °C.

The estimation of the dry matter milk intake (DMM) by calves was the average of the estimation obtained on days 30, 82 and 110 of the experimental period. The procedures were performed similarly to the description by Valente et al. (2012).

Chemical analysis

Forage, faeces and supplement ingredient samples were analysed for dry matter (DM), nitrogenous compounds, ash and ether extract (EE) according to the Association of Official Analytical Chemists (AOAC 1990). For analysis of neutral detergent fibre (NDF), samples were treated with alpha thermostable amylase without sodium sulfite and corrected for ash residue (Mertens 2002) and residual nitrogen compounds (Licitra et al. 1996). Indigestible neutral detergent fibre (iNDF) was analysed as described by Valente et al. (2011b). Faecal samples were evaluated for chromium and titanium dioxide content by using atomic absorption (Williams et al. 1962) and colorimetric methods (Myers et al. 2004), respectively.

The faecal excretion was estimated by rationing the quantity of chromic oxide offered and the concentration in faeces.

Individual intake of the supplement was estimated (DMS) by relation of excretion of TiO_2 in faeces and marker concentration in the supplement.

The dry matter forage intake (DMF) was carried out using an internal iNDF, using the following equation:

$$\mathrm{DMF} = [(\mathrm{FE} \times \mathrm{iNDFfaeces}) - \mathrm{DMSi} \times \mathrm{iNDFsup}]\ \mathrm{iNDFforage}$$

where FE=faecal excretion (kilograms per day), iNDFfaeces=concentration of iNDF in the faeces (kilograms per kilogram), DMSi=dry matter supplement intake (kilograms per day), iNDFSup=concentration of iNDF in the supplement (kilograms per kilogram) and iNDF forage=concentration of iNDF in the forage (kilograms per kilogram).

The total intake of DM was calculated by the sum of DMF intake, DMS intake and DMM intake.

Analysis of serum levels of urea and urinary levels of urea, creatinine, uric acid and allantoin, as well as production of microbial nitrogen compounds (MICN) was performed similarly to the description by Valente et al. (2011a). Efficiency of protein microbial synthesis was determined by dividing protein microbial production by the TDN intake.

Statistical analysis

The experiment was conducted according to a completely randomised design, including the fixed effect of treatments and using the initial body weight of the calves as a covariate. The comparisons among the treatments were performed by using a set of orthogonal contrasts, which encompassed a comparison between the control treatment and the treatments with supplementation, and the linear, quadratic, and cubic effects of CP concentration in the supplements. All statistical procedures were performed adopting 0.1 as the critical level of probability for the type I error and the GLM procedure of the Statistical Analysis System 9.1 (SAS Institute, Inc.).

Results

The average availability of DM during the experiment was 3.7 t/ha. The forage samples collected by the hand-plucked method had an average content of 11.6 % CP (Table 2), being above the critical threshold of 9–10 % CP reported by Lazzarini et al. (2009) for maximum voluntary intake of tropical forage by grazing cattle.

There were no differences ($P>0.1$) in total DM intake (Table 3). The supplemented animals showed lower DMF intake ($P<0.1$) than the non-supplemented animals. Among the supplemented animals, a cubic effect ($P<0.1$) of the level of CP in the supplements on the DMF intake was observed. Similar behaviour was observed for the intakes of NDFap and iNDF expressed in grams per kilogram of BW. The CP intake was higher in the supplemented animals, and an increasing linear effect was found on them ($P<0.1$) (Table 3).

Table 2 Chemical composition of the supplements and forage

Item	Level of crude protein				*U. decumbens*[a]
	8	19	30	41	
DM	91.7	91.9	92.2	92.5	27.0±0.8
OM[b]	94.9	93.5	92.1	90.6	91.1±0.2
CP[b]	7.7	18.3	29.0	39.6	11.6±0.8
EE[b]	2.6	2.3	2.0	1.7	1.7±0.1
NDFap[b]	9.2	8.5	7.7	7.0	54.4±0.9
NFC[b]	75.4	64.3	53.3	42.3	23.4±0.8
iNDF[b]	0.6	1.1	1.5	1.9	16.3±0.7
NDIN[c]	17.6	18.4	19.2	20.0	17.6±3.6

DM dry matter, *OM* organic matter, *CP* crude protein, *EE* ether extract, *NDFap* neutral detergent fibre correct for ash and protein, *NFC* non-fibrous carbohydrates, *iNDF* indigestible neutral detergent fibre, *NPN* non-protein nitrogen, *NIDN* insoluble neutral detergent nitrogen

[a] Mean ± standard error of the mean (samples from hand plucking)

[b] Percentage of DM

[c] Percentage of total nitrogen

Supplementation increased ($P<0.1$) the coefficient of digestibility of DM, OM, CP, EE, NFC and the value of TDN. On the other hand, NDFap digestibility was not affected by supplementation ($P>0.1$) or by CP levels in supplements (Table 4).

The supplemented animals had higher performance ($P<0.1$) than the non-supplemented animals. A quadratic effect ($P<0.1$) was found among the supplemented animals on average daily gain (ADG) and final body weight (FBW) for different levels of CP in supplements (Table 5). The maximum estimated ADG of 973 g occurred at the level of 35 % CP in the supplement, and the performance was approximately 34 % higher than in the non-supplemented animals.

The calves had urinary excretion of urea nitrogen (UUN) linearly increased ($P<0.1$) with CP level in supplement. By contrast, there was no difference ($P>0.1$) in the efficiency of microbial protein synthesis. The supplemented calves had higher production of microbial nitrogen compounds (MICN) ($P<0.1$) than control calves. Concentration of serum urea nitrogen (SUN) was higher in supplemented animals ($P<0.1$) in which an increasing linear effect was found (Table 6).

Discussion

The evaluation of the DM intake of milk showed no significant difference, confirming that the production of milk of the dams was similar in different groups of animals; therefore, this is not a factor that would interfere with the difference in performance among the animals.

Table 3 Adjusted means, coefficient of variation (CV) and significance indicative for intakes of nutrients for the different supplements

Item		Level of crude protein					P value[b]			
	Control[a]	8	19	30	41	CV (%)	CONT	L	Q	C
		kg/day								
DM	3.3	3.6	3.7	4.0	3.7	22.2	ns	ns	ns	ns
DMF	2.3	1.7	1.7	2.3	2.1	27.0	*	*	ns	*
DMS	–	0.8	0.8	0.8	0.8	–	–	–	–	–
DMM	1.0	1.1	1.1	1.0	1.0	22.3	ns	ns	ns	ns
OM	3.1	3.4	3.5	3.7	3.4	23.5	ns	ns	ns	ns
CP	0.3	0.6	0.6	0.7	0.7	26.1	*	*	ns	ns
CPM	0.2	0.2	0.2	0.2	0.2	24.5	ns	ns	ns	ns
EE	0.3	0.4	0.4	0.4	0.4	26.0	*	ns	ns	ns
NFC	0.5	1.0	0.9	0.9	0.7	13.8	*	*	*	*
		Intakes (g/kg of BW)								
DM	19.0	19.1	18.8	21.9	20.1	18.3	ns	ns	ns	ns
DMF	13.2	8.9	8.3	12.3	11.3	23.3	*	*	ns	*
NDFap	7.6	5.6	5.3	7.2	6.6	23.0	*	ns	ns	*
iNDF	2.3	1.5	1.5	2.1	2.0	22.8	*	*	ns	*

DM dry matter, *DMF* forage dry matter, *DMS* supplement dry matter, *DMM* milk dry matter, *OM* organic matter, *CP* crude protein, *CPM* milk crude protein, *EE* ether extract, *NDFap* neutral detergent fibre corrected for ash and protein, *NFC* non-fibrous carbohydrates, *iNDF* indigestible neutral detergent fibre

*significant at level of 0.1 of probability ($P<0.1$), ns non-significant ($P>0.1$)

[a] Mineral mixture ad libitum

[b] CONT = contrast between supplemented and non-supplemented; L, Q and C are effects of linear, quadratic and cubic order concerned to the levels 8, 19, 30 and 41 % of CP in the supplements

The lack of effect of total DM intake for the different treatments and the finding of a cubic effect for the intake of DMF show the occurrence of the substitutive effect of the intake of the offered supplement on the intake of forage. This context refers to a nutritional imbalance occurring when using multiple supplements with extreme levels of protein. In this case, the substitution was about 0.8, 0.8, 0.1 and 0.3 g of the intake of forage/gram of ingested supplements at levels of 8, 19, 30, and 41 % of CP in supplements. Valente et al. (2012) observed shorter grazing times in calves that received supplementation with 15.4 % CP, showing the occurrence of substitutive effect.

The cubic behaviour of the levels of CP in supplements on the intake of DMF seems to be related to the physiological

Table 4 Adjusted means, coefficient of variation (CV) and significance indicative for digestibility of nutrients for the different supplements

Item		Level of crude protein					P value[b]			
	Control[a]	8	19	30	41	CV (%)	CONT	L	Q	C
DM	68.2	71.5	72.0	71.7	69.9	5.9	*	ns	ns	ns
OM	70.7	74.3	75.4	74.7	73.2	6.1	*	ns	ns	ns
CP	45.1	67.8	69.1	72.9	73.5	12.1	*	ns	ns	ns
EE	86.0	90.2	91.0	85.9	90.9	5.3	*	ns	ns	ns
NDFap	69.1	70.3	70.9	71.8	71.4	5.4	ns	ns	ns	ns
NFC	42.5	66.7	65.9	67.3	55.5	16.0	*	ns	*	ns
TDN	60.5	73.9	77.3	73.9	72.7	14.2	*	ns	ns	ns

DM dry matter, *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

*significant at level of 0.1 of probability ($P<0.1$), ns non-significant ($P>0.1$)

[a] Mineral mixture ad libitum

[b] CONT = contrast between supplemented and non-supplemented; L, Q and C are effects of linear, quadratic and cubic order concerned to the levels 8, 19, 30 and 41 % of CP in the supplements

Table 5 Adjusted means, coefficient of variation (CV) and significance indicative for performance of calves for the different supplements

Item		Level of crude protein					*P* value[b]			
	Control[a]	8	19	30	41	CV (%)	CONT	L	Q	C
FBW[c]	222.5	235.6	250.5	245.5	245.1	5.5	*	*	*	ns
GMD[d]	727	820	926	889	886	10.6	*	*	*	ns

FBW final body weight in kilograms, *ADG* average daily gain in grams

*significant at level of 0.1 of probability ($P<0.1$), ns non-significant ($P>0.1$)

[a] Mineral mixture ad libitum

[b] CONT = contrast between supplemented and non-supplemented; L, Q and C are effects of linear, quadratic and cubic order concerned to the levels 8, 19, 30 and 41 % of CP in the supplements

[c] $\acute{y}=221.55+2.23X-0.04X^2$ ($R^2=0.716$)

[d] $\acute{y}=0.728+0.014X-0.0002X^2$ ($R^2=0.924$)

mechanisms regulating intake, since the values of NDF intake were low and NDFap digestibility was not affected by supplementation, indicating a low rumen fill effect.

The greater availability of energy substrates from supplementation with low protein levels (8 and 19 % CP) may have caused an imbalance and extended the formation of heat. This expansion of heat may be implicated in the reduced intake for adequacy of the animals in terms of thermal comfort, meaning that the use of basal forage is not optimal (Detmann et al. 2010).

There was also a reduction of the intake in animals that received supplementation with 41 % CP, which may have been caused by an excess of protein. The action of metabolic mechanisms of intake regulation was also checked under conditions of excessive nitrogen in the rumen that would imply a reduction in the consumption for animal fitness in terms of thermal comfort (Detmann et al. 2010).

In this study, the higher digestibility of DM can be associated with the inclusion of concentrated supplements (easy digestion) in the diet, instead of increasing the digestibility of forage, since the digestibility of NDFap was not affected by supplementation or by the levels of CP in supplements.

The increase in CP digestibility observed with supplementation may be associated with the dilution of the faecal metabolic fraction resulting from greater CP intake by animals that received multiple supplementation compared to non-supplemented animals. In addition, the apparent protein digestibility may have been magnified by higher ruminal N losses (Barros et al. 2011).

The improved performance observed in the supplemented animals may be a result of the inclusion of multiple supplements (digestible) (Porto et al. 2009). The highest performance estimated with supplements containing 35 % CP reinforces the priority of protein supplementation even in conditions where the forage does not apparently present deficiency. However, Detmann et al. (2005) pointed out that, unlike in dry season, the focus of supplementation in rainy season becomes to correct metabolic deficiencies, and not more dietary deficiencies.

The urea concentration found in the urine is positively correlated to the concentration of nitrogen in the plasma and with CP intake (Van Soest 1994). Thus, the UUN is an indicator of efficient nitrogen utilization in the rumen and of the balance in the protein/dietary energy ratio.

Table 6 Adjusted means, coefficient of variation (CV) and significance indicative for levels of nitrogen for the different supplements

Item	Level of CP in the supplement (%)						*P* value[b]			
	Control[a]	8	19	30	41	CV (%)	CONT	L	Q	C
UUN	28.5	24.2	38.3	50.1	52.1	31.5	*	*	ns	ns
MICN	46.1	57.2	58.9	75.4	69.6	25.7	*	*	ns	*
MEF	143.9	140.3	150.3	159.2	160.8	19.9	ns	ns	ns	ns
SUN	11.0	9.3	13.1	15.5	17.5	22.7	*	*	ns	ns

UUN urea nitrogen excretion in the urine grams per day, *MICN* production of microbial nitrogen compounds grams per day, *MEF* efficiency of microbial protein synthesis (microbial CP synthesis/TDN intake grams per kilogram), *SUN* serum urea nitrogen in milligrams per deciliter

*significant at level of 0.1 of probability ($P<0.1$), ns non-significant ($P>0.1$)

[a] Mineral mixture ad libitum

[b] Contrast between supplemented and non-supplemented; L, Q and C are effects of linear, quadratic and cubic order at to the levels 8, 19, 30 and 41 % of CP in the supplements

Most MICN synthesis, in response to nitrogen supplementation, indicates that the quantity of CP supplied by the forage and milk were insufficient to supply nitrogen compounds to optimise microbial growth.

Conclusions

Multiple supplementations optimise the performance of beef calves on creep feeding providing greater weight at weaning. The use of multiple supplements containing 35 % of crude protein for beef calves provides greater weight gain for the animals. The intake of supplements with CP levels between 8 and 30 % at the amount of 0.5 % of BW replaces part of the pasture ingested by calves and increases the digestibility of the diet.

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