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# Blood parameters as a possible indicator of feed efficiency in Nellore bulls

# Parâmetros sanguíneos como possíveis indicadores de eficiência alimentar em touros Nelore

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## Highlights

Inefficient animals had an increased amount of hemoglobin per erythrocyte.
Mean corpuscular hemoglobin (MCH) was correlated with RFI and gain-to-feed ratio.
Hemoglobin concentration was correlated with RFI and gain-to-feed ratio.

## Abstract

The objective of this study was to investigate the relationships between energy, protein, enzymatic metabolism, and residual feed intake (RFI) in purebred Nellore bulls. A total of 120 Nellore bulls, individually housed, underwent performance trials while being fed a high-concentrate diet. The study utilized data from the 10 most efficient, least efficient, and moderately efficient animals. Blood samples were collected via venipuncture for a complete blood count, and serum was analyzed for albumin, total protein, urea, creatinine, glucose, cholesterol, gamma GT, AST, and alkaline phosphatase. The data were subjected to a completely randomized design with three treatments and 10 replicates, and treatment means were compared using the Tukey test. Pearson's linear correlation analysis was performed. The most efficient animals demonstrated a 27.62% reduction in feed intake compared to the least efficient ones. No significant differences were observed in the serum biochemistry between different RFI classes. Inefficient animals exhibited elevated mean corpuscular hemoglobin (MCH), a red blood cell index, which correlated with RFI, gain-to-feed ratio, and dry matter intake (r = 0.46, 0.42, and -0.42, respectively). Hemoglobin concentration showed correlations with RFI and gain-to-feed ratio (r = 0.36, -0.41, respectively). These findings suggest potential variations in oxygen-carrying capacity. Red blood cell parameters could serve as biomarkers for identifying inefficient animals.

**Key words:** Intake. MCH. Hemoglobin. RFI.

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## Resumo

O objetivo deste estudo foi investigar as associações entre o metabolismo energético, proteico e enzimático com o consumo alimentar residual (CAR) de touros Nelore puros. Um total de 120 touros Nelore, alojados individualmente, passaram por provas de desempenho enquanto eram alimentados com uma dieta rica em concentrado. O estudo utilizou dados dos 10 animais mais eficientes, menos eficientes e moderadamente eficientes. Amostras de sangue foram coletadas por punção venosa para hemograma e o soro foi analisado para albumina, proteína total, ureia, creatinina, glicose, colesterol, gama GT, AST e fosfatase alcalina. Os dados foram submetidos a um delineamento completamente casualizado com três tratamentos e 10 repetições, e as médias dos tratamentos foram comparadas usando o teste de Tukey. Foi realizada uma análise de correlação linear de Pearson. Os animais mais eficientes demonstraram uma redução de 27,62% no consumo de materia seca em comparação com os menos eficientes. Não foram observadas diferenças significativas na bioquímica serica entre diferentes classes de RFI. Animais ineficientes apresentaram aumento na hemoglobina corpuscular média (HCM), um índice hematimetrico, que se correlacionou com RFI, eficiencia alimentar e consumo de matéria seca (r = 0,46; 0,42 e -0,42; respectivamente). A concentração de hemoglobina mostrou correlações com RFI e eficiencia alimentar (r = 0,36; -0,41, respectivamente). Esses achados sugerem variações potenciais na capacidade de transporte de oxigênio. O hemograma e seus parâmetros de células vermelhas do sangue podem servir como biomarcadores para identificar animais ineficientes.
**Palavras-chave:** Consumo. HCM. Hemoglobina. CAR.

## Introduction

The genetic enhancement of feed efficiency in cattle, both cumulative and permanent, represents a promising avenue for achieving gains in efficiency. Residual feed intake (RFI), an indicator independent of growth rate and body size, is a measure of feed efficiency. Genetic variability in feed efficiency exists, with a pooled heritability for RFI estimated at 0.33 (Berry & Crowley, 2013). In addition, RFI is a feasible criterion in selective breeding programs for Nellore cattle, the predominant breed in tropical regions (Santana et al., 2014).

Residual feed intake is calculated by subtracting the expected feed intake from the actual feed intake of each animal. To determine this value, daily feed intake must be measured and recorded over long-term feeding trials, typically lasting 70 to 84 days. As such, obtaining RFI data is labor-intensive and costly, limiting its widespread use as a feed efficiency metric (Sainz & Paulino, 2004). Therefore, there is a growing interest in developing early-life, easily measured, and cost-effective methodologies for the broad-scale identification of feed-efficient animals (Kelly et al., 2011). For instance, serum cholesterol (Alexandre et al., 2015; Montanholi et al., 2017), creatinine (Fitzsimons et al., 2013; Montanholi et al., 2017), and aspartate aminotransferase (Richardson et al., 2004) have been proposed as indicators of an animal's potential RFI status.

Further investigations are necessary to better understand the biological mechanisms governing feed efficiency and to identify biomarkers for ranking individuals for genetic and management purposes without the need for direct feed intake measurements. Metabolic profiling offers an accessible, economical, and reliable method for assessing the nutritional status and health conditions of animals.

This study aims to explore differences in whole blood count and metabolic profiles related to energy (glucose and cholesterol), protein (total serum protein, albumin, creatinine, and urea), and enzymatic metabolism (gamma GT, aspartate aminotransferase, and alkaline phosphatase) in Nellore bulls exhibiting divergent feed efficiency; and to identify the main associations between RFI and blood metabolism.

## Material and Methods

The study received approval from the Ethics Committee on Animal Use (approval no. 078/12), at the Federal University of Goias.

A total of 120 pedigree bulls aged 20 months, with an initial body weight of 394.33 ± 37.42 kg, participated in the study as part of the Nelore Qualitas® genetic improvement program. The animals were dewormed and then randomly assigned to individual soil-surface outdoor pens, each measuring 12.5 m².

The feeding period lasted 84 days, following a 14-day step-up dietary adaptation (Barducci et al., 2019). The diet was formulated to achieve a predicted daily gain of 1.6 kg, in accordance with the National Research Council (2000). Daily records were maintained for feed offered and orts, with the DMI of each animal calculated as the difference between feed offered and orts. Approximately 10% orts were allowed in the diet. Feed and orts were collected weekly, and samples were composited over 28 days. Table 1 describes the chemical and nutrient compositions of the diet.

**Table 1**
**Diet composition and nutritional characteristics**

| Ingredients | g/kg DM[a] |
|---|---|
| Corn silage [b] | 180 |
| Sugarcane bagasse | 50 |
| Sorghum meal | 468 |
| Soybean hulls | 243 |
| Soybean meal | 35 |
| Urea | 8 |
| Mineral premix[c] | 16 |
| Chemical composition (g/kg DM) | |
| Dry matter (g/kg as fed) | 618 |
| Crude protein | 147 |
| Neutral detergent fiber | 360 |
| Acid detergent fiber | 240 |
| Ether extract | 25 |
| Mineral salts | 45 |
| Total carbohydrates | 780 |
| Nonfibrous carbohydrates | 420 |
| Total digestible nutrients | 669 |

[a] dry matter; [b] corn silage without cob, [c] composition (g/Kg or mg/Kg): Calcium (min) 230g; Sulfur (min) 24.5g; Phosphorus (min) 13.5g; Magnesium (min) 19g; Sodium (min) 61.5g; Cobalt (min) 1mg; Cupper (min) 556mg; Iron (min) 371mg; Iodine (min) 28mg; Manganese (min) 1668mg; Selenium (min) 7.4mg; Zinc (min) 2224mg;

To determine the RFI class of the animals, a feed efficiency trial was conducted by regressing dry matter intake (DMI) against average daily gain (ADG) and metabolic weight. The following linear regression model was fitted to estimate DMI (eDMI) according to Archer et al. (1997):

$$eDMI = \beta_0 + (\beta 1 \times ADG) + (\beta 2 \times BW^{0.75}) + \varepsilon i,$$

where $\beta_0$ and $\beta_1$ are the partial regression coefficients of DMI against ADG and metabolic weight (BW0.75); and ε is the random error (RFI). Residual feed intake was calculated as the difference between observed DMI and eDMI.

At the conclusion of the trial, the 10 most efficient bulls, 10 least efficient bulls, and 10 with medium efficiency were categorized as low-RFI, high-RFI, and medium RFI, respectively. Blood samples were collected from these animals through puncture of the coccygeal vein into 10 mL vacuum tubes containing EDTA for blood count, fluoride for glucose analysis, and clot activator for biochemistry analysis. An automated analyzer (BC 2800 Vet, Mindray do Brasil, Sao Paulo, Brazil) was used for white blood cell, red blood cell, and platelet counts. Biochemistry analysis, encompassing albumin, total protein, urea, creatinine, glucose, cholesterol,

γ-glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), was conducted on a semi-automated biochemistry analyzer (Bio 2000, Bioplus, Sao Paulo, Brazil).

### *Statistical analysis*

The data were analyzed as a completely randomized design with three treatments and 10 replicates, using the easyanova package in RStudio (Arnhold, 2013). The experimental unit for all analyses was the animal. The statistical model was expressed as follows:

$$Yij = \mu + Ti + eij,$$

where Yij is the dependent variable; μ is the overall mean for each parameter, Ti is the treatment effect; and eij is the residual error.

Tukey's test was employed for comparing treatment means, and significant differences were declared at $P<0.05$. Pearson's linear correlation analysis was also performed.

To approximate normality, glucose data were log-transformed. Data from one animal that displayed signs of hemoconcentration were excluded from the analysis of blood count and protein.

## Results and Discussion

Dry matter intake was estimated through the following equation:

$$eDMI = -3.123 + 2.014*ADG + 0.089*BW^{0.75};$$
$$(R^2 = 0.71; P<0.01).$$

where eDMI is the estimated DMI (kg DM $day^{-1}$); ADG stands for average daily gain (kg $day^{-1}$); and BW represents body weight (kg). Table 2 shows performance data.

**Table 2**
**Performance of Nellore cattle with different residual intake**

| Variables [a] | Residual Feed Intake | | | SEM [b] | *p-value* |
|---|---|---|---|---|---|
| | Low n=10 | Medium n=10 | High n=10 | | |
| RFI [a], *kg/d* | -1.13[c] | -0.07[b] | 0.96[a] | | |
| Initial BW, *kg* | 400.2 | 409.6 | 413.3 | 5.34 | 0.69 |
| Final BW, *kg* | 555.9 | 556.5 | 572.9 | 6.77 | 0.54 |
| $BW^{0.75}$, *kg* | 102.6 | 103.3 | 105.0 | 1.70 | 0.60 |
| Average daily gain, *kg/d* | 1.86 | 1.75 | 1.89 | 0.05 | 0.48 |
| Dry matter intake, *kg/d* | 8.58[b] | 9.49[b] | 10.95[a] | 0.23 | <0.01 |
| Dry matter intake, *% BW* | 1.78[c] | 1.96[b] | 2.21[a] | 0.04 | <0.01 |
| Gain: Feed, *kg ADG/ kg DMI* | 0.22[a] | 0.18[b] | 0.17[b] | 0.05 | <0.01 |
| Feed conversion, *kg DMI/ kg ADG* | 4.61[b] | 5.43[a] | 5.79[a] | 0.13 | <0.01 |

ABC Means with a common letter did not differ (P> 0.05) from each other.
[a] RFI = residual feed intake; BW = body weight; ADG = average daily gain, DMI = dry matter intake;
[b] standard error of the mean.

Feed use efficiency differed between treatment groups, with low-RFI animals consuming 1.13 kg/day less feed and high-RFI animals consuming 0.960 kg/day less than predicted. The mean RFI difference between low, medium, and high RFI was 2.09 kg DM/day. The animals displayed significant differences in performance during the trial.

In terms of the percentage of body weight, the most efficient animals consumed 9.18% less feed (1.78% of BW) than medium-efficiency animals (1.96% of BW) and 19.46% less than low-efficiency animals (2.21% of BW). Gain-to-feed ratio was higher for low-RFI animals. Feed conversion was similar between medium- and high-RFI groups, both being, on average, 18% less efficient than low-RFI animals.

No differences were found for white blood cells, red blood cells, or platelet counts; however, high-RFI animals had a higher mean corpuscular hemoglobin (MCH), indicating the average quantity of hemoglobin present in a single red blood cell (Table 3). Weak correlations were detected between RFI and MCH (0.54, $p<0.05$), feed intake and MCH (0.45, $p<0.05$), and feed conversion and MCH (0.52, $p<0.05$), whereas gain-to-feed ratio and MCH correlated negatively (-0.44, $p<0.05$). Hemoglobin concentration was associated with RFI (0.42, $p<0.05$), feed conversion (0.49, $p<0.05$), and gain-to-feed ratio (-0.41, $p<0.05$) but not with DMI (0.20, $p>0.05$). There was no evidence of associations between red blood cell count and efficiency metrics. There were no differences between classes in serum biochemistry analysis (Table 4).

**Table 3**
**Blood count of Nellore cattle with different residual intake**

| Variables | Residual Feed Intake | | | CV, % [h] | *p-value* |
|---|---|---|---|---|---|
| | Low | Medium | High | | |
| RBC × $10^6$/µL[a] | 8.36 | 8.82 | 8.39 | 8.0 | 0.27 |
| HCT, %[b] | 35.68 | 36.95 | 37.39 | 7.4 | 0.37 |
| Hemoglobin, *g/dL* | 11.65 | 12.13 | 12.17 | 7.15 | 0.35 |
| MVC, *fL*[c] | 42.83 | 41.99 | 43.93 | 7.18 | 0.44 |
| MCHC, %[d] | 32.53 | 32.79 | 32.49 | 2.81 | 0.74 |
| MCH, *pg*[e] | 13.88b | 13.71b | 14.71a | 4.70 | <0.01 |
| RBW *(%)*[f] | 18.69 | 19.06 | 18.89 | 7.43 | 0.84 |
| WBC × $10^9$/L[g] | 10.44 | 9.28 | 9.69 | 14.32 | 0.20 |
| Platelets, $10^3$/*µL* | 156 | 146.44 | 143.33 | 17.9 | 0.55 |

[a] RBC = red blood cell; [b] HCT = hematocrit; [c] MCV = mean corpuscular volume; [d] MCHC = mean corpuscular hemoglobin concentration; [e] MCH= mean corpuscular hemoglobin; [f] RBW= red blood cell distribution width; [g] WBC =white blood cell); [h] coefficient of variation, [i] According to (Kaneko et al., 2008). AB Means with a common letter did not differ (P>0.05) from each other.

**Table 4**
**Biochemistry of Nellore cattle with different residual intake**

| Variables | Residual Feed Intake | | | CV, % [h] | *p-value* |
|---|---|---|---|---|---|
| | Low | Medium | High | | |
| Albumin, *g/dL* | 2.72 | 2.71 | 2.54 | 10.63 | 0.32 |
| Total serum protein, *g/dL* | 6.96 | 7.2 | 6.55 | 11.43 | 0.20 |
| Glucose, *mg/dL* | 75.18 | 71.52 | 81.45 | 3.39 | 0.18 |
| Cholesterol, *mg/dL* | 115.10 | 119.70 | 112.03 | 19.36 | 0.75 |
| Urea, *mg/dL* | 25.11 | 26.10 | 21.90 | 26.87 | 0.34 |
| Creatinine, *mg/dL* | 1.83 | 1.83 | 1.78 | 10.90 | 0.80 |
| ALP[b], UI/L | 266.1 | 266.2 | 255.4 | 23.78 | 0.90 |
| AST[c] , UI/L | 59.6 | 66.1 | 64.9 | 13.51 | 0.21 |
| GGT, *UI/L* | 19.89 | 19.13 | 17.6 | 30.22 | 0.66 |

[a] coefficient of variation; [b] Alkaline phosphatase, [c] aspartate aminotransferase.

Blood metabolite levels serve as indicators of the extent of energy, protein, and nutrient metabolism in animals. Glucose, cholesterol, non-esterified fatty acids, protein, urea, creatinine, albumin, globulin, minerals, liver enzymes, and hematology are objective, reliable, and routine measures used to assess the nutritional status of cattle (Ndlovu et al., 2007).

A hematologic examination is employed to detect structural changes and/or variations in the number of different blood cell types, including immature cells. Typically utilized for diagnosing anemia, inflammatory processes, immune responses, and blood clot disorders, it can collectively evaluate the nutritional status of cattle, particularly through hematocrit and hemoglobin levels. Low hematocrit may signal malnutrition, while low hemoglobin could indicate a deficiency in amino acids, iron, and vitamins, especially vitamins B12, E, folic acid, and niacin (Ndlovu et al., 2007). In the present study, no variations were observed in red cell, white cell, and platelet counts between divergent-RFI animals, aligning with similar findings in Nellore cattle (Nascimento et al., 2015).

In the current investigation, high-RFI animals exhibited an elevated mean corpuscular hemoglobin (MCH). Mean corpuscular hemoglobin reflects the average weight of hemoglobin in a red blood cell and is instrumental in classifying anemia as normochromic, hypochromic, or hyperchromic. Mean corpuscular volume (MCV), a measurement of the average size of red blood cells, classifies anemia as normocytic, macrocytic, or microcytic. Mean corpuscular hemoglobin results typically mirror MCV results, as larger red blood cells tend to contain more hemoglobin, while smaller cells have less. Mean corpuscular hemoglobin concentration (MCHC) calculates the amount of hemoglobin per unit volume in a single red blood cell, considering the cell size, unlike MCH (Sarna, 1990). In this study,

none of these indices showed correlations with RFI, except for MCH.

Mean corpuscular hemoglobin is not measured directly but through calculation, involving the measured hemoglobin concentration and red blood cell count. No significant associations were found between red blood cell counts and efficiency measured during this trial. However, hemoglobin concentration exhibited correlations with RFI, feed conversion, and gain-to-feed ratio. Our initial hypothesis posited that increased intake might lead to higher iron intake, potentially explaining the elevated MCH in inefficient animals. Surprisingly, daily DMI did not show a relationship with hemoglobin levels. This suggests that physiologically inefficient animals require more hemoglobin per erythrocyte to fulfill the same function. The biological functions of hemoglobin and the variations in blood indices may be linked to oxygen-carrying capacity, and may help explain the phenotypic differences in feed efficiency. This discussion delves into the biological economic design theory known as symmorphosis. According to symmorphosis, the most economically designed biological systems closely match the demands placed on them, possessing minimal spare capacity. This is attributed to the fact that structures supporting spare capacity bear the energetic costs of construction, maintenance, and load (Hudson, 2009).

The primary role of hemoglobin is to transport oxygen from the lungs to tissues, binding and releasing oxygen (Ahmed et al., 2020). However, it serves other functions, including catalytic roles such as nitrite reductase, NO dioxygenase, monooxygenase, alkyl hydroperoxidase, esterase, and lipoxygenase, as well as involvement in nitric oxide metabolism, metabolic reprogramming, pH regulation, and maintaining redox balance (Kosmachevskaya & Topunov, 2018).

Consistent with our findings, prior research indicated that MCH was higher for the least efficient animals, a parameter noted to be highly repeatable over time in European breeds (Richardson et al., 1996). In Nellore cattle, Chaves et al. (2015) reported that inefficient steers, determined by the G: F ratio, exhibited higher hematocrit percentages and hemoglobin concentrations. Baldassini et al. (2018) conducted a proteomic investigation in the liver of Nellore cattle, identifying 71 spots differentially abundant, with 28 proteins found exclusively in high-RFI animals, including the hemoglobin subunit beta. These findings align with the results of our present study.

The variation in blood parameters between animals is minimal and could serve as an indicator of feed efficiency. In our study, measurements were taken at the end of the test; ideally, measurements at the test's outset could be used as predictors of subsequent performance.

Blood glucose has a moderate diagnostic value in assessing the energy status of cattle due to tight hormonal homeostatic control (Wittwer, 2000). Glucose regulates insulin activity and glucagon, hormones influencing the deposition and mobilization of energy reserves. In ruminants, energy balance is accurately assessed by associating lipid profile with blood glucose (Fernandes et al., 2012). Triglycerides, β-hydroxybutyrate (BHB), cholesterol, and non-esterified fatty acids (NEFA) are the most commonly used metabolites. β-hydroxybutyrate and NEFA

are typically employed in dairy cattle to evaluate negative energy balance, while cholesterol, triglycerides, and blood glucose indicate a positive energy balance, activating the metabolic pathway of lipogenesis (Fernandes et al., 2012).

In our study, cholesterol levels fell within the species reference range (Kaneko et al., 2008), and no differences were observed between divergent-RFI animals. Lower cholesterol has been associated with higher feed efficiency in European breeds (Montanholi et al., 2017) and Nellore bulls (Alexandre et al., 2015). It was hypothesized that increased intake leads to higher serum cholesterol levels and greater fat deposition (Alexandre et al., 2015), potentially explaining efficiency differences, as the energy costs of depositing the same weight of lean tissue and fat vary. Blood glucose for high-RFI animals was slightly higher than the reference, likely due to increased intake. Similar results concerning blood glucose in divergent-RFI animals were observed in continental breeds (Kelly et al., 2011), Simmental heifers (Fitzsimons et al., 2013), and Nellore cattle (Nascimento et al., 2015).

To assess protein status in cattle, common metabolites include total protein, albumin, globulins, albumin-globulin ratio, non-essential-essential amino acid ratio, urea, creatinine, and urea-creatinine ratio. Differences may exist between animals with varying feed efficiency potential in terms of biochemical processes governing nutrient partitioning, fat and protein accretion, and maintenance (Fitzsimons et al., 2018). The impact of protein turnover on explaining differences in RFI has been controversial. Richardson et al. (2004) suggested that high-RFI steers have a higher rate of protein degradation in muscle and liver, coupled with a less efficient mechanism for protein deposition. Fitzsimons et al. (2013) observed higher concentrations of urea and lower concentrations of creatinine in the plasma of high-RFI vs. low-RFI heifers, with a negative association between RFI and creatinine, indicating increased muscle metabolism in efficient animals. In contrast, Nascimento et al. (2015) did not find any association, consistent with the present study. Discrepancies between literature reports are exacerbated by variations in breed, sex, and stage of physiological maturity, combined with substantial disparities in methodologies and result reporting (Cantalapiedra-Hijar et al., 2018).

The gastrointestinal tract and liver play important roles as energy sinks in cattle. Changes in feed intake lead to alterations in the metabolic rate of visceral organs. It was hypothesized that biological markers related to liver function could indicate variation in feed efficiency (Montanholi et al., 2017). In the current study, products related to liver function (albumin, cholesterol, urea) and enzymes primarily related to liver function (GGT, ALP, ALT) were similar between RFI classes.

Enzymatic diagnostics have practical applications in liver, heart, pancreas, muscle, blood, and neoplastic diseases (Puppel et al., 2022). Increased plasma enzymatic activity aids in identifying the location and degree of cell damage, given the organ-specific nature of many enzymes. Typically, aspartate AST, alkaline ALP, and GGT are used to assess liver functions. It is noteworthy that ALP levels exceeded the reference range (Kaneko et al., 2008) for all treatments. Serum ALP activity increases in cases of hepatitis, biliary

disorders, or during growth due to active bone metabolism (Sato et al., 2005).

Aspartate aminotransferase exhibited a moderate and positive correlation with RFI in European breeds (Richardson et al., 2004) and is a key enzyme in amino acid metabolism. In a proteomic investigation, several proteins were differentially expressed among Nellore bulls with high and low RFI, and AST was associated with high-RFI animals (Baldassini et al., 2018). Serum GGT was higher for inefficient animals associated with inflammation, confirmed by liver histopathology (Alexandre et al., 2015). The authors hypothesized that liver lesions were due to increased lipogenesis and/or higher bacterial infection originating from ruminitis. Microstructural evaluation of the liver parenchyma revealed direct associations between larger hepatocyte and larger hepatocyte nuclei size with feed efficiency (Montanholi et al., 2017).

## Conclusion

An elevated mean corpuscular hemoglobin serves as an indicator of low feed efficiency. This is primarily due to the positive correlation observed between hemoglobin concentration and residual feed intake, coupled with an inverse correlation with other efficiency metrics. Future studies should employ complementary techniques to elucidate the physiological mechanisms underlying this difference. Additionally, it is recommended that measurements be taken at the onset of the test to predict future performance. Hematological tests could serve as biomarkers for identifying inefficient animals, as these parameters exhibit minimal variation across individuals.

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