

Blood parameters of Angus and Nellore young bulls fed diets with or without forage

Luciana Navajas Rennó¹ , Rafael Aparecido Gomes^{2*} , Taiane da Silva Martins¹ , Karina Costa Busato¹ , Marcio Machado Ladeira³ , Maria Helena de Oliveira³ , Jarbas Miguel da Silva Júnior¹ , Mario Luiz Chizzotti¹ 

¹ Universidade Federal de Viçosa, Departamento de Zootecnia, Viçosa, MG, Brasil.

² Instituto Federal de Educação, Ciência e Tecnologia do Pará, Campus Castanhal, Castanhal, PA, Brasil.

³ Universidade Federal de Lavras, Departamento de Zootecnia, Lavras, MG, Brasil.

ABSTRACT - We evaluated blood parameters of Angus and Nellore bulls fed diets with and without forage. Forty animals with initial body weight (BW) of 380 ± 16.2 kg were housed in individual stalls. Eight bulls of each breed were fed a whole shelled corn (WSC) diet [850 g kg^{-1} of WCS and 150 g kg^{-1} of a pellet based on soybean meal, dry mater (DM) basis] or a ground corn with silage (GC) diet (300 g kg^{-1} of silage and 700 g kg^{-1} of a concentrate based on corn and soybean meal, DM basis), *ad libitum*, and four animals of each breed were limited-fed the GC diet [feed restriction (FR), 55% of the DM intake of bulls fed *ad libitum*, adjusted for the metabolic BW]. Intake was measured daily, and a metabolism trial was conducted with total collection of feces and urine to estimate the ruminally degraded and undegraded protein (intake and nitrogen balance). Blood samples for determination of hormones and metabolites were collected. The data were analyzed using the GLM procedure of SAS adopting a significance level of 0.05. The diet affected only the serum concentrations of triglycerides, HDL, and VLDL, with limited-fed animals presenting smaller concentrations than *ad libitum*-fed bulls. No difference in blood parameters was observed between animals fed WSC or GC diets. Nellore bulls presented greater T4, cholesterol, HDL, LDL, and serum urea nitrogen concentrations than Angus. Feeding finishing beef cattle with no-roughage diets does not alter their blood parameters. Also, our results support that Zebu cattle might have lower serum removal of nutrients due to a lesser demand for fat and protein deposition than *Bos taurus taurus*.

Keywords: blood metabolites, *Bos taurus indicus*, *Bos taurus taurus*, feed restriction, hormones

***Corresponding author:**

rafaelzoo84@yahoo.com.br

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Introduction

Bos taurus indicus, commonly used in tropical livestock, share a common ancestor with *Bos taurus taurus* (Loftus et al., 1994). However, both subspecies have undergone separate evolution for thousands of years, and during this period, Zebu cattle breeds have been exposed to harsh environments, resulting in acquired adaptations to survive, adapting to hot and humid weather and poor-quality diets (Turner, 1980).

Diets with whole shelled corn (WSC) without forage have been used in the United States since 1970. Depending on corn prices and availability, feeding cattle for fattening with no-forage diets can be cheaper, since roughage production requires large areas and specific and expensive agricultural

equipment. Furthermore, comparing with forage, grains are higher energy-dense and easier to transport and stock. In South America, the use of WSC diets is more recent, but it has been increasing in the last years. Previous studies have shown that animals fed WSC diets are more efficient (Traxler et al., 1995; Turgeon et al., 2010). However, most of the studies on WSC diets used taurine cattle, and only a few were carried out using *Bos taurus indicus*. Besides, when fed concentrate-based diets, *Bos taurus taurus* have greater intake relative to their maintenance requirements and higher average daily gain than Zebu cattle (Krehbiel et al., 2000; Carvalho et al., 2016). On the other hand, *Bos taurus indicus* utilize low-quality forage diets more efficiently than taurine cattle (Krehbiel et al., 2000).

Blood metabolites are a useful indicator of the metabolic status of animals and can be used to predict nutrient utilization. Most of the studies on high-grain diets has focused only on animal production, evaluating weight gain, feed efficiency, and carcass characteristics, but not on indicators of metabolic status, and to the best of our knowledge, other studies have not analyzed blood metabolites in cattle fed WSC diets. We hypothesize that serum parameters are different between animals fed or not forage and that the magnitude of the changes are different between Angus and Nellore bulls. Therefore, the objective of this work was to compare blood parameters of finishing Angus and Nellore bulls fed either a WSC diet or a ground corn with silage diet on an *ad libitum* or restricted basis.

Material and Methods

All animal care and use procedures were evaluated and approved by the Bioethic Committee in Utilization of Animals, case no. 048/2012. The study was conducted in Lavras (Minas Gerais, Brazil), located at 21°14' South latitude, 45°00' West longitude, and at an altitude of 918 m. During the experimental period, the average temperature was 23 °C, the maximum temperature was 36 °C, the minimum temperature was 20 °C, and the average relative humidity was 75%.

The animals were housed outdoors in individual covered pens measuring 1×5 m, with concreted floor. The cover was enough to provide shade to the animals and to the feed and water troughs.

Forty bulls were used (20 Nellore and 20 Angus) with an initial body weight (BW) of 380 (±16.2) kg. Animals were fed the same diet (ground corn with corn silage – GC), *ad libitum*, for 28 d before the beginning of the experiment. Then, eight animals of each breed were fed a WSC diet or the GC diet on an *ad libitum* basis (Table 1), and four animals of each breed were limited-fed the GC diet (feed restriction – FR, 55% of the DM intake of *ad libitum*-fed bulls, adjusted for the metabolic BW). The GC diet was formulated according to NRC (2000) for an average daily gain of 1.4 kg d⁻¹. The animals were individually fed two times a day, at 07.30 and 15.30 h. Feed and orts were weighed and sampled daily to quantify dry matter intake (DMI). Ingredients and orts samples were oven-dried at 55 °C for 72 h. Animals fed *ad libitum* were offered enough feed to ensure about 5% of orts daily. The experimental period lasted for 84 d after the adaption period.

A digestion trial was conducted with all animals. Feed intake, feed refusals, feces, and urine were recorded daily during three days. Feces excreted were collected directly from the floor, immediately after the animal had defecated, and urine excreted was collected using a rubber funnel tied to the body of the animal and connected to a coitaner filled with 200 mL of 20% H₂SO₄ by a drainage hose. Daily, a 10% sample of orts, feces, and urine was collected. The urine samples were stored at –20 °C, and feces samples were immediately dried at 55 °C for 72 h.

Animals were slaughtered after 84 d. Prior to the slaughter, bulls were feed-fasted for 12 h. Then, they were desensitized with a non-penetrating stunner and harvested by exsanguination using conventional humane procedures. Blood samples for determination of hormones and metabolites were collected during the slaughter, immediately after exsanguination with vacuum tubes without anticoagulant and immediately centrifuged (3000 × g, 15 min). Serum was separated and stored at –20 °C until analysis.

The chemical analysis of the diet ingredients was described by Gomes et al. (2017). Ruminally undegraded protein (RUP) intake (g kg⁻¹ d⁻¹ of BW) was estimated following the NRC (2001) models. Values for digestion rate (kd) of protein fraction B and RUP digestibility of each feed ingredient were

Table 1 - Ingredients and chemical composition of diets, in g kg⁻¹ of dry matter

Item	GC diet	WSC diet
Ingredient		
Corn silage	300	-
Corn grain	580	850
Soybean meal	100	-
Mineral premix ¹	20	-
Pelleted suplement ²	-	150
Chemical composition		
Dry matter ³	589	878
Crude protein	117	136
Protein fraction A	27.1	45.2
Protein fraction B	87.4	87.9
Protein fraction C	2.5	2.9
Neutral detergent fiber	267	151
Non-fibrous carbohydrates	541	639
Starch	504	618
Ether extract	23.0	28.0
Ash	50.1	46.0
Metabolizable energy ⁴	10.8	12.4

¹ Assurance level per kilogram of product: calcium, 170 g; phosphorus, 31 g; sodium, 155 g; zinc, 2 mg; manganese, 515 mg; copper, 15 mg; iodine, 29 mg; selenium, 2.4 mg.

² Assurance level per kilogram of product: calcium, 45 g; phosphorus, 11 g; magnesium, 7.5 g; copper, 104 mg; zinc, 344 mg; selenium, 0.83 mg; virginiamycin, 140 mg; monensin, 120 mg.

³ In g kg⁻¹ as fed.

⁴ MJ kg⁻¹ DM; Carvalho et al. (2016).

taken from NRC (2001) tables. It was assumed that the pellet had the same kd of protein fraction B and RUP digestibility as soybean meal. Ruminally degraded protein (RDP) intake (g kg⁻¹ d⁻¹ of BW) was calculated as the difference between crude protein intake (g kg⁻¹ d⁻¹ of BW) and RUP intake. Nitrogen balance (NB) was calculated as the difference between nitrogen intake (g kg⁻¹ d⁻¹ of BW) and nitrogen excreted in feces and urine (g kg⁻¹ d⁻¹ of BW).

Serum low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were estimated following the method proposed by Friedewald et al. (1972). Bioclin/Quibasa (Minas Gerais, Brazil) kits were used to analyze the serum concentration of glucose (kit #K082), total protein (kit #K031), albumin (kit #K040), urea (kit #K056), triglycerides (kit #K117), cholesterol (kit #K083), and high-density lipoprotein (HDL; kit #K071), through an automatic biochemical analyzer (Mindray BS-200E; Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China). Insulin (kit #2425-300A), total triiodothyronine (T3; kit#125-300A), and total thyroxine (T4; kit #225-300A) were analyzed by Enzyme Linked ImmunoSorbent Assay method through Monobind Inc. (California, United States) kits, using Biolisa Reader and Biolisa Washer (Bioclin/Quibasa, Minas Gerais, Brazil). Globulins were calculated by subtracting the albumin quantified from the total protein level. Serum urea nitrogen (SUN) was estimated as 46.67% of total serum urea.

Statistical analyses were performed using SAS (Statistical Analysis System, version 9.2). Data of performance, intake, nitrogen balance, and blood parameters were analyzed as completely randomized design in a factorial scheme (three diets × two breeds), using GLM procedures and considering animal as the experimental unit. The least squares means statement was used to calculate the adjusted means for treatments, and differences were considered statistically significant when P ≤ 0.05; tendencies were discussed when 0.05 < P ≤ 0.10. The general mathematical model was represented by:

$$Y_{ij} = \mu + T_i + M_j + (TM)_{ij} + \varepsilon_{ij}$$

in which μ is a constant, T_i is the effect of breed, M_j is the effect of diet, $(TM)_{ij}$ is the interaction between breed and diet, and ε_{ij} is the residual random error.

Results

The initial BW was not different between breeds or diets (Table 2). However, Angus and Nellore bulls fed *ad libitum* performed better than limited-fed animals, with a final BW 19 and 7% greater ($P < 0.001$) for Angus and Nellore, respectively, with no difference between WSC and GC diets. Angus bulls presented greater DM, average daily gain (ADG), RDP, and RUP intake and nitrogen balance (Table 2; $P < 0.001$) than Nellore. Angus bulls fed the GC diet presented greater RDP and RUP intake ($P < 0.001$) than bulls fed the WSC diet. However, for Nellore cattle, only RDP was different between the two diets, with no difference in the RUP intake ($P = 0.357$). In addition, no difference was observed in the nitrogen balance between WSC and GC diets. The DMI, in kg d^{-1} , was not different ($P > 0.05$) between Nellore bulls fed WSC or FR diets, while the animals fed GC and WSC diets had the same RUP intake.

There was no difference in the blood parameters of bulls fed WSC or GC diets. Limited-fed animals presented smaller serum concentrations of triglycerides, HDL, and VLDL ($P \leq 0.039$) than *ad libitum*-fed bulls, and bulls fed the FR diet tended to have lower T3 concentration ($P = 0.057$) (Table 3). Nellore

Table 2 - Performance and intake of Angus and Nellore cattle fed diets with and without forage

Item	Angus			Nellore			SEM	P-value		
	GC	WSC	FR	GC	WSC	FR		Breed	Diet	Breed × diet
Initial BW (kg)	386	399	385	374	365	389	12.9	0.143	0.960	0.485
Final BW (kg)	540a	535a	454b	461a	431a	415b	17.1	<0.001	0.004	0.225
Average daily gain (kg d^{-1})	1.73a	1.53a	0.86b	0.97a	0.74a	0.42b	0.15	<0.001	<0.001	0.572
Dry matter intake (kg d^{-1})	13.7A	10.3B	6.5C	9.7B	6.8C	5.3C	0.484	<0.001	<0.001	0.032
RDP intake ($\text{g kg}^{-1} \text{d}^{-1}$ of BW)	1.79a	1.66b	1.06c	1.44a	1.33b	0.93c	0.109	<0.001	<0.001	0.146
RUP intake ($\text{g kg}^{-1} \text{d}^{-1}$ of BW)	1.63A	1.37B	0.72D	1.16C	1.04C	0.60D	0.0546	<0.001	<0.001	0.048
Nitrogen balance ($\text{g kg}^{-1} \text{d}^{-1}$ of BW)	0.386a	0.376a	0.174b	0.268a	0.288a	0.140b	0.0409	<0.001	<0.001	0.084

GC - ground corn with silage diet; WSC - whole shelled corn diet; FR - feed restriction diet; BW - body weight; RDP - ruminally degraded protein; RUP - ruminally undegraded protein.

A,B,C,D - Different uppercase letters in the same row differ at $P < 0.05$ by least squares means for breed × diet effect.

a,b,c - Different lowercase letters in the same row, within diet, differ at $P < 0.05$ by least squares means.

Table 3 - Blood parameters of Angus and Nellore cattle fed diets with and without forage

Item	Angus			Nellore			SEM	P-value		
	GC	WSC	FR	GC	WSC	FR		Breed	Diet	Breed × diet
Insulin ($\mu\text{IU mL}^{-1}$)	3.50	4.12	2.01	3.10	5.94	5.81	1.14	0.074	0.252	0.216
Glucose (mg dL^{-1})	103.3	120.2	89.2	101.8	112.7	110.6	10.1	0.623	0.205	0.408
T3 (ng mL^{-1})	1.65	1.58	1.29	1.77	1.63	1.48	0.123	0.248	0.057	0.870
T4 ($\mu\text{g dL}^{-1}$)	7.10	7.77	7.63	9.65	9.44	11.11	0.731	<0.001	0.439	0.522
Total protein (g dL^{-1})	7.82	7.75	7.51	7.75	8.02	7.78	0.224	0.392	0.619	0.639
Albumin (g dL^{-1})	3.35	3.27	3.41	3.33	3.25	3.41	0.0780	0.862	0.205	0.994
Globulin (g dL^{-1})	4.47	4.48	4.10	4.42	4.76	4.37	0.238	0.396	0.340	0.688
SUN (mg dL^{-1})	13.4	13.9	15.9	15.1	17.5	18.2	1.48	0.052	0.203	0.735
Cholesterol (mg dL^{-1})	95.2	93.5	82.4	133.1	112.4	120.6	10.8	0.004	0.490	0.634
Triacylglycerol (mg dL^{-1})	18.5a	18.7a	14.7b	19.5a	15.6a	15.1b	1.45	0.638	0.039	0.271
HDL (mg dL^{-1})	55.8a	61.7a	46.8b	65.6a	64.1a	55.0b	3.83	0.039	0.020	0.543
LDL (mg dL^{-1})	20.4	13.1	19.2	40.9	30.5	41.5	8.82	0.010	0.488	0.962
VLDL (mg dL^{-1})	3.70a	3.75a	2.94b	3.89a	3.13a	3.02b	0.290	0.638	0.039	0.271

GC - ground corn with silage diet; WSC - whole shelled corn diet; FR - feed restriction diet; SUN - serum urea nitrogen; HDL - high density lipoproteins; LDL - low-density lipoproteins; VLDL - very low-density lipoproteins.

a,b,c - Different lowercase letters in the same row, within diet, differ at $P < 0.05$ by least squares means.

bulls presented greater T4, cholesterol, HDL, and LDL concentrations ($P \leq 0.039$) than Angus, while insulin ($P = 0.074$) and SUN ($P = 0.052$) concentrations tended to be higher in Zebu cattle. No other metabolites differed between treatments.

Discussion

Blood parameters were not different between bulls fed GC or WSC diets. This may have happened because, despite the lower DM intake of bulls fed the WSC diet compared with animals fed the GC diet, the WSC diet had higher energy and protein levels, and this could result in a similar intake of these two compounds between animals fed both diets, which in turn would not alter the energy and protein metabolism.

Insulin concentration values found in this study were around the range proposed by Kaneko et al. (2008), while glucose concentration was above the range reported by the authors. This could be a result of the high starch concentration in both GC and WSC diets, since increasing the amount of starch in the rumen may increase propionate production and gluconeogenesis (Russell and Gahr, 2000).

Other works have also found no difference in T4 plasma concentration between animals subjected or not to feed restriction, finding greater T3 concentration in animals fed *ad libitum* diets (Ekpe and Christopherson, 2000; Campanile et al., 2010). According to Reist et al. (2003), the impact of low energy intake in T3 blood levels is more pronounced and longer lasting than in T4.

Heat stress could be associated to the lower T4 concentration observed in Angus, reflecting the smaller tolerance of *Bos taurus taurus* to heat when compared with Zebu cattle. Thyroid hormones play an important role in the regulation of thermogenesis (Kahl et al., 2015), and some evidence suggest that thermal exposition acts on the hypothalamic-pituitary axis, reducing the thyroid-stimulating hormone secretion (Rasouli et al., 2004; Kahl et al., 2015), which is responsible to stimulate both synthesis and secretion of thyroid hormones (Kaneko et al., 2008).

Total protein, albumin, and globulin concentrations were similar to those described by other authors (Gandra et al., 2011; Egea et al., 2015). This similar serum protein levels between *ad libitum*-fed and limited-fed animals could indicate that even under feed restriction, the bulls had their protein requirements met. In fact, the bulls subjected to feed restriction showed positive nitrogen balance and, as observed in a parallel study by Gomes et al. (2017), they showed positive protein retention. If not required for protein synthesis, amino acids are deaminated by mitochondrial enzymes, and the amino group are transferred into urea for excretion (Kaneko et al., 2008). Then, the greater SUN levels of Nellore bulls suggest differences in protein utilization between the genotypes, indicating that Zebu cattle are less efficient in using absorbed protein (Myer and Elzo, 2010). Despite the difference in the RDP and RUP intake between the diets, the SUN levels were not affected.

The triacylglycerol concentration found in this study was slightly greater than that proposed by Kaneko et al. (2008), while the cholesterol concentration was within the range. The smaller triacylglycerol, HDL, and VLDL concentration in limited-fed animals was probably caused by feed restriction. Other authors also observed smaller concentrations of metabolites related to fat metabolism in animals under feed restriction (Bonnet et al., 2000; Caldeira et al., 2007). In ruminants, after absorption in small intestine, triacylglycerols, along with apolipoproteins, cholesterol, and phospholipids, are packaged into VLDL lipoproteins to deliver long-chain fatty acids to peripheral tissues, and HDL serves to deliver cholesterol to steroidogenic tissues or to membrane synthesis (Drackley, 2000). We did not observe difference in cholesterol levels between animals subjected or not to feed restriction, which is in agreement with other studies (Dashtizadeh et al., 2008; Silva et al., 2015).

Gandra et al. (2011) also observed a greater concentration of lipidogram parameters in Nellore than in taurine cattle, attributing this to the dairy ability of Holstein heifers, in which lipid metabolism is directed to the formation of the mammary gland and milk yield, differently from what happens to beef cattle, in which the lipidogram constituents are deposited in the carcass and are not secreted in the milk. However, both Angus and Nellore are beef breeds. Then, the difference found in this study could be the effect of the greater fat deposition in the muscle of Angus, which is supported by the results

of Martins et al. (2015), who reported slightly enhanced adipogenesis in the *longissimus* muscle of Angus bulls, and Ladeira et al. (2016), who observed greater gene expression of PPAR γ and SREBF1, transcription factors related to lipogenesis, in Angus than in Nellore cattle.

Conclusions

This study demonstrated that feeding beef cattle for fattening with no-roughage diets does not alter their blood parameters. Furthermore, Nellore have greater lipidogram parameters and serum urea nitrogen levels, which indicate that Zebu cattle may have lower serum removal of nutrients due to a lower demand for fat and protein deposition than Angus bulls.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: M.M. Ladeira and M.L. Chizzotti. Data curation: R.A. Gomes and T.S. Martins. Formal analysis: T.S. Martins. Funding acquisition: L.N. Rennó, M.M. Ladeira and M.L. Chizzotti. Investigation: L.N. Rennó, R.A. Gomes, T.S. Martins, K.C. Busato, M.M. Ladeira, M.H. Oliveira, J.M. Silva Júnior and M.L. Chizzotti. Methodology: L.N. Rennó, R.A. Gomes, T.S. Martins, M.M. Ladeira and M.L. Chizzotti. Project administration: L.N. Rennó, R.A. Gomes, M.M. Ladeira and M.L. Chizzotti. Resources: R.A. Gomes, M.M. Ladeira and M.L. Chizzotti. Supervision: M.M. Ladeira and M.L. Chizzotti. Validation: L.N. Rennó, R.A. Gomes and T.S. Martins. Visualization: L.N. Rennó, R.A. Gomes and T.S. Martins. Writing-original draft: L.N. Rennó, R.A. Gomes, T.S. Martins and K.C. Busato. Writing-review & editing: L.N. Rennó, R.A. Gomes, T.S. Martins and K.C. Busato.

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