Tropical Animal Health and Production (2018) 50:1779–1785
https://doi.org/10.1007/s11250-018-1619-2

REGULAR ARTICLES

Supplementation strategies for Nellore female calves in creep feeding to improve the performance: nutritional and metabolic responses

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Received: 7 October 2017 / Accepted: 9 May 2018 / Published online: 21 May 2018

Abstract
The objective of this study was to evaluate the effects of supplementation strategy on performance, nutritional and metabolic characteristics of Nellore suckling female calves, on grazing *Urochloa decumbens* during the rainy-dry transition period. Forty-four Nellore female calves, averaging 147.6 ± 1.34 kg (4 months), were used. A single supplement 20% of crude protein of dry matter was provided, consisting of daily supplement at 0.0, 0.2, 0.4, or 0.6% of body weight. A positive linear effect ($P < 0.05$) on final body weight and average weight gain of female calves was observed with increased supplementation. Supplementation increased consumption, in kilograms per day, of dry matter (DM), organic matter (OM), crude protein (CP), digested dry matter, and total digestible nutrients (TDN), all calculated as % of dry matter. There was no effect of supplementation on the consumption in kilograms per day of neutral detergent fiber corrected for ash and protein (apNDF) ($P > 0.05$). Supplementation increased, in a quadratic way, the total apparent digestibility coefficient of DM, OM, CP, apNDF, and TDN ($P < 0.05$). There was no effect of supplementation ($P > 0.05$) on non-esterified fatty acid concentrations. There was a positive linear effect ($P < 0.05$) of supplementation on insulin concentrations. In this study, it is concluded that linearly increasing the feeding level of a supplement with 20% crude protein (% of DM) in the range of 0.2–0.6% of body weight improves the performance, nutritional and metabolic characteristics of the animals, considering these pasture characteristics.

Keywords *Bos indicus* · Creep feeding · Insulin · Tropical pasture

Introduction

Weaning heavier cattle is important because it allows the slaughter of males and the mating of heifers at ages of less than 16 months (Paulino et al. 2012). In many parts of the tropics, calving is generally concentrated in the month of October before the first rains begin. Suckling calves are more efficient in nutrient uptake and have a higher potential for weight gain.

However, due to physiological changes in calves and their dams during the suckling phase, the performance of the calf can be compromised. During this period, the milk production of the dam is decreased after reaching the lactation peak and the quality of the pasture may be compromised, due to the period of rainy-dry transition, characterized by the lower incidence of rainfall and, consequently, reduction of growth and quality of forage. These factors may limit the animal's performance, since the availability of nutrients from milk and pasture may not be sufficient to meet the animal's demands (Porto et al. 2009).

Traditionally, creep-feeding systems have been used to increase weight at weaning (Valente et al. 2012). Furthermore, creep-feeding systems reduce grazing pressure, alleviate the pressure on the dam, and increase feed intake at weaning. However, most of the results from these studies were obtained using male calves, and therefore literature data on beef female suckling calves is lacking.

In the case of bovine females, studies have shown different results on when is the best time to accelerate growth. Some authors report the occurrence of precocious puberty with

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increased rate of gain at early stages of development (Buskirk and Faulkner, 1995; Cardoso et al. 2014; Rodríguez- Sánchez et al. 2015).

Brito and Sampaio (2001) suggested that age and level of consumption are factors that affect the performance of the animals. Thus, it is possible that increased supplementation as a function of body weight (BW) may increase the productive performance of female calves more than fixed amounts of supplement over a long period of time.

In this way, we hypothesized that supplementation of suckling zebu female calves would improve their performance, nutritional and metabolic characteristics. Secondly, we hypothesized that dam's milk yield will affect calves' forage intake and nutrient digestibility. The objective of this study was to evaluate the influence of increasing supplementation and the influence of dam's milk yield on performance, and nutritional and metabolic characteristics of Nellore suckling female calves.

Material and methods

Animals, experimental design, and supplements

The experiment was conducted at the Federal University of Viçosa, Brazil, between March and July, referring to the rainy-dry transition period. Forty-four Nellore female calves, with age and initial mean weight, of 4 months and 147.6 ± 1.34 kg, respectively, were followed, accompanied by their respective mothers, with age and initial mean weight of 6 years and 453.2 ± 10.3 kg, respectively. The experiment lasted 123 days, divided into three experimental periods with 41 days each.

An experimental area of 35 ha was assigned to the 44 animals. The area consisted of four paddocks measuring 8.75 ha each. The soil was covered uniformly with the *Urochloa decumbens* grass and equipped with water dispensers and feeders. Each experimental group grazed one paddock. Every 7 days, the animals were rotated between the paddocks. This was done to control any paddock effects on the treatments (pasture availability, water dispenser and feeder locations, relief, shading, and so on).

The experimental design was a completely randomized design with four treatments, and all treatments with 11 calves. A single formulated supplement (Table 1) (63% corn grain, 34% soybean meal, and 3% molasses) was provided through the feed composition data provided by BR-CORTE 2.0 (Valadares et al. 2010). The supplement contained 20% crude protein (% of dry matter, DM) in different amounts as a function of BW. Thus, supplements were provided at 0.0, 0.2, 0.4, or 0.6% of BW. All animals received a mineral mix ad libitum. This mineral mix consisted of 50% dicalcium phosphate, 47.2% sodium chloride, 1.5% zinc sulfate, 0.7% copper sulfate, 0.05% cobalt sulfate, 0.05% potassium iodate, and 0.5% manganese sulfate.

Experimental procedures and sampling

Evaluations of the average milk yield of the cows were performed by analyzing two samples of the production at 41 and 82 days after the beginning of the experiment. Cows were separated from their offspring at 17:30 p.m. and remained in the paddock. They were milked at 05:30 a.m. and 17:30 p.m. on the following day. The total production consisted of the sum of the collections done at both collection moments. The total milk produced by the cow corresponds to the daily milk intake by the calf. The milk secretion was stimulated with 2-mL oxytocin (10 IU/mL, Ocitovet ®, Brazil) in the mammary artery, initiating milking immediately after oxytocin administration. Samples were collected for analysis of milk protein (MP), milk fat (MF), milk lactose (ML), and total solids (ST).

Forage samples were randomly taken every 15 days to evaluate the forage mass per hectare. In each paddock, four forage samples were randomly selected using a metal square (0.5 × 0.5 m) and cut at approximately 1 cm above the soil. Additionally, every 7 days, a hand-plucking sample collection was performed to evaluate the chemical composition of the forage consumed by the animals (Silva et al. 2017). In total, six hand-plucking samples were collected per period. All samples were dried at 60 °C for 72 h, grounded with 1- and 2-mm knife mills (Willye® TE-680), and proportionally subsampled to a composite sample per period.

To evaluate the nutritional characteristics of female calves from the 60th day of the experimental period, a digestibility assay was performed with duration of 9 days. To estimate the fecal excretion, the external chromium oxide (Cr_2O_3) indicator, packed in paper cartridges, corresponding to 10 g per female calf/day, was fed to the 44 calves using a metal probe directly in the esophagus, always at 10:00 a.m. To estimate the individual intake of supplementation by female calves, titanium dioxide (TiO_2), provided via supplement, was used in the proportion of 10 g of indicator/kg of supplement (Titgemeyer et al. 2001). To estimate the DM intake of pasture, the iNDF was used as an internal indicator (Detmann et al. 2001).

From the 9 days of the test, six were destined to the adaptation of the animals to Cr_2O_3 and TiO_2. During the last 3 days, feces samples were collected at different times, at 15:00 p.m., 11:00 a.m., and 07:00 a.m., respectively. Feces samples were collected immediately after defecation or directly into the rectum of the animals, in an approximate amount of 200 g. They were identified by animal and oven dried with forced air circulation (60 °C/72 h) and milled with a knife mill (1 and 2 mm).

On the last day of the digestive test, spot samples of blood were obtained, via jugular vein puncture, performed 4 h after supplementation. Blood samples were collected to measure

Table 1 Chemical composition of the supplement and forage

Item		Forage				
	Supplement	(1–41 days)[d]	(42–82 days)[d]	(83–123 days)[d]	Dig[e]	Mean
Dry matter[a]	898.5	289.2	303.8	294.7	298.8	299.5
Organic matter[b]	910.4	919.1	910.8	912.3	911.2	910.6
Crude protein[b]	208.2	87.0	83.0	63.9	84.2	71.4
NDIN[c]	143.2	251.1	232.1	214.2	230.1	232.4
Ether extract[b]	24.4	19.0	16.2	15.9	16.8	17.1
apNDF[b]	152.1	690.3	638.5	656.4	643.5	653.5
NFC[b]	525.7	122.8	173.1	176.1	166.7	168.6
iNDF[b]	26.4	248.3	193.3	251.5	214.5	244.4

NDIN neutral detergent insoluble nitrogen, *apNDF* neutral detergent fiber corrected for residual ash and protein, *NFC* non-fibrous carbohydrates, *iNDF* indigestible neutral detergent fiber

[a] In grams per kilogram of natural matter

[b] In grams per kilogram of dry matter

[c] In grams per kilogram of total nitrogen

[d] Mean values of the samples obtained by hand-plucking

[e] Sample obtained by hand-plucking in the digestibility assay

the levels of non-esterified fatty acids (NEFA), glucose (GLUC), and insulin (INS). The animals were weighed at the beginning and at the end of the experiment after a fasting period of 12 h.

Chemical analysis and calculations

The supplement and forage samples obtained by the hand-plucking method were quantified with regard to DM (INCT-CA G-003/1), crude protein (CP; INCT-CA N-001/1), ether extract (EE; INCT-CA G-004/1), neutral detergent fiber corrected for ash, and protein (apNDF; INCT-CA F-002/1), using thermostable α-amylase, without using sodium sulfite; nitrogen insoluble in neutral detergent (NDIN; INCT-CA N-004/1) according to Detmann et al. (2012); iNDF, according to Valente et al. (2011), was obtained after in situ incubation in (F57 Ankom®) bags for 288 h. Milk was analyzed for protein, fat, lactose, and total solid content, using spectroscopy (Foss MilkoScan FT120, Hillerød, Denmark).

The pdDM (Paulino et al., 2008) was calculated by the following equation:

$$\mathrm{pdDM}(\%) = 0.98 \times (100-\mathrm{NDF}) + (\mathrm{NDF}-\mathrm{iNDF})$$

where

pdDM potentially digestible DM
NDF neutral detergent fiber
iNDF indigestible neutral detergent fiber

Approximately 200 g of fecal samples was collected immediately after defecation or directly from the rectum of the animals. A sample composed of feces from the 3 days of collection was assessed individually and later analyzed for the contents of chromium and titanium. The analysis was conducted by using atomic absorption (Souza et al. 2013) and colorimetry (Titgemeyer et al. 2001), respectively. We also evaluated the contents of DM, CP, EE, apNDF, and iNDF, as previously described. Fecal excretion and individual supplement intake were calculated according to equations described by Almeida et al. (2015). Individual forage DM was estimated using iNDF as internal marker in the following equation:

$$\mathrm{DMI(kg/day)} = \{|(\mathrm{FE} \times \mathrm{CMF})\text{-}\mathrm{MS}]/\mathrm{CMFO}\} + \mathrm{SDMI} + \mathrm{MDMI}$$

where

FE fecal excretion (kg/day)
CMF concentration of the marker in the feces (kg/kg)
MS intake of marker from supplement (kg)
CMFO concentration of the marker in the forage (kg/kg)
SDMI supplement DM intake (kg/day)
MDMI milk dry matter intake (kg/day)

Metabolites were analyzed following the biochemistry analyzer manufacturer's instructions (Mindray, BS200E, Shenzhen, China). The concentrations of glucose (K082) were measured using kits from Bioclin Diagnostics (Belo Horizonte, Brazil). Serum concentrations of non-esterified fatty acids (NEFA) were quantified by a colorimetric method (FA115, Randox Laboratories Ltd., São Paulo, Brazil). Insulin was analyzed by chemiluminescence using Access Ultrasensitive Insulin Reagent (Ref. Number 33410, Beckman Coulter Brea, USA) in the Access 2 Immunoassay System (Beckman Coulter Inc., Brea, USA).

Table 2 Performance and blood concentration of hormone and metabolites of supplemented and non-supplemented female calves during creep feeding

Item	Supplementation strategy[a]						*P* value[b]				
	0.0	0.2	0.4	0.6	DMYc	SEM	SS	DMY	Int	L	Q
iBW (kg)	147.5	145.8	149.4	147.8	–	–	–	–	–	–	–
fBW (kg)	227.5	229.8	236.5	240.0	–	–	–	–	–	–	–
ADG (kg)	0.650	0.682	0.708	0.749	0.01	0.03	0.149	0.193	0.354	0.011	0.644
NEFA (mmol/L)	0.212	0.210	0.209	0.208	0.01	0.02	0.813	0.147	0.120	0.854	0.965
GLUC (mg/dL)	67.75	75.12	80.89	82.41	1.00	2.79	0.002	0.308	0.234	0.001	0.289
INS (mg/dL)	26.42	29.63	45.23	47.59	1.58	4.62	0.003	0.338	0.363	0.001	0.859

iBW initial body weight, *fBW* final body weight, *ADG* average daily gain, *NEFA* non-esterified fatty acids, *GLUC* glucose, *INS* insulin, *DMYc* parameter estimation of the effect of dam's milk yield on the response variable

[a] 0.0 mineral mix, 0.2 supplementation with 0.2% of BW, 0.4 supplementation with 0.4% of BW, and 0.6 supplementation with 0.6% of BW

[b] Supplementation strategy (SS), dam's milk yield (DMY), interaction (Int) between DMY and SS, and indicative of significance for linear (L) and quadratic (Q) effects of progressive increase of supplementation

Statistical analyses

The results were assessed using analysis of variance, adopting the initial BW as a covariable when significant. The linear and quadratic effects of the multiple supplement amounts were evaluated by decomposing the sum of the squares of the treatments using orthogonal contrasts. In addition, dam's milk yield and its interaction with supplementation strategy were included in the model as quantitative variables and were excluded thereafter when found to be non-significant. The PROC MIXED procedure of SAS software (SAS Institute Inc 2015) was used for all statistical analyses. For all statistical procedures, $\alpha = 0.05$ was adopted as the critical level of type I error probability.

Results

The mean total DM and pdDM availability of forage *Urochloa decumbens* throughout the experiment was 3297 and 2468 kg/ha, respectively. The forage *Urochloa decumbens* was obtained by hand-plucking and presented an average content of 78 g of CP/kg of DM (Table 1).

Supplementation improved the performance of the heifer calves and this fact can be verified by the increasing linear effect ($P < 0.05$) on the final BW (fBW) and the average daily gain (ADG) of the female calves with increased supplementation (Table 2).

Supplementation did not affect ($P > 0.05$) the concentrations of NEFA. However, there was an increasing linear effect ($P < 0.05$) of supplementation on INS and GLUC concentrations ($P < 0.05$) (Table 2).

Supplementation of female calves did not affect the milk yield (MY) of their dams ($P > 0.05$; Table 3). In this study, the average milk yield was 5.43 kg/day and included 3.61% of crude protein, 4.58% of fat, 4.31% of lactose, and 13.36% of total solids.

Multiple supplementation increased intake (kg/day) (Table 4) of DM, organic matter (OM), CP, dDM and total digestible nutrients (TDN). There was an increasing linear effect on total DM and DM in grams per kilogram of BW ($P < 0.05$). Supplementation supported a quadratic effect on forage DM intake in grams per kilogram of BW and was higher in non-supplemented female calves. There was no effect of supplementation on intake in kilograms per day of apNDF ($P > 0.05$). However, supplementation supported a quadratic effect on the consumption of apNDF in grams per kilogram of BW and dNDF. The maximum intake was 9.96 g/kg of BW of apNDF ($P < 0.05$) and 1.37 kg/day of dNDF ($P < 0.05$), both values observed for the treatment 0.2. Multiple supplementation allowed a linear effect on the intake and digestibility of DM ($P < 0.05$; Table 4).

There were no interactions between dam's milk yield and supplementation strategy on intake and digestibility of the nutrients ($P > 0.05$; Table 4). The dam's milk yield positively

Table 3 Milk yield and its components in function of the different treatments

Item	Supplementation strategy					*P* value[a]	
	0.0	0.2	0.4	0.6	SEM	L	Q
MY (kg)	5.72	5.37	5.20	5.42	0.44	0.591	0.444
MF (g/kg)	47.0	46.9	43.8	45.3	1.40	0.188	0.369
MP (g/kg)	35.2	35.9	37.2	36.0	0.90	0.384	0.225
ML (g/kg)	43.0	43.5	42.2	43.8	0.60	0.719	0.503
TS (g/kg)	136.5	131.8	135.1	131.1	5.20	0.576	0.826

MY milk yield, *MF* milk fat, *MP* milk protein, *ML* milk lactose, *TS* total solids

[a] Indicative of significance for linear (L) and quadratic (Q) effects of progressive increase of supplementation

Table 4 Intake and total apparent digestibility of the constituents of the diet of supplemented or non-supplemented female calves during creep feeding

Item	Supplementation strategy						*P* value[a]				
	0.0	0.2	0.4	0.6	DMYc	SEM	SS	DMY	Int	L	Q
Intake (kg/day)											
DM	4.01	4.25	4.36	4.48	0.06	0.17	0.036	0.189	0.314	0.046	0.343
FDM	3.14	3.10	2.99	2.70	− 0.01	0.13	0.027	0.855	0.329	0.006	0.673
MDM	0.87	0.85	0.81	0.86	–	0.04	0.762	–	–	0.354	0.554
OM	3.65	3.94	4.05	4.14	0.10	0.15	0.001	0.115	0.217	0.001	0.430
CP	0.41	0.47	0.51	0.58	0.03	0.02	0.001	0.001	0.214	0.001	0.176
apNDF	1.94	2.09	2.00	1.87	− 0.02	0.08	0.310	0.506	0.198	0.184	0.378
dDM	2.59	2.73	2.84	3.14	0.11	0.12	0.008	0.009	0.219	0.003	0.778
dNDF[1]	1.10	1.37	1.39	1.13	− 0.04	0.07	0.003	0.103	0.100	0.344	0.001
TDN	2.65	2.89	2.97	3.05	− 0.10	0.11	0.001	0.489	0.767	0.001	0.335
Intake (g/kg) of BW											
DM	19.10	20.28	20.43	20.67	− 0.11	0.36	0.018	0.427	0.821	0.004	0.194
FDM	14.91	14.55	13.93	12.11	− 0.27	0.35	0.001	0.038	0.172	0.465	0.043
apNDF[1]	9.23	9.96	9.59	8.68	− 0.24	0.23	0.001	0.003	0.397	0.378	0.001
Digestibility coefficients											
DM	0.646	0.640	0.650	0.698	0.60	0.01	0.001	0.028	0.197	0.567	0.001
OM	0.543	0.660	0.669	0.667	− 0.74	0.01	0.001	0.083	0.130	0.187	0.001
CP	0.539	0.583	0.625	0.610	0.71	0.01	0.001	0.018	0.549	0.221	0.034
apNDF	0.588	0.657	0.663	0.659	− 0.81	0.02	0.001	0.188	0.200	0.234	0.029
TDN	0.521	0.673	0.677	0.707	0.00	0.01	0.001	0.995	0.222	0.124	0.001

DM dry matter, *FDM* forage dry matter, *MDM* milk dry matter, *OM* organic matter, *CP* crude protein, *apNDF* neutral detergent fiber corrected for residual ash and protein, *dDM* digested dry matter, *dNDF* digested apNDF, *TDN* total digestible nutrients and *DMYc* parameter estimation of the effect of dam's milk yield on the response variable

[a] Supplementation strategy (SS), dam's milk yield (DMY), interaction (Int) between DMY and SS, and indicative of significance for linear (L) and quadratic (Q) effects of progressive increase of supplementation

affected the CP and dDM intake (kg/day) and DM and CP digestibility, and negatively affected FDM and apNDF intake (g/kg BW; $P < 0.05$; Table 4).

Supplementation had a quadratic effect on the apparent digestibility coefficient of DM, OM, CP, NDF, and TDN ($P < 0.05$; Table 4). The maximum digestibility point for the treatment of DM (69.78), OM (66.68), and TDN (70.71) was observed to be 0.6%. In addition, for treatment, the maximum point of CP (62.48) and apNDF (66.27) was observed at 0.4%.

Discussion

Paulino et al. (2008), aiming to associate production per animal and per area, suggested the supply of 4 to 5% of BW in pdDM (between 40 and 50 g of pdDM/kg of BW), from pasture to satisfactory animal performance of animals under grazing conditions. In this research, the mean mass of pdDM was 57.3 g/kg of BW, a value above that was recommended by Paulino et al. (2008), demonstrating that the amount of forage available did not compromise animal performance. The percentage of CP was below the 10% reported by Sampaio et al. (2009) as the level that optimizes the use of energetic substrates of the forage, which justifies the supplementation with nitrogen compounds to optimize the use of the forage and, consequently, the animal performance.

The increase of ADG (0.65–0.75) and fBW (227.5–240.0) of the female calves (Table 2) confirmed our hypothesis that improvement in the performance of the female calves can be attributed to the higher intake of nitrogen compounds (Table 4) and to the greater availability of readily available energy with the increase of the supplement supply. This may have had a beneficial effect on the ruminal environment, maximizing ammonia utilization and dNDF (Table 4), resulting in more efficient use of the consumed diet with an increase in the amount of energy extracted from the pasture by the supplemented animals and, consequently, greater weight gain.

Similar to the results reported in this work, Lopes et al. (2016) found no difference in milk production and its components (Table 3) among cows that had their calves supplemented or did not receive multiple supplements. Therefore, the milk production of cows did not influence intake and performance of female calves. The differences observed in the ADG and fBW are attributed to greater supplementation levels. Thus, the higher the supplementation, the lower the forage DM intake in kilograms per day due to the substitutive effect caused by the supplement intake (Porto et al. 2008).

The observed substitutive effect on forage intake is not desired because the main purpose of concentrate supplementation is the optimization forage use, minimizing possible substitutive effects (Detmann et al. 2005). However, supplementation promotes greater total DM intake by supplemented calves (Table 4).

There was a positive effect of the dam's milk yield on CP intake mainly due to the high milk protein when compared to the forage protein. Additionally, dam's milk yield negatively affected the intake of FDM and apNDF (g/kg BW) due to the substitutive effect of forage intake by their dam's milk. As milk protein had greater digestibility than forage and concentrate CP, the dam's milk yield positively affected the DM and CP digestibility (Table 4).

Supplementation also increased the dDM intake by female calves that received multiple supplements (Table 4). This greater digestibility is attributed to the greater intake of easily digestible nutritional compounds as well as to the increased digestibility of the pasture. Supplementation increased the TDN intake and this was reflected in the increase of the CP and apNDF digestibility coefficients.

In the case of DM, OM, and TDN, the increase in digestibility may be due to the progressive presence of more easily digestible compounds in the diet of animals receiving multiple supplements (Table 4). On the other hand, the significant increase in the digestibility of apNDF may be due to the supply of the necessary substrates for the rumen microorganisms, in response to supplement consumption, allowing the increase of fibrolytic bacteria in number and the degradability of the fiber (Doyle et al. 2005).

All treatments had similar NEFA concentrations, with mean of 0.210 mmol/L (Table 2). This is likely because in young suckling animals, with high ADG, the mobilization of adipose tissue is not expected to cause energy deficiencies. However, this value represents a reference of the basal level of NEFA in young calves for future use in studies of negative energy balance in beef cattle. Interestingly, the NEFA observed in the present study is close to those observed for dairy cows in a positive energy balance (Rabelo et al. 2005; Van Knegsel et al. 2007; Loiselle et al. 2009).

In this study, supplementation increased the concentration of GLUC and INS likely due to the effect of increased energy intake from supplement intake and increased digestibility of nutrients, as discussed previously. Diets rich in starch increase the production of propionate, and this is converted to glucose in the liver, stimulating the release of insulin. Additionally, increased insulin concentration may be the result of amino acid stimulation in insulin secretion (Harmon 1992). Insulin, in turn, as well as insulin-like growth factor 1 (IGF-1), functions as a potent stimulus for cell proliferation and cell hypertrophy (Lawrence et al. 2012), indicating the metabolic, positive effect of increased supplementation on the higher ADG of these animals.

In summary, it is concluded that linearly increasing the feeding level of a supplement with 20% crude protein (% of DM) in the range of 0.2 to 0.6% of BW improves the performance, nutritional and metabolic characteristics of the animals, considering these pasture characteristics. In addition, the dam's milk yield positively affects CP and dDM intake and DM and CP digestibility and negatively affects intake of FDM and apNDF (g/kg BW). Furthermore, further studies are still necessary to evaluate long-term effects of such supplementation strategies. Creep-feeding supplementation will increase weaning costs but may be linked to early parturition and a faster production cycle. However, to our knowledge, no such data is available in the literature.

Acknowledgements We are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), and Instituto de Ciência e Tecnologia de Ciência Animal (INCT-CA) for supporting this study. We acknowledge Ward van der Zee for proof reading.

Compliance with ethical standards

All animal care and handling procedures were approved by the Animal Care and Use Committee of the Universidade Federal de Viçosa, Brazil (protocol CEUAP-UFV 0008).

Conflict of interest The authors declare that they have no conflict of interest.

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