BLOOD PARAMETERS OF NELLORE STEERS FED A HIGH CONCENTRATE DIET IN TWO RESTRICTED FEEDING REGIMES FOLLOWED BY *AD LIBITUM* FEEDING[1]

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Abstract: Blood parameters of 18 Nellore steers submitted to high concentrate diet in two sequential feeding regimes (phase 1: restriction and phase 2: *ad libitum* feeding) were evaluated. In the first phase, steers were fed for 56 days three intake levels of dry matter (*ad libitum*, 75 g dry matter (DM)/kg metabolic body weight ($BW^{0.75}$) or 60 g DM/kg $BW^{0.75}$. In the second phase, from the 57th day on feed, animals fed 60 g DM/kg $BW^{0.75}$ and 75 g DM/kg $BW^{0.75}$ were fed *ad libitum* until the 136th day. In the 8th, 27th, 55th (first phase), 71st, 84th, 112th and 132nd (second phase) day of experimental period, blood samples were collected and insulin-like growth factor I (IGF-I), triiodothyronine (T3), thyroxine (T4), glucose, plasma ureic nitrogen (PUN) and total protein (TP) were determined. In the first phase IGF-I, T4, glucose and total protein concentration was not different among treatments (P>0.05) and there was no interaction between treatment and time (P>0.05). There was an interaction among treatments and time on T3 and plasma ureic nitrogen (P<0.01). In the second phase IGF-I, T3, T4, glucose and total protein concentration was not different among treatments and there was no interaction between treatment and time (P>0.05). For plasma ureic nitrogen concentration there was an interaction among treatments and time (P=0.02). Lower levels of diet intake can be used to improve animal performance in feedlot.

Keywords: glucose, IGF-I, T3, T4, urea nitrogen

PARÂMETROS SANGUÍNEOS DE NOVILHOS NELORE SUBMETIDOS À DIETA DE ALTO CONCENTRADO EM DOIS REGIMES ALIMENTARES SEQUENCIAIS (FASE 1: RESTRIÇÃO E FASE 2: ALIMENTO AD LIBITUM)

Resumo: Foram avaliados parâmetros sanguíneos de 18 novilhos Nelore submetidos a dois regimes sequenciais de alimentação (fase 1: restrição e fase 2: alimento *ad libitum*) com dieta de alto teor de concentrado. Na primeira fase, os animais foram alimentados por 56 dias em três níveis de ingestão de matéria seca (MS) representando os tratamentos: *ad libitum*, 75 g MS/kg peso corporal metabólico ($PV^{0,75}$) e 60 g MS/kg $PV^{0,75}$. Na segunda fase, a partir do 57º dia de confinamento, os animais alimentados com 60 g MS/kg $PV^{0,75}$ e 75 g MS/kg $PV^{0,75}$ receberam alimentação *ad libitum* até o 136º dia. No 8º, 27º, 55º, primeira fase, e 71º, 84º, 112º e 132º dia de confinamento, segunda fase, foram colhidas amostras de sangue para determinação das concentrações de fator de crescimento semelhante à insulina I (IGF-I), triiodotironina (T3), tiroxina (T4), glicose, nitrogênio ureico plasmático e proteína total. No período de restrição alimentar as concentrações plasmáticas de IGF-I, T4, glicose e proteína total não foram diferentes entre os tratamentos (P>0,05) e não houve interação entre tratamento e tempo (P>0,05). Foi observada interação entre tratamento e tempo para a concentração plasmática de T3 e nitrogênio ureico no plasma (P<0,01). No período de alimento *ad libitum* as concentrações de IGF-I, T3, T4, glicose e proteína total não foram diferentes entre os tratamentos e não houve interação entre tratamento e tempo (P>0,05). Para a concentração de nitrogênio ureico no plasma houve interação entre os fatores tratamentos e tempo (P=0,02). Níveis mais baixos de ingestão de dieta podem ser usados para melhorar o desempenho animal em confinamento.

Palavras-Chave: glicose, IGF-I, nitrogênio ureico, T3, T4

INTRODUCTION

The assessment of the nutritional status of a production herd can be carried out by determination of some blood parameters, acting as an auxiliary method in the evaluation of animals and herds with different productive rates (PEIXOTO and OSÓRIO, 2007; KANG et al., 2017). The biochemical composition of the blood plasma reflects the metabolic state of animal tissues permitting to assess tissue damage, organ dysfunction, adaptation of animals to nutritional and physiological challenges, and specific metabolic or nutritional imbalances (GONZÁLEZ and SCHEFFER, 2003; TAO et al., 2017).

The blood chemistry became of greater interest for animal nutrition from the appearance of the term metabolic profile (PEIXOTO and OSÓRIO, 2007). The evaluation of the concentration of hormones and other metabolites in cattle has been used in nutrition studies related to reproduction (SARTORI and GUARDIEIRO, 2010) and feedlot performance with different cattle production strategies (ELSASSER et al., 1989; DAVIS and SIMMEN, 2006; CERVIERI et al., 2005; MATURANA FILHO et al., 2010) and as an auxiliary tool to select animals with better feed efficiency (BOURGON et al., 2017).

After a feed restriction period followed by a period of *ad libitum* feeding in beef cattle FIEMS et al. (2007) verified changes in the plasma metabolite concentrations in steers, heifers, bulls and cows.

Therefore, blood concentrations of IGF-I, T3, T4, glucose, urea nitrogen and total plasma protein were evaluated with Nellore steers fed two feed restriction levels followed by *ad libitum* feeding period.

MATERIAL AND METHODS

The feedlot was carried out in the Department of Animal Science of Faculty of Animal Science and Food Engineering of University of São Paulo (FZEA/USP), Pirassununga, SP. The procedures were approved by the Research Ethics Committee of the FZEA/USP, under Nº 6706080515.

Eighteen Nellore steers averaging 20 months old and initial weight of 359 ± 13 kg were submitted to 21 days of adaptation to feedlot and diets. The animals were individually housed in pens provided with covered troughs and individual drinking fountains, with six steers for each treatment.

The experiment was divided into two distinct and consecutive periods with different levels of dry matter intake (DMI). In the first period, the animals were fed for 56 days at three levels of consumption: *ad libitum* (TAL), 75 g DMI/kg $BW^{0.75}$ (T75) or 60 g DMI/kg $BW^{0.75}$ (T60). In the second period, from the 57^{th} day of feedlot, animals with feed restrictions were changed to *ad libitum* diet for 77 days, totaling 136 days of confinement.

The food supply was calculated according to the NRC (1996) to promote minimum weight gain of 250 g/day, intermediate weight gain of 650 g/day and maximum gain of 1,200 g/day for treatments T60, T75 and TAL , respectively, during the first period. For this purpose, the same diet was used throughout the confinement period for all treatments, and offered *ad libitum* or with two levels of feed restriction in the first phase, and *ad libitum* in the second phase for treatments T60 and T75.

Concentrated and roughage feeds (Table 1) were weighed, mixed and distributed daily in the morning. The orts were collected before feeding three times a week, weighed and sampled for dry matter determination. In the adaptation period the orts were adjusted to 5% of the offered. The dietary adjustment offered to the *ad libitum* group was performed according to the orts. The diet supply for the treatments 75 g DM/kg $BW^{0.75}$ and 60 g DM/kg $BW^{0.75}$ was adjusted after weighing performed on the 28^{th} day of feedlot, since it was determined as a function of the initial metabolic weight of the period for each animal.

At 8^{th}, 27^{th}, 55^{th}, 71^{st}, 84^{th}, 112^{th} and 132^{nd} day of experimental period, blood samples were collected by venipuncture of the outer jugular in 10 ml heparinized vacuum tubes. Samples were collected before feeding the animals, cooled and centrifuged at 1,500 x g for 15 minutes at 10ºC to obtain plasma, which was stored in Eppendorf tubes at -20ºC for subsequent dosing.

Plasma insulin-like growth factor I (IGF-I) concentrations were obtained by post-extraction immunoassay with commercial kits (ACTIVE® IGF-I ELISA DSL-10-5600, DSL Diagnostic Systems Laboratories, Inc. USA). The determinations of triiodothyronine (T3 - ACTIVE® Total T3 EIA DSL-10-3100S, DSL

Table 1 - Diet composition in dry matter basis

Ingredient	%
Sorghum silage	20.00
Corn grain	20.00
Soybean hull	57.29
Soybean meal	0.71
Urea	0.92
Minerals*	0.60
Limestone	0.49
Monensin, mg/kg	27.00
Nutrient	
Total digestible nutrients	76.43
Crude protein	13.62
Rumen degradable protein**	8.53

*Minerals: commercial formula for beef cattle. **Rumen degradable protein: calculated using common feedstuffs for Brazilian feedlots.

Diagnostic Systems Laboratories, Inc. USA) and thyroxine (T4 - ACTIVE® THYROXINE T4 EIA DSL-10-3200, DSL Diagnostic Systems Laboratories, Inc. USA) were performed by enzyme-immunoassay with commercial kits. The glucose concentration was determined by the enzymatic method (LABORLAB, CAT Nº 02200), the total protein by colorimetry (LABORLAB, CAT Nº 03800) and the urea concentration by the enzymatic method (LABORLAB, CAT Nº 02800) with commercial kits.

The experimental design was a completely randomized design with three treatments and six replicates. Statistical analysis was performed using the Mixed procedure of the SAS program (SAS Institute Inc., Cary, NC, USA). The main effects of treatment, feedlot time and interaction between the time and treatment factors were evaluated by analysis of variance, and the means compared by the Student t test. All the studied variables were analyzed as repeated measures over time. The T3 and T4 data were analyzed in logarithmic form and the results expressed in original values.

RESULTS

There was no interaction between feeding time and treatments for the plasma concentration of IGF-I, T4, glucose and total protein during the period of feed restriction, as well as no differences (P>0.05) were observed between treatments (Table 2).

No differences were observed among treatments for IGF-I concentrations both during the first phase (P=0.13) and in the *ad libitum* feed period (Table 3). In the *ad libitum* feeding phase, a linear reduction (P=0.01) was observed in the IGF-I plasma concentration for treatment with 75 g DM/kg $BW^{0.75}$ (Figure 1).

In the feed restriction period, an interaction between treatments and time was observed for the plasma concentration of T3 (Table 4). The feeding level at the 8^{th} day of experimental period showed linear effect (P=0.01). The time effect in the feeding level T60 (P<0.01) and T75 (P=0.02) was linear (Figure 2).

There was no difference among treatments for T4 concentration during the feed restriction

Table 2 - Mean, standard error (SE), probability (Pr<F) and interaction between treatments and time in plasma concentrations of IGF-I, T3, T4, glucose, total protein and plasma ureic nitrogen (PUN) during the restriction period.

Parameters	Treatment			SE	Pr	Interaction
	T60	T75	TAL			
IGF-I, ng/mL	487.91	463.06	401.80	31.20	0.15	0.89
T3, ng/dL	**1	**1	**1	**1	**1	<0.01
T4, ng/dL	15.19	16.71	15.70	1.65	0.84	0.25
Glucose, mg/dL	119.95	129.23	110.29	9.77	0.40	0.63
Total Protein, g/dL	8.38	7.75	7.94	0.29	0.30	0.31
PUN, mg/dL	**2	**2	**2	**2	**2	<0.01

**Interaction data presented in the tables: [1]Table 4 and [2]Table 5. T60: 60 g Dry Matter Intake/kg Body $Weight^{0.75}$, T75: 75 g Dry Matter Intake/kg Body $Weight^{0.75}$ or TAL: *ad libitum* intake.

Table 3 - Mean, standard error (SE), probability (Pr<F) and interaction between treatment and time in plasma concentrations of IGF-I, T3, T4, glucose, total protein and plasma ureic nitrogen (PUN) during the *ad libitum* feed period

Parameters	Treatment			SE	Pr	Interaction
	T60	T75	TAL			
IGF-I, ng/mL	472.38	447.05	--	43.16	0.33	0.19
T3, ng/dL	188.28	171.42	--	13.26	0.35	0.11
T4, ng/dL	12.45	12.50	--	1.33	0.99	0.73
Glucose, mg/dL	134.67	134.55	--	7.56	0.98	0.63
Total protein, g/dL	6.90	6.85	--	0.59	0.90	0.36
PUN, mg/dL	**1	**1	**1	**1	**1	0.02

**1 Interaction data presented in Table 5. T60: 60 g Dry Matter Intake/kg Body Weight$^{0.75}$, T75: 75 g Dry Matter Intake/kg Body Weight$^{0.75}$ or TAL: *ad libitum* intake.

phase (Table 2) and *ad libitum* feeding (Figure 3).

The mean values of plasma glucose concentrations did not differ among treatments and times (Figure 4), both in the restriction phase (Table 2) and in the *ad libitum* feeding (Table 3).

The concentration of total protein was not different among treatments in both the restriction (Table 2) and in the *ad libitum* feeding (Table 3). During the feed restriction phase, there was a linear reduction in the total protein concentration (P<0.01) and in the *ad libitum* phase there was a linearly increased for the treatment 60 g DM/kg BW$^{0.75}$ (P=0.04), which remained low in the treatment with 75 g DM/kg BW$^{0.75}$ (Figure 5).

For the concentration of plasma ureic nitrogen (PUN), there was interaction of the factors treatments and time in the restriction phase as well as in the *ad libitum* feeding (Table 5). The PUN values during the restriction period (Figure 6) had a higher variation how much more the feed intake level, with a linear increase (P=0.03).

DISCUSSION

IGF-I is considered to be an important mediator of metabolic action and stimulus to growth, provided by somatotropin, correlating positively with the rate of live weight gain and feed efficiency (CERVIERI et al., 2005), indicating that the growth rates obtained were not sufficiently different to express the negative correlation between the circulating IGF-I concentration and the growth rate (SCANES, 2003).

The plasma concentration of IGF-I can be altered by the nutritional plan as well as the diet composition (ELSASSER et al., 1989), which may be responsible for divergence in the literature data for animals in feed restriction followed by *ad libitum* feed (ELLENBERGER et

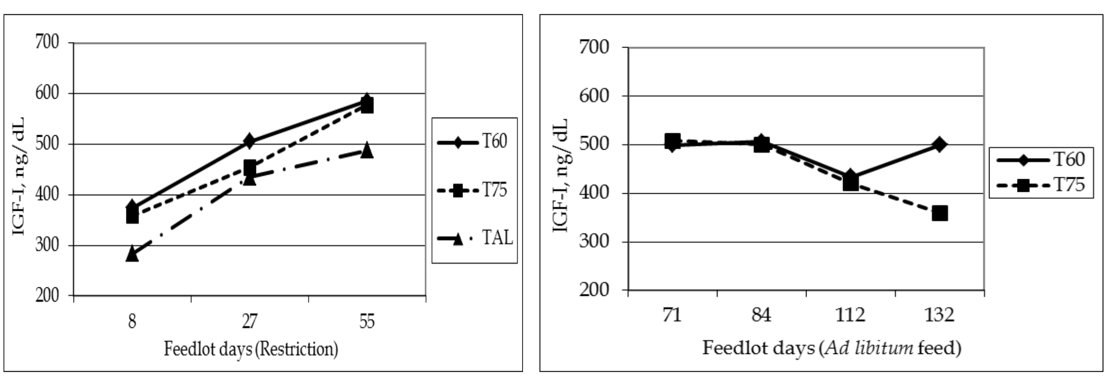

Figure 1. Concentration of IGF-I during the restriction and *ad libitum* feed periods for T60: 60 g Dry Matter Intake/kg Body Weight$^{0.75}$, T75: 75 g Dry Matter Intake/kg Body Weight$^{0.75}$ or TAL: *ad libitum* intake.

Table 4 - Mean, standard error (SE), probability (Pr<F) and interaction between treatments and time in T3 plasma concentrations in the restriction period

Parameters	Treatment			SE	Pr	Interaction
	T60	T75	TAL			
T3, ng/dL						<0.01
Day 8	113.92	130.22	153.05	10.26	0.01	
Day 27	165.03	159.22	137.20	12.88	0.11	
Day 55	218.55	204.45	177.94	27.67	0.41	
Pr	<0.01	0.02	<0.05	--	--	

T60: 60 g Dry Matter Intake/kg Body Weight$^{0.75}$, T75: 75 g Dry Matter Intake/kg Body Weight$^{0.75}$ or TAL: *ad libitum* intake.

al., 1989; HAYDEN et al., 1993; HORNICK et al., 1998), as by the effect of breed (CABARAUX et al., 2005).

The concentrations of IGF-I (Figure 1) during the restriction phase indicated that through this hormone either directly or indirectly, anabolic processes such as cell division, skeletal growth and protein synthesis are stimulated (AMORIM et al., 2007).

Thus, due to the short period of feed restriction and the nutrient balance provided by the diet, probably the animals were in growth during the feedlot period, since IGF-I regulates cell growth and metabolism during the entire development phase (DAVIS and SIMMEN, 2006).

However, data from the literature (ELLENBERGER et al., 1989; HAYDEN et al., 1993; HORNICK et al., 1998) with growing steers slower than normal indicate that the IGF-I level decreases. In this case, the concentration of IGF-I would possibly be reflecting more the weight gain occurred than indicating the future weight gain (DAVIS and SIMMEN, 2006) since the evaluation periods were long, close to 100 days.

The same could have occurred in the *ad libitum* phase where there was a reduction in plasma IGF-I concentration for treatment with 75 g DM/kg BW$^{0.75}$, which was not observed for treatment with 60 g DM/kg BW$^{0.75}$. Probably, this reduction in IGF-I was a consequence of the lower weight gain in the restriction phase, which would allow a longer time for weight recovery, as reported by NRC (1996) for animals in subsequent *ad libitum* feeding.

Thyroid hormones, T3 and T4, have among their actions in the body the stimulus to protein synthesis (REECE, 1996) that occurs with greater intensity than the synthesis of fat in animals with moderate weight gains or that are in the deposition phase of fat, as can be observed in the present study. Thus, there seems to be a greater effect of feedlot time in a

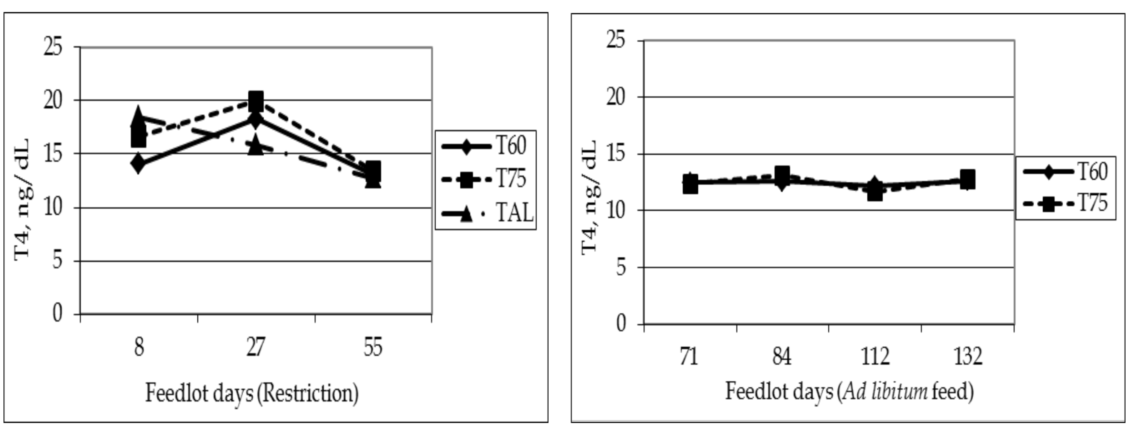

Figure 3. Concentration of T4 during the restriction and *ad libitum* feed periods for T60: 60 g Dry Matter Intake/kg Body Weight$^{0.75}$, T75: 75 g Dry Matter Intake/kg Body Weight$^{0.75}$ or TAL: *ad libitum* intake.

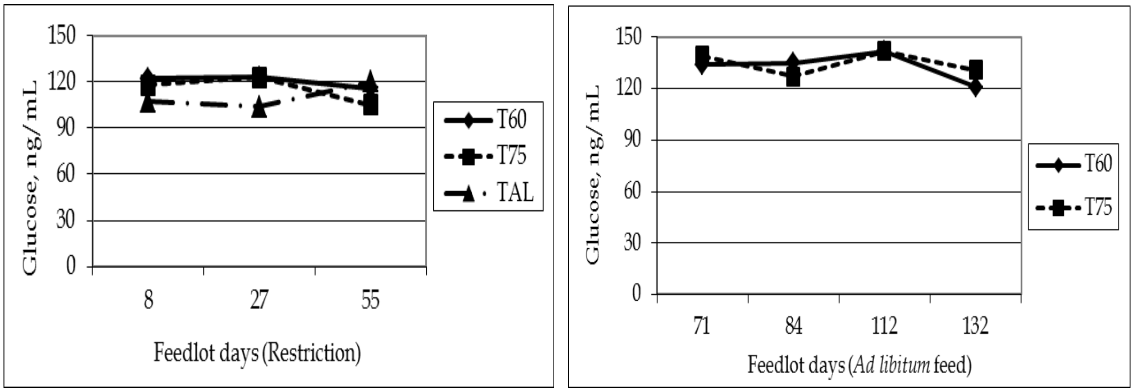

Figure 4. Glucose concentration during the restriction and *ad libitum* feed periods for T60: 60 g Dry Matter Intake/kg Body Weight$^{0.75}$, T75: 75 g Dry Matter Intake/kg Body Weight$^{0.75}$ or TAL: *ad libitum* intake.

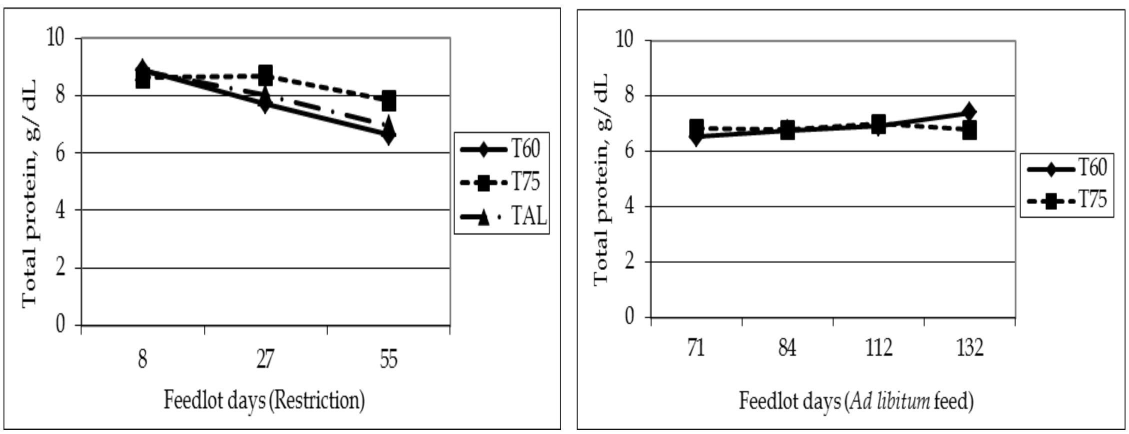

Figure 5. Total protein concentration during the restriction and *ad libitum* feed periods for T60: 60 g Dry Matter Intake/kg Body Weight$^{0.75}$, T75: 75 g Dry Matter Intake/kg Body Weight$^{0.75}$ or TAL: *ad libitum* intake.

given feeding condition (HAYDEN et al., 1993) than an effect of different rates of weight gain (ELLENBERGER et al., 1989).

The decrease in T3 concentrations with the reduction in feed level at day 8 of feedlot was an indicator of the nutritional restriction effect in animals (CHRISTOPHERSON et al., 1979) and the higher metabolism in animals fed *ad libitum*, since this hormone reflects the metabolic rate and nutritional pattern of the animals (LIMA et al., 2011). In the restricted intake animals, the T3 concentrations increased during the restriction phase, following the same pattern of IGF-I concentration, a hormone that correlates with T3 concentration (LIMA et al., 2011).

The T4 secretion seems to have been increased by the need for the active form of thyroid hormone T3, in the initial phase of the feedlot, returning to lower values already at the end of the restriction phase, remaining at this level also in the *ad libitum* feeding phase.

Differences were observed in the results found for T4, as well as for T3 concentrations, in the restriction and *ad libitum* feeding studies (ELLENBERGER et al., 1989; HAYDEN et al., 1993; HORNICK et al., 1998). Although they may also be related to variations in factors such as breed, stress, age, diet, management and climate (GONZÁLEZ and SCHEFFER, 2003).

A blood glucose concentration required for normal physiological processes in ruminants vary of 30 to 60 mg/dL (SWENSON, 1996). However, for zebu animals fed high concentrate diets values greater than 60 mg/dL can be observed (OLIVEIRA JUNIOR et al., 2004; MENDES et al., 2005) and still higher than 120 mg / dL for newborn calves (RIBEIRO et al., 2006), since the age and activity of the animal may alter the values of the blood metabolites (SWENSON, 1996).

Table 5 - Mean, standard error (SE), probability (Pr<F) and interaction between treatment and time in plasma urea nitrogen concentrations (PUN)

Parameters	Treatment			SE	Pr	Interaction
	T60	T75	TAL			
PUN, mg/dL						
Restriction						<0.01
Day 8	13.22	10.13	16.48	0.46	<0.01	
Day 27	11.48	10.87	15.84	0.51	0.03	
Day 55	11.29	14.13	25.75	1.06	<0.01	
Pr	0.33	0.03	<0.01	--	--	
Ad libitum feed						0.02
Day 71	21.95	23.27	--	1.48	0.50	
Day 84	25.24	19.61	--	1.29	<0.01	
Day 112	16.00	16.46	--	4.76	0.82	
Day 132	13.91	13.73	--	1.68	0.93	
Pr	0.33	<0.01	--	--	--	

T60: 60 g Dry Matter Intake/kg Body Weight$^{0.75}$, T75: 75 g Dry Matter Intake/kg Body Weight$^{0.75}$ or TAL: *ad libitum* intake.

The glucose concentrations observed in the present study may be a result of higher rumen production of propionic acid, a precursor of glucose, which is associated with high concentrate intake (HORNICK et al., 1998) and by the use of the monensin ionophore in the diet (MATURANA FILHO et al., 2010) which by increasing the production of propionic acid in the rumen favors hepatic gluconeogenesis (DUFFIELD et al., 2008).

The absence of significant variation in glucose behavior over time and in treatments was expected (HORNICK et al., 1998) since the blood glucose content has few variations in the ruminants due to the very efficient homeostatic mechanisms of the organism, except in animals with severe malnutrition (GONZÁLEZ and SCHEFFER, 2003) or subjected to intense heat (KIM et al., 2018), cases in which a decrease in blood glucose concentration may be observed

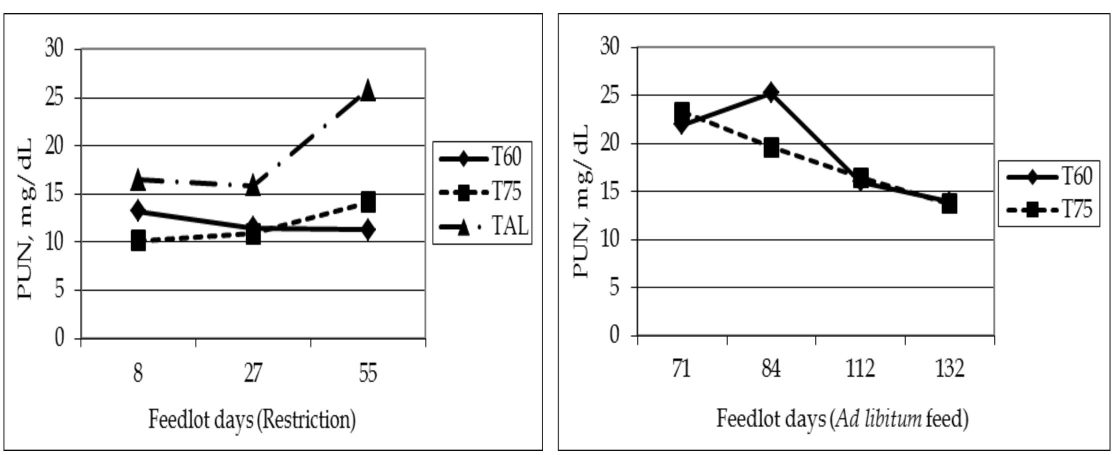

Figure 6. Concentration of plasma urea nitrogen (PUN) during the restriction and *ad libitum* feed periods for T60: 60 g Dry Matter Intake/kg Body Weight$^{0.75}$, T75: 75 g Dry Matter Intake/kg Body Weight$^{0.75}$ or TAL: *ad libitum* intake.

(ELLENBERGER et al., 1989).

In cases where the feed restriction has been imposed for long periods, the responses of the animals can be quite variable (NRC, 1996) as a function of the metabolic changes provided by the compensatory weight gain (BLUM et al., 1985; ELLENBERGER et al., 1989; HAYDEN et al., 1993).

The rate of synthesis of blood proteins is directly related to the nutritional status of the animal (PEIXOTO and OSÓRIO, 2007) with normal values between 5.0 and 8.5 g/dL normal (REECE, 1996; SWENSON, 1996).

Although reduction in total protein concentration may indicate malnutrition (GONZÁLEZ and SCHEFFER, 2003), it may be also an influence of energy-rich diets on animal metabolism, because similar values with high concentrate diets were observed for Nellore steers by BONILHA et al. (2015), and in the present study, both in *ad libitum* and restricted fed animals. In this sense, there are reports of higher values of total protein for animals in pastures (BARBOSA et al., 2003) or in feedlot with greater proportion of roughage (MENDES et al., 2005).

The two main indicators of protein metabolism in ruminants are serum levels of urea and albumin. Urea level indcates the protein status of the animal in the short term, while the albumin indicates it in the long term (PEIXOTO and OSÓRIO, 2007). Thus, it can be suggested that the variation observed in *ad libitum* fed animals after the restriction phase corresponded to the greater feed availability in the treatments, reflecting changes in the short-term intake.

Plasma metabolites change rapidly in favor of protein anabolism in steers during the *ad libitum* feeding period (HAYDEN et al., 1993), as observed with plasma urea nitrogen concentration (PUN) for treatment with 60 g DM/kg $BW^{0.75}$ in the 84^{th} feedlot day. This reflects high hepatic synthesis resulting from increased production of microbial ammonia in the rumen or degradation of amino acids in the liver (HORNICK et al., 1998) due to increased demand for nutrients for the growth of visceral tissue (ELLENBERGER et al., 1989). Different from what was observed in the treatment with 75 g DM/kg $BW^{0.75}$, which had a linear decrease in the PUN concentration that may be indicative of the efficient use of nutrients in the feedback period and the lower demand for visceral recovery in this phase.

The observed values for plasma urea nitrogen concentration during restriction and *ad libitum* feeding phases were close to the expected maximum limit for these characteristics ranging from 10 to 30 mg/dL (SWENSON, 1996). This can be related to the increase of glucose and N ureic plasma levels in bovines that are supplemented with ionophores (DUFFIELD et al., 2008) and fed diets with a high proportion of concentrate (OLIVEIRA JUNIOR et al., 2004) without representing an imbalance of protein (PUTRINO et al., 2006).

Another important aspect in the evaluation of the PUN concentration is that the protein metabolic pathway depends not only on the protein source, but also on the dietary energy intake, because if the energy consumption is low, there is a change in the metabolism of the ruminal microorganisms (AMORIM et al., 2007). Thus, the way in which energy is supplied, by roughage or concentrate, may influence the observed values for animals in feedlot with feed restriction (ELLENBERGER et al., 1989) or not (MENDES et al., 2005; OBEID et al., 2006).

CONCLUSION

The concentrations of the major blood metabolites used to evaluate the nutritional status of Nellore steers fed diets with high proportion of concentrate in different levels of feed intake showed variable values, as a function of time and level of diet intake. Therefore, lower levels of diet intake can be used to improve feedlot performance.

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