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REGULAR ARTICLES

Performance, endocrine, metabolic, and reproductive responses of Nellore heifers submitted to different supplementation levels pre- and post-weaning

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Abstract The present study was conducted to evaluate the effects of high and low supplementation levels pre- and post-weaning on performance, endocrine, metabolic, and reproductive responses of Nellore heifers. Fifty Nellore heifers with 132 ± 9.9 kg average body weight (BW) and 138 ± 19 days of age were supplemented from 4 to 14 months. The heifers were distributed into five supplementation plans: HH—6 g/kg of BW of supplement pre- and post-weaning, HL—6 g/kg of BW of supplement pre-weaning and 3 g/kg post-weaning, LH—3 g/kg of BW pre-weaning and 6 g/kg of BW post-weaning, LL—3 g/kg of BW pre- and post-weaning, and CC—control, no supplementation. Interactions were not significant ($P > 0.10$). The level of supplement fed pre-weaning did not affect any of the performance variables evaluated at the end of the experiment ($P > 0.10$). There was a significant effect of supplementation and level of supplementation fed post-weaning on average daily gain (ADG) and final BW ($P < 0.05$). Overall ADG was also affected only by supplementation and level of supplement fed post-weaning ($P < 0.05$) with animals receiving 6 g/kg of BW post-weaning gaining more weight. Follicular diameter was greater in animals that received 6 g/kg of BW post-weaning ($P < 0.05$). In summary, performance, endocrine, metabolic, and reproductive variables evaluated in the current study were improved by the level of supplement fed post-weaning. Heifers receiving supplementation of 6 g/kg of BW post-weaning had greater responses, independent of the level received during the pre-weaning phase.

Keywords Biometry · Growth hormone · Insulin · Ruminant metabolism

Introduction

The occurrence of puberty depends on the growth rate and development of the animal to support endocrine mechanisms controlling the first ovulation. Studies have shown different results on when is the best time to accelerate growth in bovine females. Some authors have reported the occurrence of early puberty with increased rate of gain in the early stages of development (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 (Gojjam et al. 2011; Barcellos et al. 2014; Rodríguez-Sánchez et al. 2015).

However, a target average daily gain (ADG) should not be established based solely on animal's physiological response. The existence of complex interactions between genotype and environment, seasonality in forage production, and economic efficiency should also be considered when planning a nutritional strategy to develop heifers.

Thus, this study was conducted to evaluate the effects of high and low supplementation levels pre- and post-weaning on performance, endocrine, and metabolic responses and characteristics related to reproduction of Nellore heifers.

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Material and methods

Experimental design and treatments

Experiments were conducted at the Universidade Federal de Viçosa, Brazil, from February to November of 2013. Fifty Nellore heifers with 132 ± 9.9 kg average body weight (BW) and 138 ± 19 days of age were used (ten experimental units per treatment).

The completely randomized 2 × 2 + 1 factorial design had two levels of concentrate supplementation fed in two phases (pre- and post-weaning), plus a control group that received no concentrate. In this way, the heifers were distributed into five supplementation plans: HH, animals received 6 g/kg of BW of supplement pre- and post-weaning; HL, animals received 6 g/kg of BW of supplement pre-weaning and 3 g/kg of BW post-weaning; LH, animals received 3 g/kg of BW of supplement pre-weaning and 6 g/kg of BW post-weaning; LL, animals received 3 g/kg of BW of supplement pre- and post-weaning; and CC, control, no concentrate supplement was fed.

The supplement was formulated to contain approximately 25% crude protein (CP; Table 1). The animals from all the treatments had unlimited access to mineral supplement throughout the experiment. The mineral supplement was composed of 8.7% calcium, 9.0% phosphorus, 18.7% sodium, 9.0% sulfur, 2400 mg/kg of zinc, 800 mg/kg of copper, 1600 mg/kg of manganese, 40.0 mg/kg of iodine, 8.00 mg/kg of cobalt, and 8.16 mg/kg of selenium.

The pre-weaning phase lasted 120 days, from February to May, and the post-weaning phase lasted 180 days, from June to November.

In the pre-weaning phase, the animals were placed in an experimental area of *Brachiaria decumbens*, divided into five paddocks of 7.0 ha each, with free access to water and creep feeders. Heifer calves were weaned at 240 days of age. After weaning, the calves were transferred to another area of *B. decumbens* and distributed into five paddocks of 2.5 ha each, with water dispensers and feeders.

Table 1 Ingredients and composition of supplements

Item	Supplement	
	Pre-weaning	Post-weaning
Ingredients (%; as-fed basis)		
Corn	26.0	27.5
Sorghum	26.0	27.5
Soybean meal	45.0	45.0
Molasses	3.0	–
Chemical composition (g/kg; dry matter basis)		
OM	961	967
CP	298	270
apNDF	139	136

OM organic matter, *CP* crude protein, *apNDF* neutral detergent fiber corrected for ash and protein residue

Forage analysis and intake trial

Pasture chemical composition was assessed by hand-plucked samples every 2 weeks. In the middle of every experimental month, a second pasture sample was collected to estimate the forage potentially digestible dry matter (pdDM; Detmann et al. 2016). The samples were weighed and oven dried at 60 °C for 72 h. After that, mill ground to pass through a 2-mm screen for indigestible neutral detergent fiber (iNDF) analysis (Valente et al. 2011). A 20-g subportion of each sample was ground to pass through a 1-mm screen for analyses of dry matter (DM), ash, crude protein (CP), and neutral detergent fiber (NDF).

A 9-day intake trial was carried out in each phase. Chromium oxide (Cr_2O_3) was used as external marker to estimate fecal excretion (in the amount of 10 and 15 g/animal in pre- and post-weaning phases, respectively). The chromium oxide was packed in paper cartridges and delivered via the esophagus with a metal probe once daily at 10 a.m. Individual intake of the supplement was estimated using titanium dioxide (TiO_2) mixed in the supplement in a proportion of 10 g/kg of supplement. Finally, iNDF was used as internal marker to estimate DM intake. Six days were allowed for stabilization of marker excretion, and fecal samples were subsequently collected at 3 p.m. on the seventh day, at 11 a.m. on the eighth day, and at 7 a.m. on the ninth day of the intake trial.

The fecal samples were collected immediately after defecation or directly from the rectum of the animals in amounts of approximately 200 g, dried (60 °C/72 h), and mill ground as described for the forage samples. The ground samples were proportionally combined to a pooled 3-day sample per animal per phase.

Milk intake by the calves was estimated on days 20, 60, and 100 of the experiment following procedures described by Lopes et al. (2016). The milk produced was corrected to 4% of fat ($milk_{4\%}$; NRC 2001).

The samples of forage, feces, and supplement were analyzed following procedures described by Detmann et al. (2012) for DM (index INCT-CA G-003/1), CP (index INCT-CA N-001/1), ash (index INCT-CA M-001/1), and neutral detergent fiber corrected for contaminant ash and protein (apNDF; indexes INCT-CA F-002/1, INCT-CA M-002/1, and INCT-CA N-004/1). The iNDF was evaluated using F57 filter bags (Ankom®) by a 288-h in situ incubation procedure (Valente et al. 2011). The fecal samples were also analyzed for levels of chromium by atomic absorption spectrophotometry (index INCT-CA M-005/1) and titanium dioxide by colorimetry (index INCT-CA M-007/1) as recommended by Detmann et al. (2012). Milk was analyzed for protein, fat, lactose, and

total solids content, using spectroscopy (Foss MilkoScan FT120, Hillerød, Denmark).

Fecal excretion, individual supplement intake, and dry matter intake were calculated according to equations described by Detmann et al. (2016).

Blood sampling and analysis

Blood samples were collected at 8:00 a.m. every 40 days during the pre-weaning phase and every 36 days in the post-weaning phase. Two blood samples were collected from the jugular vein of each heifer with vacuum tubes. One of the samples was collected in tubes with clot activator and gel for serum separation (BD Vacuntainer® SST II Plus, São Paulo, Brazil) for analyses of GH, insulin, total cholesterol, HDL, triglycerides, total protein, albumin, and urea. The sample collected in the second tube, with EDTA and sodium fluoride (BD Vacutainer® Fluoreto/EDTA, São Paulo, Brazil), was used for glucose analysis. The collected samples were centrifuged at 3600×*g* for 20 min, and serum and plasma were immediately frozen at −20 °C in triplicate until further analysis.

Growth hormone and insulin were analyzed by chemiluminescence using Access Ultrasensitive hGH Reagent (Ref. Number 33580, Beckman Coulter®, Brea, USA) and 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, Bioclin® Quibasa, Belo Horizonte, Brazil), total cholesterol (Ref. Number K083, Bioclin® Quibasa, Belo Horizonte, Brazil), HDL (Ref. Number K071, Bioclin® Quibasa, Belo Horizonte, Brazil), triglycerides (Ref. Number K117, Bioclin® Quibasa, Belo Horizonte, Brazil), and urea (Ref. Number K056, Bioclin® Quibasa, Belo Horizonte, Brazil) were quantified by enzymatic-colorimetric method and total protein (Ref. Number K031, Bioclin® Quibasa, Belo Horizonte, Brazil) and albumin (Ref. Number K040, Bioclin® Quibasa, Belo Horizonte, Brazil) by colorimetric method. The LDL levels were estimated according to the Friedewald equation (Tietz 1986). Globulins were calculated by subtracting the albumin quantified from the total protein level. Serum urea N (SUN) was estimated as 46.67% of the total serum urea. Metabolites were analyzed in accordance with the manufacturer's instructions in an automatic biochemistry analyzer (Mindray BS200E, Shenzhen, China). The results were averaged by phase, resulting in the serum and plasma concentrations of hormones and metabolites for each animal per phase (pre- and post-weaning).

Performance, body measures, and carcass characteristics

After 14 h of solid fasting, the animals were weighed at the beginning and the end of each phase.

Body measures (BM) were taken at weaning and at the end of the experiment. 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 height stick. The heart girth (the body circumference immediately posterior to the front legs) was measured with a flexible tape.

At weaning and at the end of the experiment, *Longissimus* muscle area (LMA) and fat thickness over the *Longissimus* muscle were measured by ultrasound scan (Aloka SSD 500; 3.5-MHz linear probe) of the area between the 13th and 14th ribs. Vegetable oil was used to ensure adequate acoustic contact.

Follicle diameter

At the end of the experiment, an ultrasound Aloka SSD500 with trans-rectal transducer of 5 MHz was used to measure the diameter of the dominant follicle.

Statistical analysis

Statistical analyses were performed using the PROC GLIMMIX in SAS 9.4. The treatments were compared using orthogonal contrasts. Within the pre-weaning phase, the effects of supplementation and the linear effect of level were evaluated. For the whole experimental period, contrasts were constructed in order to evaluate the effects of supplementation (CC vs. HH, HL, LH, and LL), level of supplementation fed pre-weaning (HH and HL vs. LH and LL) and post-weaning (HH and LH vs. HL and LL), and interaction (HH and LL vs. HL and LH). Initial BW was used as covariate. Significant difference was considered at $P < 0.05$, and tendency was declared when $0.05 < P < 0.10$.

Results

Average pdDM and CP content in the forage were 3984 kg/ha and 105 g/kg in the pre-weaning phase and 4070 kg/ha and 77 g/kg in the post-weaning phase (Table 2).

Supplementation increased intake of DM, OM, and CP ($P < 0.05$; Table 3) in the pre-weaning and post-weaning phases. The level of supplementation also positively affected intake of DM, OM, and CP post-weaning ($P < 0.01$).

Pre-weaning, growth hormone levels were higher for the heifers receiving 6 g/kg of BW of supplement ($P < 0.01$; Table 4). Post-weaning, growth hormone was lower in the heifers receiving supplement (30.2 ng/mL) compared to that in the control heifers (35.2 ng/mL; $P < 0.05$).

Table 2 Potentially digestible forage mass and chemical composition of forage

Item	Phase							
	Pre-weaning				Post-weaning			
	Experimental month							
	1	2	3	4	5	6	7	8
pdDM (kg/ha)	3730	5070	3150	6350	3350	3220	2240	5080
OM (g/kg)	926	914	921	913	920	908	910	926
CP (g/kg)	102.9	118.4	93.8	81.4	97.8	74.8	59.6	71.5
NDIN (% of total N)	31.9	26.1	30.2	36.3	37.1	34.2	17.2	16.0
apNDF (g/kg)	558	581	603	608	588	625	630	610
iNDF (g/kg)	172	183	236	232	198	278	284	214

pdDM was estimated for forage sampled in the area delimited by a metal square 0.5 × 0.5; chemical composition was evaluated in the hand-plucked forage sample

pdDM potentially digestible forage dry matter, *OM* organic matter, *CP* crude protein, *NDIN* neutral detergent insoluble N, *apNDF* neutral detergent fiber corrected for ash and protein residue, *iNDF* indigestible neutral detergent fiber

No differences in insulin levels were observed pre-weaning ($P > 0.10$). Post-weaning, insulin levels were affected only by the level of post-weaning supplementation ($P < 0.01$), the animals receiving high supplementation level post-weaning had higher insulin concentrations (1.52 μIU/mL) in comparison to the heifers that had received low supplementation level (1.19 μIU/mL).

Pre-weaning, most metabolites were not affected by the treatment ($P > 0.10$). Exception was SUN, which increased with supplementation and linearly increased with the level of supplement offered ($P < 0.01$), and triglycerides which tended to be higher in the control heifers ($P < 0.10$).

Table 3 Intake in the pre- and post-weaning phases

Item	Pre-weaning[a]					SEM	*P* value[b]					
	H		L		C		Pre-weaning		Overall			
	Post-weaning						S	L	S	L-pre-weaning	L-post-weaning	L-pre-weaning × L-post-weaning
	H	L	H	L	C							
Pre-weaning												
$Milk_{4\%}$ (kg/day)	6.70		6.60		7.60	0.69	0.21	0.82	–	–	–	–
DM (kg/day)	4.22		3.88		3.12	0.4	0.04	0.40	–	–	–	–
Forage DM (kg/day)	2.78		2.61		2.19	0.34	0.18	0.60	–	–	–	–
OM (kg/day)	3.90		3.57		2.87	0.37	0.04	0.37	–	–	–	–
CP (g/day)	743		687		499	63.6	<0.01	0.40	–	–	–	–
Post-weaning												
DM (kg/day)	4.01	3.31	4.25	3.28	2.68	0.32	–	–	<0.01	0.74	0.01	0.68
Forage DM (kg/day)	2.43	2.54	2.66	2.51	2.68	0.22	–	–	0.58	0.64	0.94	0.56
OM (kg/day)	2.40	1.68	2.51	1.64	1.06	0.20	–	–	<0.01	0.85	<0.01	0.70
CP (g/day)	595	335	560	315	118	44.2	–	–	<0.01	0.54	<0.01	0.86

Milk intake is presented as natural basis; all other variables as dry matter basis

DM dry matter, *OM* organic matter, *CP* crude protein

[a] H—animals received 6 g/kg of BW; L—animals received 3 g/kg of BW; and C—control, no supplement offered

[b] L—linear effect of level of supplementation offered in the pre-weaning phase; Q—quadratic effect of level of supplementation offered in pre-weaning phase; S—supplementation effect in the pre-weaning phase; L-pre-weaning—effect of supplementation level in the pre-weaning phase; L-post-weaning—effect of supplementation level in the post-weaning phase; L-pre-weaning × L-post-weaning—interaction between supplementation level pre- and post-weaning

Table 4 Endocrine and metabolic profile in the pre- and post-weaning phases

Item	Pre-weaning[a]					SEM	*P* value[b]					
	H		L		C		Pre-weaning		Overall			
	Post-weaning						S	L	S	L-pre-weaning	L-post-weaning	L-pre-weaning × L-post-weaning
	H	L	H	L	C							
Pre-weaning												
GH (ng/mL)	29.8		25.3		25.9	1.62	0.38	<0.01	–	–	–	–
Insulin (μIU/mL)	1.45		1.43		1.37	0.14	0.67	0.92	–	–	–	–
Glucose (mg/dL)	84.3		80.6		80.2	3.99	0.61	0.36	–	–	–	–
Cholesterol (mg/dL)	172		160		175	6.80	0.38	0.22	–	–	–	–
HDL (mg/dL)	103.8		98.5		97.9	3.53	0.42	0.14	–	–	–	–
LDL (mg/dL)	61.3		54.6		69.4	7.83	0.20	0.39	–	–	–	–
Triglycerides (mg/dL)	34.0		34.4		40.6	2.98	0.06	0.91	–	–	–	–
Total protein (g/dL)	6.46		6.32		6.44	0.11	0.73	0.21	–	–	–	–
Albumin (g/dL)	3.28		3.17		3.20	0.08	0.76	0.16	–	–	–	–
Globulins (g/dL)	3.19		3.15		3.24	0.12	0.59	0.76	–	–	–	–
SUN (mg/dL)	19.4		15.4		11.3	0.80	<0.01	<0.01	–	–	–	–
Post-weaning												
GH (ng/mL)	30.5	33.0	29.9	27.4	35.2	1.80	–	–	0.02	0.09	0.97	0.17
Insulin (μIU/mL)	1.51	1.22	1.52	1.16	1.29	0.11	–	–	0.57	0.82	<0.01	0.78
Glucose (mg/dL)	74.3	70.2	67.1	70.5	65.1	2.51	–	–	0.06	0.17	0.87	0.14
Cholesterol (mg/dL)	102	102	91	103	107	4.12	–	–	0.11	0.26	0.14	0.13
HDL (mg/dL)	72.1	70.3	66.3	70.5	65.5	2.73	–	–	0.17	0.31	0.68	0.28
LDL (mg/dL)	24.8	25.3	18.6	27.4	37.0	3.16	–	–	<0.01	0.52	0.15	0.19
Triglycerides (mg/dL)	26.6	30.8	31.2	30.3	29.0	1.75	–	–	0.70	0.24	0.36	0.16
Total protein (g/dL)	6.31	5.91	6.10	5.97	5.90	0.11	–	–	0.18	0.51	0.02	0.24
Albumin (g/dL)	3.27	2.91	3.16	2.93	2.83	0.06	–	–	<0.01	0.47	<0.01	0.31
Globulins (g/dL)	3.04	3.00	2.94	3.04	3.07	0.12	–	–	0.62	0.80	0.79	0.55
SUN (mg/dL)	16.9	13.2	14.4	12.4	11.4	0.69	–	–	<0.01	0.02	<0.01	0.21

[a] H—animals received 6 g/kg of BW; L—animals received 3 g/kg of BW; and C—control, no supplement offered

[b] L—linear effect of level of supplementation offered in the pre-weaning phase; Q—quadratic effect of level of supplementation offered in pre-weaning phase; S—supplementation effect in the pre-weaning phase; L-pre-weaning—effect of supplementation level in the pre-weaning phase; L-post-weaning—effect of supplementation level in the post-weaning phase; L-pre-weaning × L-post-weaning—interaction between supplementation level pre- and post-weaning

The post-weaning animals receiving supplementation tended to have higher glucose levels compared to the control animals (70 vs. 65 for supplemented and not supplemented, respectively; $P = 0.06$).

All metabolites related to fat metabolism were reduced post-weaning; however, only LDL differed significantly among treatments (supplementation effect; $P < 0.01$), with the control heifers (CC) having the highest LDL levels (37 mg/dL).

There was a positive effect of supplementation level offered post-weaning on total protein concentrations ($P < 0.05$). The post-weaning supplementation was associated with increased albumin levels ($P < 0.05$), and the animals that received high supplement levels also had higher albumin levels ($P < 0.05$). The difference observed in total serum protein levels was due to albumin, whereas no significant difference was observed in globulin concentrations ($P > 0.10$).

The concentrations of SUN were higher in the supplemented heifers post-weaning ($P < 0.01$), and they were also affected by supplement levels received pre-weaning ($P < 0.05$). The heifers that have received high pre-weaning supplementation level had higher post-weaning levels of SUN (means were 15.1 for HH and HL vs. 13.4 for LH and LL). Supplementation level offered post-weaning also affected SUN levels ($P < 0.01$); the HH and LH heifers had averages of 15.6 vs. 12.8 mg/dL in the HL and LL heifers.

Wither and rump heights did not differ among the treatments at weaning or at the end of the experiment ($P > 0.05$; Table 5). At weaning, only rib depth was positively affected

Table 5 Body measurements of heifers at 8 and 14 months of age

Item	Pre-weaning[a]					SEM	*P* value[b]					
	H		L		C		Pre-weaning		Overall			
	Post-weaning						S	L	S	L-pre-weaning	L-post-weaning	L-pre-weaning × L-post-weaning
	H	L	H	L	C							
8 months—weaning												
Rump width (cm)	33.1		32.2		32.8	0.64	0.82	0.21	–	–	–	–
Rump length (cm)	36.1		36.0		35.5	0.66	0.46	0.88	–	–	–	–
Rib depth (cm)	51.3		49.7		48.7	0.75	0.03	0.04	–	–	–	–
Body length (cm)	113		112		109	1.87	0.07	0.52	–	–	–	–
Heart girth (cm)	135		134		136	2.07	0.51	0.58	–	–	–	–
Height at withers (cm)	113		113		112	1.48	0.62	0.83	–	–	–	–
Rump height (cm)	119		120		120	1.59	0.98	0.63	–	–	–	–
BW:height at withers (kg/cm)	2.01		2.03		1.93	0.07	0.22	0.84	–	–	–	–
14 months—breeding season												
Rump width (cm)	39.1	38.7	38.9	38.6	36.4	0.80	–	–	0.01	0.85	0.66	0.90
Rump length (cm)	41.2	40.4	40.7	40.8	38.3	0.63	–	–	<0.01	0.94	0.58	0.53
Rib depth (cm)	56.3	55.7	55.9	55.6	51.4	0.82	–	–	<0.01	0.76	0.59	0.81
Body length (cm)	124	124	128	123	118	1.78	–	–	<0.01	0.42	0.19	0.21
Heart girth (cm)	153	150	154	150	141	2.07	–	–	<0.01	0.89	0.09	0.99
Height at withers (cm)	125	123	124	124	121	1.81	–	–	0.12	0.99	0.56	0.86
Rump height (cm)	131	129	132	130	127	1.73	–	–	0.10	0.39	0.25	0.98
BW/height at withers (kg/cm)	2.40	2.28	2.48	2.29	2.08	0.07	–	–	<0.01	0.55	0.04	0.58

BW body weight

[a] H—animals received 6 g/kg of BW; L—animals received 3 g/kg of BW; and C—control, no supplement offered

[b] L—linear effect of level of supplementation offered in the pre-weaning phase; Q—quadratic effect of level of supplementation offered in pre-weaning phase; S—supplementation effect in the pre-weaning phase; L-pre-weaning—effect of supplementation level in the pre-weaning phase; L-post-weaning—effect of supplementation level in the post-weaning phase; L-pre-weaning × L-post-weaning—interaction between supplementation level pre- and post-weaning

by supplementation and level of supplementation ($P < 0.05$); body length tended to be positively affected by the supplementation ($P < 0.10$). At the end of the experiment, important BM were improved by supplementation and level of supplementation offered post-weaning ($P < 0.05$). Rump width, rump length, rib depth, body length, heart girth, and BW/height ratio were greater for the supplemented animals. Heart girth tended to be higher for the animals receiving high supplementation levels post-weaning ($P = 0.09$), and BW/height ratio was also greater for the animals receiving high post-weaning supplementation levels ($P < 0.05$).

No significant difference was observed in BW, ADG, and LMA at weaning ($P > 0.10$; Table 6). The heifer calves were weaned with an average of 219 kg of BW across the treatments. Despite the lack of difference in BW and ADG, there was a positive effect of supplementation on fat thickness at weaning ($P < 0.01$) and a tendency for greater fat thickness in the animals receiving higher pre-weaning supplementation levels ($P < 0.10$).

An interaction between supplementation level fed pre-weaning and level of supplementation fed post-weaning was not significant for any of the performance variables evaluated ($P > 0.10$). Supplement level fed pre-weaning did not influence any of the performance variables evaluated at the end of the experiment ($P > 0.10$). There was a significant effect of supplementation and level of supplementation offered post-weaning on post-weaning ADG ($P < 0.01$). Post-weaning ADG was 410 g/day for the heifers receiving high supplementation level, 315 g/day for the heifer receiving low level, and 157 g/day for the control heifers. Overall, ADG was also affected only by supplementation and level of supplementation offered post-weaning ($P < 0.01$), with the animals receiving 6 g/kg of BW after weaning gaining more weight. The average BW at the beginning of the breeding season (at the end of the experiment) differed among the strategies ($P < 0.01$) and was 297 kg for the heifers in supplementation strategies HH and LH, 275 for the heifers in HL and LL, and 244 kg for the CC heifers.

The supplemented animals had higher LMA values at the end of the experiment ($P < 0.05$). The animals receiving 6 g/kg of supplement post-weaning also had higher fat thickness (3.43 and 2.98 mm for the HH and LH heifers, respectively;

Table 6 Performance, carcass characteristics, and reproductive performance

Item	Pre-weaning[a]					SEM	*P* value[b]					
	H		L		C		Pre-weaning		Overall			
	Post-weaning						S	L	S	L-pre-weaning	L-post-weaning	L-pre-weaning × L-post-weaning
	H	L	H	L	C							
8 months of age—weaning												
BW (kg)	218		221		216	3.53	0.30	0.51	–	–	–	–
Pre-weaning ADG (g/day)	706		727		678	29.4	0.25	0.48	–	–	–	–
LMA (cm^2)	38.7		39.1		36.9	1.68	0.29	0.82	–	–	–	–
Fat thickness (mm)	2.06		1.48		0.51	0.30	<0.01	0.06	–	–	–	–
14 months of age—breeding season												
BW (kg)	296	275	297	274	244	6.60	–	–	<0.01	0.98	<0.01	0.88
Post-weaning ADG (g/day)	416	323	406	307	157	23.8	–	–	<0.01	0.58	<0.01	0.89
Overall ADG (g/day)	537	470	541	467	364	21.7	–	–	<0.01	0.97	<0.01	0.87
LMA (cm^2)	49.8	46.7	49.0	46.4	42.4	2.10	–	–	0.02	0.75	0.11	0.90
Fat thickness (mm)	3.43	2.54	2.98	2.27	2.52	0.33	–	–	0.43	0.21	<0.01	0.75
Follicular diameter (mm)	11.5	9.5	11.3	7.7	9.9	0.78	–	–	0.91	0.24	<0.01	0.38

Carcass characteristics accessed by ultrasonography

BW body weight, *ADG* average daily gain, *LMA Longissimus* muscle area, *Fat thickness* fat thickness over the *Longissimus* muscle

[a] H—animals received 6 g/kg of BW; L—animals received 3 g/kg of BW; and C—control, no supplement offered

[b] L—linear effect of level of supplementation offered in the pre-weaning phase; Q—quadratic effect of level of supplementation offered in pre-weaning phase; S—supplementation effect in the pre-weaning phase; L-pre-weaning—effect of supplementation level in the pre-weaning phase; L-post-weaning—effect of supplementation level in the post-weaning phase; L-pre-weaning × L-post-weaning—interaction between supplementation level pre- and post-weaning

$P < 0.01$), while the HL and LL heifers had fat thickness comparable to those of the control heifers (2.54 for the HL and 2.27 for the LL vs. 2.52 for the control).

Follicular diameter was greater for the animals receiving 6 g/kg of BW post-weaning ($P < 0.01$).

Discussion

Important performance, endocrine, and metabolic variables were affected by supplementation level offered post-weaning, with pre-weaning supplementation level having little or no effect on animals' performance at weaning and at the end of the experiment.

Roberts et al. (2009) demonstrated that puberty was much more affected by variation in growth rates up to approximately 8 months of age than subsequent growth up to the start of breeding. Gasser et al. (2006) and Cardoso et al. (2014) reported that during early calfhood development, heifers between 4 and 6.5 months of age are more sensitive to nutritional programming. The majority of studies demonstrating this positive effect of early nutrition on heifer development were conducted using level of feeding in order to change ADG, most of them using heifers weaned early (Gasser et al. 2006; Cardoso et al. 2014) or in a controlled pre-weaning regimen (Rodríguez-Sánchez et al. 2015).

Results from these studies contributed to our hypothesis that creep-feeding heifers accompanied by their dams with high supplementation levels could improve heifer's performance. This hypothesis was refuted in the present study; creep-feeding heifer calves in ad libitum suckling was not efficient to improve their performance at weaning and had no effect on performance variables evaluated later in their lives.

There is an inverse association between genetic potential for milk production and age of puberty (Martin et al. 1992), indicating that inherent differences, especially genetics and milk supply, contribute to the influence of pre-weaning growth on subsequent attainment of puberty. As such, it would not be advantageous to implement management strategies to increase pre-weaning growth in an attempt to increase pubertal proportions because this would result in the retention of more heifers with less desirable genetic characteristics for growth and reproduction (Roberts et al., unpublished data).

Average CP content of forage during the pre-weaning phase of 105 g/kg combined with milk ingested ad libitum was probably enough to achieve the heifers' CP requirement for maintenance and genetically programmed growth in all the

treatments. The increase in intake of DM, OM, and CP pre-weaning was reflected in an increased fat thickness at weaning instead of muscle deposition, as no difference in LMA or ADG was observed at weaning. The CP content of forage was lower in the post-weaning phase than that in the pre-weaning phase. Therefore, providing a higher supplementation level efficiently improved heifers' post-weaning ADG, and consequently, their overall ADG and final BW.

The concentrations of GH are elevated during feed restriction because GH plays a catabolic role in mobilizing lipid from adipose tissue in order to conserve glucose. The post-weaning phase of heifers' development is characterized by the dry season that usually takes place in Central Brazil. In the present study, the unsupplemented animals had higher GH and lower insulin concentrations compared to the animals receiving high supplement levels. The endocrine profile of control heifers is consistent with their lower nutritional level.

The heifers receiving high supplementation levels post-weaning had higher intake of CP and OM and consequently energy; therefore, the supplemented heifers had higher glucose levels. Glucose transporter 4 (GLUT4), an insulin-activated transporter, may play a supporting role in the bovine follicle (Nishimoto et al. 2006), providing more glucose to follicle metabolism when more glucose is available in the blood stream. In accordance with these findings, the animals receiving high supplementation levels post-weaning had higher insulin concentrations and follicle diameter.

Albumin is the main serum protein synthesized by the liver, and its concentration can be influenced by amino acid and nutrient availability. Lower values for albumin concentrations in the control heifers and heifers receiving low supplementation levels post-weaning further indicate that animals from high-supplementation strategy had a greater nutritional status post-weaning.

Severe nutritional restriction can cause permanent impairment to a heifer's growth. The height at withers is primarily a composite of the long bone measurement of the forelimb and is a good indicator of skeletal development. While other authors have reported difference in height due to nutritional treatment applied (Roberts et al. 2009; Rodríguez-Sánchez et al. 2015), the lack of difference in height indicates that the feed level or nutrient restriction applied in the present study was not detrimental to skeletal development and subsequent mature size, reflected by height.

The BW/height ratio reflects animals' body condition. The supplemented heifers had greater BW/height ratios, and within the supplemented heifers, those receiving 6 g/kg of BW had greater BW/height ratio. Therefore, higher supplementation level post-weaning improves animal's development of muscle and fat deposition.

The heart girth is the BM that correlates most with BW. In the current study, heart girth was influenced only by feeding treatment applied post-weaning, with the heifers receiving higher supplementation levels after weaning tending to have higher heart girths.

Reserves of body fat may act as a marker of energy available for reproductive activity (Hall et al. 1995), a permissive signal allowing first ovulation and pregnancy. In the present study, the animals receiving high supplementation level post-weaning had more fat thickness at the end of the experiment.

In summary, performance, endocrine, metabolic, and reproductive variables evaluated in the current study were improved by supplementation level applied post-weaning. Due to higher nutrient intake provided via concentrate supplement, the heifers receiving 6 g/kg of BW post-weaning had greater responses, independently of the supplementation level received pre-weaning.

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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 0011).

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

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