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Protein-energy supplementation during pre-weaning on the performance and metabolism of crossbred female calves

Abstract – The objective of this work was to evaluate the effect of dietary supplementation with a protein-energy concentrate during the pre-weaning period on the nutritional and productive performance and on the metabolic response of crossbred female calves fed medium-quality forage in a dual-purpose system. Twelve Gyr×Holstein suckling female calves, with a body weight of 123.0±0.23 kg and an initial average age of 5.7±0.38 months, were distributed in a completely randomized design. Two treatments were carried out: animals without supplementation (control); and animals supplemented with 5.0 g kg^{-1} body weight of a protein-energy concentrate, containing 23.01% crude protein and 78% total digestible nutrients. Supplementation increased the intake of crude protein, nonfibrous carbohydrates, and neutral detergent fiber, but had no effect on dry matter intake. There was also an increase in the blood concentration of serum urea nitrogen. In addition, supplementation decreased forage intake time and increased idle time. The final body weight of the supplemented female calves was the heaviest, but did not influence the body measurements of the animals. Supplementation with 5.0 g kg^{-1} body weight of a protein-energy concentrate during the pre-weaning period improves the productive performance of crossbred female calves.

Index terms: *Pennisetum*, forage, metabolism, nutrient intake, ruminants.

Suplementação proteico-energética durante o pré-desmame no desempenho e no metabolismo de bezerras mestiças

Resumo – O objetivo deste trabalho foi avaliar o efeito da suplementação dietética com concentrado proteico-energético durante o período de pré-desmame sobre o desempenho nutricional e produtivo e a resposta metabólica de bezerras mestiças lactentes alimentadas com forragem de qualidade média, em sistema de duplo propósito. Doze bezerras Gir×Holandesa lactentes, com peso corporal de 123,0±0,23 kg e idade média inicial de 5,7±0,38 meses, foram distribuídas em delineamento inteiramente casualizado. Foram realizados dois tratamentos: animais sem suplemento (controle); e animais suplementados com 5,0 g kg^{-1} de peso corporal de concentrado proteico-energético, com 23,01% de proteína bruta e 78% de nutrientes digestíveis totais. A suplementação aumentou o consumo de proteína bruta, carboidratos não fibrosos e fibra em detergente neutro, mas não teve efeito no consumo de matéria seca. Também houve aumento na concentração sanguínea de nitrogênio ureico sérico. Além disso, a suplementação diminuiu o tempo de consumo de forragem e aumentou o tempo de ócio. O peso corporal final das bezerras suplementadas foi o maior, mas não influenciou as medidas corporais dos animais. A suplementação com 5,0 g kg^{-1} de peso corporal de

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concentrado proteico-energético durante o período de pré-desmame melhora o desempenho produtivo de bezerras mestiças.

Termos para indexação: *Pennisetum*, forragem, metabolismo, consumo de nutrientes, ruminantes.

Introduction

In Colombia, 35% of cattle production is carried out in a dual-purpose system for beef and milk, commonly using crossbred animals (*Bos taurus* × *Bos indicus*) with a low milk production (< 3.8 L per cow per day), low daily weight gain of calves (< 350 g per day), low birth rate (0.53 calves per cow per year), high production costs, and a low profitability (Ortiz-Valdes et al., 2023). However, the adoption of feeding strategies can optimize animal performance and favor the development of the replacement females necessary to maintain herd size and productive efficiency (Grings et al., 2006; Moreno et al., 2023).

In most dual-purpose systems, the farmers sell the milk instead of using it to feed the calves, whose potential for a high growth rate and weight gain due to their more efficient nutrient absorption is affected by the low amount of energy and protein provided (Henriques et al., 2011; Almeida et al., 2018). In this scenario, it is important to meet the consumption requirements of the calves during the pre-weaning period via milk and also pasture (Almeida et al., 2018). However, tropical pastures cannot be considered nutritionally balanced diets due to deficiencies in several nutrients (Paulino et al., 2008), which restrict nutrient intake and digestibility, affecting negatively the metabolic efficiency of the animals (Detmann et al., 2014a).

An alternative to improve feeding is supplementation, whose positive effects have been shown under tropical conditions, specifically on animal productive and nutritional performance (Cardenas et al., 2015; Márquez et al., 2014; Rodríguez-Sánchez et al., 2015; Almeida et al., 2018; de Paula et al., 2022; Moreno et al., 2022), as well as metabolic characteristics (Rodríguez-Sánchez et al., 2015; de Paula et al., 2022). Moreno et al. (2022) concluded that the low gain rate of female calves consuming a low-quality forage might be compensated after weaning when 5.0 or 7.5 g per kg body weight (BW) of a protein supplement is provided. However, other studies on suckling female calves did not report an increased performance or improvement in the metabolic status of the animals fed different supplements (Silva et al., 2017; Ortega et al., 2020).

Despite the divergences observed in the literature, most results presented are an indicative that the profitability of the bovine production systems could be benefited by nutritional strategies to increase nutrient intake and digestibility, optimizing the growth rate and metabolic status of crossbred female calves. Therefore, in the present study, it was hypothesized that dietary supplementation with a protein-energy concentrate in the pre-weaning phase would improve both the nutritional and productive performance and the metabolic response of crossbred calves fed medium-quality pasture in a dual-purpose system.

The objective of this work was to evaluate the effect of dietary supplementation with a protein-energy concentrate during the pre-weaning period on the nutritional and productive performance and on the metabolic response of crossbred female calves fed medium-quality forage in a dual purpose system.

Materials and Methods

All procedures applied to the animals were approved by the ethics and environmental impact committee of Universidad de Pamplona according to Act n.008 of October 25, 2023.

The experiment, covering 84 days between February and May 2020 during the dry season, was conducted at the Villa Marina experimental farm of Universidad de Pamplona, in the municipality of Pamplonita, in the department of Norte de Santander, Colombia (7°32'05"N, 72°38'02"W, at an altitude of 1,174 m). According to Köppen-Geiger's classification, the climate of the region is Cwa, i.e., humid temperate, with a dry winter and a hot summer. During the experimental period, average rainfall was 1,400 mm and average temperature was 20°C.

A total of 12 Gyr×Holstein suckling female calves with an average BW of 123.0±0.23 kg and an initial age of 5.7±0.38 months were evaluated, accompanied by their respective multiparous dams, with 5 years of age and an initial average body weight (BW) of 485.4 kg. The animals were distributed in a completely randomized design with six replicates, being subjected to two treatments: feed without supplement (control);

and feed supplemented with 5.0 g kg^{-1} BW of a protein-energy concentrate.

The protein-energy concentrate, offered in an as-fed basis, was composed of ground corn grain, rice bran, wheat meal, and soybean meal plus urea, being formulated to contain 230 g kg^{-1} dry matter (DM) of crude protein (CP) and 78% of total digestible nutrients (TDN) (Table 1). The quantify of concentrate supplemented was chosen to meet 35 and 25% of the nutritional requirements of CP and TDN, respectively, for crossbred female calves with 150 kg BW, with an average gain of 0.5 kg per day when consuming pasture with 90 g kg^{-1} CP on a DM basis according to the nutrient levels recommended for Zebu and crossbred cattle (BR-CORTE, 2016). All animals received a mineral mixture with the following composition: 500 g kg^{-1} $CaHPO_4$, 471.9 g kg^{-1} NaCl, 15 g kg^{-1} $ZnSO_4$, 7.0 g kg^{-1} Cu_2SO_4, 500 mg kg^{-1} $CoSO_4$, 500 mg kg^{-1} KIO_3, 100 mg kg^{-1} Na_2SeO_3, and 5.0 g kg^{-1} $MnSO_4$.

The animals underwent 14 days of adaptation to the diet and to the experimental area, being fed a basal supplementation of 2.0 g kg^{-1} BW per animal before the evaluation phase. At the beginning of the experiment, the female calves were weighed after 14 hours of solids fasting and, then, were kept in individual 12 m^2 pens equipped with drinkers and feeders, with unrestricted access to water. The calves were fed chopped *Pennisetum* sp. ad libitum, as the basal diet, at 7 a.m. and 4 p.m., allowing 10% orts. The concentrate was supplied simultaneously with the forage, being divided into two parts of equal weight.

Table 1. Ingredients and chemical composition of the concentrate and forage consumed by crossbred female calves (Gyr×Holstein) during the experimental period.

Ingredient (% as-fed)	Concentrate	Forage
Ground corn grain	37.5	–
Rice bran	22.0	–
Wheat meal	22.0	–
Soybean meal	17.0	–
Urea	1.5	–
Chemical composition (g kg^{-1} DM)(1)		
Dry matter (g kg^{-1} natural matter)	965.7	944.8
Organic matter	929.4	880.2
Crude protein	230.1	93.5
Ether extract	77.4	15.4
NFC	395.1	122.7
NDF	245.0	648.6
TDN	780	504

(1)NFC, nonfibrous carbohydrates; NDF, neutral detergent fiber; and TDN, total digestible nutrients.

The female calves were also subjected to restricted suckling, which is an experimental way to mimic the normal procedure in the dual-purpose system in Colombia. The animals were fed for 30 min at 6 a.m. (first suckling) and at 3 p.m. (second suckling), being weighed at 6 a.m. every 28 days (without fasting) in order to monitor their performance, allowing the adjustment of the amount of supplement to be offered.

During the experimental period, three seven-day trials (from days 21 to 27, 42 to 48, and 63 to 69) were performed to evaluate voluntary nutrient intake and digestibility. This was done by measuring the amount of feed offered (forage ad libitum and 5.0 g kg^{-1} BW of concentrate) during the first six days of each trial, as well as the quantity of orts from the second day to the last. Subsequently, samples of forage, concentrate, and orts were collected, partially dried in an oven at 60°C for 72 hours, ground to 1.0 mm in the TE-650 Wiley-type mill (Tecnal, Piracicaba, SP, Brazil), and stored in plastic containers for further analysis.

To estimate the excretion of fecal DM, all feces were collected immediately after defecation in individual pens on the third, fifth, and seventh day of each trial during 24 hours, beginning at 6 a.m. At the end of each collection day, the feces of each animal were weighed, a 100 g sample was taken and dried at 60°C for 72 hours, and a composite sample was obtained from each animal for further analysis.

Samples of concentrate, forage, and feces were analyzed for: DM, dried overnight at 105°C; ash, through complete combustion in a muffle furnace at 600°C for 4 hours; nitrogen, by the Kjeldahl procedure; ether extract, with the Randall procedure; and neutral detergent fiber (NDF). The respective methods used for the analyses were: G-003/1, M-001/1, N-001/1, G-005/1, 002/1 of Instituto Nacional de Ciência e Tecnologia de Ciência Animal (Detmann et al., 2021).

Nonfibrous carbohydrates (NFC) were quantified according to Detmann & Valadares Filho (2010), as follows:

$$NFC = 100 - [(\%CP - \%CP\ urea + \%\ urea) + \%NDF + \%EE + \%MM],$$

where EE is ether extract, and MM is mineral matter.

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Throughout the study period, the cows were immobilized in a squeeze chute and milked every day, in the same order, at 7 a.m., using the Km03061 milking machine (Kurtsan, Istanbul, Turkey). In addition, to estimate the quantity and composition of the milk consumed daily by the female calves, two milking were performed, one at 7 a.m. and the other at 3 p.m. on the third and fourth day of each trial. The milk produced by each cow was immediately weighed after each milking, and a milk sample was collected in 50 mL plastic bottles for later analysis of density, lactose, fat, protein, nonfat solids, and fat solids in the Julie C3 milk analyzer (Scope Electric Instruments, Razgrad, Bulgaria/Regensburg, Germany). The exact time when each cow was milked was recorded, and milk yield over 24 hours was estimated according to Almeida et al. (2020). The milk produced by the dams was corrected to 4% fat ($Milk_{4\%}$) using the following equation of National Research Council (NRC, 2001):

Milk 4% (kg) = 0.4 × (milk production) + [15 × (fat production × milk production/100)].

On the first and second day of each trial, the diurnal ingestive behavior of the female calves was evaluated through direct observation, from 6 a.m. to 6 p.m. (12 hours per day), by 12 trained evaluators located at a distance of 6.0 m from the pens (Lima et al., 2020). The observations covered: forage intake, measuring the time that the animals spent at the feeder consuming the forage; concentrate intake, measuring the time that the animals spent at the feeder consuming the concentrate; rumination time, measuring the time that the animals spent ruminating, including the process of regurgitation and remastication of the feed; milk intake, measuring the time that the calves spent suckling directly from a nipple; and leisure, measuring the time that the animals were not consuming feed or ruminating.

On days 28, 56, and 84, blood samples were collected to quantify the serum concentration of urea nitrogen, total proteins, albumin, globulins, glucose, triglycerides, and total cholesterol. All samples were collected at 6 a.m. before supplementation, via jugular venipuncture using two types of vacuum tubes: the BD Vacutainer SST II Advance (BD, São Paulo, SP, Brazil), with clot activator and gel, for serum separation; and the BD Vacutainer Fluoreto/EDTA, containing ethylenediamine tetraacetic acid and sodium fluoride (BD, São Paulo, SP, Brazil) as a glycolytic inhibitor and anticoagulant, respectively, for plasma preparation. Immediately after collection, the samples were centrifuged at 3,600 rpm for 20 min, and serum and plasma were frozen at -20°C for later analysis.

Urea, glucose, triglycerides, and total cholesterol were quantified via enzymatic-colorimetric methods using kits K056, K082, K117, and K083, respectively (Quibasa-Bioclin, Belo Horizonte, MG, Brazil). Total proteins and albumin were determined through colorimetric methods using kits K031 and K040, respectively (Quibasa-Bioclin, MG, Belo Horizonte, Brazil). In addition, globulin concentrations were calculated as the difference between total protein and albumin content. Serum urea nitrogen was estimated as 46.67% of total serum urea. All blood parameters were evaluated using the BS-200E automatic biochemistry analyzer, following the manufacturer's instructions (Mindray, Shenzhen, China).

For growth performance evaluation, all female calves were weighed on days 1 and 84 of the experimental period after 14 hours of solids fasting. In parallel with the last weighing, the following body measures were taken using a height stick (cm) and recorded to evaluate animal body growth: rump width, maximum distance between the iliac tuberosities; rump length, length from the ischial tuberosity to the iliac tuberosity; width between ischium, maximum distance between the ischial tuberosities; rib width, distance from the outermost point between the thirteenth ribs; rib depth, vertical depth from the highest point over the scapulae to the endpoint of the ribs; thorax width, transverse maximum distance between the retroscapulae; diagonal body length, length from the anterior point of the scapulae vertically to the iliac tuberosity; total body length, length from the anterior point of the scapulae vertically to the posterior midline; height at the withers, highest point of the shoulder blade to the ground; and rump height, height from the iliac tuberosity to the ground. Heart girth, the body circumference immediately posterior to the front legs, was measured with a flexible tape.

Response variables were analyzed using the MIXED procedure of the SAS, version 9.4, software (SAS Institute Inc., Cary, NC, USA). The effect of treatments on all measured variables was evaluated by the one-way analysis of variance and Student's t-test through the following mathematical model: $Y_{ij} = \mu + T_i + \varepsilon_{ij}$, where Y_{ij}

is the dependent variable corresponding to individual j undergoing treatment i; μ is the general mean; T_i is the fixed effect of the treatment; and ε_{ij} is the unobservable random error associated with each observation j subjected to treatment i under the assumption of normal and independent distributions (0, σ^2).

The blood metabolites were analyzed as time-repeated measures, considering day of collection as the repeated variable. The assumptions of linearity, sphericity, normality, independence of errors, and homoscedasticity were checked at α=0.05 using correlation and coefficient of determination, Mauchly's test, Shapiro-Wilk's test, Durbin-Watson's test, and Lavene's test, respectively. All assumptions were met. The choice of the most appropriate covariance structure was based on the lowest value of the corrected Akaike information criterion. The degrees of freedom were estimated according to Kenward-Roger's method.

Results and Discussion

The forage and supplement consumed by the animals contained 9.35 and 23.01% CP, respectively (Table 1). According to Detmann et al. (2014a), the consumed forage presents medium-quality, that is, >7.0% CP and < 65% NDF, both in DM.

The supplementation offered to the female calves did not affect milk production and composition (p>0.05) (Table 2), although the former showed a decreasing behavior over time. Moreno et al. (2022) and Ortega et al. (2020) reported similar results.

Table 2. Effect of supplementation with a protein-energy concentrate during the pre-weaning period on the milk yield and composition of crossbred female calves (Gyr×Holstein) fed medium-quality forage.

Milk production and composition	Treatment[1]		SEM[2]	p-value
	Control	Supplemented		
Milk (kg per day)	6.7	5.3	0.97	0.544
$Milk_{4\%}$ (kg per day)[3]	5.9	4.6	0.88	0.555
Protein (%)	3.4	3.4	0.10	0.657
Fat (%)	3.1	3.2	0.22	0.780
Lactose (%)	4.9	5.0	0.16	0.668
Total solids (%)	11.4	11.7	0.18	0.338

(1)Control, animals without supplementation; and Supplemented, animals supplemented with 5.0 g kg^{-1} body weight of a protein-energy concentrate. (2)Standard error of the mean. (3)$Milk_{4\%}$, milk yield corrected to 4% fat.

Supplementation (p>0.05) also had no effect on the voluntary intake of DM (kg per day and g kg^{-1} BW), milk DM (kg per day), organic matter (kg per day), ether extract (kg per day), digested organic matter (kg per day), digested NDF (kg per day), and total digestible nutrients (Table 3). However, the supplemented animals showed a decrease (p<0.05) in the intake of forage DM (kg per day and g kg^{-1} CP) and NDF (kg per day and g kg^{-1} CP), as well as an increase in the intake of CP and NFC.

Supplementation increased (p<0.05) the CP:digested organic matter dietary ratio, which was 186 and 222 g kg^{-1}

Table 3. Effect of supplementation with a protein-energy concentrate during the pre-weaning period on the voluntary intake and total digestibility coefficients of crossbred female calves (Gyr×Holstein) fed medium-quality forage.

Intake[1] (kg per day)	Treatment[2]		SEM[3]	p-value
	Control	Supplemented		
DM	3.92	3.83	0.294	0.824
FDM	3.48	2.67	0.312	<0.001
CDM	-	0.70	0.033	-
MDM	0.44	0.45	0.058	0.866
OM	3.50	3.46	0.236	0.889
CP	0.45	0.56	0.019	0.003
EE	0.18	0.22	0.013	0.054
NDF	2.26	1.90	0.202	<0.001
NFC	0.62	0.80	0.025	<0.001
DOM	2.44	2.53	0.151	0.696
DNDF	1.51	1.32	0.133	0.317
TDN	2.63	2.78	0.151	0.465
CP:DOM	186	222	5.60	0.001
Digestibility (g g^{-1})				
DM	0.686	0.721	0.0144	0.112
OM	0.699	0.735	0.0155	0.142
CP	0.765	0.793	0.0144	0.206
EE	0.882	0.889	0.0140	0.714
NDF	0.671	0.694	0.0164	0.345
NFC	0.693	0.750	0.0176	0.044
DOM (g kg^{-1} DM)	626	668	19.20	0.149

(1)DM, dry matter; FDM, forage DM; CDM, concentrate DM; MDM, milk DM; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; NFC, nonfibrous carbohydrates; DOM, digested OM; DNDF, digested NDF; TDN, total digestible nutrients; and CP:DOM, CP and DOM ratio. (2)Control, animals without supplementation; and Supplemented, animals supplemented with 5.0 g kg^{-1} body weight of a protein-energy concentrate. (3)Standard error of the mean.

for the control and supplemented treatments, respectively. According to Detmann et al. (2014b), a CP:digested organic matter ratio of 288 g kg^{-1} indicates maximum forage intake, meaning that the values obtained in the present study were lower than those recommended by these authors, possibly explaining the lack of differences observed in DM intake. Similar results were found by Batista et al. (2016), but opposite ones by Barros et al. (2015), Moreno et al. (2023), and Ortega et al. (2020).

The lower forage and NDF intake by the supplemented female calves are an indicative that there was a negative effect of the substitution of a quantity of pasture (31.5% less than the control group) by the protein-energy concentrate. This response may be a consequence of the multiple interactive effects observed between forage and supplement in supplemented cattle, with a possible reduction in forage intake due to the inclusion of supplements in the diet (Paulino et al., 2004). This can explain the lack of difference in total DM intake between the tested treatments, despite the additional CP and NFC provided to the supplemented animals through the concentrate. Márquez et al. (2014), Almeida et al. (2018), Ortega et al. (2020), and Moreno et al. (2022) reported similar results when supplementing suckling female calves with different amounts of supplements under tropical conditions.

Supplementation did not affect ($p>0.05$) the total digestibility coefficients of DM, organic matter, CP, ether extract, NDF, and digested organic matter (Table 3). These responses are associated with the 9.35% CP content in the forage consumed by all animals during the experiment, a value higher than minimum of 7.0–8.0% required for an adequate degradation of fibrous carbohydrates by fibrolytic bacteria (Detmann et al., 2014a). This may explain the similarities in the digestibility of NDF and in the extraction and use of other dietary components such as DM, organic matter, CP, ether extract, and digested organic matter.

An increase in the intake of NFC ($p<0.05$) was observed in the female calves fed the supplement, whose digestibility is greater than that of forages (Moura et al., 2020; Moreno et al., 2022).

Forage intake time ($p<0.05$) was decreased in 64 min per day due to supplementation (Table 4), whereas idle time was increased ($p<0.05$). However, there was no effect of supplementation ($p>0.05$) on the time of animal rumination, break, and milk intake, which suggests the involvement of complex metabolic mechanisms, whose reflexes on animal behavior were not observed with the supplementation strategy used in the present study.

In comparison with the control animals, the supplemented cattle spent most diurnal time (16 min) consuming the concentrate, spending less time on other daily activities, such as forage intake, and more time standing idle, which could be attributed to a longer digestion time (Lima et al., 2020). According to Detmann et al. (2014b), supplementation can improve the ruminal environment, increasing the animal's ability to digest the diet. The obtained results show how bovines adjust their behavior in response to changes in their environment, dividing their time between grazing activities, rumination, social interactions, and idleness to maintain feed intake according to their nutritional requirements (Martins et al., 2012).

There was an interaction effect ($p<0.05$) between treatment and sample collection day for serum urea nitrogen concentrations (Table 5). These concentrations were higher on the second and third days of collection in the supplemented female calves (Figure 1). However, no interaction effect ($p>0.05$) was detected between treatment and collection day regarding the serum urea nitrogen concentrations of total proteins, albumin, globulins, glucose, triglycerides, and total cholesterol during the experiment.

According to Van Soest (1994), serum urea nitrogen concentrations are positively associated with CP intake, suggesting a greater absorption of ammonia through the rumen wall, an increase in the synthesis of hepatic

Table 4. Effect of supplementation with a protein-energy concentrate in the pre-weaning period on ingestive behavior of crossbred female calves fed with medium-quality forage.

Ingestive behavior	Treatment[(1)]		SEM[(2)]	p-value
	Control	Supplemented		
Forage intake (min day^{-1})	251	187	12.9	0.005
Ruminating (min day^{-1})	98	85	5.4	0.121
Break (min day^{-1})	75	71	6.1	0.622
Idle (min day^{-1})	97	149	13.0	0.017
Concentrate (min day^{-1})	–	16	1.0	–
Milk intake (min day^{-1})	16	16	0.7	0.705

[(1)]Control, animals without supplementation; and Supplemented, animals supplemented with 5.0 g kg^{-1} body weight of a protein-energy concentrate. [(2)]SEM, standard error of the mean.

urea, and a greater concentration of serum urea. In the present study, those concentrations were higher in the supplemented calves due to their higher CP intake.

The higher CP intake was insufficient to increase the synthesis of protein precursors, total proteins, or albumin, which may be related to imbalances in the protein:energy ratio observed in the present study, i.e., CP:digested organic matter ratio < 288 g kg^{-1}. This result may have prevented the use of nitrogen compounds to maximize microbial growth and to extract nutrients from fiber, limiting the formation of protein products in the post-absorptive metabolism of the animals (Ortega et al., 2016; Hanigan et al., 2018; Detmann et al., 2014b; Mora-Luna et al., 2023).

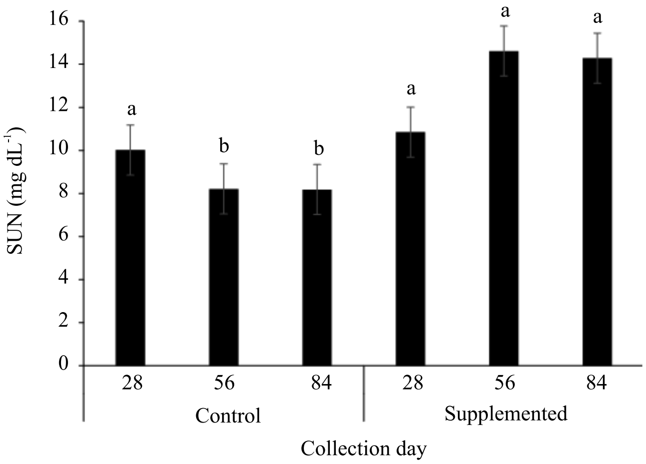

Figure 1. Serum urea nitrogen (SUN) concentration in crossbred lactating female calves fed medium-quality forage plus a protein-energy concentrate. Control, animals without supplement and; Supplemented, animals supplemented with 5 g kg^{-1} of BW of a protein-energy concentrate. Means of treatments × collection day without a common lowercase letter differ significantly ($p \leq 0.05$). Error bars represent standard error of the mean.

The blood concentrations of total proteins, globulins, and glucose were affected by collection day ($p<0.05$) (Table 5). During the experiment, the concentrations of total proteins (Figure 2 A) and globulins (Figure 2 C) were higher on the last collection day ($p<0.05$). Contrastingly, blood glucose levels presented a decreasing behavior as a function of collection day ($p<0.05$), being higher on the first day, 28 days after the beginning of the experiment (Figure 2 B). However, there was no effect of collection day on the serum levels of albumin, triglycerides, and total cholesterol during the experimental period ($p>0.05$).

The lower blood glucose concentration on the last collection day may be associated with the lower milk production of the dams and with the decrease in milk intake by the calves during this period. Similarly, Henriques et al. (2011) found that, after three months of lactation, there was a gradual decrease in the milk production of the dams and, consequently, a lower participation of milk in the total diet of the calves, resulting in a lower intestinal absorption of glucose.

Supplementation increased blood globulin concentrations ($p<0.05$) in comparison with the control (Table 5). However, the supplied concentrate did not affect total proteins, albumin, glucose, triglycerides, and total cholesterol serum levels ($p>0.05$). Ortega et al. (2020) found a higher serum urea nitrogen concentration of 18.4 mg dL^{-1} for female calves supplemented with 4.0

Table 5. Effect of supplementation with a protein-energy concentrate during the pre-weaning period on the metabolic prolife of crossbred female calves (Gyr×Holstein) fed medium-quality forage.

Metabolic profile	Treatment(1)		SEM(2)	p-value(3)		
	Control	Supplemented		T	D	T × D
Serum urea nitrogen (SUN, mg dL^{-1})	8.8	13.3	4.21	<0.001	0.384	0.003
Total proteins (g dL^{-1})	5.5	6.4	3.16	0.091	0.049	0.652
Albumin (g dL^{-1})	3.2	3.4	1.52	0.369	0.214	0.765
Globulins (g dL^{-1})	2.4	3.0	1.86	0.037	0.024	0.612
Glucose (mg dL^{-1})	84.1	87.1	6.69	0.766	0.043	0.954
Triglycerides (mg dL^{-1})	26.3	27.0	2.448	0.861	0.351	0.119
Total cholesterol (mg dL^{-1})	162.7	168.0	2.29	0.126	0.875	0.669

(1)Control, animals without supplementation; and Supplemented, animals supplemented with 5.0 g kg^{-1} body weight of a protein-energy concentrate. (2)Standard error of the mean. (3)T, treatment effect; D, effect of collection day; and T × D, interaction of treatment × effect of collection day.

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or 6.0 g kg^{-1} BW of concentrate. Likewise, Moreno et al. (2022) also reported a higher value for the supplemented group of animals (14 mg dL^{-1}) when compared with those of the control (8.3 mg dL^{-1}).

An indicative of a similar energy status between animals are the similar values obtained for blood concentrations of glucose, cholesterol, and triglycerides due to the absence of differences in the intake of DM, organic matter, digested organic matter, digested NDF, and TDN. Silva et al. (2017), Ortega et al. (2020), and Moreno et al. (2022) reported similar results for female calves fed up to 6.0 g kg^{-1} supplement under tropical conditions.

The final BW and average daily gain (ADG) were greater ($p<0.05$) for the supplemented female calves (Table 6). However, these values did not affect any of the body measurements of the animals during the experiment ($p>0.05$).

The greater final BW and ADG can be justified by the greater intake of the easily digestible elements CP and NFC by the supplemented calves, which optimizes their growth performance. Paulino et al. (2004) recommended an ADG of > 600 g per day for the animals to achieve their first conception at 14–15 months of age. Similar results were highlighted by Moreno et al. (2022) for female beef calves supplemented during the pre-weaning period. However, Ortega et al. (2020) observed an opposite

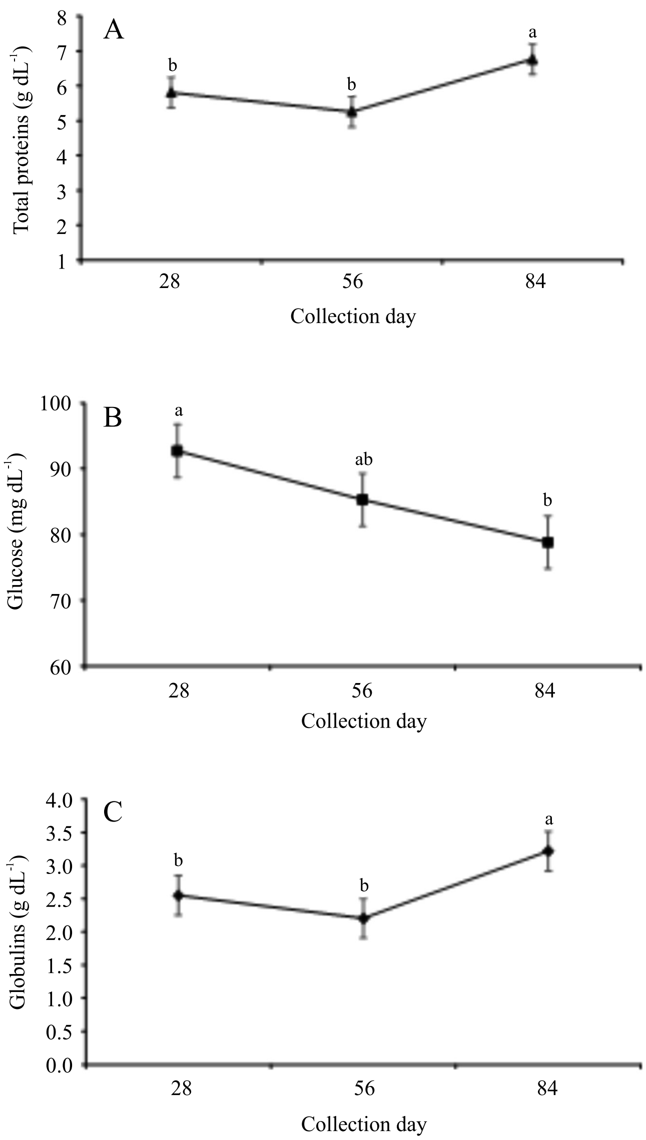

Figure 2. Total proteins (A), glucose (B), and globulins (C) concentration in crossbred female calves fed medium-quality forage plus a protein-energy concentrate in the pre-weaning period. Means of collection day over the line followed by different lowercase letters differ significantly ($p\leq0.05$). Error bars represent standard error of the mean.

Table 6. Effect of supplementation with a protein-energy concentrate during the pre-weaning period on the productive performance of crossbred female calves (Gyr×Holstein) fed medium-quality forage.

Productive performance(1)	Treatment(2)		SEM(3)	p-value
	Control	Supplemented		
Initial body weight (kg)	127.7	118.7	11.31	0.586
Final body weight (kg)	175.5	190.3	4.26	0.037
ADG (kg per day)	0.624	0.800	0.0507	0.037
HW (cm)	107.0	105.0	1.73	0.627
Thorax width (cm)	26.2	27.1	3.78	0.354
Rib depth (cm)	48.6	48.1	3.42	0.752
Heart girth (cm)	131.8	131.3	1.62	0.792
Rib width (cm)	39.2	39.0	3.98	0.904
DBL (cm)	81.3	79.7	3.33	0.501
Total body length (cm)	112.3	111.3	3.58	0.779
Rump length (cm)	34.4	34.2	3.83	0.864
Rump height (cm)	112.0	111.7	4.35	0.944
Rump width (cm)	29.6	30.1	4.62	0.668
Ischium width (cm)	9.9	10.1	4.73	0.587
BW:HW	1.68	1.76	6.44	0.408

(1)ADG, average daily gain; HW, height at the withers; DBL, diagonal body length; and BW:HW, body weight and height at the withers ratio. (2)Control, animals without supplementation; and Supplemented, animals supplemented with 5.0 g kg^{-1} body weight of a protein-energy concentrate. (3)Standard error of the mean.

response, where female beef calves fed different levels of supplement (4.0 or 6.0 g kg^{-1} BW) presented a similar growth performance.

Regarding body measurements, the long bones in bovines (rear and front legs, i.e., height at the withers and height at the rump) are optimal indicators of skeletal development (Rodríguez-Sánchez et al., 2015). In this sense, the lack of difference in these variables in the present study indicates that both evaluated treatments promoted an adequate skeletal development of the animals.

Heart girth, a predictor of animal body weight, and rump width and length, used to estimate internal pelvic area, have an important relationship with the distribution of primal cuts in the hindquarter and the incidence and difficulty in calving in primiparous heifers (Rodríguez-Sánchez et al., 2015). Therefore, the lack of difference in these measurements in the present work is an indicative that both treatments provided the necessary nutrients for the adequate growth of the soft tissues of the studied animals. Silva et al. (2017), Ortega et al. (2020), and Moreno et al. (2022) reported similar results when feeding suckling beef calves with different amounts of supplement under tropical conditions. However, Rodríguez-Sánchez et al. (2015) found differences in these measurements according to the applied treatments, observing a greater growth for the animals that received a higher amount of supplement.

Conclusions

1. Crossbred female calves (Gyr × Holstein) supplemented with 5.0 g kg^{-1} body weight of a protein-energy concentrate in the pre-weaning period show an improved productive performance and increased metabolic indicators of their short-term protein status.

2. The level of supplementation interferes with the ingestive behavior of the female calves, which should be considered when delineating supplementation plans.

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