ROMÁN ENRIQUE MAZA ORTEGA

SUPPLEMENTATION STRATEGIES FOR BEEF HEIFERS IN TROPICAL PASTURES IN THE PREWEANING AND REARING PERIODS

Thesis submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae.*

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APPROVED: February 26, 2018.

Lívia Vieria de Barros

Ériton Egídio Lisboa Valente

Edenio Detmann

Luciana Navajas Rennó

Mário Fonseca Paulino
(Adviser)

Bom mesmo é ir à luta com determinação, abraçar a vida com paixão, perder com classe e vencer com ousadia... Pois o triunfo pertence a quem se atreve.

Charles Chaplin

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BIOGRAPHY

Román Enrique Maza Ortega, son of Román Maza Buelvas and Luz Enith Ortega Júlio, was born in Maria La Baja, Bolívar - Colômbia, on July 2, 1983.

In March of 2000 he started the undergrad in Veterinarian and Animal Science at Universidad de Córdoba, Colombia and became a Bachelor in Veterinarian and Animal Science on December 19, 2005.

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In March of 2014 he started the doctorade programe in Animal Science with major on ruminant nutrition and beef cattle production in the same University. On February 26 th of 2018 Ms. Ortega defended his doctoral thesis to obtain the Doctor Scientiae degree in Animal Science.

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ABSTRACT

MAZA ORTEGA, Román Enrique, D.Sc., Universidade Federal de Viçosa, February, 2018. **Supplementation strategies for beef heifers in tropical pastures in the preweaning and rearing periods**. Adviser: Mário Fonseca Paulino.

This research was conducted from two studies with beef heifers grazing submitted different supplementation strategies and the results are here presented in two manuscripts. **In the first manuscript**, effects of supplement amounts on productive and nutritional performance and metabolic profile were evaluated in suckling female calves under grazing in the tropics. The treatments were 1) 4 g/kg body weight (BW) of supplement or 2) 6 g/kg BW of supplement. Forage and organic matter (OM) intake did not affect (P>0.12) by amounts of supplement, though crude protein and non-fibrous carbohydrates intake were greater (P<0.04) by increasing supplement amount. There was no effect (P>0.23) the amounts of supplement on OM and CP digestibility. In addition, production of microbial nitrogen was similar (P>0.15) between treatments, however, the excretion of urea nitrogen in the urine increased (P<0.01) by increasing supplement amount. The metabolic profile of the animals was not affected (P>0.99) by amounts of supplement. Mean daily gain, *longissimus dorsi* area, fat thickness over rump of the animals did not affect (P>0.26) by amounts of supplement. However, there was trend of increasing (P=0.074) in fat thickness over loin by increase the supplement amount. Although the body growth of animals was similar (P>0.18) between treatments, there was observed a trend of increase (P=0.064) in ratio BW:Height at the withers by increasing supplement amount. The results indicate that increasing supplement amounts does not improve the productive and nutritional performance and metabolic status in female calves on creep-feeding system. **In the second experiment,** effects of supplement amounts pre-weaning and rearing on productive and nutritional performance, metabolic profile, and ovarian activity were evaluated in heifers under grazing in the tropics. Forty Nellore heifers averaging 8.5±0.06 months and 248.6±3.3 kg body weight (BW) were distributed in a completely randomized design in a 2 × 2 factorial scheme with four treatments and ten replicates. The treatments were: 1) 4 g/kg of BW of supplement pre-weaning and rearing; 2) 4g/kg of BW of supplement pre-weaning and 6 g/kg rearing; 3) 6 g/kg of BW pre-weaning and 4 g/kg of BW rearing and; 4) 6 g/kg of BW pre-waning and rearing. Crude protein (CP), organic matter (OM) intake were increased (P≤0.02) by increasing the amounts of supplement in the rearing. Additionally, increasing supplements amounts in the rearing increased the digestibility the OM and CP (P<0.05). Means insulin and

glucose concentrations were greater (P<0.03) for heifers that received greater amounts of supplement in the rearing. Daily gain and fat thickness in the rump were increased (P<0.01) by increasing supplement amounts in the rearing. Amounts of supplement did not influence (P>0.15) body growth of heifers. However, follicular number, diameter and progesterone concentration were greater (P<0.02) for heifers that received greater amounts of supplement in the rearing. The results indicate that increasing supplement amounts in the rearing has greater positive impact on performance, metabolic status, and ovarian activity of heifers under grazing than increasing supplement amounts in the pre-weaning.

RESUMO

MAZA ORTEGA, Román Enrique, D.Sc., Universidade Federal de Viçosa, fevereiro de 2018. **Estratégias de suplementação para novilhas de corte em pastagens tropicais nos períodos de cria e recria**. Orientador: Mário Fonseca Paulino.

Esta pesquisa foi elaborada a partir de dois estudos com novilhas de corte em pastejo submetidas a diferentes estratégias de suplementação, e os resultados estão aqui apresentados em dois manuscritos. No primeiro manuscrito foram avaliados os efeitos das quantidades de suplemento sobre desempenho produtivo e nutricional e perfil metabólico em bezerras lactentes alimentadas com forragem tropical. Quarenta bezerras (com média de 3,5±0,06 meses e 127,3±2,68 kg) e suas respectivas mães foram distribuídas em delineamento inteiramente casualizado com dois tratamentos e vinte repetições. As estratégias de suplementação foram 1) 4 g/kg de peso corporal (PC) de suplemento ou 2) 6 g/kg PC de suplemento. O consumo de matéria seca e matéria orgânica (OM) não foram afetadas (P>0,12) pelas quantidades de suplemento, embora o consumo de proteína bruta e carboidratos não-fibrosos foi maior (P<0,04) pelo aumento da quantidade de suplemento. Não houve efeito (P>0,23) das quantidades de suplemento sobre a digestibilidade da MO e PB. Além disso, a produção de nitrogênio microbiano foi semelhante (P>0,15) entre os tratamentos, a excreção de nitrogênio ureico na urina incrementou (P<0.01) pelo aumento da quantidade de suplemento. O perfil metabólico dos animais não foi afetado (P>0,99) pelas quantidades de suplemento. O ganho médio diário, área do músculo *longisimus dorsi*, espessura de gordura sobre a garupa dos animais não foram afetados (P>0,26) pelas quantidades de suplemento. No entanto, observou-se uma tendência de aumento (P=0,074) na espessura de gordura sobre o lombo pelo aumento da quantidade de suplemento. Embora o crescimento o corporal dos animais foi semelhante (P>0,18) entre os tratamentos, observou-se uma tendência de aumento (P=0,064) na proporção PC: Altura na cernelha pelo aumento da quantidade de suplemento. Os resultados indicam que o aumento das quantidades de suplemento não melhora o desempenho produtivo e nutricional e estado metabólico em bezerras lactentes no sistema creep-feeding. No segundo manuscrito foram avaliados os efeitos das quantidades de suplemento na cria e recria sobre desempenho produtivo e nutricional, perfil metabólico e atividade ovariana em novilhas de corte em pastejo nos trópicos. Quarenta novilhas Nelore com 8,5±0,06 meses idade e 248,6±3,32 kg de peso corporal médio foram distribuídas em delineamento inteiramente casualizado em esquema fatorial 2 × 2, com quatro tratamentos e dez repetições. Os tratamentos foram: 1) g/kg de PC de

suplemento na cria e na recria; 2) 4 g/kg de PC de suplemento na cria e 6 g/kg na recria; 3) 6 g/kg de PC na cria e; 4) 4 g/kg na recria e 6 g/kg de peso PC na cria e na recria. O consumo de proteína bruta (PB) e matéria orgânica (MO) foram aumentados ($P \leq 0,02$) pelo aumento das quantidades de suplemento na recria. Além disso, o aumento das quantidades de suplemento na recria aumentou a digestibilidade da OM e CP ($P < 0,05$). Concentrações médias de insulina, glicose e progesterona foram maiores ($P < 0,03$) para novilhas que receberam maiores quantidades de suplemento na recria. O ganho diário e espessura da gordura na garupa foram incrementados ($P < 0,01$) pelo aumento das quantidades de suplemento na recria. As quantidades de suplemento não influenciaram ($P > 0,15$) de crescimento corporal das novilhas. No entanto, o número folicular e o diâmetro foram maiores ($P < 0,02$) para novilhas que receberam maior quantidade de suplemento na recria. Os resultados indicam que o incremento das quantidades de suplemento na recria tem maior impacto positivo no desempenho, estado metabólico e atividade ovariana de novilhas em pastejo que o aumento das quantidades de suplemento na cria.

GENERAL INTRODUCTION

The developing of nutritional strategies aiming at optimizing growth rate and time to sexual maturity of heifers lending maximum lifetime productivity is still one of the main goals of the beef industry (Kluyts *et al.,* 2003; Cardoso *et al.,* 2014). The onset of puberty and age at first calving are events that have the greatest impact on productivity, reproduction, and economic efficiency in beef cattle production (Patterson *et al.,* 1992; Kluyts *et al.,* 2003; Monteiro *et al.,* 2013). By contrast, developing replacement heifers are necessary to maintain stable herd size, represent genetic improvement and production efficiency (Bagley 1993).

Thus, the beef cattle industry should be able to provide an adequate number of heifers that reach puberty and regularly cycle before the breeding season to improve conception rate at the first artificial insemination (AI). To reach this goal, heifers should reach puberty between 12 and 13 months of age, be pregnant between the ages of 14 and 15 months, and give birth at approximately 2 years old (Schillo *et al.,* 1992).

The probability of conception at the beginning of the breeding season is increased in heifers that present multiple oestrus cycles before the mating season begins. Thus, it has been shown that the heifer conception rate increases by approximately 21% (Byerley *et al.,* 1987; Bagley, 1993) from the first ovulation to the third estrous cycle.

The mating of heifers at 14-15 years of age aims to reduce requirements for animal replacement, to eliminate unproductive categories and to reduce the generational interval (Nogueira, 2004). Animals that give birth at 2 years of age have a higher potential for life-productive than heifers who give birth at more advanced ages.

Age at puberty varies depending on numerous factors including body weight, genetics, nutrition, and management. The occurrence of puberty depends on the growth

rate and development of the animal to support the endocrine mechanisms that result in first ovulation (Maquivar and Day 2009). Schillo *et al.* (1992) suggested that hormonal and metabolic changes resulting from parallel changes in nutrition and body fat may regulate the releasing luteinizing hormone (LH). Hence, hormonal metabolic changes before puberty may be associated with decreased negative feedback of estradiol, increased secretion of luteinizing hormone (LH), and subsequent ovulation. Accordingly, several hormones and metabolites have been studied as nutritional signals to reproduction, for example, IGF-I, insulin, leptin, glucose and others (Steiner, 1987; Schillo *et al.,* 1992).

Studies have shown different results on when is the best time to increase growth and decrease the age to puberty in bovine females. Some authors have reported the occurrence of early puberty with increased rate of gain in early stages of development (Patterson *et al.,* 1992; Gasser *et al.,* 2006; Cardoso *et al.,* 2014; Rodríguez-Sánchez *et al.,* 2015), while others observed a reduction in age at puberty with higher weight gain post-weaning (Barcellos *et al.,* 2014; Rodríguez-Sánchez *et al.,* 2015; da Silva *et al.,* 2017).

In Brazil, the beef cattle industry is based mainly on the pasture model, which represents more than 90% of the cattle diet. However, cattle in the pre- and post-weaning periods are characterized by deficient feed in both quality and quantity of forage which causes a slow growth rate and low gains or losses weight of the animals, limiting the continuous growth and leading to a delay in puberty. As consequence the average age at first calving is over 40 months (Pereira, 2000).

During the suckling stage, beef calves under grazing meet their nutritional requirements through nutrients originating from maternal milk and from the pasture. However, Bartle *et al*. (1984) and Henriques *et al.* (2011) 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.

On the other hand, tropical grasses, which constitute the basal feed source for cattle in the tropics and represents the main source of energy for animals under tropical conditions (Detmann *et al.,* 2010), which is the primary dietary consideration for heifer development (Maas, 1987), protein is considered the limiting nutrient (DelCurto *et al.,* 2000). However, tropical forage do not represent a balanced diet for animal production, as multiple nutritional constraints (i.e. protein, energy and minerals) are observed throughout year (Paulino *et al.,* 2008), which may restrict pasture intake and the digestibility of the forage (Detmann *et al.,* 2010; 2014). Such as pattern implies a lacking of necessary nutrients to support the recommended growth rates during rearing (Roberts *et al.,* 2009) and results in suboptimal animal performance, allowing heifers do not to reach puberty and sexual maturity at the genetically defined ages.

During the dry season, crude protein (CP) content of tropical forage under grazing is usually less than 70-80 g/kg dry matter (DM), which limits the use of fibrous carbohydrates from basal forage by ruminal microorganisms. During the rainy season, although the CP content is higher than 70-80 g/kg DM, tropical forages present an unbalanced energy to protein ratio, exhibiting a relative excess of energy in relation to available CP, which limits the forage intake by the animals (Detmann *et al.,* 2010).

In this context, nutritional strategies that optimize the body growth and development of animals during the suckling stages and consequently improve its production in the post-weaning period to increase the percentage of heifers that initiate ovarian activity at lower ages and give birth at 24 months of age should be developed.

In such scenarios, in the intensive livestock production systems, where there is a need to a greater nutrient input, it is visualized the animals supplementation in pre-

weaning (creep-feeding system) and post-weaning (Paulino *et al.,* 2012) periods. Protein and multiple supplementation for cattle fed tropical forage is great importance for increasing nutrient intake, leading to high rates of weight gain from birth to puberty, anticipating puberty, and age at first calving (Yelich *et al.,* 1996).

According to Patterson *et al.* (1992), suckling heifers fed diets with larger amounts of supplement and protein have higher daily gain rates, reach puberty earlier, and have increased probabilities of pregnancy. This is possibly due to the early phase of development may be critical for the establishment of many components of the reproductive axis, thus the possibility that pre-weaning nutritional status have more influence on puberty is consistent with the dynamic changes of this period.

Rodríguez-Sánchez *et al.* (2015) found that animals with a weight gain rate of 1 kg/day during the pre and post-weaning periods reached puberty 129 days earlier compared to animals that had a weight gain rate of 0.7 kg/day.

Thus, improving energy and protein intake in the pre and post-weaning periods through multiple supplementation may favors the growth rate and reproductive development such as weaning weight, adult weight, age at onset of puberty, fertility at first mating of heifers in tropical conditions. In this context, finding the best strategy of supplementation for developing of replacement heifers is necessary to increase the productivity, reproduction fertility, and economic efficiency in beef cattle industry.

In this way, studies were conducted aiming to:

1- Evaluate the effects of supplementation strategies on productive and nutritional performance and metabolic profile in suckling female calves under grazing in the tropics and;

2) Evaluate the effects of supplementation strategies on productive and nutritional performance, metabolic profile and ovarian activity in heifers under grazing in the tropics.

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Effects of supplementation strategies in female calves under grazing in the tropics: I. Productive and nutritional performance and metabolic profile in the preweaning period

Abstract. Effects of supplement amounts on productive and nutritional performance and metabolic profile were evaluated 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 body weight (BW) of supplement or 2) 6 g/kg BW of supplement. Forage and organic matter (OM) intake did not affect (P>0.12) by amounts of supplement, though crude protein and non-fibrous carbohydrates intake were greater (P<0.04) by increasing supplement amount. There was no effect (P>0.23) the amounts of supplement on OM and CP digestibility. In addition, production of microbial nitrogen was similar (P>0.15) between treatments, however, the excretion of urea nitrogen in the urine was greater by increasing supplement amount. The metabolic profile of the animals was not affected (P>0.99) by amounts of supplement. Mean daily gain, *longissimus dorsi* area, fat thickness over rump of the animals did not affect (P>0.26) by amounts of supplement. However, there was trend of increasing (P=0.074) in fat thickness over loin by increase the supplement amount. Although the body growth of animals was similar (P>0.18) between treatments, there was observed a trend of increase (P=0.064) in ratio BW:Height at the withers by increasing supplement amounts. These results indicate that increasing supplement amounts does not improve the productive and nutritional performance and metabolic status in female calves on creep-feeding system.

Additional keywords: Body growth, *Bos indicus*, female calves, intake, supplements, tropical forage.

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 observe 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 *et al.,* 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 *et al.,* 2008), as several nutrient deficiency or unbalance may restrict pasture intake, digestibility of the forage and metabolic efficiency (Detmann *et al.,* 2010; 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 profitability of the beef industry. Thus, supplementation of suckling calves in creep-feeding can therefore enable the exploration of optimum of each individual, resulting in better performance and higher body weight (BW) at weaning (Paulino *et al.* 2012).

Studies in not tropical conditions have shown that suckling heifers fed diets with larger amounts of supplement and protein have higher daily gain rates and, reach puberty earlier (Patterson *et al.,* 1992; Rodirguez-Sánchez *et al.,* 2014; 2015). By contrast, several studies in tropical condition on creep-feeding have consistently shown an increase in the weaning weight of calves (Lopes *et al.,* 2014; Cardenas *et al.,* 2015; Almeida *et al.,* 2017). However, questions remain about the optimal point among the amounts 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.

The objective of this study was to evaluate the effects of supplement amounts on productive performance, intake, digestibility, efficiency of microbial protein synthesis, metabolic profile, and carcass characteristics of 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).

Location

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.

Animals, experimental design and diets

Forty Nellore female calves with 127.3±2.7 kg average body weight (BW) and 3.5±0.1 m old 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 BW of supplement or 2) 6 g/kg 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 amounts of 4g/kg BW (210 g CP/d) and 6 g/kg BW (315 g CP/d) 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 kg/d (Costa e Silva *et al.*, 2016).

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 plots 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 (Table 1) 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 animals. 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.

To evaluate the microbial protein production and urine urea nitrogen (UUN) excretion, spot urine samples were collected on the last day of the trial, during spontaneous urination, four hours after the supplement delivery. After the collection, 10 mL of urine were diluted in 40 mL H_2SO_4 (0.036 N) and frozen at -20 ºC for later analysis.

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 Vacuntainer® SST II Advance, Phymouth, UK) and vacuum tubes containing sodium fluoride and EDTA (BD Vacutainer® Fluoreto/EDTA, São Paulo, Brasil) 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 analysed in the BioSoft 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) and titanium dioxide by colorimetry (Titgemeyer *et al.*, 2001).

The pdDM in forage available on pasture was estimated using the following equation described (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).

Daily urinary volume was calculated using the relationship between the daily creatinine excretion (CE), taking as reference the equation proposed by Costa e Silva *et al.* (2012), and its concentration in the spot samples:

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

where: BW = body weight

Excretion of the purine derivatives in urine was calculated by the sum of the allantoin and uric acid excretions, which were obtained by the product between their concentrations in urine by the daily urinary volume. Absorbed purines were calculated from the excretion of purine derivatives according to Chen and Gomes (1992).

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

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

Ruminal synthesis of nitrogen compounds was calculated as a function of the absorbed purines using the equation described by Barbosa *et al.* (2011).

$$NMIC = 70 \times AP / 0.93 \times 0{:}137 \times 1.000$$

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

Efficiency of protein microbial synthesis (EFM) was determined by dividing protein microbial production by the DOM intake.

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 and urine by the enzymatic kinetic method (Ref. Número K056-1, Bioclin® Quibasa, Belo Horizonte, Brasil) 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. Urinary creatinine by kinetic colorimetric method (Ref. Número K067-1, Bioclin® Quibasa, Belo Horizonte, Brasil) and, urinary uric acid by enzymtic-colorimetric method (Ref. Número K139-1, Bioclin® Quibasa, Belo Horizonte, Brasil). 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 trends were considered at $0.05 < P \leq 0.10$. The data of intake, digestibility and microbial protein production from one animal were lost during the analysis.

Results

Forage samples and nutritional characteristics

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 DM (Table 1).

The average milk yield and composition were not affected ($P > 0.20$) by the amounts of supplement delivery to calves (Table 2).

The voluntary intake (kg/day) of DM, forage, and milk were not influenced ($P > 0.10$) by the amounts of supplement. However, the intake of supplement, organic matter (OM), CP, non-fibrous carbohydrates (NFC), and digested organic matter (DOM) were greater ($P < 0.04$) for the greatest supplement amount (Table 3). Likewise, a trend of increasing was noted ($P = 0.060$) in EE intake as a consequence of the greater supplement amount. Treatments were similar ($P > 0.60$) regarding intake of NDFap, digestible NDF (DNDF), and iNDF (Table 3).

In the evaluation of intake as g/kg BW, the amounts 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 3).

Supplement amounts effects were not observed (P>0.20) on the total digestibility and dietary DOM content (Table 4).

The microbial nitrogen (NMIC) production, relative production of microbial nitrogen in the rumen (NMICR), or the efficiency of microbial protein synthesis (EMS) did not affect (P>0.15) by the amounts of supplement (Table 4). However, urine urea nitrogen (UUN) excretion was grater (P<0.01) for the greatest supplement amount.

Metabolic profile

No interaction (P>0.50) was observed between amounts of supplement and collection days for glucose, insulin, SUN, albumin, and total proteins. Only, an increase trend was detected (P=0.068) for the interaction between amounts of supplements and collection days on blood cholesterol. The study of this effect the study of this effect did not show a difference between treatments (P>0.10). Blood concentration of insulin, glucose, cholesterol, albumin and total proteins were not affected (P>0.50) by the supplement amounts (Table 5). Only, mean SUN concentration was greater (P<0.01) for the greatest supplement amount (Table 5). In addition, a difference was observed in glucose concentration between the collection days (Fig.1a), with a downward trend in relation to the first collection. An effect was observed (P<0.01) in albumin concentration between collection days, with the lowest value observed in the second collection (Fig. 1b).

Performance, body measures and carcass characteristics

The ADG and FBW of the animals were not affected (P>0.17) by the amounts of supplement (Table 5). At the end of the experimental period, no treatment effect was

detected (P>0.36) on LMA and SFTR. By contrast, an increase trend was observed (P=0.074; Table 5) on SFTL being greater for the greatest supplement amount (Table 5).

In general, the body growth of the animals was not affected (P>0.18) by the amounts of supplement. Only a trend of increasing was observed (P=0.064) in BW: HW ratio which increasing the supplement amount (Table 5).

Discussion

The forage intake is determined by integration of different mechanisms physical constraints and metabolic feedbacks. Among these last, the adequacy of dietary protein-to-energy ratio has been pointed out as one of the main parameters that regulates of the 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 (Poppi e McLennan 1995; Reis *et al.* 2016). The dietary CP:DOM for calves hat received 4 and 6 g/kg BW of supplement was 237 and 248 g/kg, 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 2). A similar pattern was reported in the tropics (Marquez *et al.*, 2014; Lima *et al.*, 2016).

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 amounts of supplement used did not affect the milk yield of cows (Table 2). As a consequence, the calves milk intake (Table 3) did not differ between treatments.

On the other hand, the greater CP, EE, and NFC intake for calves that received greater amount of supplement was a result of the increased supply of a multiple

supplement (Table 3). 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 amount of supplement, the lack of an effect on total digestibility and dietary DOM content between treatments (Table 4), may be associated with the inclusion of multiple supplements (easily digested) in both treatments and the high participation of milk (high digestible components) in the total diet.

The rumen availabilities of carbohydrates, ammonia, peptides, aminoacids, sulfur, and branched-chain fatty acids are the nutritional factors that affect microbial growth (Clark *et al.*, 1992; Van Soest 1994). Thus, lack of difference in NMIC and EMS values (Table 3), indicate that the provision of substrates essential for the growth of rumen microorganisms were similar between treatments. Although CP intake was higher with the increase in the amount of supplement provided, this was not sufficient to change NMICR in the different treatments.

According to Van Soest (1994), the UUN concentration is positively associated with SUN and CP intake. In this study, the greater excretion UUN and SUN concentration for the greatest supplement amount may be attributed to their higher CP intake, indicating that small amount of N is not efficiently used by the animal (Table 4). In addition, the optimal SUN concentrations for growing beef cattle fluctuate between 15 to 19 mg/dL (Hammond 1997), this results indicate that the diet of calves in this study did not have deficient or excess of protein (Table 5).

The similar blood concentration of glucose and insulin between treatments can be justified by lack of differences in DM intake (Table 3). According to Hersom *et al.* (2004) and Huntington *et al.* (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 *et al.*,

1998). On the other hand, the blood cholesterol levels it can be influenced by the physiological and nutritional statuses. In this way, equal blood concentrations of cholesterol between treatments reflect a similar energy status between animals (Table 5). Similar results are reported by (da Silva *et al.*, 2017) found no difference in blood concentration of insulin, glucose and, cholesterol in Nellore female calves under grazing.

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 (Fig. 1a).

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 consumed by the animals led to similar nutritional statuses (Table 5). However, the lower albumin concentration in the second collection (Fig. 1b) may possibly be due to the lower CP content from the pasture consumed at that time (91.4 g CP/kg DM) in relation to the first and third collections (106.9 g CP/kg DM and 101.7 g CP/kg 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 6). 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 amount 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 *et al.*, 2008; Rodríguez-Sánchez

et al., 2015). The lack of an effect on this variable indicates that the supplement amounts tested did not compromise the skeletal development of the animals (Table 6).

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 *et al.*, 1996; Swali *et al.,* 2008; Rodríguez-Sánchez *et al.,* 2015). In the current study, the absence of difference in these variables indicates that the amounts provided in this experiment did not limit 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 *et al.,* 2013). The upward trend in BH: HW ratio indicates higher supplement amount produced some benefit in the muscle and adipose tissue deposition ability (Table 6)**.**

Conclusions

Increasing supplement amounts on creep-feeding system, does not improve the productive and nutritional performance, metabolic and energetic status in suckling female calves under grazing in the tropics.

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Tables

Table 1. Ingredients and chemical composition of supplement and forage consumed by the animals during the experimental period

NDFap: neutral detergent insoluble fiber corrected for contaminant ash and protein; NDIP: neutral detergent insoluble protein. [A]Hand-plucked samples obtained during the digestion trial.

[B]Hand-plucked samples obtained during the experimental period. [C]Means± standard error of the mean.

[D]NFC = OM – (CP + EE + NDFap).

Item	Supplement	*B. Decumbens*[A,C]	*B. Decumbens*[B,C]
Ingredient % (as fed)			
Soybean meal	54.3	-	-
Corn meal	37.7	-	-
Molasses	3.0	-	-
Mineral mixture	5.0	-	-
Chemical composition (g/kg DM)			
Dry matter (DM)	914.1	897.5 ± 0.14	896.9 ± 0.80
Organic matter (OM)	917.9	919.1 ± 3.34	903.3 ± 2.90
Crude protein (CP)	296.0	91.3 ± 0.29	98.6 ± 0.35
Ether extract (EE)	13.7	15.9 ± 0.30	15.1 ± 0.40
Non-fibrous carbohydrates[D]	467.5	274.8 ± 0.52	219.9 ± 1.00
NDFap	140.7	556.5 ± 0.25	569.8 ± 0.74
NDIP (g/kg CP)	122.9	384.4 ± 0.18	374.5 ± 0.25
Indigestible NDF (iNDF)	15.1	137.1 ± 0.33	143.8 ± 0.98

Table 2. Milk yield and its components in function of the different treatments

[A] $Milk_{4\%}$: milk production corrected to 4% of fat.

Item [A]	Supplement amount (g/kg BW)		*P*-value
	4	6	
	kg/d		
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		
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

Table 3. Effect of supplementation strategies on voluntary intake in female calves under grazing in the tropics

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. [A]Means± standard error of the mean.

Item	Supplement amount (g/kg BW)[A]		*P*-value
	4	6	
	kg/day		
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
	g/kg 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

Table 4. Effect of supplementation strategies on apparent digestibility coefficients and nitrogen levels in female calves under grazing in the tropics

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; NMIC: nitrogen production in the rumen: MICNR: microbial nitrogen: ingested nitrogen ratio; EMS: efficiency of microbial protein synthesis; UUN: urine urea nitrogen excretion. [A]Means± standard error of the mean.

Item	Supplement amount (g/kg BW)[A]		*P*-value
	4	6	
OM (g/g)	0.776 ± 0.0089	0.783 ± 0.0087	0.562
CP (g/g)	0.781 ± 0.0124	0.799 ± 0.0111	0.284
EE (g/g)	0.838 ± 0.0210	0.874 ± 0.0204	0.239
NDFap (g/g)	0.667 ± 0.0100	0.660 ± 0.0097	0.631
NFC (g/g)	0.823 ± 0.0076	0.827 ± 0.0074	0.667
DOM (g/kg DM)	710.8 ± 20.29	734.2 ± 19.78	0.412
NMIC (g/day)	50.1 ± 2.30	54.8 ± 2.34	0.150
MICNR (g/g N)	0.490 ± 2.2495	0.458 ± 2.1926	0.307
EMS (g/kg DOM)	116.2 ± 5.64	115.1 ± 5.50	0.890
UUN (g/day)	40.0 ± 1.99	53.1 ± 1.99	<0.001

Table 5. Effect of supplementation strategies on the metabolic profile in female calves under grazing in the tropics

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

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

Table 6. Effect of supplementation strategies on the productive performance, carcass characteristics and body growth in female calves under grazing in the tropics

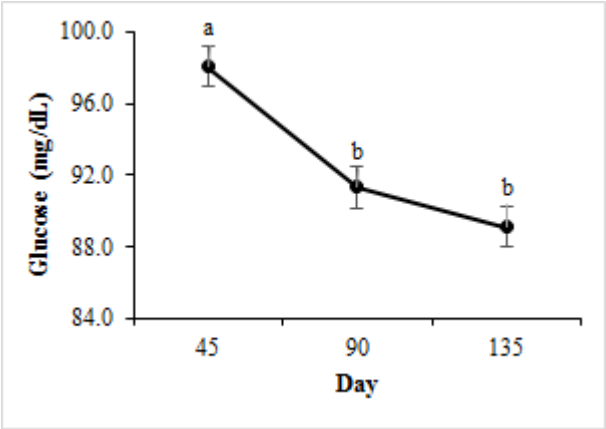
FBW: final body weight; ADG: average daily gain; LMA: Longissimus muscle area; SFTL: subcutaneous fat thickness at the Longissimis mucle; SFTR: fat thickness at the rump; BW: body weight; HW: height at the withers. [A]Means± standard error of the mean

Item	Supplement amount (g/kg BW)[A]		*P*-value
	4	6	
FBW (kg)	246.1 ± 2.67	251.2 ± 2.67	0.185
ADG (kg/day)	0.792 ± 0.0176	0.826 ± 0.0176	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
SFTR (mm)	2.54 ± 0.163	2.75 ± 0.163	0.361
Height at withers (cm)	118.9 ± 0.77	117.9 ± 0.77	0.323
Heart girth (cm)	144.0 ± 0.59	144.8 ± 0.59	0.368
Rib width (cm)	38.2 ± 0.53	38.2 ± 0.53	0.989
Rump height (cm)	126.5 ± 0.76	125.8 ± 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)	121.4 ± 1.23	123.7 ± 1.23	0.194
BW:HW (kg/cm)	2.07 ± 0.022	2.13 ± 0.022	0.064

Figures

1.

(a)



(b)

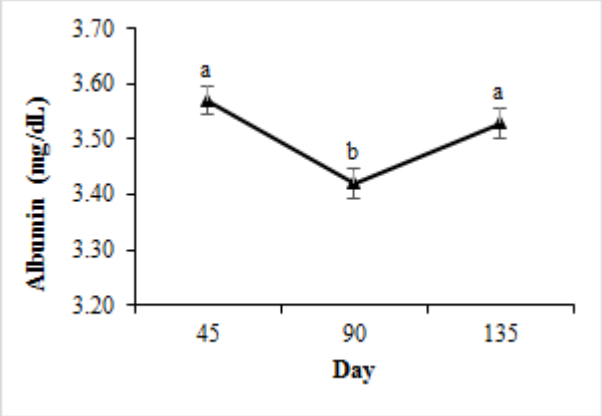


Figure 1. Blood glucose (a) and albumin (b) concentrations during the experimental period. [A]Means over the line followed by different letters differ (P<0.05).

Effects of supplementation strategies in heifers under grazing in the tropics: II. Productive and nutritional performance, metabolic profile and ovarian activity in the rearing period

Abstract. Effects of supplement amounts pre-weaning and rearing on productive and nutritional performance, metabolic profile, and ovarian activity were evaluated in heifers under grazing in the tropics. Forty Nellore heifers averaging 8.5±0.06 months and 248.6±3.3 kg body weight (BW) were distributed in a completely randomized design in a 2 × 2 factorial scheme with four treatments and ten replicates. The treatments were: 1) 4 g/kg of BW of supplement pre-weaning and rearing; 2) 4g/kg of BW of supplement pre-weaning and 6 g/kg rearing; 3) 6 g/kg of BW pre-weaning and 4 g/kg of BW rearing and; 4) 6 g/kg of BW pre-waning and rearing. Crude protein (CP), organic matter (OM) intake were increased ($P \leq 0.02$) by increasing the amounts of supplement in the rearing. Additionally, increasing supplements amounts in the rearing increased the digestibility the OM and CP ($P<0.05$). Means insulin and glucose concentrations were greater ($P<0.03$) for heifers that received greater amounts of supplement in the rearing. Daily gain and fat thickness in the rump were increased ($P<0.01$) by increasing supplement amounts in the rearing. Amounts of supplement did not influence ($P>0.15$) body growth of heifers. However, follicular number, diameter and progesterone concentration were greater ($P<0.02$) for heifers that received greater amounts of supplement in the rearing. These results suggest that increasing supplement amounts in the rearing has greater impact on optimizing productive and nutritional performance, metabolic status, and ovarian activity of heifers than increasing supplement amounts in the pre-weaning.

Additional keywords: *Bos indicus*, heifers, supplementation, puberty, tropical pastures.

Introduction

In grazing cattle systems, although forage represents the main energy source for cattle under grazing in the tropics (Detmann *et al.,* 2010), which is the primary dietary consideration for heifer development (Mass, 1987), protein is traditionally considered the limiting nutrient (DelCurto *et al.,* 2000). However, the tropical forage do not represent a balanced diet for animal production (Paulino *et al.,* 2008), lacking the necessary nutrients equilibrium to support the recommended growth rates during rearing (Roberts *et al.,* 2009) and not heifers allowing to reach puberty and sexual maturity early. During the dry season, crude protein (CP) content of tropical forage under grazing is usually less than 70-80 g/kg dry matter (DM), which limits the use of fibrous carbohydrates from basal forage by ruminal microorganisms. During the rainy season, although the CP content is higher than 70-80 g/kg DM, tropical forages present an unbalanced energy-to-protein ratio, exhibiting a relative excess of energy in relation to available CP, which limits the forage intake and metabolic efficiency by the animals (Detmann *et al.,* 2010).

In this context, protein and multiple supplementation of grazing cattle are considered the main management tools to circumvent dietary and metabolic deficiencies of tropical grasses (Detmann *et al.,* 2014). Accordingly, by improving energy and protein intake there may be favorable effects on growth, nutritional status, and reproductive development of heifers under grazing in the tropics.

Studies have shown different results on when is the best time to increase growth and decrease the age to puberty in bovine females. Some authors have reported the occurrence of early puberty with increased rate of gain in early stages of development (Patterson *et al.,* 1992; Gasser *et al.,* 2006; Cardoso *et al.,* 2014; Rodríguez-Sánchez *et al.,* 2015), while others observed a reduction in age at puberty with higher weight gain post-weaning (Barcellos *et al.,* 2014; Rodríguez-Sánchez *et al.,* 2015; da Silva *et al.,* 2017).

Thus, the supplementation of beef heifers in the pre-weaning and rearing periods under grazing and assess its carry-over effect could contribute to optimizing of growth rate and time to sexual maturity of animals, lending maximum lifetime productivity and profitability beef industry.

The objective of this study was to evaluate the effects of supplement amounts in the pre-weaning and rearing on performance, intake and digestibility, efficiency of microbial protein synthesis, metabolic profile, carcass characteristics, and ovarian activity in heifers 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).

Location

This experiment was conducted at the Department of Animal Science of the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil (20° 45′ S 42° 52′ W), between June and November 2015, which corresponded to the dry season and dry-rainy transition season. The experimental area was located in mountainous region, with 670 m of altitude and, presented an average temperature and precipitation values of 20.8°C and 304 mm, respectively.

Animals, experimental design, and diets

The animals studied in the present experiment were the same as those which were included in our companion study conducted by Ortega *et al.* (2018). These animals were weaned and transported to another area where the present study was performed.

Forty Nellore heifers (averaging 248.6±3.3 kg initial body weight (BW) of and of 8.5±0.06 months of age) were used. Animals were distributed in a completely randomized design in a 2 x 2 factorial scheme (two amounts of supplement in the pre-weaning period and two amounts of supplement in the rearing period). The animals were randomly assigned to receive one of four treatments (10 replicates per treatment). The treatments ere: 1) animals that received 4 g/kg BW of supplement during the pre-weaning and rearing periods; 2) animals receiving 4 g/kg BW of supplement during the pre-weaning period and 6 g/kg BW of supplement during the rearing period; 3) animals receiving 6 g/kg BW of supplement during the pre-weaning period and 4 g/kg BW of supplement during the rearing period; and 4) animals receiving 6 g/kg BW of supplement during the pre-weaning and rearing periods. The treatments offered during pre-weaning period were described in our companion study (Ortega *et al.,* 2018). Supplement was composed of corn meal, soybean meal, and mineral mix and, formulated to contain 30% CP as fed (Table 1). The supplement amounts of 4 g/kg BW (310 g CP/d) and 6 g/kg BW (465 g CP/d) accounted to approximately 50 and 75% of the dietary requirements of CP for Zebu heifers under grazing with BW of 300 kg and expected gain of 0.5 kg/d (Rotta *et al.*, 2016).

The duration of the study was 150 days. At the beginning of the study, animals were weighed after 14 h of solids fasting, and then divided into four groups. Animals were allocated to one of four paddocks of 2.5 hectares (one for each treatment), uniformly covered with *Brachiaria decumbens* Stapf., equipped with drinking and feeders.

Supplements were delivered daily at 10 am. Water was provided *ad libitum* during the study. Animals were weighed every 30 d without fasting and always in the morning in the order to adjust the amount of supplement provided to each group. In order to minimize potential effects of the plots on experimental treatments, animals were rotated across the four pastures every seven days with each group staying on each plot the same length of time.

Forage samples and nutritional characteristics

Pasture chemical composition (Table 1) was assessed by hand-plucked samples, collected every 15 days of each experimental period. A second pasture sample was collected every 30 d in to estimate the total availability of 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.

To evaluate voluntary intake and digestibility of animals, a nine-day trial was performed starting at the 75th experimental day. 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 and g per animal). Titanium dioxide (TiO_2) was used to estimate individual intake of supplement mixed in the supplement at the proportion of 10 g/kg of supplement. The indigestible neutral fiber (iNDF) was used as internal marker to estimate forage DM intake. Fecal samples were collected immediately after defecation or taken directly from 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. Fecal samples were identified, oven-dried at 60°C and ground as previously described. After that, samples were pooled based on each animal.

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

Blood samples

Blood samples were collected on days 45, 90, and 135 of the experiment to quantify the concentration of insulin, glucose, cholesterol, serum urea nitrogen (SUN), albumin, and total proteins. Additionally, every 15 days between 10.5 to 13.0 months of age, blood samples were collected to quantify blood progesterone concentration of heifers. Samples were collected at 7h00, via jugular venipuncture in vacuum tubes with clot activator and gel for serum separation (BD Vacuntainer® SST II Advance, Phymouth, UK) and vacuum tubes containing sodium fluoride and EDTA (BD Vacutainer® Fluoreto/EDTA, São Paulo, Brasil) 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

Methods used to assess performance, biometric measurements, and carcass characteristics of animals were previously described by Ortega *et al.* (2018). In brief, for performance evaluation, animals were weighed at both the beginning and end of the experiment after 14 h of solids fasting.

At the end of the experiment, carcass characteristics were evaluated using ultrasound (Aloka SSD 500; 3.5 MHz linear probe). The *longissimus dorsi* muscle area (LMA) and subcutaneous fat thickness of the loin (SFTL) were measured between the 12th and 13th ribs and subcutaneous fat thickness on the rump (SFTR) between the ischium and pubis. Occurring at the same time, body measurements (BM) were also taken to evaluate body growth of the animals. The rump width, rump length, rib depth, body

length, height at withers, and rump height were recorded with a measuring stick, whereas heart girth heart was recorded using a flexible tape.

Ovarian ultrasonography

On day 150 of study, transrectal evaluation was performed using ultrasound (Aloka SSD 500 of 5mHz with linear transducer, Aloka Co, Tokyo, Japan) to measure the diameter of the greatest follicle, number of follicles and corpus luteum presence.

Analytical procedures

Samples of forage, feces, and supplement (ground through 1-mm sieves) were analyzed according to standard analytical procedures of the Brazilian National Institute of Science and Technology in Animal Science (INCT-CA; Detmann *et al.*, 2012) 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), and 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). The content of iNDF in samples of feces, forage, 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).

Fecal samples were also analyzed for chromium concentration using nitroperchloric digestion and atomic absorption spectrophotometry (Souza *et al.*, 2013) and titanium dioxide by colorimetry (Titgemeyer *et al.*, 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:

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

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).

Daily urinary volume was calculated using the relationship between the daily creatinine excretion (CE), taking as reference the equation proposed by Costa e Silva *et al.* (2012), and its concentration in the spot samples:

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

where: BW = body weight

Excretion of the purine derivatives in urine was calculated by the sum of the allantoin and uric acid excretions, which were obtained by the product between their concentrations in urine by the daily urinary volume. Absorbed purines were calculated from the excretion of purine derivatives according to Chen and Gomes (1992).

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

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

Ruminal synthesis of nitrogen compounds was calculated as a function of the absorbed purines using the equation described by Barbosa *et al.* (2011).

$$MICN = 70 \times AP / 0.93 \times 0{:}137 \times 1.000$$

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

Efficiency of protein microbial synthesis (EFM) was determined by dividing protein microbial production by the DOM intake.

Blood insulin and progesterone concentrations were quantified by the indirect chemiluminescence using Access Ultrasensitive Insulin Reagent (Ref. Number 33410, Beckman Coulter®, Brea, USA), Access Progesterone Reagent (Ref. Number 33550, 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 the enzymatic-colorimetric method. Urea in serum and urine by the enzymatic kinetic method (Ref. Número K056-1, Bioclin® Quibasa, Belo Horizonte, Brasil), and albumin (Ref. Number K040-1, Bioclin® Quibasa, Belo Horizonte, Brazil) and total protein (Ref. Number K031-1, Bioclin® Quibasa, Belo Horizonte, Brazil) were quantified using the colorimetric method. Urinary creatinine was assessed using the kinetic colorimetric method (Ref. Número K067-1, Bioclin® Quibasa, Belo Horizonte, Brasil), whereas urinary uric acid was measured using the enzymtic-colorimetric method (Ref. Número K139-1, Bioclin® Quibasa, Belo Horizonte, Brasil). 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).

Statistical analyses

Experimental data was analyzed consistent with our randomized design in a 2 × 2 factorial scheme. All statistical procedures were conducted using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The performance characteristics were submitted to ANOVA tests adopting the initial BW as covariate. Supplement amount during pre-weaning and rearing periods along with respective interactions were considered fixed effects. Serum concentrations of insulin, progesterone, glucose, cholesterol, urea, albumin, and total proteins were analyzed using the procedure for repeated measures. 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 trends were considered at $0.05 \leq P \leq 0.10$. In the absence of interaction between supplement amounts in the pre-weaning and rearing periods main effects are reported.

Results

Forage samples and nutritional characteristics

Mean forage availability on pasture was 4.7±0.37 and 3.2±0.28 t/ha of total DM and MSpd, respectively. Forage samples obtained by the hand-plucked method had an average CP content of 91.3 g/kg DM (Table 1).

There was no interaction ($P>0.10$) between amounts of supplement in the pre-weaning and rearing for voluntary intake (Table 2). Additionally, there was no effect ($P>0.13$) of pre-weaning supplement amounts on voluntary intake in the rearing. There was a trend for an increase ($P=0.056$) in DM intake when the amounts of supplement

were increased in the rearing. However, there were no supplement amounts effect (P>0.13) in the rearing on forage, NDFap, and digested FDN (DNDF) intake. In addition, there was an increase (P<0.02) in intake of supplement, CP, organic matter (OM), and digested OM (DOM) with the higher amount of supplement provided in the rearing (Table 2).

Intake in relation to BW of animals (g/kg BW), there was no effect (P>0.47) the amounts of supplement in the rearing on voluntary intake. However, a decrease trend (P=0.082) in forage intake with increase the amounts of supplements in the rearing (Table 2).

No interaction effect (P>0.10) was observed between amounts of supplement in the pre-weaning and rearing on the total digestibility (Table 3). In general, there were no effects (P>0.11) the amounts of supplement in the pre-weaning on total digestibility in the rearing. There was a significant effect (P=0.023) the amounts of supplement in the pre-weaning on the total digestibility of OM in the rearing. The total digestibility of OM and CP were increased (P<0.05) by increasing the amounts of supplement in the rearing (Table 3). However, the amounts of supplement in the rearing did not affect (P>0.26) the digestibility of NDFap and MOD (g/kg DM) (Table 3).

There was no interaction effect (P>0.10) between amounts of supplement in the pre-weaning and rearing on the synthesis of nitrogen compounds (Table 3). No effects were detected (P>0.36) for pre-weaning supplement amounts on the synthesis of nitrogen compounds in the rearing. Additionally, there was no effect (P>0.12) of amounts of supplement in the rearing on synthesis of microbial nitrogenous compounds in the rumen (MICN), microbial nitrogen: ingested nitrogen ratio (MICNR) and efficiency of microbial protein synthesis (EFM) (Table 3). Th urine urea nitrogen excretion (UUN) was increased (P=0.001; Table 3) by increasing the amounts of supplement in the rearing.

Metabolic Profile

There was interaction effect (P<0.001) was observed between treatments and collection days to SUN and albumin concentrations (Table 4). Closer examination of this effect indicated SUN concentration was increased only when supplement amount was increased (P<0.001; Fig. 1a), showing the higher concentrations in the second collection day(P<0.001). Evaluation of the interaction for albumin concentration indicated that all treatments showed a decrease compared with the first collection day (P<0.01; Fig. 1b).

In general, there was no interaction effect (P>0.72) between amounts of supplement in the pre-weaning and rearing on variables evaluated in the metabolic profile. There was also no effect (P>0.16) associated with pre-weaning supplement amounts for any metabolic profile variables evaluated in the rearing (Table 4). By contrast, there was an effect (P<0.03) the amounts of supplement in the rearing on concentrations of glucose, insulin, and progesterone in blood (P<0.03) which were greater for the greatest supplement amounts (Table 4). However, no effects the amounts of supplement were observed (P<0.15) for the other metabolic profile variables evaluated (Table 4).

The blood concentration of insulin increased (P<0.001) from the first collection day (Fig 2a). Glucose and cholesterol concentrations were lowest (P<0.001) during the last collection day (Fig. 2b and Fig. 2c, respectively). Blood total protein concentration increased (P <0.001) from the first collection day (Fig. 2d). Progesterone concentration increased (P<0.001) from the first collection day, demonstrating highest values at the fourth collection day (Fig. 2e).

Performance, carcass characteristics and biometric measurements

There was no interaction effect (P>0.28) between amounts of supplement in the pre-weaning and rearing on variables associated with productive performance, carcass characteristics, and body growth (Table 5). No effect was observed (P>0.55) the pre-

weaning supplement amounts on productive performance, carcass characteristics, and body growth in the rearing. However, there was a trend (P=0.053) for pre-weaning supplement amounts to affect SFTL in the rearing period (Table 5). The ADG and SFTR were increased (P<0.01) by increasing the amounts of supplement in the rearing (Table 5). However, the amounts of supplement in the rearing did not affect (P>0.30) FBW, LMA and STFL (Table 5).

In general, the amounts of supplement did not influence (P>0.15) body growth (Table 5). There was a positive effect (P=0.023) of the increase the amounts of supplement in the rearing on BW related to Height at withers. There was also a trend (P=0.060) for increased rump length by increase the amounts of supplement in the rearing.

Ovarian ultrasonography

There was no interaction effect (P>0.19) between amounts of supplement in the pre-weaning and rearing on follicle number and diameter of the greatest follicle. In addition, there was no effect (P<0.29) the pre-weaning supplement amounts on follicle number and diameter of the greatest follicle in the rearing. The number and follicular diameter were increased (P<0.02) by increasing the amounts of supplement in the rearing period (Table 5). Only three heifers demonstrate the presence of corpus luteum (aimed the puberty), one from the treatment that received of 4 g/kg BW of supplement in the pre-weaning and 6 g/kg BW in the rearing period, one from treatment that received 6 g/kg BW of supplement in the pre-weaning and 4 g/kg BW in the rearing period and, one from the treatment that received 6 g/kg BW of supplement in the pre-weaning and rearing periods.

Discussion

Studies with cattle fed tropical forage indicate that supplementation with nitrogen compounds can elevate the dietary CP content to a level near 100 g CP/kg DM, optimizing forage intake (Lazzarini *et al.*, 2009; Sampaio *et al.*, 2010). After this level of CP in the diet, the nitrogen-compound requirements of the rumen microorganisms are met, and benefits from the forage degradation would not be further observed. In addition, voluntary intake forage is maximized when the dietary CP content up to 145 g CP/kg DM (Detmann *et al.*, 2014). In this study, average dietary CP content was 147.7 and 147.5 g CP/kg DM for heifers that received 4 g/kg BW and 4 g/kg BW of supplement in the rearing, respectively. This may explain the similar intake of forage and NDFap digestibility between treatments (Table 3). Results obtained in this study are similar to those reported by others authors who supplement cattle under tropical conditions (Costa *et al.*, 2011; Batista *et al.*, 2016; Franco *et al.*, 2017).

Higher DM and CP intake for heifers that received greater amount of supplement in the rearing appears to directly reflect the high intake of the supplement, as the forage intake was unaffected (Table 2). This pattern resulted in an increase in DOM intake for these animals.

The trend for decreasing forage intake (g/kg BW) indicates that heifers receiving a greater amount of supplement in the rearing gained more weight (Table 5) and, apparently, were heavier in the digestion trial. As a consequence, the forage intake was slightly lower in g/kg BW.

Positive effects of pre-weaning supplements amount on digestibility of OM in the rearing period indicates a carry-over effect in that heifers with greater amount of supplement in the pre-weaning had greater use of OM compared with those with lower amount of supplement pre-weaning (0.666 vs. 0.654, respectively). However, this behavior had not influence on the performance of the animals.

The higher digestibility of CP for heifers that received greater amounts of supplement in the rearing (Table 3) can be attributed to higher supplement intake, that provided higher CP intake. Supporting this rationale, higher protein intake allows lower participation of endogenous protein leading to reduced presence in fecal metabolic fraction of nitrogen compounds (Van Soest 1994). Additionally, higher digestibility of OM for heifers with greater amount of supplement appears to be directly related to greater digestibility of CP since digestibility of NDFap was unaffected (Table 3).

According to Clark *et al.* (1992), ruminal availability of energy and nitrogen are the nutritional factors that most affect microbial growth. Thus, the lack of effect on NMIC, NMICR and EMS between treatments (Table 3), can be attributed to the fact that all diets provided sufficient energy and nitrogen compounds to optimize growth of ruminal microorganisms. These results suggest that there was no deficiency of nitrogen compounds in the animals' diet.

According to Van Soest (1994), the concentration of UUN is positively associated with CP intake and SUN. Higher UUN and SUN concentration for heifers that received greater amounts of supplement (Table 3 and 4) is due to higher CP intake, which possibly decreases the efficiency of ammonia use in the rumen. In addition, SUN concentration is positively associated with CP intake, rumen degradable protein, and ruminal ammonia concentration (Broderick and Clayton 1997). The optimal SUN concentrations in beef heifers range from 11 to 15 mg/dL (Byers and Moxon 1980), indicating that the heifers in this study were not protein deficient or in excess. The highest concentration of SUN (Fig. 1d) can be attributed to increase in CP content of the forage ingested by the animals (74.89, 105.02 and 107.86 g of CP/kg DM for first, second and third collection, respectively). However, it is important to note that the second collection period coincided with the dry-rainy transition season. According to Detmann *et al.* (2010), in the dry-rainy transition season protein content of the forage rapidly rises, and, in particular, non-protein

nitrogen (NPN). Thus, higher NPN (high solubility) intake increases the concentration of ruminal ammonia and, consequently, increases the transport of nitrogen by diffusion into the blood stream (NRC 1985).

In the present study, the most prominent effects of increase in the supplement amounts were observed on energy metabolism and the nitrogen accretion in animal body, which would represent an increase in concentrations of glucose, insulin, weight gain and ovarian activity.

Thus, the greater concentrations of glucose and insulin for heifers that received greater amounts of supplement (Table 4) indicate that the higher intake of DM, CP and concentrate promoted increase of gluconeogenic precursors to be metabolized into glucose. As a consequence, higher concentrations of glucose and insulin in the blood. Consistent with this, glucose and insulin concentration in the blood is positively associated with energy intake (Huntington *et al.,* 2006).

In ruminants, glucose requirements are met primarily via hepatic gluconeogenesis (Huntington *et al.*, 2006). Thus, the decreasing pattern in glucose concentration from the second collection day (Fig. 2b) can be attributed to a reduction in the rate of gluconeogenesis caused by the increase in insulin concentrations from the second collection day (Fig 2a). Supporting of these results, the uptake of glucose precursors as well as the release of hepatic glucose are reduced by insulin (Huntington *et al.,* 2006).

Cholesterol is the main precursor for synthesis of steroid hormones (Yart *et al.,* 2014). Thus, the absence of a difference in cholesterol concentrations between treatments (Table 4) may be due to the greater use of cholesterol for progesterone synthesis by heifers with greater amounts of supplement in the rearing, which is consistent with higher plasma progesterone concentration in these animals. Rodríguez-Sánchez *et al.* (2015) reported a negative association between plasma cholesterol and progesterone. By contrast, the lower cholesterol concentration in the last collection day (Fig. 2c) is probably due to a reduction

in glucose levels from the second collection day (Fig. 2b). Consistent with our observations, Ndlovu *et al.* (2007) reported that variation in plasma cholesterol concentration can be attributed to variation in plasma glucose levels.

Blood protein and albumin concentrations may be related to the availability of amino acids and nutrients (Lawrence *et al.,* 2012). Thus, lack of effect in albumin and total proteins blood concentrations between treatments (Table 4) suggests that diets provided similar protein status in the animals. However, the increase in nutritional value of the forage ingested by the animals, suggested by the increase in CP content from the first to the third collection period, may explain the increasing pattern in relation to the first collection of total protein blood concentration (Fig. 1d).

In fact, in this study the greater ADG observed for heifers that received greater amounts of supplement in the rearing (Table 5), can be attributed to the higher intake high digestible supplements, resulting in increased animal performance.

The absence of an effect on LMA between treatments suggests that supplement amounts used in this study were not sufficient to affect deposition of muscle tissue (Table 5). Differences observed in STFR between treatments are consistent with the higher deposits of adipose tissue for heifers that received the greatest supplement amounts (Table 5). At this site, fat accumulation begins earlier than in the ribs and is more accurate for measuring fat thickness (Yokoo 2005), which may explain the lack of effect on STFL between treatments. Body reserves can act as an indicator of energy available for reproductive activity (Hall *et al.,* 1995) acting as a positive signal allowing for ovulation and pregnancy.

Collectively, body measurement results suggest that supplement amounts applied in this experiment did not limit body growth of heifers (Table 5). However, the pattern for increased rump length, higher BW: HW ratio and SFTR for heifers receiving greater

amounts of supplement in the rearing (Table 5) suggests that higher concentrate intake improves muscular and adipose tissue composition.

Higher concentrations of progesterone, follicular diameter, and number of follicles for heifers supplemented with the greatest amounts in the rearing (Table 4 and 5), reaffirms the positive effect of nutrition on reproductive function. Similar patterns have been observed by Barcellos *et al.* (2014) and da Silva *et al.* (2017). These results may be supported by higher concentrations of glucose and insulin. Insulin can directly stimulate cell proliferation and steroidogenesis by increasing estradiol from the dominant follicle or indirectly by stimulate release of insulin-like growth factor (IGF-I). The binding to its receptor results in a number of metabolic effects, the most important being the stimulation of glucose transport into cells, which may be used as the main energetic source for the ovary (Bossis *et al.*, 2000; Lawrence *et al.*, 2012). This has been suggested to be a potent stimulator of proliferation and differentiation of granulosa cells (Lawrence *et al.*, 2012), which increases progesterone concentration and diameter and follicle number of these animals.

Conclusions

Increasing supplement amounts in the rearing period has greater positive impact on performance, energy and metabolic status, and ovarian activity in beef heifers under grazing in the tropics than increasing supplement amounts in the pre-weaning period.

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Tables

Table 1. Ingredients and chemical composition of supplement and forage consumed by the animals during the experimental period

NDF: neutral detergent fiber; NDFap: neutral detergent insoluble fiber corrected for ash and protein; NDIP: neutral detergent insoluble protein. [A]Samples obtained from a grazing simulation during the digestion trial. [B]Samples obtained from a grazing simulation during the experimental period. [C]Means± standard error of the mean. [D]NFC = OM – (CP + EE + NDFap)

Item	Supplement	*B. Decumbens*[A,C]	*B. Decumbens*[A,B]
Ingredient % (as fed)			
Soybean meal	54.3	-	-
Corn meal	40.7	-	-
Mineral mixture	5.0	-	-
Chemical composition (g/kg DM)			
Dry matter (DM)	915.0	887.5 ± 0.20	885.8 ± 0.55
Organic matter (OM)	916.6	917.9 ± 0.97	911.9 ± 0.44
Crude protein	285.9	109.4 ± 5.80	91.3± 0.64
Ether Extract	16.3	19.8 ± 0.07	-
Non-fibrous carbohydrates[D] (NFC)	485.8	103.3 ± 0.46	-
NDFap	128.6	593.1 ± 2.80	621.6 ± 4.05
NDIP (g/kg de CP)	132.9	354.6 ± 0.39	329.6 ± 0.19
Indigestible NDF (iNDF)	15.2	154.4 ± 0.30	157.7 ± 0.35

Table 2. Effect of supplementation strategies on voluntary intake in heifers under grazing in the tropics

DMF: dry matter from forage; DMS: dry matter from supplement; DOM: digested organic matter; DNDF: digested neutral detergent insoluble fiber. [A]PW = pre-weaning period; R = rearing period; [B]PW = supplementation effect in the pre-weaning period; R = supplementation effect in the rearing period; PW × R = interaction between supplementation amounts pre-weaning and rearing; SEM = standard error of the mean

Item	Supplement amount (g/kg BW)[A]				SEM	*P*-value[B]		
	PW (4)		PW (6)					
	R (4)	R (6)	R (4)	R (6)		PW	R	PW × R
				kg/day				
DM	6.53	6.86	6.34	6.91	0.231	0.757	0.056	0.620
DMF	5.51	5.34	5.32	5.33	0.184	0.592	0.658	0.636
DMS	1.01	1.52	1.01	1.58	0.083	0.738	<0.001	0.738
OM	5.98	6.30	5.81	6.35	0.169	0.714	0.014	0.513
CP	0.92	1.02	0.98	1.01	0.021	0.335	0.003	0.101
EE	0.13	0.13	0.12	0.13	0.004	0.664	0.056	0.568
NDFap	3.39	3.41	3.24	3.37	0.109	0.410	0.477	0.604
INDFi	0.86	0.85	0.84	0.85	0.028	0.617	0.990	0.701
MOD	3.88	4.15	3.78	4.29	0.112	0.845	0.001	0.285
FDND	2.06	2.03	1.91	2.11	0.071	0.660	0.228	0.133
				g/kg BW				
DM	22.6	23.6	23.1	23.1	0.80	0.989	0.563	0.558
DMF	19.1	18.3	19.4	17.8	0.67	0.868	0.082	0.536
OM	20.8	21.7	21.2	21.3	0.65	0.995	0.462	0.532
NDFap	11.8	11.7	11.8	11.3	0.40	0.655	0.466	0.541
iNDF	3.0	2.9	3.1	2.8	0.10	0.915	0.172	0.472

Table 3. Effect of supplementation strategies on apparent digestibility coefficients and nitrogen levels in heifers under grazing in the tropics

MICN: nitrogen production in the rumen: MICNR: microbial nitrogen: ingested nitrogen ratio; EFM: efficiency of microbial protein synthesis; UUN: urine urea nitrogen excretion. [A]PW = pre-weaning period; R = rearing period; [B]PW = supplementation effect in the pre-weaning period; R = supplementation effect in the rearing period; PW × R = interaction between supplementation amounts pre-weaning and rearing; SEM = standard error of the mean

Item	Supplement amount (g/kg BW)[A]				SEM	*P*-value[B]		
	PW (4)		PW (6)					
	R (4)	R (6)	R (4)	R (6)		PW	R	PW × R
OM (g/g)	0.649	0.658	0.652	0.679	0.0047	0.023	0.001	0.105
CP (g/g)	0.722	0.738	0.735	0.743	0.0073	0.118	0.049	0.427
NDFap (g/g)	0.597	0.598	0.592	0.604	0.0061	0.269	0.447	0.345
DOM (g/kg DM)	596.3	605.6	600.4	621.6	10.19	0.328	0.142	0.566
NMIC (g/dia)	71.3	72.8	68.9	76.1	4.02	0.704	0.123	0.232
NMICR (g/g N)	0.486	0.447	0.441	0.472	0.0244	0.365	0.730	0.107
EFM (g/kg DOM)	115.4	110.5	107.2	110.9	6.05	0.519	0.916	0.485
UUN (g/dia)	49.6	66.4	56.7	63.9	3.35	0.489	0.001	0.156

Table 4. Effect of supplementation strategies on the metabolic profile in heifers under grazing in the tropics

[A]PW = pre-weaning period; R = rearing period; [B]PW = supplementation effect in the pre-weaning period; R = supplementation effect in the rearing period; PW × R = interaction between supplementation amounts pre-weaning and rearing; Col = collection effect; Treat × Col = interaction between treatment and collection. SEM = standard error of the mean

Item	Supplement amount (g/kg BW)[A]				SEM	*P*-value[B]				
	PW (4)		PW (6)							
	R (4)	R (6)	R (4)	R (6)		PW	R	PW x R	Col.	Treat × Col
Insulin µUI/mL	0.53	0.64	0.54	0.64	0.046	0.919	0.023	0.994	<0.001	0.677
Glucose (mg/dL)	70.9	75.0	72.4	74.8	1.35	0.489	0.001	0.394	<0.001	0.758
Cholesterol (mg/dL)	89.5	89.6	82.6	89.0	4.17	0.161	0.218	0.242	0.022	0.882
SUN (mg/dL)	13.6	15.2	12.6	16.0	0.52	0.800	<0.001	0.015	<0.001	<0.001
Total proteins (g/dL)	6.08	6.19	6.07	6.16	0.103	0.754	0.150	0.909	<0.001	0.834
Albumin (g/dL)	3.39	3.41	3.38	3.42	0.052	0.984	0.439	0.855	0.962	<0.001
Progesterone (ng/mL)	0.53	0.90	0.71	1.09	0.271	0.230	0.019	0.962	<0.001	0.162

Table 5. Effect of supplementation strategies on the productive performance, carcass characteristics, body growth and ovarian activity in heifers under grazing in the tropics

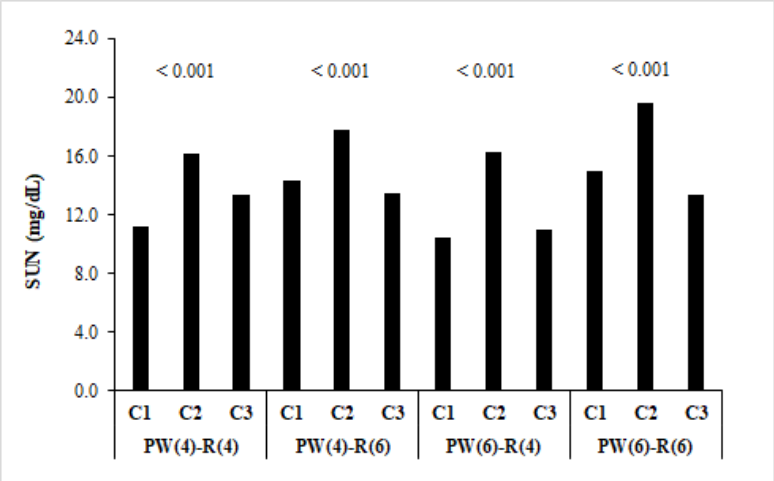
FBW: final body weight; ADG: average daily gain; LMA: longissimus muscle area; SFTL: subcutaneous fat thickness at the loin; SFTR: fat thickness at the rump; BW: body weight; HW: height at the withers. [A]PW = pre-weaning period; R = rearing period; [B]PW = supplementation effect in the pre-weaning period; R = supplementation effect in the rearing period; PW × R = interaction between supplementation amounts pre-weaning and rearing; SEM = standard error of the mean

Item	Supplement amount (g/kg BW)[A]				SEM	*P*-value[B]		
	PW (4)		PW (6)					
	R (4)	R (6)	R (4)	R (6)		PW	R	PW × R
FBW (kg)	310.0	316.9	310.9	328.0	7.33	0.418	0.110	0.490
ADG (g/day)	0.413	0.486	0.413	0.497	0.0264	0.831	0.004	0.828
LMA (cm^2)	51.3	54.00	52.1	51.7	1.47	0.611	0.429	0.303
SFTL (mm)	2.29	2.34	2.60	2.97	0.236	0.053	0.378	0.503
SFTR (mm)	3.38	3.81	3.33	4.33	0.262	0.374	0.009	0.286
Height at withers (cm)	125.7	125.4	126.9	124.3	1.21	0.934	0.233	0.353
Heart girth (cm)	157.2	158.1	157.7	160.6	1.32	0.259	0.156	0.465
Rib width (cm)	41.6	41.3	41.3	42.9	0.54	0.214	0.227	0.095
Rump height (cm)	133.4	134.2	134.1	134.3	1.37	0.757	0.724	0.858
Rump width (cm)	38.6	39.2	38.7	39.4	0.57	0.786	0.242	0.854
Rump length (cm)	43.1	43.5	42.6	43.9	0.44	0.945	0.060	0.312
Body length (cm)	138.4	137.8	138.9	138.9	1.65	0.642	0.869	0.869
BW:HW (kg/cm)	2.47	2.52	2.44	2.64	0.053	0.400	0.023	0.164
Number of follicles	9.1	12.7	12.0	12.7	0.959	0.140	0.031	0.154
Follicular diameter (mm)	11.4	12.1	11.5	13.29	0.449	0.153	0.008	0.233

Figures

1.

(a)



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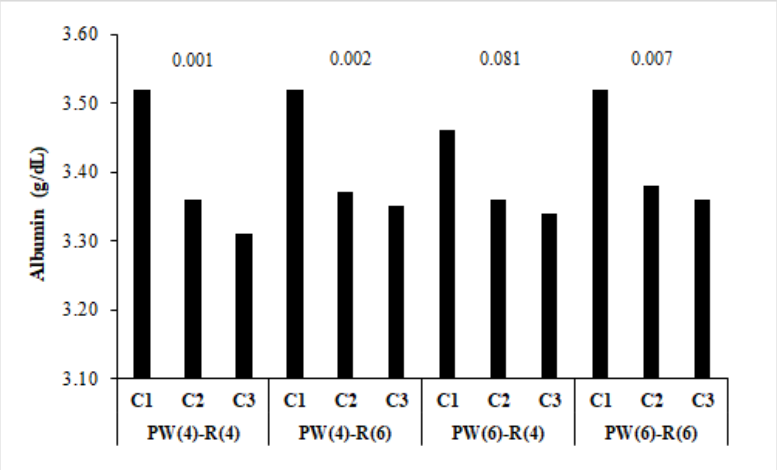
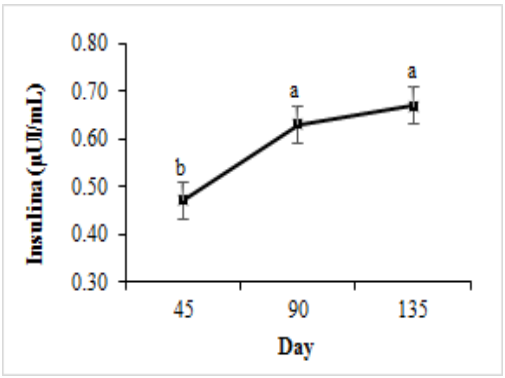


Figure 1. Serum urea nitrogen (SUN) (a) and blood albumin concentration (b) of different treatments according to collection days. C = Collection day; PW (4) = 4 g/kg BW in the pre-weaning; PW (6) = 6 g/kg BW in the pre-weaning; R (4) = 4 g/kg BW in the rearing and; R (6) = 6 g/kg BW in the rearing.

2.

(a)



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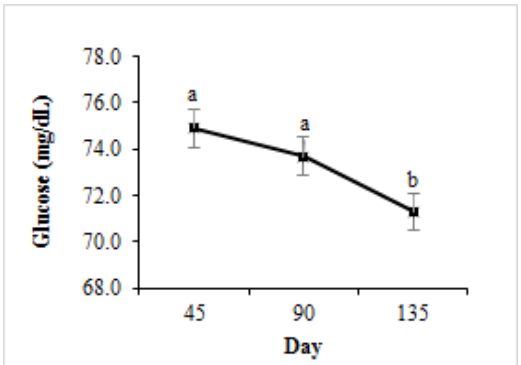

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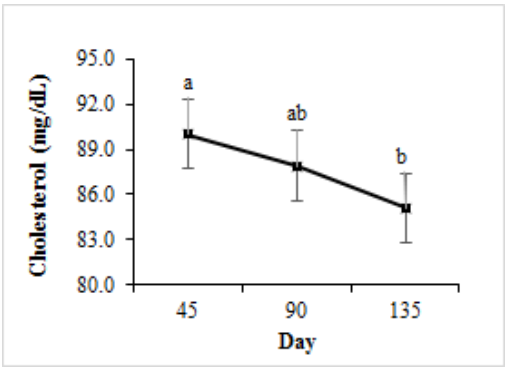
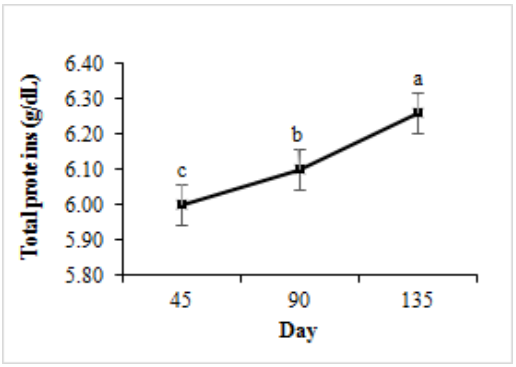


(d)



(e)

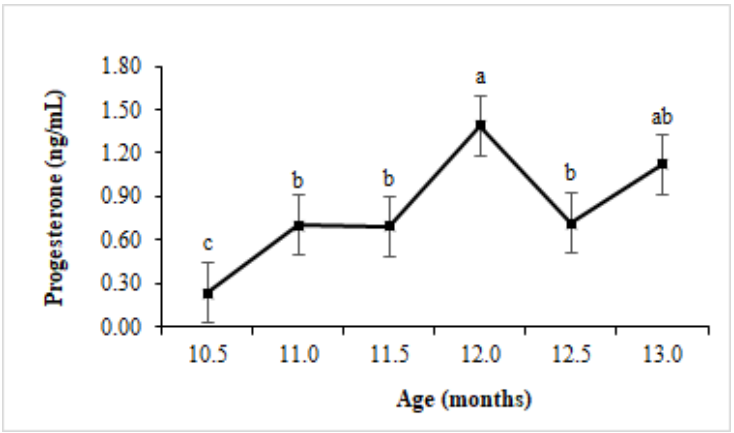


Figure 2. Blood insulin (a), glucose (b), cholesterol (c), total proteins (d) and progesterone (e) concentrations during the experimental period. [1]Means over the line followed by different letters differ (P<0.05).