Tropical Animal Health and Production (2019) 51:79–87
https://doi.org/10.1007/s11250-018-1662-z

REGULAR ARTICLES

Nutritional planning for Nellore heifers post-weaning to conception at 15 months of age: performance and nutritional, metabolic, and reproductive responses

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Received: 30 May 2018 / Accepted: 4 July 2018 / Published online: 13 July 2018

Abstract

The objective of this study was to evaluate the effects of strategic supplementation in the dry period and dry/rainy transition period on the performance and nutritional, metabolic, and reproductive responses of Nellore heifers grazing *Urochloa decumbens*. Forty-eight Nellore heifers with age and body weight (BW) of eight months and 235 ± 3.3 kg, respectively, were used. The experimental design was a completely randomized design, with four treatments, all with 12 replications. The evaluated strategies were as follows: low supplementation (LOHI; 0.2% of BW/heifer/day) in the first 90 days and high supplementation (0.6% of BW/heifer/day) in the 90 days thereafter; average supplementation (AVER) with 0.4% of BW/heifer/day for 180 days; high supplementation (HILO; 0.6% of BW/heifer/day) in the first 90 days and low supplementation (0.2% of BW/heifer/day) in the 90 days thereafter; only mineral mix (MM) ad libitum during the 180 days. Data were evaluated using orthogonal contrasts. Supplementation improved the performance of the animals during of dry period ($P < 0.05$) and dry/rainy transition period ($P < 0.05$). Supplemented animals had higher longissimus muscle area (LMA) and subcutaneous fat thickness (SFT) at the end of the experiment ($P < 0.05$). Multiple supplementation increased intake of dry matter (DM), organic matter (OM), and crude protein (CP) in kg/day throughout the experiment. The supplementation increased the digestibility of DM, OM, CP, apNDF, and TDN ($P < 0.05$). Serum urea nitrogen (SUN), glucose (GLUC), insulin (INS), and progesterone (PROG) were higher in supplemented heifers ($P < 0.05$). Supplementation reduced the concentrations of non-esterified fatty acids (NEFA) ($P < 0.05$) and increased conception rate ($P < 0.05$). In summary, the supplementation strategies adopted in this study improve the performance, metabolic status, and carcass traits of heifers under grazing, allowing an improvement in the conception rate of 15-month-old Nellore heifers.

Keywords Animal nutrition · Beef cattle · Heifers · Supplementation · Tropical pastures

Introduction

The biological efficiency of a herd it is linked to age at puberty and early mating, and the importance of these characteristics increases as the production system becomes more intensive and competitive (Menegaz et al. 2008). The reduction of the age at first calving increases the reproductive life of the dam and the number of calves and reduces the permanence of less productive categories in the production system, clearing areas for other categories and reducing energy cost per unit of product (Beretta et al. 2001). The study of sexual precocity in zebu females is still recent in scientific literature; therefore, it is rare find literature data on mating zebu females close to 14 to 16 months of age.

Nutrition is undoubtedly the main driver of age at slaughter or at first conception, which means that precocity or rate at which the animal approaches adult weight is sensitive to changes in nutritional plan (Paulino et al. 2004). In low-

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quality pastures, such as in the dry season and dry/rainy transition period, supplementation with nitrogen compounds is the most important in improving the use of forage (Franco et al. 2017) and should be initially supplemented.

Management decisions regarding replacement heifers should focus on factors that promote early onset of puberty. However, little is known about how the supply of the supplement (uniformly throughout the period, most at the beginning of the feeding period or most at the end) should be performed during post-weaning so that the animal expresses its genetic potential and can achieve puberty at the age of 15 months.

Thus, this study aimed to evaluate supplementation strategies in the dry period and dry/rainy transition period on the performance, carcass traits, hormone and metabolite concentrations, and reproductive performance of Nellore heifers.

Material and methods

Animals, experimental design, and supplements

The experiment was conducted at the Universidade Federal de Viçosa, located in the municipality of Viçosa, MG, between July and December of 2015, with a duration of 180 days, during dry period and dry/rainy transition period.

The experimental area of 32 ha was constituted by four paddocks of 8.0 ha, covered uniformly with *Urochloa decumbens*, provided with water dispensers and feeders that are covered and accessible from both sides. Animals were continuously stocked such that all four paddocks were stocked with cattle throughout the entire experiment. Specifically, all animals from a given supplement treatment were assigned to one of the 8.0 ha paddocks at all times. Each treatment group of animals was moved sequentially from one paddock to the next every 15 days in an attempt to minimize any effects of different paddock conditions on the response to supplement treatments. Forty-eight Nellore heifers with an initial age of 8 months and an initial weight of 235 ± 3.3 kg were used.

The experimental design was a completely randomized design, with four treatments. There were 12 replications per treatment, and an individual heifer was considered to be the experimental unit. Supplement for a given treatment was fed in a common feeder in each paddock to represent what is done in commercial practice. Four supplementation strategies were evaluated:

- LOHI—low supplementation (0.2% of the body weight (BW)/heifer/day) in the first 90 days and high supplementation (0.6% of BW/heifer/day) in the 90 days thereafter;
- HILO—high supplementation (0.6% of BW/heifer/day) in the first 90 days and low supplementation (0.2% of BW/heifer/day) in the 90 days thereafter;
- AVER—average supplementation with 0.4% of BW/heifer/day for 180 days; and
- MM—mineral mix ad libitum during the 180 days.

A single supplement was supplied, with 20% protein (63% corn grain, 32% soybean meal, and 5% mineral mix). The mineral mix was composed 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

Heifers were weighed at trial start, at 90 days, and at the end of the experiment, always with 12-h fasting. At beginning and at the end of the experiment, the longissimus muscle area (LMA) and subcutaneous fat thickness (SFT) 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.

Forage samples were collected every 15 days for evaluation of forage mass. At each paddock, four forage samples were randomly selected using a metal square (0.5 × 0.5 m) and cut approximately 1 cm above the ground. Additional forage samples, for chemical composition, were collected at intervals of 30 days by hand-plucking (Silva et al. 2017). Subsequently, the forage samples (200 g) were immediately oven dried at 60 °C for 72 h and milled with a knife mill (Willye® TE-680) with sieves of 1 and 2 mm.

Two 9-day digestibility trials were carried out, starting at day 45 and day 135 of the experimental period. To estimate fecal excretion, 20 g per animal of chromium oxide (Cr_2O_3) was supplied daily in paper cartridges (Detmann et al. 2001) with the aid of a metal probe. To estimate the individual intake of the supplement, titanium dioxide (TiO_2), mixed in the supplement, was used in the proportion of 10 g of indicator per animal (Titgemeyer et al. 2001). To estimate the dry matter (DM) intake of pasture, an indigestible neutral detergent fiber (iNDF) was used as the internal marker (Detmann et al. 2001).

From the 9-day digestibility trial, 5 days were used only to adapt the animals to Cr_2O_3 and TiO_2 supply. During the last 4 days, collections were held at the following times: 1600 h, 1300 h, 1000 h, and 0700 h. Fecal samples were collected directly in the rectum of the animals, in an approximate amount of 200 g, and oven dried at 60 °C for 72 h and milled with a knife mill (Willye® TE-680) with sieves of 1 and 2 mm. On the fifth day each digestibility trial, a manual grazing simulation was performed on each paddock separately, and these samples were used to estimate intake and apparent digestibility coefficients.

Blood samples were collected at days 15, 45, 75, 105, 135, and 165. The collection was performed always at 0700 h, via puncture of the jugular vein, using vacuum tubes with a clot accelerator and separator gel (BD Vacuntainer® SSTIIAdvance, São Paulo, Brazil). Subsequently, the levels of serum urea nitrogen (SUN), glucose (GLUC), insulin (INS), and non-esterified fatty acids (NEFA) were quantified. Finally, blood samples were collected at days 105, 135, and 165 for analysis of progesterone (PROG).

On the 170th day of the experiment, heifers were synchronized using a protocol. Fixed-time artificial insemination (AIrt) was performed and the diagnosis of gestation was determined by transrectal ultrasonography 30 days after AIrt. The conception rate was calculated considering all heifers.

Chemical analysis

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), and 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), obtained after in situ incubation in (F57 Ankom®) bags for 288 h. The potentially digestible dry matter (pdDM) was estimated according to Paulino et al. (2008).

The feces samples were analyzed for chromium and titanium contents using atomic absorption (Souza et al. 2013) and colorimetry (Titgemeyer et al. 2001), respectively. The contents of DM, CP, EE, apNDF, iNDF, and ashes were also evaluated, as previously described. Excretion of fecal DM was estimated using the chromic oxide marker.

Serum concentrations of urea (K056) and glucose (K082) were measured using kits from Bioclin Diagnostics (Belo Horizonte, Brazil). Serum concentrations of NEFA were quantified using a colorimetric method (FA115, Randox Laboratories Ltd., São Paulo, Brazil). An automatic biochemical apparatus (Mindray BS-200E; Shenzhen Mindray Bio-Medical Electronics Co. Ltd.) was used for all analyses. Moreover, SUN was estimated as 46.67% of the total serum urea. Progesterone and insulin were analyzed by chemiluminescence using Access Progesterone Reagent (Ref. Number 33550) and Access Ultrasensitive Insulin Reagent (Ref. Number 33410), in the Access 2 Immunoassay System (Beckman Coulter Inc., Brea, USA).

Statistical analyses

The results were submitted to analysis of variance (ANOVA), adopting a completely randomized design, being the evaluation periods included in the model with measures repeated in time. The PROC MIXED procedure of the SAS software (Statistical Analysis System) was applied for all statistical analyses. In the case of treatment significance, least square means (LS means) were compared using Student's *t* test, adopting $\alpha = 0.05$ as the critical level of type I error probability.

Treatment effects on the priming rate were determined using the PROC FREQ of the SAS software (Statistical Analysis System). In the case of significance, LS means were compared by the following orthogonal contrasts:

- Supplemented vs not supplemented: 3× MM–LOHI–AVER–HILO
- Change in supplementation vs no change in supplementation: 2× AVER–LOHI–HILO
- High initial supplementation vs low initial supplementation: LOHI–HILO

Results

The mean total dry matter (TDM) and pdDM of the *U. decumbens* forage were 4112 and 2680 kg/ha, respectively, during the dry season, and 3622 and 2744 kg/ha, respectively, during the dry/rainy transition.

Supplementation improved the performance of the animals during the first 90 days of the experiment, and this fact can be verified by the heifers' average daily gain (ADG) ($P < 0.05$) and the trend in the highest final body weight (fBW; $P = 0.077$) with increased supplementation (Table 2). The same fact was observed in the dry/rainy transition period, where supplementation improved fBW ($P < 0.05$) and ADG ($P < 0.05$; Table 2).

In the present study, for the initial LMA and SFT, there was no difference ($P > 0.05$) between the supplementation strategies (Table 2). However, the supplemented animals had higher LMA and SFT at the end of the experiment ($P < 0.05$; Table 2).

Multiple supplementation increased intake (Table 3) in kg/day of the DM, organic matter (OM), PB, dDM, and total digestible nutrients (TDN) throughout the experiment and of dNDF and apNDF only in the dry/rainy transition. There was no effect of supplementation on the intake in kg/day of forage dry matter (FDM), dNDF, and apNDF ($P < 0.05$) during the dry period. The lowest FDM intake in the dry/rainy transition period was observed for the LOHI treatment. The AVER and HILO treatments obtained greater apNDF intake than the other treatments in the dry/rainy transition period. The DM intake in g/kg of BW ($P < 0.05$) was higher in the supplemented heifers, being greater for the HILO treatment in the dry period and LOHI in the dry/rainy transition. However, FDM intake in g/kg of BW was higher in non-supplemented heifers, and the intake of apNDF in g/kg of BW was greater for MM and LOHI treatments ($P < 0.05$).

The supplementation increased the total apparent digestibility coefficient of DM, OM, CP, apNDF, and TDN ($P <$

0.05), that is, of all analyzed parameters (Table 4). There was an interaction effect of the treatment with the day of collection on the SUN, NEFA, and INS concentrations ($P < 0.05$). There was an effect of the day of collection on the concentrations of SUN, NEFA, and GLUC ($P < 0.05$). Concentrations of SUN, GLUC, INS, and PROG were higher in supplemented heifers compared to those not supplemented ($P < 0.05$). On the other hand, supplementation reduced NEFA concentrations ($P < 0.05$). The conception rate (CR) was higher for supplemented heifers ($P < 0.05$; Table 5).

Discussion

Paulino et al. (2008) aimed to associate production per animal and per area and suggested a supply between 40 and 50 g of pdDM/kg of BW of pasture for satisfactory grazing performance. In this work, the average mass of pdDM during the first 90 days was 41.4 g/kg of BW, a value that was recommended by this author, demonstrating that the amount of forage did not compromise animal performance. The percentage of CP of forage (Table 1) was below the minimum of 7% of CP in the basal diet (6.02%), reported by Lazzarini et al. (2009) as necessary for adequate utilization of the apNDF of basal forage, which is the main source of energy for animals raised on pasture. This resulted in the lower ADG reached by the MM group, to the detriment of the supplemented animals that had higher intake of nitrogen compounds (Table 3), which probably led to an improvement in the energy to diet protein adequacy, allowing a significant increase in performance (Table 2).

In the last 90 days of the experiment, the mean mass of pdDM was 30.3 g/kg of BW and the percentage of CP of the forage was 6.75 (Table 1). Since pdDM is the basal resource in the feeding of this production system, its availability below the recommended level promotes the need for additional resources. Thus, the animals remained for a prolonged period on a diet of poor protein quality with a marked neutral detergent insoluble nitrogen (NDIN) content (31%), which is protein slowly available to the animal. Moreover, iNDF, especially in the first 60 days of the dry/rainy transition period (Table 1), reduces the use of apNDF by microorganisms (Paulino et al. 2008), which is determinant for weight loss of the MM group in this phase (− 0.155 kg/day). On the other hand, the synchrony between protein adjustment and the insertion of rapidly fermentable carbohydrates provided by supplementation was sufficient to meet the needs of heifers for maintenance (HILO, 0.002 kg/day) or weight gain (AVER, 0.211 kg/day; LOHI, 0.264 kg/day), varying according to the level of supplementation adopted (Table 2).

The LMA analysis allowed us to infer that the supplemented animals, regardless of the amount offered, had less marked muscle tissue growth due to the optimization of gain during infancy. However, MM treatment animals had LMA reduction (Table 2), probably because caloric restriction tends to increase protein turnover, increasing the metabolism of amino acids to glucose via gluconeogenesis (Weindruch et al. 2001). The AVER and LOHI treatments obtained higher LMA at the end of the experiment.

Body fat reserves can be used to establish the body condition score and define nutritional status (Bruckmaier 1998). They have been related to the maintenance of the estrous cycle in

Table 1 Chemical composition of the supplement and the forage

		Forage (days of experiment)							
Item	Suppl.	+ 15	+ 45	+ 75	+ 105	+ 135	+ 165	< 90[D]	> 90[E]
Dry matter[A]	886	492	598	689	666	345	262	593	424
Organic matter[B]	911	918	916	936	926	918	924	923	923
Crude protein[B]	200	71	67	42	47	56	99	60	68
NDIN[C]	305	305	344	354	361	312	217	334	297
Ether extract[B]	29	16	4	9	10	11	12	10	11
apNDF[B]	149	701	789	806	796	688	597	765	694
NFC[B]	533	129	55	80	73	163	216	88	151
iNDF[B]	24	239	266	306	426	361	192	270	326

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

[A] g/kg of natural matter

[B] g/kg of dry matter

[C] g/kg of the total nitrogen

[D] Mean values of the samples obtained by manual grazing simulation in the first 90 days of the experiment

[E] Mean values of the samples obtained by manual grazing simulation after 90 days

Table 2 Performance and carcass traits of heifers supplemented and non-supplemented during the dry season (1–90 days) and dry/rainy transition period (91–180 days)

Item	Per	Nutrition plans[A]				SEM	*P* value[B]		
		MM	LOHI	AVER	HILO		Treatment	Per	Treatment × per
iBW (kg)	1–90	228	230	239	243	9.06	0.196	0.001	0.197
	91–180	236	250	262	273				
fBW (kg)	1–90	236	250	262	273	9.92	0.009	0.185	0.049
	91–180	226	268	276	273				
ADG (kg)	1–90	0.088	0.227	0.263	0.345	0.03	0.001	0.001	0.001
	91–180	− 0.155	0.264	0.211	0.002				
LMA (cm^2)	1–90	43.6	45.2	46.8	48.2	1.76	0.042	0.001	0.001
	91–180	38.0	47.2	48.0	43.2				
SFT (mm)	1–90	3.13	3.36	3.35	3.50	0.24	0.140	0.001	0.049
	91–180	2.23	3.14	2.88	2.89				

iBW initial body weight, *fBW* final body weight, *ADG* average daily gain, *LMA* longissimus muscle area, *SFT* subcutaneous fat thickness

[A] *MM* mineral mix, *LOHI* 0.2% of the BW of supplement in the dry season and 0.6% of the BW of supplement in the dry/rainy transition, *AVER* 0.4% of BW of supplement in dry season and dry/rainy transition, *HILO* 0.6% of BW of supplement in dry season and 0.2% of BW of supplement in the dry/rainy transition

[B] Indicative of significance ($P < 0.05$)

cattle and can act as a marker of available energy for reproductive activity (Hall et al. 1995), acting as a permissive signal that allows ovulation and future conception. In the present study, animals that received a high level of supplementation after weaning presented higher SFT at the end of the experiment (Table 2), an interesting indicator when working with heifers.

In some studies, there has been an increase in the total DM and FDM intake with the protein supplementation of low-quality tropical forages, especially when the forage presents levels of CP lower than 70 g/kg of DM (Sampaio et al. 2010). However, although supplementation allowed higher intake of total DM by supplemented heifers (Table 3), this was not observed in the intake of FDM in kg/day. The FDM intake is related to digestibility, which primarily reflects the fermentation and feed passage rates of the rumen. Thus, as the cell wall of the diet increases, both rates decrease.

Heifers receiving only mineral mix had FDM intake similar to the intake of animals supplemented during the dry season. In addition, the LOHI treatment obtained lower intake than the other treatments in kg/day and g/kg of BW in the dry/rainy transition period, probably due to the substitutive effect caused by the higher intake of supplemental DM by these heifers (Table 3). Considering the intake in g/kg of BW of FDM, MM treatment animals obtained higher intake. However, it was observed that animals supplemented with a low level of supplementation in the dry period (LOHI) obtained similar intake. This was possibly due to the positive effect on the rumen with catalytic supplement doses (0.2% of BW), greatly increasing the performance of this group (0.088 × 0.227).

According to Detmann et al. (2003), the filler effect is expected to be the main controlling mechanism of the intake of animals fed with low-quality fodder, justifying the lower DM intake of MM treatment animals. The iNDF content, which has been attributed to a high portion of the ruminal repletion effect of tropical forages, causing a reduction in intake, was on average 27 and 32.6% in the dry season and dry/water transition, respectively (Table 1). Protein supplementation had a positive effect on DM intake, probably increasing the rate of passage of the indigestible residue.

The higher CP intake by the supplemented animals, as supplementation levels increased, may be due to the higher concentrations of these nutrients in the multiple supplements in relation to the forage (Table 3). The apparent digestibility coefficient of CP was higher in animals receiving multiple supplements (Table 4), due to the higher CP intake of higher degradation, since the NDIN content in the supplement was lower than for forage (Table 1).

The increase in total digestibility can be expected with the inclusion of concentrates in the diet of grazing animals, as they have greater digestibility than forage. However, the interaction between digestion of the concentrates and the pasture may or may not alter the digestion of the fiber. In this work, the greater intake of dNDF by supplemented animals in the dry/rainy transition period signals the improvement in energy extraction from the supplemented animals (Table 3), and their greater digestibility confirms this better utilization efficiency (Table 4).

The level of SUN is affected by the nutritional level, especially in ruminants, and is a sensitive and immediate indicator of protein intake (Batista et al. 2016). On days 15, 45, and 75, the HILO treatment had higher concentrations of NUS, consistent with the greater CP intake of the same in the dry season. At day 165, the observed increase

Table 3 Nutrient intake of Nellore heifers supplemented or non-supplemented in the dry season (1–90 days) and dry/rainy transition period (91–180 days)

Item	Per	Nutrition plans				SEM	*P* value[A]		
		MM	LOHI	AVER	HILO		Treatment	Per	Treatment × per
DM (kg)	1–90	4.24	4.81	4.95	5.56	0.20	0.003	0.143	0.001
	91–180	4.23	5.07	5.26	5.16				
FDM (kg)	1–90	4.24	4.39	4.00	4.09	0.17	0.574	0.087	0.001
	91–180	4.23	3.74	4.26	4.67				
OM (kg)	1–90	3.94	4.47	4.62	5.19	0.19	0.002	0.012	0.001
	91–180	3.95	4.77	4.94	4.84				
CP (kg)	1–90	0.21	0.30	0.39	0.50	0.02	0.001	0.001	0.001
	91–180	0.38	0.60	0.58	0.52				
apNDF (kg)	1–90	2.82	2.98	2.80	2.94	0.12	0.427	0.001	0.001
	91–180	3.00	2.86	3.17	3.39				
dDM (kg)	1–90	2.41	2.91	3.16	3.68	0.13	0.001	0.001	0.001
	91–180	2.10	3.00	2.80	2.61				
dNDF (kg)	1–90	1.76	1.85	1.81	1.96	0.08	0.147	0.402	0.003
	91–180	1.69	1.74	1.87	2.02				
TDN (kg)	1–90	2.37	2.83	3.14	3.69	0.13	0.001	0.001	0.001
	91–180	2.12	2.98	2.89	2.66				
		g/Kg of BW							
DM	1–90	18.6	20.0	19.6	20.5	0.19	0.001	0.306	0.001
	91–180	19.3	20.1	19.9	19.8				
FDM	1–90	18.6	18.2	15.8	15.1	0.15	0.001	0.181	0.001
	91–180	19.3	14.8	16.1	18.0				
apNDF	1–90	12.4	12.5	11.1	10.8	0.10	0.001	0.001	0.001
	91–180	13.7	11.3	12.0	13.1				

DM dry matter, *FDM* forage dry matter, *OM* organic matter, *CP* crude protein, *apNDF* neutral detergent fiber corrected for ash and protein, *dDM* digested dry matter, *dNDF* digested neutral detergent fiber, *TDN* total digestible nutrients

[A] Indicative of significance ($P < 0.05$)

was influenced by the greater CP content of the forage (Table 1; Fig. 1c). The higher SUN concentration of the supplemented heifers (Table 5) can be attributed to the higher CP intake.

The higher plasma concentrations of NEFA in the non-supplemented animals (Table 5) are indicative of adipose tissue mobilization due to inadequate nutrition (Chapinal et al. 2011). The behavior in the NEFA concentrations over the

Table 4 Total apparent digestibility of the constituents of the diet in function of the different treatments

Item	Per	Nutrition plans				SEM	*P* value[A]		
		MM	LOHI	AVER	HILO		Treatment	Per	Treatment × per
DM (%)	1–90	56.8	60.4	63.7	66.1	0.35	0.001	0.001	0.001
	91–180	49.4	59.2	53.1	50.6				
OM (%)	1–90	59.8	62.4	66.7	70.0	0.46	0.001	0.001	0.001
	91–180	53.6	62.6	58.5	55.9				
CP (%)	1–90	40.5	46.4	59.3	62.5	1.13	0.001	0.005	0.001
	91–180	44.7	59.2	50.5	47.2				
apNDF (%)	1–90	62.6	62.1	64.5	66.6	0.59	0.001	0.001	0.001
	91–180	56.4	60.8	58.8	59.5				
TDN (%)	1–90	56.0	58.7	63.2	66.2	0.65	0.001	0.001	0.001
	91–180	50.0	58.7	54.8	51.5				

DM dry matter, *OM* organic matter, *CP* crude protein, *apNDF* neutral detergent fiber corrected for ash and protein, *TDN* total digestible nutrients

[A] Indicative of significance ($P < 0.05$)

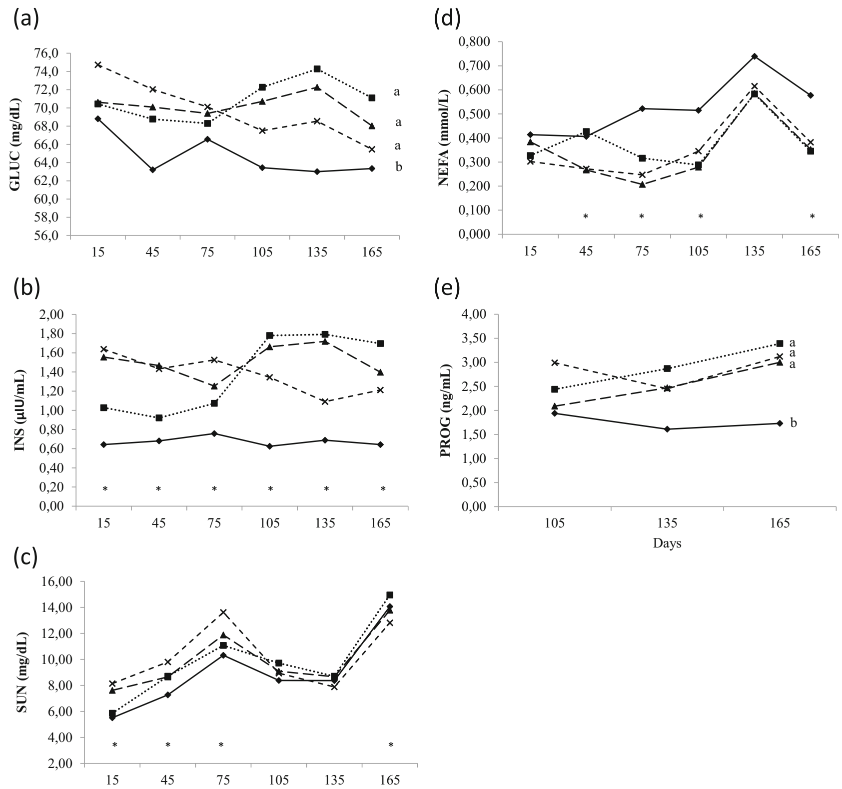

Fig. 1 Concentrations of GLUC (**a**), INS (**b**), SUN (**c**), NEFA (**d**), and PROG (**e**) in heifers with the following treatments: LOHI (⋯■⋯), AVER (–▲–), HILO (- -×- -), or MM (—◆—) in the dry season and dry/rainy transition. **a**, **b** Means of treatment followed by different letters, differed by Student's *t* test ($P < 0.05$). *Indicative of significance of interaction of the treatment × day ($P < 0.05$)

days (Fig. 1d) shows a similar rate of lipolysis of the adipose tissue, but is more pronounced in the non-supplemented animals, which can be sustained by the lower SFT in the end of the experiment (Table 2). The increase in NEFA concentrations from day 75 to day 135 in non-supplemented animals is possibly due to the ingestion of low-quality forage and was compensated by the mobilization of these metabolites of adipose tissue. The NEFA peak at day 135 (Fig. 1d) coincides with the worst quality of forage. The decrease observed after this period may be indicative of the recovery of the pastures through the first rains. However, the supplemented animals presented a mean NEFA of 0.360 against 0.577 of the animals not supplemented. This fact may be associated with the greater CR after the AIrt for the supplemented animals (Walsh et al. 2007).

The supplemented heifers had a greater intake of CP and OM and, consequently, energy due to the better quality of the supplement over forage. This explains the greater levels of glucose presented by these animals (Table 5; Fig. 1a) since starch-rich diets increase the production of propionate, which is converted to glucose in the liver and stimulates the release of insulin.

The concentration of insulin in the blood is often associated with the nutritional status of the animal since animals under a

Table 5 Concentration of metabolites, hormones, and reproductive performance in Nellore heifers non-supplemented or supplemented during the dry season and dry/rainy transition period

Item	Nutrition plans					*P* value[A]		
	MM	LOHI	AVER	HILO	SEM	Treatment	Day	Treatment × day
SUN (mg/dL)	8.98	9.79	9.95	9.91	0.58	0.017	0.001	0.001
NEFA (mmol/L)	0.53	0.38	0.35	0.36	0.04	0.001	0.001	0.033
GLUC (mg/dL)	64.7	70.9	70.2	69.7	1.82	0.001	0.040	0.094
INS (µIU/mL)	0.68	1.45	1.50	1.32	0.16	0.001	0.268	0.003
PROG (ng/mL)	1.76	2.90	2.52	2.85	0.32	0.006	0.225	0.701
CR[B] (%)	0.00	33.3	58.3	50.0	–	0.020	–	–

SUN serum urea nitrogen, *NEFA* non-esterified fatty acids, *GLUC* glucose, *INS* insulin, *PROG* progesterone, *CR* conception rate

[A] Indicative of significance ($P < 0.05$)

[B] Performed with the chi-square test ($P < 0.05$)

restricted diet have lower blood levels of insulin compared to animals fed without food restriction (Pires et al. 2010); this explains the behavior of the data of the present experiment (Fig. 1b). It was observed that the levels of this metabolic hormone varied according to the days of collection, and this variation followed the amount of supplement provided in each period. The behavior of the insulin levels is also in agreement with the circulating levels of the hormone progesterone, and the increase of insulin levels was accompanied by greater levels of progesterone (Table 5).

Physiologically, puberty is characterized by an increase in the concentration and pulsatile frequency of LH and a decrease in the sensitivity of the hypothalamus to gonadal steroids, which results in the first ovulation (Hafez and Hafez 2004) and consequent elevation of serum progesterone levels. At day 105, the values of progesterone for the supplemented animals were greater than the non-supplemented animals (Table 5; Fig. 1e), indicating that the supplemented animals were more amenable to reproduction. The mean progesterone concentration of the MM treatment was 1.76 ng/mL. However, this was not enough for these heifers to become pregnant.

Supplemented heifers had a mean CR of 47.2% while heifers of the MM treatment did not conceive (0.0%; Table 5). This CR observed for supplemented heifers to be higher than those reported in the literature involving Nellore animals, ranging from 10 to 18% (Eler et al. 2004; Silva et al. 2005).

In summary, the supplementation strategies adopted in this study improve the performance, nutrient digestibility, metabolic status, and carcass traits of heifers under grazing, allowing an improvement in the conception rate of 15-month-old Nellore heifers.

Funding information We are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for supporting this study.

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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