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Supplementation levels for suckling female calves under grazing: productive and nutritional performance and metabolic profile

Níveis de suplementação para bezerras lactentes sob pastejo: desempenho produtivo e nutricional e perfil metabólico

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Highlights:
Scarce literature shows the optimal supplementation level to be used in female calves.
Our study evaluates to productive and nutritional performance and metabolic profile.
We evidenced differences in fat thickness over loin in the animals.
Our study not evidence differences in body growth and nutritional performance.
Our study not evidence differences in metabolic profile in the animals.

Abstract

The objective in this study was to evaluate the effects of supplementation levels on productive and nutritional performance and metabolic profile in suckling female calves under grazing. Forty female calves (averaging 3.5±0.06 months and 127.3±2.68 kg), and their respective dams were distributed in a completely randomized design with two treatments and twenty replicates. The treatments were 1) 4 g kg^{-1} body weight (BW) of supplement or 2) 6 g kg^{-1} BW of supplement. Forage and organic matter (OM) intake did not affect ($P > 0.05$) by levels of supplement, though crude protein and non-fibrous carbohydrates intake were greater ($P < 0.05$) by increasing supplementation level. There was no effect ($P > 0.05$) the supplementation levels on OM and CP digestibility. The metabolic profile of the animals was not affected ($P > 0.05$) by supplementation level. Average daily gain, *longissimus dorsi* area, fat thickness over rump of the animals did not affect ($P > 0.05$) by levels of supplement. However, there was trend of increasing ($P=0.074$) in fat thickness over loin by increase the supplementation level. Although the body growth of animals was similar ($P > 0.05$) between treatments, there was observed a trend of increase ($P=0.064$) in ratio BW:Height at the withers by increasing supplementation levels. In conclusion**,** increasing the supplementation level of 4 to 6 g kg^{-1} of BW, not improve the productive and nutritional performance and metabolic status in female calves under grazing on creep-feeding system.
Key words: Body growth. *Bos indicus*. Intake. Supplements. Tropical forage.

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Resumo

O objetivo deste estudo foi avaliar os efeitos dos níveis de suplementação sobre o desempenho produtivo e nutricional e perfil metabólico de bezerras lactentes sob pastejo. Quarenta bezerras lactentes (média de 3,5 ± 0,06 meses e 127,3 ± 2,68 kg) e suas respectivas foram distribuídas em delineamento inteiramente casualizado com dois tratamentos e vinte repetições. Os tratamentos foram 1) 4 g kg^{-1} de peso corporal (PC) de suplemento ou 2) 6 g kg^{-1} PC de suplemento. O consumo de forragem e matéria orgânica (MO) não foi influenciado (P > 0,05) pelos níveis de suplemento, porém, o consumo de proteína bruta e carboidratos não-fibrosos foi aumentado (P < 0,05) pelo incremento do nível de suplemento. Não houve efeito (P > 0,05) dos níveis de suplementação sob a digestibilidade da MO e PB. O perfil metabólico dos animais não foi afetado pelo nível de suplementação (P > 0,05). O ganho médio diário, área de *longissimus dorsi*, espessura de gordura sobre a garupa dos animais não foram afetados (P > 0,05) pelos níveis de suplementação. No entanto, houve tendência de aumento (P=0,074) na espessura de gordura no lombo pelo aumento do nível de suplementação. Embora o crescimento corporal dos animais foi similar (P > 0,05) entre os tratamentos, observou-se uma tendência de aumento (P=0,064) na relação BW: Altura na cernelha, com o aumento do nível de suplementação, Concluindo, o aumento do nível de suplementação de 4 a 6 g kg^{-1} de PC, não melhora o desempenho produtivo e nutricional e estado metabólico em bezerras lactentes sob pastejo no sistema de creep feeding.

Palavras-chave: *Bos indicus*. Consumo. Crescimento corporal. Forragem tropical. Suplementos.

Introduction

During the suckling stage, beef calves under grazing meet their nutritional requirements through nutrients originating from maternal milk and from the pasture. However, it is observed that after 65 - 90 days of age, the growth rate of calves may be limited by the milk production of their dams and by the amount of energy and protein in the maternal milk (Bartle, Males, & Preston, 1984; Henriques et al., 2011). On the other hand, tropical grasses, which constitute the basal feed source for cattle in the tropics, can not be considered a balanced diet (Paulino, Detmann, Valente, & Barros, 2008), as several nutrient deficiency or unbalance may restrict pasture intake, digestibility of the forage and metabolic efficiency (Detmann, Paulino, & Valadares, 2010; Detmann, Valente, Batista, & Huhtanen, 2014). As a consequence, the animal may not be able to attain an optimal weight gain rates, and so supplemental nutrients may be needed.

This context, identifying nutritional strategies that optimize the calf performance during the suckling stages and, consequently, improve its production in the post-weaning period may be an important means to increase the beef industry profitability. Studies have consistently shown that supplementation of suckling heifers fed diets with larger levels of supplement and protein have higher daily gain rates resulting in better performance and greater body weight (BW) at weaning (Patterson et al., 1992; Cardenas et al., 2015; Rodríguez-Sánchez, Sanz, Tamanini, & Casasús, 2015). However, questions remain about the optimal point among the amounts or levels of supplements that are used, which may influence the biological response of beef female calves and provide better development in the post-weaning period under grazing in the tropics that are subjected to different supplementation strategies.

Thus, the objective of this study was to evaluate the effects of supplementation levels on productive performance, intake, digestibility, metabolic profile, body growth and carcass characteristics of suckling female calves under grazing in the tropics.

Materials and Methods

All practices involving the use of animals were approved by the Institutional Animal Care and Use Committee of the Universidade Federal de Viçosa (protocol CEUAP-UFV number 10/2016).

Animals, experimental design, and diets

This experiment was carried out at the Department of Animal Science of the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil (20° 45′ S 42° 52′ W), between January and June 2015, which corresponded to the rainy season and rainy-dry transition season. The experimental area was located in mountainous region, with 670 m of altitude and, presented an average temperature and precipitation values of 22.6ºC and 521 mm, respectively.

Forty Nellore female calves with 127.3±2.7 kg average body weight (BW) and 3.5±0.1 months and their dams (averaging 7 years old and 505.3±7.8 kg BW) were used in this experiment.

The cow-calf were distributed in a completely randomized design with two treatments and twenty replicates. The treatments were 1) 4 g kg^{-1} BW of supplement or 2) 6 g kg^{-1} BW of supplement. Supplement was composed of corn meal, soybean meal, molasses and mineral mix and formulated to contain 30% crude protein as fed (CP; Table 1). The supplement levels of 4 g kg^{-1} BW (210 g CP d^{-1}) and 6 g kg^{-1} BW (315 g CP d^{-1}) corresponded to approximately 40 and 60%, respectively, of the dietary requirements of CP for Zebu young female under grazing with BW of 200 kg and expected gain of 1 kgd^{-1} (Costa e Silva et al., 2016).

Table 1
Composition of supplement consumed by the animals during the experimental period

Item	Supplement
Ingredient % (as fed)	
Soybeanmeal	54.3
Cornmeal	37.7
Molasses	3.0
Mineral mixture[1]	5.0

[1]Centesimal composition: dicalciumphosphate, 50.00; sodiumchloride, 47.15; zinc sulfate, 1.50; copper sulfate, 0.75; cobalt sulfate, 0.05; potassiumiodate, 0.05 andmanganese sulfate: 0.05.

Animals were submitted to 14 d of adaptation to the diet and to the experimental area. The experiment lasted 150 d. At the beginning of the experiment the animals were weighed after 14 h of solids fasting. The cow-calf units were allocated in two paddocks of 15 hectares each (one for each treatment), uniformly covered with *Brachiaria decumbens* Stapf., equipped with drinkers and feeders.

The supplement was delivered daily at 10h00, on creep-feeding system. Cows received mineral mixture *ad libitum*. Animals had unrestricted access to water throughout the experiment. Animals were weighed every 30 d without fasting (and always in the morning at 6h00), in order to adjust the amount of supplement to be provided to each group. In order to minimize the possible effects of the paddocks on the experimental treatments, animals were rotated among the two pastures every seven days, so each group stayed for the same period of time on each plot.

Forage samples and nutritional characteristics

Forage chemical composition was assessed by hand-plucked samples, collected every 15 days. Every 30 d 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 × 0.5 m). 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 on the experimental period.

A nine-day trial was carried out from the 75 days of experiment to evaluate the intake and digestibility of the nutrients. The first five days were used for the adaptation of animals to the markers (stabilization of markers excretion). Chromium oxide (Cr_2O_3) was used as external maker to estimate fecal excretion (in the amount of 10 g per animal). The chromium oxide was packed in paper cartridges and delivered via esophagus with a metal probe once daily, at 10 a.m. Individual intake of supplement was estimated using titanium dioxide (TiO_2) mixed in the supplement at the proportion of 10 g/kg of supplement. The indigestible neutral detergent fiber (iNDF) was used as internal marker to estimate forage DM intake. Feces samples were collected immediately after defecation or directly into the rectum of animals (at amounts of approximately 200 g) on the last four days of the trial, at different times according to the following schedule: Day 6 - 18h00, Day 7 - 14h00, Day 8 - 10h00 and Day 9 - 06h00. Samples feces were identified, oven-dried at 60°C and ground as previously described. After that, samples were pooled based on each animal.

The dams were milked on days 25, 75, and 125 of experiment to estimate the quantity and composition of daily milk intake by the calves following procedures described by Lopes et al. (2016). The milk production obtained at day 75 was used to estimate intake in the digestion trail.

Blood samples

On days 45, 90 and 135 of study, blood was collected to quantify the concentration of insulin, glucose, cholesterol, serum urea nitrogen (SUN), albumin and total proteins. Samples were collected at 7h00, via jugular venipuncture in vacuum tubes with clot activator and gel for serum separation (BD Vacutainer® SST II Advance, São Paulo, Brazil) and vacuum tubes containing sodium fluoride and EDTA (BDVacutainer® Fluoreto/EDTA, São Paulo, Brazil) as glycolytic inhibitor and anticoagulant, respectively, for glucose analysis. Samples collected with separator gel and clot accelerator were immediately after collection, centrifuged (3,600 × g for 20 min). Samples collected with glycolytic inhibitor were immediately and centrifuged (2,600 × g for 10 min) and plasma was frozen at -20°C for later analysis.

Performance, body measures, and carcass characteristics

For performance evaluation, the animals were weighed at the beginning and end of the experiment after 14 h of solids fasting. The last weighed coincided with weaning.

At the end of the experiment, body measures (BM) were taken to evaluate the body growth of the animals. The rump width (the maximum distance between iliac tuberosities), rump length (from the ischial tuberosity to the iliac tuberosity), rib depth (vertically from the highest point over the scapulae to the end point of the rib), body length (from the anterior point of the scapulae vertically to the posterior midline), height at withers (from the highest point of the shoulder blade to the ground) and rump height (from the iliac tuberosity vertically to the ground) were recorded with a measuring stick. The heart girth (the body circumference

immediately posterior to the front legs) was measured with a flexible tape. In parallel, carcass characteristics were evaluated by ultrasound (Aloka SSD 500; 3.5 MHz linear probe; Aloka Co, Tokyo, Japan). Carcass images were obtained between the 12th and 13th ribs over the longissimus muscle to measures the *longissimus dorsi* muscle area (LMA) and fat thickness over the longissimus muscle and, between the ischium and pubis to measures the fat thickness over the rump. Vegetable oil was used to ensure adequate acoustic contact. Images were analyzed in the Bio Soft Toolbox® II for Beef software (Biotronics Inc., Ames, IA, USA).

Analytical procedures

Samples of forage, feces and supplement (ground 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 4 h; method INCT-CA number M-001/1), N (Kjeldahl procedure; method INCT-CA number N-001/1), ether extract (Randall procedure; method INCT-CA number G-005/1), neutral detergent fiber corrected for ash and protein (NDFap; 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 the standard analytical procedures of the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA) (Detmann et al., 2012). The content of iNDF in samples of feces, forages and supplement (ground through 2-mm sieves) was estimated as the residual NDF remaining after 288 h of ruminal in situ incubation using F57 filter bags (Ankom Technology Corp., Macedon, NY) according to Valente et al. (2011).

Feces samples were also analyzed for chromium concentration using nitroperchloric digestion and atomic absorption spectrophotometry (Souza et al., 2013)titanium dioxide by colorimetry (Titgemeyer, Armendariz, Bindel, Greenwood, & Löest, 2001).

The pdDM in forage available on pasture was estimated using the following equation described by Paulino et al. (2008):

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

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 relation 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:

$$DMI = [(FE \times iNDFfeces - iNDFsupplement) \div iNDFforage] + SI + MI$$

Where FE = fecal excretion (kg/day), iNDFfeces = concentration of iNDF in the feces (kilograms per kilogram), iNDFsupplement = concentration of iNDF in the supplement (kg/kg) and iNDFforage = amount of iNDF form forage (kg/kg), SI = Supplement intake (kg DM/d) and, MI = Milk intake (kg DM/d).

The milk produced was corrected to 4% of fat ($Milk_{4\%}$) calculated by the following equation (NRC, 2001):

$$Milk_{4\%}\ (kg) = 0.4 \times (milk\ production) + [15 \times (fat\ production \times milk\ production/100)]$$

The blood insulin concentration was quantified by the indirect chemiluminescence method using Access Ultrasensitive Insulin Reagent (Ref. Number 33410, Beckman Coulter®, Brea, USA) in the Access® 2 Immunoassay System (Beckman Coulter Inc., Brea, USA). Glucose (Ref. Number K082-2, Bioclin® Quibasa, Belo Horizonte, Brazil) and total cholesterol concentrations (Ref. Number

K083-2, Bioclin® Quibasa, Belo Horizonte, Brazil) were quantified by enzymatic-colorimetric method. Urea in serum by the enzymatic kinetic method (Ref. Número 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 colorimetric method. Serum urea N (SUN) was estimated as 46.67% of total serum urea. Metabolites were analyzed in accordance with an automatic biochemistry analyzer (Mindray BS200E, Shenzhen, China).

The milk lactose, protein, fat and total solids content was analyzed using an infrared spectrophotometer (Foss MilkoScan FT120, Hillerød, Denmark).

Statistical analyses

The experiment was analysed according to completely randomized design. All statistical procedures were conducted using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Performance characteristics were submitted to ANOVA test adopting the initial BW as covariate. Serum concentrations of insulin, glucose, cholesterol, urea, albumin, and total proteins were analyzed using the procedure for repeated measures according to collection days. The best (co) variance structure was chosen based on Akaike´s information criterium with correction. The degrees of freedom were estimated according to Kenward-Roger method. Statistical significance was considered at $P \leq 0.05$, and tendencies were considered at $0.05 < P \leq 0.10$.

Results

In this study, the average availability of total DM and pdDM of the forage on pasture were 5.5±0.20 and 3.9±0.18 t/ha, respectively. The forage samples collected by the hand-plucked method had an average CP content of 98.3 g kg^{-1} DM (Table 2).

Table 2
Chemical composition of supplement and forage consumed by the animals during the experimental period

Item	Supplement	*B. Decumbens*[1,3]	*B. Decumbens*[2,3]
		g kg^{-1} DM	
Dry matter (DM)	914	897 ± 0.14	897± 0.80
Organic matter (OM)	918	919 ± 3.34	903 ± 2.90
Crude protein (CP)	296	91 ± 0.29	99 ± 0.35
Ether extract (EE)	14	16 ± 0.30	15 ± 0.40
Non-fibrous carbohydrates[4]	468	275 ± 0.52	220 ± 1.00
NDFap	141	557 ± 0.25	570 ± 0.74
NDIP (g/kg CP)	122.9	384 ± 0.18	375 ± 0.25
Indigestible NDF (iNDF)	15	137 ± 0.33	144 ± 0.98

NDFap: neutral detergent insoluble fiber corrected for contaminant ash and protein; NDIP: neutral detergent insoluble protein.
[1]Hand-plucked samples obtained during the digestion trial.
[2]Hand-plucked samples obtained during the experimental period.
[3]Means± standard error of the mean.
[4]NFC = OM - (CP + EE + NDFap).

The average milk yield and composition were not affected ($P > 0.05$) by supplementation levels delivery to female calves (Table 3).

Table 3
Milk yield and its components yield and composition of cows according to the supplement level delivered to the calves

Item [1]	Supplementation level (g kg^{-1} BW)		P-value
	4	6	
	kg d^{-1}		
Milk	6.56 ± 0.335	7.15 ± 0.335	0.216
$Milk_{4\%}$	7.32 ± 0.439	8.16 ± 0.439	0.181
	g kg^{-1}		
Fat	47.6 ± 0.22	49.1 ± 0.22	0.640
Protein	36.2 ± 0.06	35.5 ± 0.06	0.387
Lactose	45.5 ± 0.05	44.9 ± 0.05	0.401
Total solids	140.9 ± 0.25	140.7 ± 0.25	0.954

[1]Milk4%: milk production corrected to 4% of fat.

The voluntary intake (kg day^{-1}) of DM, forage, and milk were not influenced (P>0.05) by the level of supplement. However, the intake of supplement, organic matter (OM), CP, non-fibrous carbohydrates (NFC), and digested organic matter (DOM) were greater (P<0.05) for the greatest supplementation level (Table 4). Likewise, a trend of increasing was noted (P=0.060) in EE intake as a consequence of the greater supplementation level. Treatments were similar (P>0.05) regarding intake of NDFap, digestible NDF (DNDF), and iNDF (Table 4).

Table 4
Effect of supplementation levels on voluntary intake in suckling female calves under grazing in the tropics

Item	Supplementation level (g kg^{-1} BW)[1]		P-value
	4	6	
	kg day^{-1}		
DM	3.82 ± 0.186	4.23 ± 0.181	0.127
DMF	2.22 ± 0.145	2.19 ± 0.141	0.917
DMS	0.70 ± 0.085	1.01 ± 0.083	0.012
DMM	0.92 ± 0.048	1.01 ± 0.046	0.231
OM	3.49 ± 0.124	3.88 ± 0.121	0.031
CP	0.64 ± 0.139	0.75 ± 0.135	<0.001
EE	0.35 ± 0.021	0.41 ± 0.020	0.060
NDFap	1.32 ± 0.082	1.37 ± 0.080	0.637
NFC	0.89 ± 0.036	1.02 ± 0.035	0.013
iNDF	0.31 ± 0.019	0.32 ± 0.019	0.814
DOM	2.71 ± 0.089	3.03 ± 0.087	0.013
DNDF	0.88 ± 0.054	0.90 ± 0.052	0.750
CP:DOM	237 ± 3.6	248 ± 3.5	0.030

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	g kg^{-1} BW		
DM	18.4 ± 0.92	20.0 ± 0.90	0.201
DMF	10.7 ± 0.77	10.4 ± 0.75	0.825
OM	16.8 ± 0.67	18.4 ± 0.66	0.092
NDFap	6.4 ± 0.43	6.5 ± 0.42	0.769
iNDF	1.5 ± 0.10	1.5 ± 0.10	0.951

DM: dry matter; DMF: dry matter from forage; DMS: dry matter from supplement; DMM: dry matter from milk; OM: organic matter; CP: crude protein: EE: ether extract; NDFap: neutral detergent insoluble fiber corrected for contaminant ash and protein; NFC: non-fibrous carbohydrates; NDF: neutral detergent fiber; iNDF: indigestible NDF; DOM: digested OM; DNDFap: digested NDFap. [1]Means± standard error of the mean.

In the evaluation of intake as g kg^{-1} BW, the levels of supplement did not affect (P>0.20) the intake of DM or forage, although a trend of increasing was observed (P=0.092) in OM intake which increased as the supplementation amount increased (Table 4).

Supplementation levels effects were not observed (P>0.05) on the total digestibility of OM, CP, EE, NFDap, NFC and dietary DOM content (Table 5).

Table 5
Effect of supplementation levels on apparent digestibility coefficients levels in suckling female calves under grazing in the tropics

Item	Supplementation level (g kg^{-1} BW)[1]		P-value
	4	6	
OM (g g^{-1})	0.776 ± 0.0089	0.783 ± 0.0087	0.562
CP (g g^{-1})	0.781 ± 0.0124	0.799 ± 0.0111	0.284
EE (g g^{-1})	0.838 ± 0.0210	0.874 ± 0.0204	0.239
NDFap (g g^{-1})	0.667 ± 0.0100	0.660 ± 0.0097	0.631
NFC (g g^{-1})	0.823 ± 0.0076	0.827 ± 0.0074	0.667
DOM (g kg^{-1} DM)	710.8 ± 20.29	734.2 ± 19.78	0.412

OM: organic matter; CP: crude protein: EE: ether extract; NDFap: neutral detergent insoluble fiber corrected for contaminant ash and protein; NFC: non-fibrous carbohydrates; DOM: digested OM. [1]Means± standard error of the mean.

No interaction (P>0.05) was observed between supplementation levels and collection days for glucose, insulin, SUN, albumin, and total proteins (Table 6). Only an increase trend was detected (P=0.068) for the interaction between supplementation levels and collection days on blood cholesterol. The study of this effect the study of this effect did not show a difference between treatments (P>0.05). Blood concentration of insulin, glucose, cholesterol, albumin and total proteins were not affected (P>0.05) by the supplementation levels (Table 6). Only mean SUN concentration was greater (P<0.01) for the greatest supplement level (Table 6). In addition, a difference was observed in glucose concentration between the collection days (Figure 1a), with a downward trend in relation to the first collection. An effect was observed (P<0.05) in albumin concentration between collection days, with the lowest value observed in the second collection (Figure 1b).

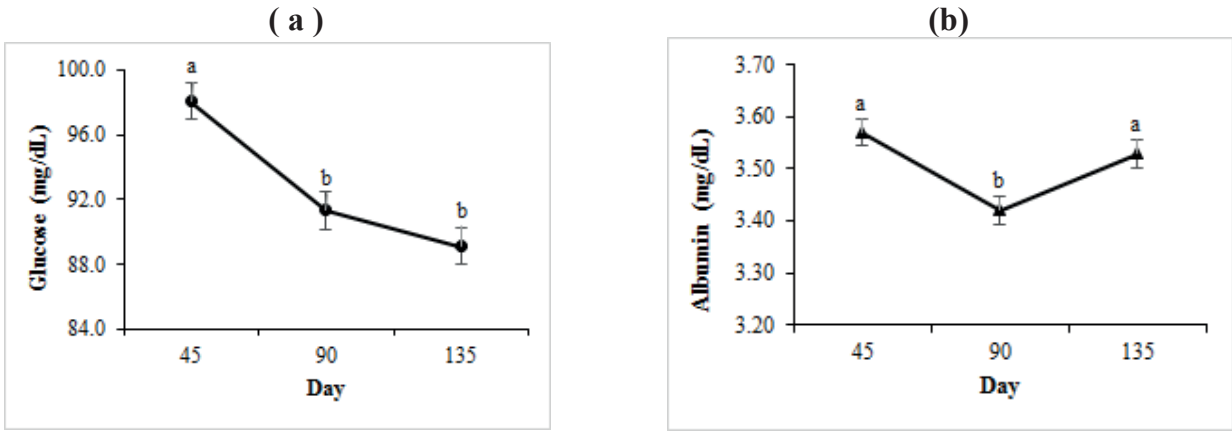

Figure 1. Blood glucose (a) and albumin (b) concentrations during the experimental period. [1]Means over the line followed by different letters differ (P<0.05).

Table 6
Effect of supplementation levels on the metabolic profile in suckling female calves under grazing in the tropics

Item	Supplementation level (g kg^{-1} BW)		SEM	P-value[1]		
	4	6		Treat	Col	Treat × Col
Insulin µ IU mL^{-1}	1.12	1.33	0.178	0.424	0.129	0.546
Glucose (mg dL^{-1})	92.8	92.8	1.07	0.999	<0.001	0.269
Cholesterol (mg dL^{-1})	164.3	158.4	4.40	0.348	0.693	0.068
SUN (mg dL^{-1})	16.3	18.4	0.46	0.001	0.370	0.793
Total proteins (g dL^{-1})	5.99	6.08	0.055	0.245	0.756	0.390
Albumin (g dL^{-1})	3.49	3.51	0.025	0.639	<0.001	0.999

SEM = standard error of the mean.
[1]Treat = treatment effect; Day = collection day effect; Treat × Col = interaction between treatment and collectionday.

The ADG and FBW of the animals were not affected (P>0.05) by the levels of supplement (Table 7). At the end of the experimental period, no treatment effect was detected (P>0.05) on LMA and SFTR. By contrast, an increase trend was observed (P=0.074; Table 5) on SFTL being greater for the greatest supplementation level (Table 7).

Table 7
Effect of supplementation levels on the productive performance, carcass characteristics and body growth in suckling female calves under grazing in the tropics

Item	Supplementation level (g kg^{-1} BW)[1]		P-value
	4	6	
FBW (kg)	246.1 ± 2.67	251.2 ± 2.67	0.185
ADG (g/day)	792 ± 0.02	826 ± 0.02	0.179
LMA (cm²)	47.7 ± 1.11	47.1 ± 1.11	0.707
SFTL (mm)	1.52 ± 0.127	1.85 ± 1.127	0.074

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SFTR (mm)	2.54 ± 0.163	2.75 ± 0.163	0.361
Height at withers (cm)	119 ± 0.77	118 ± 0.77	0.323
Heart girth (cm)	144 ± 0.59	145 ± 0.59	0.368
Rib width (cm)	38.2 ± 0.53	38.2 ± 0.53	0.989
Rump height (cm)	127 ± 0.76	126 ± 0.76	0.486
Rump width (cm)	35.4 ± 0.34	34.7 ± 0.34	0.180
Rump length (cm)	38.3 ± 0.40	38.3 ± 0.40	0.964
Body length (cm)	121 ± 1.23	124 ± 1.23	0.194
BW:HW (kg/cm)	2.07 ± 0.022	2.13 ± 0.022	0.064

FBW: final body weight; ADG: average daily gain; LMA: Longissimus muscle area; SFTL: subcutaneous fat thickness at the Longissimis mucle; SFTR: fat thickness at the rump; BW: body weight; HW: height at the withers.
[1]Means± standard error of the mean.

In general, the body growth of the animals was not affected (P>0.05) by the supplementation levels of supplement. Only a trend of increasing was observed (P=0.064) in BW: HW ratio which increasing the supplement level (Table 7).

Discussion

The forage intake is determined by integration of different mechanisms physical constraints and metabolic feedbacks. The adequacy of dietary protein-to-energy ratio has been pointed out as one of the main parameters that regulates intake of cattle fed tropical forages (Detmann et al., 2014). The maximum forage intake is observed with dietary CP:DOM at 210 to 216 g kg^{-1}(Poppi & Mclennan, 1995; Reis et al., 2016). The dietary CP:DOM for calves received 4 and 6 g kg^{-1} BW of supplement was 237 and 248 g kg^{-1}, respectively. Therefore, they both was slightly higher than suggested by abovementioned authors to maximize forage intake by grazing cattle and the nitrogen utilization efficiency, showing a slightly unbalanced dietary protein-to-energy ratio when adequacy of intake is considered, which seems to support the similar forage intake among treatments (Table 4). A similar pattern was reported by Marquez et al. (2014) and Lima et al. (2016) when supplemented suckling calves in the tropics.

In this study, the similar DM intake between treatments indicate that differences in supplement, CP, OM, NFC and EE intake were not sufficient to affect the DM intake. Additionally, the levels of supplement used did not affect the milk yield of cows (Table 3). Thus, the calves milk intake (Table 4) did not differ between treatments.

On the other hand, the greater CP, EE, and NFC intake for calves that received greater supplementation level was a result of the increased supply of supplement (Table 4). Consequently, the increase in the intake of CP, EE, and NFC allowed a higher DOM intake.

In this study, although an increase in OM, CP, and NFC intake was observed for calves that received greater supplementation level, the lack of an effect on total digestibility and dietary DOM content between treatments (Table 5), may be associated with the inclusion of supplements (easily digested) in both treatments and the high participation of milk (high digestible components) in the total diet.

The similar blood concentration of glucose and insulin between treatments can be justified by lack of differences in DM intake (Table 4). According to Hersom, Wettemann, Krehbiel, Horn, & Keisler (2004) and Huntington, Harmon, & Richards (2006), the blood glucose and insulin concentration are positively associated with DM intake and

weight gain rates. In addition, the concentration of circulating insulin is positively regulated by the blood glucose level (Vizcarra, Wettemann, Spitzer, & Morrison, 1998). On the other hand, similar blood concentrations of cholesterol between treatments reflect a similar energy status between animals (Table 6). Similar results are reported by Silva et al. (2017) found no difference in blood concentration of insulin (1.45 vs. 1.43 μIU mL^{-1}), glucose (84.3 vs. 80.6 mg dL^{-1}) and, cholesterol (172 vs. 160 mg dL^{-1}) in Nellore female calves submitted to different supplementation levels under grazing in the tropic.

According to Henriques et al. (2011), after three months of lactation, there is a gradual decrease in milk production by cows and consequently less participation of milk in the total diet of calves, which results in a lower intestinal absorption of glucose. This may support the decrease behavior shown by blood glucose across the collection days (Figure 1a).

According to Van Soest (1994), the SUN concentration positively associated with CP intake. Thus, the greater SUN concentration for the greatest supplementation level may be attributed to their higher CP intake. In addition, the optimal SUN concentrations for growing beef cattle fluctuate between 15 to 19 mg dL^{-1}(Hammond, 1997), these results indicate that the diet of calves in this study did not have deficient or excess of protein (Table 6).

The blood concentrations of albumin and total proteins can be influenced by the availability of amino acids and nutrients. Thus, similar values between treatments indicate that the diet intake by the animals led to similar nutritional statuses (Table 6). However, the lower albumin concentration in the second collection (Figure 1b) may possibly be due to the lower CP content from the pasture consumed at that time (91.4 g CP kg^{-1} DM) in relation to the first and third collections (106.9 g CP kg^{-1} DM and 101.7 g CP kg^{-1} DM, respectively).

ADG is positively associated with DM intake; thus, the lack of effects on DM intake may explain the similar ADG and FBW of the animals (Table 7). This patter resulted in similar LMA and SFTR between treatments (Table 6). However, a trend of increasing in SFTL seems indicate some benefit of the greater level of supplement (Table 6).

In this study, body measures were taken to obtain skeletal growth indices, in addition to soft tissues. In cattle, the height at the withers and the rump height are composed mainly of the measurement of long bones in the animal (hind and fore legs) and are good indicators of skeletal development (swali, Cheng, Bourne, & Wathes, 2008; Rodríguez-Sánchez et al., 2015). The lack of an effect on this variable indicates that the supplement levels tested did not compromise the skeletal development of the animals (Table 7).

The heart girth is an individual predictor of the animals' BW and, the rump width and length provide an estimate of the internal pelvic area and have an important relationship with the distribution of prime cuts in the hindquarter and the incidence and difficulty of calving in primiparous heifers (Fernandes, Magnobosco, Ojala, Caetana, & Famula, 1996; Swali et al., 2008; Rodríguez-Sánchez et al., 2015). In the current study, the absence of difference in these variables indicates that the both supplementation levels provided in this experiment promoted an adequate tissue development in the animals (Table 6).

The BW: HW ratio is a measure used to estimate the difference in body condition between animals (Eborn, Cushman, & Echternkamp, 2013). The upward trend in BH: HW ratio indicates higher supplementation level produced some benefit in the muscle and adipose tissue deposition ability (Table 7)**.**

Conclusions

Increasing the supplementation level of 4 to 6 g kg^{-1} of BW on creep-feeding system, does not improve the productive and nutritional performance and, metabolic status in suckling female calves under grazing in the tropics.

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Conflict of Interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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