**ANA CLARA BAIÃO MENEZES**

# NITROGEN BALANCE AND NUTRIENT REQUIREMENTS OF YOUNG NELLORE BULLS FED WITH STATIC OR OSCILLATING CRUDE PROTEIN LEVELS, AND FEEDING BEHAVIOR, WATER INTAKE AND REQUIREMENTS OF BULLS WITH DIFFERENT RESIDUAL FEED INTAKES

Thesis submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of Doctor Scientiae.

Adviser: Sebastião de Campos Valadares Filho

Co-advisers: Fabyano Fonseca e Silva
Mário Fonseca Paulino

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# ABSTRACT

MENEZES, Ana Clara Baião, D.Sc., Universidade Federal de Viçosa, November, 2019. **Nitrogen balance and nutrient requirements of young Nellore bulls fed with static or oscillating crude protein levels, and feeding behavior, water intake and requirements of bulls with different residual feed intakes.** Adviser: Sebastião de Campos Valadares Filho. Co-advisers: Fabyano Fonseca e Silva and Mário Fonseca Paulino.

Our objectives with this study were 1) to evaluate the effects of dietary crude protein (CP) supply on intake, digestibility, performance, N balance, and requirements of young Nellore bulls, and 2) to determine feeding behavior, water intake, and requirements of high and low residual feed intake (RFI) Nellore bulls. 42 young bulls (initial BW of 260 ± 8.1 kg; age of 7 ± 1.0 mo) were fed ad libitum and were randomly assigned to receive one of six diets with different CP concentrations for 140 d: 105 (LO), 125 (MD), or 145 g CP /kg DM (HI), and LO to HI (LH), LO to MD (LM), or MD to HI (MH) oscillating CP at a 48-h interval for each feed. The bulls were housed in a feedlot in group pens that contained electronic feeders, waterers, and a scale connected to the waterers. At the end of the experiment, bulls were slaughtered to evaluate carcass characteristics. There was no alteration in the performance of growing Nellore bulls fed with oscillating CP diets versus a static level of 125 g CP/kg DM, nor static LO and HI levels; however, there may be undesirable increases in environmental N excretion when the average dietary CP content is increased. The results suggest that dietary CP concentrations of 105, 125 g/kg DM, or within this range can be indicated for finishing young Nellore bulls, since it reaches the requirements, reduces the environmental footprint related to N excretion, and may save on costs of high-priced protein feeds. Regarding requirements, the net energy requirements for maintenance and metabolizable energy (ME) for maintenance were 77 and 122.75 Kcal/EBW$^{0.75}$/d, respectively. The efficiency of ME utilization for maintenance was 62.7%. The equation obtained for net energy for gain (NEg) was: NEg (Mcal/EBW$^{0.75}$/d) = 0.0535 × EBW$^{0.75}$ × EBG$^{0.7131}$, where EBG is the empty body gain, and the efficiency was 24.25%. Net protein for gain (NPg) was: NPg (g/d) = 227.372 × EBG – 19.479 × RE. There was a linear increase for carcass, CP, and water present in the EBW as the animal grew. The EE deposition exponentially increased as EBW increased. Low RFI bulls had lower DMI intake than high RFI bulls, and no differences were observed between the two groups regarding performance and feeding behavior measurements. The net energy requirements for maintenance, metabolizable energy for maintenance, and efficiency of metabolizable energy utilization were 63.4, 98.6 kcal/EBW$^{0.75}$/d, and 64.3%, respectively for low RFI bulls, and 78.1, 123.9 kcal/EBW$^{0.75}$/d, and 63.0% for high RFI bulls. We did not observe any difference regarding the composition of gain in terms of protein or fat deposition between the two groups. Both groups presented also similar carcass and non-carcass traits. Therefore, our study shows that low RFI Nellore bulls eat less, grow at a similar rate, and have lower maintenance energy requirements than high RFI bulls. We also suggest that the lower feed intake did not compromises the carcass traits of more efficient animals, which would reduce production costs and increase the competitiveness of the Brazilian beef sector on the world market.

Keywords: Nellore. Nitrogen. Performance. Requirements. Residual Feed Intake.

# RESUMO

MENEZES, Ana Clara Baião, D.Sc., Universidade Federal de Viçosa, novembro de 2019. **Balanço de nitrogênio e exigências nutricionais de machos Nelore super precoces não castrados alimentados com níveis estáticos ou oscilantes de proteína bruta, e comportamento alimentar e exigências de bovinos Nelore de diferentes consumos alimentar residual.** Orientador: Sebastião de Campos Valadares Filho. Coorientadores: Fabyano Fonseca e Silva e Mário Fonseca Paulino.

Os objetivos deste estudo foram 1) avaliar os efeitos da suplementação dietética de proteína bruta (PB) sobre o consumo, digestibilidade, desempenho, balanço de N e exigências de machos Nelore não castrados super precoces; e 2) determinar o comportamento alimentar, o consumo de água e as exigências nutricionais de touros Nelore com alto e baixo consumo alimentar residual (CAR). 42 machos Nelore não castrados (PC inicial de 260 ± 8,1 kg; idade de 7 ± 1,0 mês) foram alimentados ad libitum e aleatoriamente distribuídos para receber uma das seis dietas com diferentes concentrações de PB por 140 d: 105 (LO), 125 (MD) ou 145 g CP / kg de MS (HI), e oscilando de LO para HI (LH), LO para MD (LM) ou MD para HI (MH) a cada 48 horas. Os animais foram alojados em um confinamento em baias em grupo que continham alimentadores eletrônicos, bebedouros e uma balança conectada aos bebedouros. Ao final do experimento os touros foram abatidos para avaliar as características da carcaça. Não foi observada alteração no desempenho de animais alimentados com dietas oscilantes de PB versus um nível estático de 125 g CP / kg MS, nem níveis estáticos LO e HI; no entanto, pode haver aumentos indesejáveis na excreção ambiental de N quando o teor médio de PB na dieta é aumentado. Os resultados sugerem que concentrações de PB na dieta de 105, 125 g / kg de MS ou dentro dessa faixa podem ser indicadas para bovinos Nelore super precoces em crescimento, pois atendem às exigências, reduzem a pegada ambiental relacionada à excreção de N e podem economizar os altos custos relacionados à alimentos proteicos. Com relação às exigências nutricionais, as exigências de energia líquida e energia metabolizável (EM) de mantença foram 77 e 122,75 Kcal/PCVZ$^{0,75}$/d, respectivamente. A eficiência da utilização de EM de mantença foi de 62,7%. A equação obtida para a energia líquida para ganho (ELg) foi: ELg (Mcal/PCVZ$^{0,75}$/d) = 0,0535 × PCVZ$^{0,75}$ × GPCVZ$^{0,7131}$, em que GPCVZ é o ganho de corpo vazio, e a eficiência foi de 24,25%. A proteína líquida para ganho (PLg) foi: PLg (g/d) = 227.372 × GPCVZ - 19.479 × ER. Foi observado um aumento linear para carcaça, PB e água presentes no PCVZ à medida que o animal crescia, já a deposição de gordura aumentou exponencialmente à medida que o PCVZ aumentou. Touros com baixo CAR apresentaram menor consumo de MS que touros com alto CAR, e não foram observadas diferenças entre os dois grupos quanto ao desempenho e comportamento alimentar. As exigências de energia líquida de mantença, energia metabolizável de mantença e eficiência da utilização de energia metabolizável foram 63,4, 98,6 kcal/PCVZ$^{0,75}$/d e 64,3%, respectivamente, para touros com baixo CAR e 78,1, 123,9 kcal/PCVZ$^{0,75}$/d e 63,0% para touros com alto CAR. Com relação à composição do ganho não foi observada nenhuma diferença entre os dois grupos, assim como também não foi observada nenhuma diferença com relação aos componentes carcaça e não carcaça. Nosso estudo mostra que animais baixo CAR comem menos, apresentam mesmo ganho e tem menor exigência de energia para mantença que animais alto CAR. Nós também sugerimos que o menor consumo de matéria seca não compromete negativamente características de carcaça de animais mais eficientes, o que pode resultar em redução dos custos de produção e aumento da competitividade do Brasil no mercado internacional.

Palavras-chave: Consumo Alimentar Residual. Desempenho. Exigências. Nelore. Nitrogênio.

# SUMMARY

**2 CHAPTER 1 - Oscillating and static dietary crude protein supply: I. Impacts on intake, digestibility, performance, and nitrogen balance in young Nellore bulls.**

**3 CHAPTER 2 - Oscillating and static dietary crude protein supply: II. Energy and protein requirements of young Nellore bulls**

**4 CHAPTER 3 - Feeding behavior, water intake, and energy and protein requirements of young Nellore bulls with different residual feed intakes**

# 1 INTRODUCTION

Since 2003 Brazil has been the largest beef exporter with the largest commercial cattle herd in the world (USDA, 2019). Due to an increasing external beef demand the number of feedlots in Brazil has increased, with recent reports forecasting an expansion in 2019 by 10 percent (USDA, 2018). However, greater concentration of livestock results in greater local pollutant emissions from manure during housing, storage, and land application (Petersen et al., 2007; Li et al., 2012). These pollutants are mainly linked to methane ($CH_4$), nitrous oxide ($N_2O$), carbon dioxide ($CO_2$), and ammonia ($NH_3$) emissions, and to eutrophication of water bodies (Chadwick et al., 2011; Mathot et al., 2012). If livestock intensification continues, technology and strategies need to be developed to control the associated environmental challenges.

Environmental regulations in developed countries have addressed the need to reduce the excretion of certain compounds, especially nitrogenous compounds (N). Netherlands, for instance, have put limits on N excretion and N fertilization because of public concern for the environment (Børsting et al., 2003). Therefore, nutritionists and other scientists have been researching different ways to reduce N emissions from production animal systems and increase N use efficiency. It is well known that the efficiency of nitrogen assimilation by animals is low, beef cattle for instance may convert 20 to 30% of their dietary N into animal protein, consequently, about 70 to 80% is excreted in the urine and feces. According to Menezes et al. (2016), the nitrogen metabolism is affected by the levels of dietary CP, and urinary and fecal N excretion increases linearly with protein intake. If protein contents in the diet are higher than the animal nutritional requirements, it results in an increase of N excretion, mainly via urine.

Therefore, one of the strategies that can be adopted is the appropriate formulation of diets to meet the nutritional requirements of cattle, reducing the excretion of polluting compounds without decreasing animal performance. If more N could be retained as a percentage of total N fed, more N would be available to maximize growth and production in the animals. In this context, a feeding strategy to potentially increase N use efficiency may be to oscillate crude protein concentrations in the diet. Initially this strategy was adopted in grazing systems, as a way to reduce labor and protein supplementation costs. Subsequently, experiments with confined animals, such as growing sheep (Cole, 1999; Kiran and Mutsvangwa, 2009; Doranalli et al., 2011), finishing cattle (Cole et al., 2003; Archibeque et al., 2007ab), and dairy cows (Brown, 2014, and Kohler, 2016) were developed to evaluate the effect of this feeding strategy on productivity, N retention, and N excretion.

Literature data (Archibeque et al., 2007b) suggests that alternating the dietary CP concentration from high (13.9%) to low (9.1%) in a 48-hour interval improves N retention in finishing cattle. Then, Archibeque et al. (2007c) worked with growing Dorset × Suffolk wethers surgically implanted with catheters in the abdominal aorta, a mesenteric vein, a hepatic vein, and the portal vein, to test the hypothesis that oscillating the dietary CP of finishing ruminants would improve N retention by altering the uptake of endogenous urea by portal drained viscera. Data of Archibeque et al. (2007c) indicate that oscillating dietary protein may improve N retention by increasing endogenous urea N uptake by the gastrointestinal tract, suggesting also that the excretion of nitrogenous compounds in urine would be reduced. However, Archibeque et al. (2007a) found that slurries (feces, urine, soil, and water, incubated for 35 d), from steers fed high (13.9%) or oscillating (9.1% to 13.9%) CP had greater concentrations of total aromatics and ammonia than those from steers fed low (9.1%) or medium (11.8%) CP. On the other hand, Kiran and Mutsvangwa (2009) observed less urinary and fecal N from the lambs fed and oscillating diet (9.5 to 15.5% CP on a 48h basis) than those fed a medium level (12.5%). Thus, it is not clear if the effects of oscillating diets on N retention and excretion are due to timing or the average CP content provided by the diets.

Additionally, dietary nutrient oscillation seems to affect the homeostatic and homeorhetic processes of host animal and ruminal microbial population in a manner that promotes a period of accelerated microbial growth due to an increase in N assimilation by ruminal microorganisms (Amaral et al., 2016). Therefore, the reduction in N excretion by meeting the ruminally degradable protein and metabolizable protein requirements of animals, without decreasing performance, has great potential to reduce the environmental impact of beef cattle production and increase economic returns for producers. However, to our knowledge, no systematic empirical research exists addressing the question of how oscillating CP dietary content affects N excretion and productive performance of growing and finishing Bos indicus animals in tropical conditions.

Indeed, Bos indicus represent a large portion of the global cattle herd as more than half of the cattle in the world are maintained in tropical environments (Cundiff et al., 2012). Bos indicus are predominant in the Brazilian herd, the world's largest commercial herd (ANUALPEC, 2017), where the Nellore (Bos indicus) breed represents 80% of the herd (Oliveira and Millen 2014). The Nellore genetics also play a critical role in providing heterosis for beef production, since the use of crossbred animals could be an alternative to reduce the feedlot period. The crossbred F1 Bos taurus × Bos indicus has greater growth potential, due to the effects of breed complementarity, which leads to improvements in performance, carcass traits, and productivity (Amaral et al., 2018). However, it is important to note that B. indicus type differs markedly from B. taurus in terms of feed intake, growth rate, body composition, temperament and feeding behavior (Lunstra and Cundiff, 2003, Almeida, 2005, and Schutt et al., 2009), which supports the need for investigations specific to this breed. Such metabolic and physiologic differences imply potential differences in nutrient utilization, which is supported by currently available separate requirement systems (the BR-CORTE system, Valadares Filho et al., 2016; NASEM, 2016, respectively).

Also, the appropriate formulation of diets to meet the nutritional requirements of zebu cattle during growing and finishing phases may result in reduced excretion of polluting compounds, like N, without decreasing cattle performance (Menezes et al., 2016), as discussed before. Furthermore, it is known that animal requirements change over time (Robertson et al. 1970), therefore, considering the adoption of feed systems, like oscillation of dietary CP levels, that adjust diets according to the animals' growth stage is an essential tool to improve production systems from both economic and environmental perspectives. A more effective diet formulation to optimize the use of protein can reduce dietary costs, since protein is considered the most expensive nutrient in beef cattle diets (Appuhamy et al., 2014). Equally important, the reduction in N excretion by meeting the ruminally degradable protein and metabolizable protein requirements of animals, without decreasing performance, has great potential to reduce the environmental impact of beef cattle production and increase economic returns for producers. Despite this interest, to the best of our knowledge, there are limited data about metabolizable energy and protein requirements of feedlot young Nellore bulls. Therefore, there is a need to improve estimates of metabolizable energy and protein requirements in order to accurately formulate diets to Bos indicus cattle, considering the global importance of this genetic.

In addition to improve nutrient requirements estimate, another important tool to potentially lower the environmental footprint of beef production is to improve feed efficiency. Furthermore, there is considerable interest in improved feed efficiency as a means of improving the economic sustainability of beef production systems, since one of the major economic factors influencing the profitability of beef cattle enterprises is the provision of feed, which represents up to three-quarters of total direct costs (Nielsen et al., 2013). At the animal level, many alternative definitions of feed efficiency exist, each differing in their application (Berry and Crowley, 2013). Traditionally, feed conversion ratio (i.e. feed:gain) or its mathematical inverse, feed conversion efficiency (i.e. gain:feed), was widely used. However, the selection for classical measures of feed efficiency may lead to an increase in mature size, which is undesirable in many circumstances (Archer et al., 1998).

Therefore, although there are many different approaches to measuring feed efficiency, residual feed intake (RFI) has increasingly become the measure of choice. Defined as the difference between an animal's actual and predicted feed intake (based on weight and growth), RFI is conceptually independent of growth and body size (Kenny et al., 2018). Postweaning tests for RFI have demonstrated that genetic variation exists, and that the trait is moderately heritable (Archer et al., 1999). However, before adopting RFI as selection criterion in genetic breeding programs, it is necessary to understand the genetic and phenotypic correlations between the traits to guarantee gains in efficiency without causing alterations in production parameters.

Even though RFI has gained popularity in recent years, mainly among geneticists (Cantalapiedra-Hijar et al., 2018), few studies have investigated genetic parameters and the effects of selection for efficiency traits in Zebu cattle (Grion et al., 2014, Fidelis et al., 2017). Nascimento et al. (2015) reported associations of RFI with biological processes affecting economically important traits in Nellore cattle, such as DMI and G:F. While, Fidelis et al. (2017) reported no differences between RFI classes (low and high RFI Nellore bulls) for dressing percentage, ribeye area, rib fat thickness, and rump fat thickness, suggesting a lack of phenotypic associations between RFI class and carcass traits in young Nellore bulls. Fidelis et al. (2017) also did not found any difference between the two classes regarding internal organ, internal fat, and body tissue weights. However, Gomes et al. (2012) observed that low RFI Nellore steers presented less fat on the gastrointestinal tract than the high RFI steers, and also tended to have lower KPI fat. Thus, published research evaluating the association between RFI status and efficiency traits in Nellore cattle is equivocal, and may be partly due to the diversity of diet types offered, and cattle age or sex.

Additionally, there exists a considerable amount of animal-to-animal variation around the average feed efficiency observed in beef cattle reared in similar conditions, which is still far from being understood (Cantalapiedra-Hijar et al., 2018). So far, the main physiological mechanisms identified and related to RFI are tissue metabolism, heat increment, feeding behavior and activity, and feed digestibility (Richardson and Herd, 2004; Nkrumah et al., 2006; Cruz et al., 2010; Lancaster et al., 2009). Yet much more research is warranted, since the results are equivocal. Another relevant question for the beef industry is the beef cattle water demand, and if it could be associated with RFI because it co-varies with feed intake. To the extent of our knowledge there are no data regarding feeding behavior, water intake, and metabolizable energy and protein requirements of feedlot young Nellore bulls diverging for RFI. In addition, there is a need to clarify if body composition and organ size would differ between the two RFI classes.

Therefore, the objectives of this thesis were to 1) evaluate the effects of dietary crude protein supply on intake, digestibility, performance, and N balance in young Nellore bulls consuming static or oscillating CP concentrations; 2) to evaluate the whole body chemical composition and establish the energy and protein requirements for maintenance and gain of young Nellore bulls, and 3) to determine feeding behavior, water intake, energy and protein requirements of high and low residual feed intake Nellore bulls.

In summary, this thesis is made up by three chapters, where chapters 1 and 2 were published at Translation Animal Science, and the third chapter was written according to the Journal of Animal Science guidelines, and is in the process of being submitted.

The two published papers are complementary and can be referred respectively as:

1. Menezes, A. C. B.; Valadares Filho, S. C.; Pacheco, M. V. C.; Pucetti, P.; Silva, B. C.; Zanetti, D.; Paulino, M. F.; Silva, F. F.; Neville, T. L.; Caton, J. S. 2019. Oscillating and static dietary crude protein supply: I. Impacts on intake, digestibility, performance, and nitrogen balance in young Nellore bulls. Translational Animal Science. doi: 10.1093/tas/txz138

2. Menezes, A. C. B.; Valadares Filho, S. C.; Pucetti, P.; Pacheco, M. V. C.; Godoi, L. A.; Zanetti, D.; Alhadas, H. M.; Paulino, M. F.; Caton, J. S. 2019. Oscillating and static dietary crude protein supply: II. Energy and protein requirements of young Nellore bulls. Translational Animal Science. doi: 10.1093/tas/txz139

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# 2 CHAPTER 1

Running head: Dietary protein in young Nellore bulls

## Oscillating and static dietary crude protein supply: I. Impacts on intake, digestibility, performance, and nitrogen balance in young Nellore bulls.[1]

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**ABSTRACT:** Effects of dietary crude protein (**CP**) supply on intake, digestibility, performance, and N balance were evaluated in young Nellore bulls consuming static or oscillating CP concentrations. Forty-two young bulls (initial BW of 260 ± 8.1 kg; age of 7 ± 1.0 mo) were fed ad libitum and were randomly assigned to receive one of six diets with different CP concentrations for 140 d: 105 (**LO**), 125 (**MD**), or 145 g CP /kg DM (**HI**), and LO to HI (**LH**), LO to MD (**LM**), or MD to HI (**MH**) oscillating CP at a 48-h interval for each feed. At the end of the experiment, bulls were slaughtered to evaluate carcass characteristics. Linear and quadratic effects were used to compare LO, MD, and HI, and specific contrasts were applied to compare oscillating dietary CP treatments vs. MD (125 g CP/kg DM) static treatment. Dry matter intake (**DMI**) was not affected ($P > 0.26$) by increasing or oscillating dietary CP. As dietary N concentration increased, there was a subsequent increase in apparent N compounds digestibility ($P = 0.02$), and no significant difference ($P = 0.38$) was observed between oscillating LH and MD. Daily total urinary and fecal N increased ($P < 0.01$) in response to increasing dietary CP. Significant differences were observed between oscillating LM and MH vs. MD, where bulls receiving the LM diet excreted less ($P < 0.01$; 71.21 g/d) and bulls fed MH excreted more ($P < 0.01$) urinary N (90.70 g/d) than those fed MD (85.52 g/d). A quadratic effect was observed ($P < 0.01$) for retained N as a percentage of N intake, where the bulls fed LO had greater N retention than those fed HI, 16.20% and 13.78%, respectively. Both LH and LM had greater ($P < 0.01$) daily retained N when compared to MD. Performance and carcass characteristics were not affected ($P > 0.05$) by increasing or oscillating dietary CP. Therefore, these data indicate that although there is no alteration in the performance of growing Nellore bulls fed with oscillating CP diets versus a static level of 125 g CP/kg DM, nor static low (105 g CP/kg DM) and high (145 g CP/kg DM) levels; there may be undesirable increases in environmental N excretion when the average dietary CP content is increased. The results suggest that dietary CP concentrations of 105, 125 g/kg DM, or within this range can be indicated for finishing young Nellore bulls, since it reaches the requirements, reduces the environmental footprint related to N excretion, and may save on costs of high-priced protein feeds.

**Key words:** bulls, crude protein, Nellore, nitrogen, performance

## INTRODUCTION

There are growing concerns about the effects of feedlot operations on air and water quality. Ammonia (**$NH_3$**) is the main gas emitted into the atmosphere from manure decomposition that impact environmental ecosystems and represent an unproductive loss of dietary nutrients (Liu et al., 2017). Several factors can affect the excretion of nitrogen such as feed intake, chemical composition of the diet, and efficiency of nutrient utilization (Muñoz et al., 2015). Strategies that increase production efficiency can conserve resources, improve environmental stewardship, and represent a great opportunity for mitigating N emissions per unit of livestock product.

Beef cattle may convert 20 to 30% of their dietary N into animal protein, consequently, about 70 to 80% is excreted in the urine and feces. The excess dietary N that is excreted accumulates in the atmosphere, soil, and groundwater and is detrimental to the ecosystem (NASEM, 2016). Promising strategies to alleviate N excretion and improve N retention involve manipulating the dietary crude protein (**CP**) content. Reducing the dietary CP content in finishing diets can decrease N excretion, mainly via urine, without a negative impact on performance (Amaral et al., 2014; Menezes et al., 2016). Additionally, oscillating CP concentration can enhance N retention in growing sheep (Cole, 1999; Kiran and Mutsvangwa, 2009; Doranalli et al., 2011) and finishing cattle (Cole et al., 2003; Archibeque et al., 2007a).

Dietary nutrient oscillation seems to affect the homeostatic and homeorhetic processes of host animal and ruminal microbial population in a manner that promotes a period of accelerated microbial growth due to an increase in N assimilation by ruminal microorganisms (Amaral et al., 2016). Therefore, the reduction in N excretion by meeting the ruminally degradable protein and metabolizable protein requirements of animals, without decreasing performance, has great potential to reduce the environmental impact of beef cattle production and increase economic returns for producers. However, to our knowledge, no systematic empirical research exists addressing the question of how oscillating CP dietary content affects N excretion and productive performance of growing and finishing Bos indicus animals in tropical conditions.

We hypothesized that (1) oscillation of the dietary CP concentration would enhance growth performance, reduce N excretion, and improve N retention; and (2) it is possible to reduce CP during feedlot stages, without adversely affecting animal performance and efficiency. These hypotheses were tested by evaluating three static dietary CP concentrations; and three oscillating CP concentrations versus a static level of 125 g CP/kg DM, by determining intake, apparent digestibility, performance, feed efficiency and carcass characteristics of young Nellore bulls.

## MATERIALS AND METHODS

### Dietary Treatments and Animals

The experiment was conducted at the Experimental Feedlot of the Animal Science Department at the Universidade Federal de Viçosa (**UFV**), Viçosa, Minas Gerais, Brazil. Animal care and handling followed guidelines set by the UFV (process 59/2016). Dietary CP levels were determined according to the protein requirements for Nellore bulls suggested by the BR-CORTE system (Valadares Filho et al., 2016), where 125 g CP/kg DM was established as the adequate CP concentration for bulls in this age and weight category. Therefore, we used

125 g CP/kg DM as our medium, or average, treatment, and the oscillating treatments were compared to this static treatment.

Forty-two weaned Nellore bulls (initial BW of 260 ± 8.1 kg; age of 7 ± 1.0 mo) were fed ad libitum and were randomly assigned to receive one of six dietary treatments (n = 7 bulls per treatment) with different CP concentrations for 140 d, either: 1) Low (**LO**; 105 g CP/kg DM), 2) Medium (**MD**; 125 g CP/kg DM), 3) High (**HI**; 145 g CP/kg DM), 4) Low to High (**LH**; Oscillating dietary CP concentration of 105 to 145 g CP/kg DM at a 48-h interval), 5) Low to Medium (**LM**; oscillating dietary CP concentration of 105 to 125 g CP/kg DM at a 48-h interval), and 6) Medium to High (**MH**; Oscillating dietary CP concentration of 125 to 145 g CP/kg DM at a 48-h interval). The chemical composition of the three diets used in this experiment is presented in Table 1. Briefly, the Low diet (105 g CP/ kg DM), provided 673.2 g RDP /kg CP, and 326.8 g RUP /kg CP; the Medium diet (125 g CP/ kg DM) provided 696.7 g RDP/kg CP, and 303.3 g RUP / kg CP; and the High diet (145 g CP / kg DM) provided 713.7 g RDP/kg CP, and 286.3 g RUP / kg CP.

Each treatment was group-housed in a feedlot pen (48.0 $m^2$) with one electronic feeder (model AF-1000Master; Intergado Ltd., Contagem, Minas Gerais, Brazil) and one electronic waterer per pen (model WD-1000 Master; Intergado Ltd.). Before the experiment, each bull was fitted with an ear tag (left ear) containing a unique radio frequency transponder (FDX- ISO 11784/11785; Allflex, Joinville, Santa Catarina, Brazil). The bulls were allowed a 21-d adaptation period to the experimental conditions and treated against internal and external parasites by administration of injectable ivermectin (Ivomec; Merial, Paulinia, Brazil). The experiment was divided into five 28-d experimental periods, where the bulls were weighed at the beginning and end of the experiment after undergoing 16 h of fasting to measure initial and final BW, and weighed every 28 d to evaluate and monitor average daily gain (**ADG**) and BW. Diets were formulated according to the BR-CORTE system (Valadares Filho et al., 2016) to achieve an ADG of 1.1 kg. The diets (50:50 forage to concentrate ratio) consisted of corn silage and a concentrate that was formulated with ground corn, wheat bran, soybean meal, urea, ammonium sulfate, sodium bicarbonate, salt, and mineral mix. Chemical composition and amount of feed in diets are shown in Table 1. The RDP was calculated according to Brazilian Tables of Chemical Composition of Feeds described by Valadares Filho et al. (2015), and the RUP was estimated by difference.

The total mixed rations were provided twice a day, at 0700 and 1600 h. Feed delivery was adjusted daily to maintain minimum refusals the next day and ad libitum intake. The appropriate feed delivery for each group was based on refusal weight each morning. Electronic feeders were evaluated at 0600 h daily to quantify orts and adjust daily feed delivery to a maximum of 2.5% orts. According to the amount of refusals, the total mixed ration was reduced (more than 2.5% orts at morning evaluation) or increased (less than 2.5% orts at morning evaluation) to reach ad libitum intake. Each treatment was delivered to the electronic feeder and consequently provided unique access to individual animals. Using the electronic identification tags, individual daily feed intake was recorded and measured using electronic equipment (model AF-1000 Master; Intergado Ltda., Contagem, Minas Gerais, Brazil; Chizzotti et al., 2015).

### Sample Processing and Chemical Analysis

Feeds offered and refused were weighed daily, sampled and frozen. Weekly, corn silage and refused feeds were pooled, oven-dried at 55ºC for 72 h and ground at 2 mm to determinate the indigestible neutral detergent fiber (iNDF) concentration and 1 mm for other analyses, with a Wiley mill (TECNAL, SP, Brazil). The total DM was evaluated using a drying oven at 105 °C for 16 h. Based on the amount of DM from each animal refusal, pooled samples were made for each 28-d period. Samples of each one of the concentrate ingredients were collected directly at the feed mill, and corn silage samples were collected daily and stored in a freezer at -20ºC.

To evaluate apparent total-tract nutrient digestibility, grab samples of feces were obtained from each bull over two 5-d periods, from d 36 to 40 and 98 to 102. Within each period, collections were conducted at 1800 h on d 1, at 1200 h on d 2, at 0600 h and 1800 h on d 3, at 1200 h on d 4, and at 0600 h on d 5. These collection times were used in order to obtain proportional and representative samples to the oscillating and fixed treatments. A composite sample from each animal was created per period and processed as described for silage and orts. Indigestible NDF was used as a marker to estimate fecal DM excretion.

Pooled samples of corn silage, concentrate ingredients, refusals, and feces were quantified in terms of dry matter (**DM**), organic matter (**OM**), N, and ether extract (**EE**) according to the AOAC (2012, method numbers 934.01, 930.05, and 981.10; 2006, method number 945.16, respectively). Neutral detergent fiber (**NDF**) was analyzed according to the technique described by Mertens et al. (2002) without the addition of sodium sulfite, but with the addition of thermostable alpha-amylase to the detergent (Ankom Tech. Corp., Fairport, NY). The analyses of NDF were performed by using a fiber analyser (Ankom®200, Ankon Technology, Macedon, NY, USA). The NDF content corrected to ash (Mertens 2002) and protein (Licitra et al. 1996) content was estimated. The fecal DM excretion was obtained by dividing the iNDF intake by the fecal iNDF concentration. To quantify iNDF, the fecal samples, concentrate, refusals, and corn silage were placed in filter bags (model F57, Ankon®) and incubated in the rumen of a rumen-cannulated animal for 288 h (Valente et al. 2011). Non-fiber carbohydrates (**NFC**) were calculated according to Detmann and Valadares Filho (2010), where NFC (% DM) = 100 - [CP - (CP derived from urea + urea) + NDF + EE + ash].

## Blood Sampling

Jugular blood samples were obtained on d 56 and 112 prior to morning feed delivery, placed into evacuated tubes (Labor Import, Osasco, São Paulo, Brazil), and immediately cooled in ice. Blood samples were transported on ice to the laboratory and centrifuged to separate plasma (1,200 ×g for 10 min at 4°C). Once separated, plasma was removed by pipetting, aliquoted into 2-mL tubes, and immediately frozen at −40°C until analysis could be performed. Plasma was analyzed for plasma urea N using an automated biochemistry analyzer (ModelBS200E; Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China).

## Slaughter and Sampling

Prior to slaughter, bulls were fasted from feed for 16-h to estimate shrunk body weight (**SBW**). Bulls were slaughtered via captive bolt stunning followed by exsanguination. After slaughter, the carcass of each bull was separated into 2 halves, weighed to quantify hot carcass weight and dressing percentage, and then chilled at 4ºC for 18 h. Next, half-carcasses were removed from the cold chamber, weighed, and cold carcass yields were calculated. Subcutaneous fat thickness was then measured using a digital caliper in the region between 11th and 12th rib cut.

## Statistical Analyses

The experiment was carried out under a completely randomized design, where the bulls were the experimental units. Constant CP concentration treatment comparisons followed the decomposition of orthogonal polynomials in linear and quadratic effects to compare 105, 125, and 145 g CP/kg DM. Moreover, specific contrasts were applied to compare oscillating dietary CP treatments vs. MD (125 g CP/kg DM) static treatment. Hartley's Fmax test was used to account for treatments homogeneity of variances; and the residual normality was investigated by Shapiro–Wilk test. Both ANOVA assumptions were verified for all variables. The MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) software was used to perform all statistical analyses assuming the significance level of 0.05.

## RESULTS AND DISCUSSION

### Voluntary Intake and Digestibility

Voluntary intake and digestibility data are presented in Table 2. Dry matter, OM, NDF, and NFC intake were not affected ($P > 0.05$) by increasing dietary CP, nor by oscillating dietary CP compared with MD. Mean intake of DM and other constituents, except for the planned difference in N (Table 3), were not different between dietary treatments, suggesting that neither the dietary CP content nor the way CP is delivered in diet restricts or stimulates intake parameters. As such, the absence of the effects of dietary CP content in voluntary intake suggests that the dietary inclusion of 105 g CP/kg DM, even though considered the lowest CP content relative to other treatments, fulfilled minimum requirements for microbial growth and feed degradation in the rumen (Kidane et al., 2018). It is suggested (Marini and Van Amburgh, 2003; Brake et al., 2010) that under such low CP diets, it is expected that the higher turnover rate of urea N with reduced clearance in the kidneys and increased clearance from the digestive tract would compensate for the low level of dietary CP for rumen microbes. Additionally, the oscillation frequency of 48 hours is probably in synchrony with retention time of digesta in the rumen, which would ensure that greater rates of urea-N recycling with the low CP diet, as in the treatments LM and LH, would occur when ruminal $NH_3$-N concentration is sub-optimal in terms of supporting microbial growth. Similar findings are reported in finishing Bos taurus steers (Archibeque et al., 2007a; Westover., 2011), ram lambs (Kiran and Mutsvangwa., 2009), and dairy cows (Brown., 2014) fed oscillating CP diets.

In addition to the above intake parameters, no significant differences for DM and OM digestibility ($P > 0.05$) were observed with increasing dietary CP content. However, significant effects for DM ($P = 0.01$) and OM digestibility ($P = 0.02$) were observed between oscillating MH and MD static CP concentration, where the DM and OM digestibilities were reduced when oscillating 125 to 145g CP/kg DM at a 48-h interval. According to Westover (2011), digestibility accounts for a large portion of variation in nutrient utilization in feedlot cattle and increased dietary CP concentration can be utilized to improve DM digestibility in roughage and mixed rations. However, the results from this experiment showed that increasing dietary CP had no significant effect on DM or OM digestibilities, probably because of similar DM and OM intake among treatments.

Previous studies with sheep reported that DM digestibility was not altered by CP concentration and oscillation (Ludden et al., 2002; Kiran and Mutsvangwa, 2009; Doranalli et al., 2011). In contrast, Archibeque et al. (2007a) observed that DM digestibility increased from low (91 g CP/kg DM) to medium (118 g CP/kg DM), high (139 g CP/kg DM), and oscillating CP diets (91 to 139 g CP/kg DM) in steers. Cole (1999) reported that apparent DM digestibility tended to decrease with increasing dietary CP and oscillating dietary CP concentration at 24 and 48 h basis for lambs. These results likely differ due to different protein concentrations and sources, timing of CP oscillations, animal species, and other confounding components of the ration, such as the inclusion level of concentrate and forage.

No significant difference was observed ($P > 0.05$) for NDF digestibility when dietary CP increased, however, there was a significant difference between MD vs. MH ($P < 0.01$), where the NDF digestibility was lower when oscillating MH compared to MD. These differences can be explained by the proportion of wheat bran in the diets (122.4, 61.4, and 0 g/kg DM for LO, MD, and HI CP diets, respectively) and the NDF content of wheat bran that consequently resulted in a reduction of NDF content from LO to HI CP diets. Kiran and Mutsvangwa (2009) reported a linear increase in NDF digestibility from low, medium, high, and oscillating CP pelleted diets fed to ram lambs. Amaral et al. (2016) observed in an in vitro assay that apparent ruminal digestibility of NDF was not affected by increasing dietary CP, nor by oscillating dietary CP (100 to 140 g CP/kg of DM) compared with a static supply of 120 g CP/kg DM.

A quadratic effect ($P < 0.01$) was observed in NFC digestibility when dietary CP increased. This performance apparently contradicts the patterns observed on NFC intake estimates, even though no significant difference was observed, the NFC intake decreased as the CP content in the diet increased. The NFC intake pattern seemed to be caused by decreasing NFC levels in the diet as nitrogen supplementation increased. A significant difference was observed between MD vs. LM ($P = 0.03$), where the NFC digestibility was reduced when oscillating LO to MD CP content compared to static MD. The biological responses observed in this study are not normally observed, as an illustration, Menezes et al. (2016) and Cavalcante et al. (2005) did not observe any influence of dietary CP level on NFC digestibility, on the other hand, Lazzarini et al. (2009) reported a quadratic pattern on the digestibility coefficient of NFC, with a decrease in the NFC digestibility according to increase in CP diet levels, associated with a linear reduction in NFC intake. A possible explanation for the variation in NFC digestibility between studies are differences in feed processing, dietary CP concentrations and source, feed additives, interactions among feedstuffs, and levels of feed intake (Westover, 2011).

## Nitrogen Balance

This study was designed to provide a linear increase in dietary N from sub-adequate (low) to adequate (medium) and excessive (high) concentration, based on protein requirements for young Nellore bulls estimated according to BR-CORTE (2016). The nitrogen balance is reported in Table 3, and was calculated according to Cole et al. (2006) and Cole and Todd (2009) where urine N was obtained based on the difference of N intake, fecal N, and retained N. A quadratic effect was observed ($P < 0.05$) for N intake with increasing dietary CP levels.

As would be expected, steers fed the HI CP diet had greater N intakes (197.6 g/d) than steers fed the MD (149.7 g/d) or LO CP diets (135.2 g/d). A significant difference was observed between oscillating dietary CP treatments and the MD static treatment, where N intake was greater ($P \leq 0.02$) for bulls fed LH and MH in comparison to those receiving the MD treatment, while bulls fed LM consumed less ($P < 0.01$) CP than those fed MD.

As dietary N concentration increased, there was a subsequent increase in apparent nitrogenous compounds digestibility, as stated by the quadratic effect ($P < 0.01$) yielding a greater apparent digestibility in the bulls fed HI (730.2 g/kg of DM) than those fed MD (704.9 g/kg of DM) or LO diets (679.1 g/kg of DM). No significant difference ($P > 0.37$) was observed between oscillating LH and MD. A significant difference was observed between oscillating LM and MD ($P < 0.01$) and between oscillating MH and MD ($P = 0.03$), and in both situations, the greater digestibility value was obtained by the static MD treatment. A similar pattern was observed by Archibeque et al. (2007a) where the steers fed high (139 g CP/kg DM) or oscillating (139 to 91 g CP/kg DM) diets had greatest apparent CP digestibility than steers fed medium (118 g CP/kg DM) or low (91 g CP/kg DM). According to Rufino et al. (2016), the differences in CP digestibility could be because the CP apparent digestibility coefficient is proportional to CP intake and can be considered a direct consequence of the dilution of the metabolic fecal fraction.

There was a significant quadratic effect ($P < 0.01$) on fecal N when dietary CP increased, and a significant difference ($P < 0.01$) between oscillating MH and MD was observed; however, no significant difference ($P > 0.06$) was observed between oscillating LH, oscillating LM, and the MD static treatment. Significant effects of CP concentration on fecal N were reported by Vasconcelos et al. (2009) and Hales et al. (2013) due to increasing CP intake. However, some authors (Menezes et al., 2016; Jennings et al., 2018) reported a lack of a dietary effect on fecal N excretion for finishing bulls. In a study involving static and oscillating CP concentration for finishing steers, Archibeque et al. (2007a) observed that daily fecal N did not differ between steers fed high (139 g CP/kg DM) or medium (118 g CP/kg DM), but was reduced when steers were fed oscillating (139 to 91 g CP/kg DM each 48h) or low (91 g CP/kg DM). The variation in fecal N excretion can be associated with increased microbial protein synthesis (Prates et al., 2017), as according to the NASEM (2016), 20% of microbial N is indigestible and can be excreted in feces. As observed by Cole et al. (2005) 30 to 50% of N intake is excreted in feces by beef cattle fed "typical" finishing diets, thus the appropriate formulation of diets to meet the nutritional requirements of cattle to reduce the excretion of polluting compounds without decreasing animal performance is of fundamental importance.

The rate of environmental emission of N, such as losses as ammonia volatilization to the atmosphere, nitrate diffusion in soil and groundwater, and denitrification and nitrous oxide emission in the atmosphere, is influenced by the source (fecal or urinary N). Fecal N (mainly undigested dietary, microbial, and endogenous proteins) differs substantially from N in the urine (mainly urea, allantoin, hippuric acid, creatinine, ammonia, and uric acid); the latter is more soluble and rapidly metabolized by microorganisms, affecting the severity of the environmental impact (Chizzotti et al., 2016). Daily total urinary N was greatest in the bulls fed HI (116.1 g/d) compared to those fed MD (85.5 g/d) and LO diets (67.7 g/d) as evidenced by quadratic effects ($P < 0.01$). There was no significant difference ($P = 0.33$) in urinary N between bulls fed oscillating LH and those fed MD static diets. Significant differences ($P < 0.01$) were observed between oscillating LM and MH vs. MD, where bulls receiving the LM diet excreted less (71.2 g/d) and bulls fed MH excreted more urinary N (90.7 g/d) than those fed MD (85.5 g/d), suggesting that the apparent effect of oscillating dietary CP content is more associated with dietary CP than with the way CP is delivery in diet.

The route of N excretion, such as fecal N and urinary N, was dependent on diet composition and greater than 75% of N excretion that was found in urine when high protein and high concentrate-based diets were used (Swensson, 2003; Cole et al., 2005; Hristov et al., 2011). Cole et al. (2003) evaluated three CP concentrations in diets where steers were fed as follows: constant 120 g CP/kg DM, constant 140 g CP/kg DM, and oscillating 100 or 140 g CP/kg DM at 2-d intervals. Greater N excretion was reported for steers fed constant 140 g CP/kg DM compared to all other steers (Cole et al., 2003). Archibeque et al. (2007a) reported that daily total urinary N was greatest for the steers fed high (139 g CP/kg DM), intermediate for steers fed medium (118 g CP/kg DM) or oscillating (139 to 91 g CP/kg DM), and least for steers fed low CP diets (91 g CP/kg DM). In this study, considering the results described above, the low and oscillating LM diets resulted in an average 22.52% and 40.15% less urinary N losses, respectively than the medium, oscillating MH, oscillating LH, and high CP-based diets resulting in a smaller environmental impact, which is explained due to the average CP content of the diets as stated before. According to Menezes et al. (2016), the efficiency of N utilization is affected by dietary CP content, and urinary and fecal N excretion increases linearly with protein intake. If protein contents in the diet are higher than the animal nutrient requirements, then increased N excretion results, mainly via urine. The reduction in N excretion by meeting the nutritional requirements of animals, without decreasing performance, has great potential to reduce the environmental impact of beef cattle production and increase economic returns to producers (Prados et al., 2016).

There was a quadratic effect ($P < 0.01$) on N retained (g/d) with increasing dietary CP levels where the bulls fed the HI and MD CP diet had greater N retained (29.1 and 29.0 g/d, respectively) than those fed low (23.8 g/d). A significant difference for N retention (g/d; $P < 0.04$) was observed between oscillating LM and MH vs. MD, where bulls receiving the MD diet retained more N. No difference ($P = 0.13$) was observed on N retention between oscillating LH and MD. When we consider retained N as a percentage of N intake we observed a quadratic effect ($P < 0.01$, where the bulls fed with LO CP had greater N retention than those fed HI

(16.20% and 13.78%, respectively). Significant differences ($P < 0.01$) were observed between LH vs. MD and LM vs. MD, and no difference ($P = 0.23$) was observed between MH and MD.

It is known that the efficiency of nitrogen utilization by animals is low; this results in high amounts of nitrogen excretion (Steinfeld et al., 2006). According to Hutchings et al. (1996), nitrogen use efficiency of beef cattle is approximately 10%, and the nitrogen retention in animal product ranges from 5 to 20% of the total consumed. Some causes of low nitrogen retention can be related to a grazing system with low quality of forage (low N supply) or feedlot diets that are excessive in nitrogen due to overestimation of the animal's requirements or use of inconsistent requirement systems to the climate conditions and genetic groups (Detmann et al., 2014). According to Cole et al. (2003), oscillating CP does not seem to affect N retention when supplemental CP was highly degradable (i.e., urea) but do affect N retention when supplemental CP contained appreciable amounts of ruminally undegradable CP (Cole, 1999) suggesting that some ruminally undegradable CP could potentially be fermented in the large intestine and the N recycled to the rumen. Archibeque et al. (2007a) observed that nitrogen retention was greater in steers fed oscillating (139 to 91 g CP/kg DM with 2 days interval) and medium (118 g CP/kg DM) diets compared with steers fed low (91 g CP/kg DM) or high (139 g CP/kg DM).

It has been reported that plasma urea N (**PUN**) concentration are correlated with CP intake (Valadares et al., 1997). In the present study, there was a significant quadratic effect ($P = 0.01$) in PUN levels when dietary CP increased and a difference ($P < 0.01$) between oscillating MH and LM vs. MD. No difference ($P = 0.84$) was observed between oscillating LH and the MD static CP diet. The increase observed for PUN concentrations with increased concentration of dietary CP (11.21, 21.45, and 23.76 mg/dL for LO, MD, and HI treatments) can be explained by the increase in daily N intake, as described by Prates et al. (2017). A linear increase of serum urea-N concentration with an increased supply of dietary CP was described in Nellore heifers and bulls (Prates et al., 2017), British x Continental steers (Gleghorn et al., 2004), and in Bos grunniens (yak; Guo et al., 2012).

## Animal Performance and Carcass Characteristics

Animal performance and carcass characteristics were not affected (P > 0.05) by dietary CP content or oscillating dietary CP (Table 4), suggesting that oscillating CP diets were not detrimental as well as do not bring any evident benefit to bull performance. This response is similar to the results of previous research demonstrating that feeding supplemental protein at 48 h intervals to ruminants has no negative impact on animal performance and carcass characteristics (Ludden et al., 2002; Ludden et al., 2003; Archibeque et al., 2007b; Westover, 2011). In contrast to this study, Cole et al. (2003) observed a greater ADG in steers fed oscillating CP concentrations (10 to 14% CP at 48-h intervals) than in steers fed the same quantity of CP on a continuous basis (12% or 14% CP). As discussed by this author, the variability in results could be caused by several factors including timing of CP oscillations, CP concentrations in diets vs. animal requirements, degradability of CP, or diet composition/fermentation ability.

Data from the literature suggests that for growing animals, dietary CP content influence weight gain (Winchester et al., 1957), and an optimal CP for beef cattle is approximately 13% over the grow out period (Gleghorn et al., 2004) and is greater during early feeding and less during the finishing phase, as cattle approach final weight (Todd et al., 2008). Amaral et al. (2018) developed a study with Nellore and crossbred Nellore x Angus bulls divided into three groups receiving diets with 100, 120, and 140 g CP/kg DM. This author observed that calves that were weaned and thereafter finished in feedlot should receive diets with CP content of approximately 120 g CP/kg DM during the initial growing phase (84 days). At the end of this period, or during the finishing phase (56 days), dietary CP content could be reduced to 100 g

CP/kg DM without affecting animal performance during this phase. In agreement with Amaral et al. (2018), this study shows that reducing CP from 145 to 105 g CP/kg DM or oscillating CP supply did not affect animal performance and carcass characteristics of growing Nellore bulls.

A possible explanation for the lack of effect of dietary treatments obtained in the present study is that the total dietary CP content of the low CP treatment (105 g CP/kg DM) may be sufficient to supply degradable CP for rumen microbial activity and MP for muscle production, as proposed by Hynes et al. (2016). The present study found that increasing dietary CP concentration had no significant effect on total DMI or ADG, however it increased N retention (Table 3). It is important to highlight that protein and fat are components of the gain, and protein has a lower energetic efficiency of deposition than fat, likely because it is influenced by the mix of amino acids available and the energy cost associated with body protein turnover (Baldwin, 1995). Additionally, when averaged over the entire feeding period and animals are fed to a constant endpoint, the body composition of gain and the diluting effects water gain on cost of lean weight gain may minimize the effects of protein versus fat gain (Tedeschi et al., 2010). Data regarding the chemical body composition of the bulls, and its pattern of deposition can be found in a complimentary paper (Menezes et al., 2019 submitted).

Therefore, these data indicate that although there is no alteration in the performance of growing Nellore bulls fed with oscillating CP diets versus a static level of 125 g CP/kg DM, nor static low (105 g CP/kg DM) and high (145 g CP/kg DM) levels; there may be undesirable increases in environmental N excretion when the average dietary CP content is increased. The results suggest that dietary CP concentrations of 105, 125 g/kg DM, or within this range can be indicated for finishing young Nellore bulls, since it reaches the requirements, reduces the environmental footprint related to N excretion, and may save on costs of high-priced protein feeds.

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**Table 1.** Proportion of ingredients and nutrient composition of the experimental diets

| Item | Experimental Diets[1] | | |
|---|---|---|---|
| | Low | Medium | High |
| Proportion | | | |
| Corn Silage | 50.0 | 50.0 | 50.0 |
| Ground corn | 39.4 | 39.4 | 39.4 |
| Soybean meal | 2.38 | 4.92 | 7.46 |
| Wheat bran | 6.09 | 3.05 | 0.00 |
| Urea | 0.47 | 0.98 | 1.49 |
| Salt | 0.30 | 0.30 | 0.30 |
| Limestone | 0.06 | 0.06 | 0.06 |
| Mineral mix[2] | 0.29 | 0.29 | 0.29 |
| Sodium bicarbonate | 0.75 | 0.75 | 0.75 |
| Magnesium oxide | 0.25 | 0.25 | 0.25 |
| Total | 100 | 100 | 100 |
| Chemical composition | | | |
| Dry matter, g/kg as-fed | 406.0 | 406.1 | 406.2 |
| Organic matter, g/kg DM | 944.1 | 943.8 | 943.5 |
| Crude protein, g/kg DM | 102.7 | 122.3 | 141.9 |
| Rumen degradable protein, g/kg CP | 673.2 | 696.7 | 713.7 |
| Rumen undegradable protein, g/kg CP | 326.8 | 303.3 | 286.3 |
| Ether extract, g/kg DM | 42.8 | 42.1 | 41.4 |
| Neutral detergent fiber, g/kg DM | 321.3 | 314.2 | 307.1 |
| Indigestible neutral detergent fiber, g/kg DM | 98.09 | 95.7 | 93.4 |
| Non-fiber carbohydrates, g/kg DM | 480.6 | 476.2 | 471.8 |

[1] Low = 105 g CP/kg DM; Medium = 125 g CP/kg DM; High =145 g CP/kg DM [2] Mineral mix = 7.83 g S/kg; 5,950 mg Co/kg; 10,790 mg Cu/kg; 1,000 mg Mn/kg; 1,940 mg Se/kg; 1,767.4 mg Zn/kg.

**Table 2.** Voluntary intake and apparent digestibility by bulls fed with differing crude protein concentrations.

| Items | Treatments[1] | | | | | | SEM[2] | Contrasts[3] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LO | MD | HI | LH | LM | MH | | Linear | Quadratic | M vs. LH | M vs. LM | M vs. MH |
| | Dry matter | | | | | | | | | | | |
| Intake, kg/d | 7.71 | 7.62 | 7.29 | 7.54 | 7.51 | 7.62 | 0.21 | 0.27 | 0.33 | 0.51 | 0.77 | 0.77 |
| Apparent digestibility, g/kg DM | 732.4 | 730.1 | 734.5 | 737.5 | 731.9 | 716.4 | 4.37 | 0.54 | 0.99 | 0.94 | 0.72 | 0.01 |
| | Organic matter | | | | | | | | | | | |
| Intake, kg/d | 7.28 | 7.19 | 6.88 | 7.11 | 7.08 | 7.19 | 0.20 | 0.27 | 0.33 | 0.51 | 0.77 | 0.77 |
| Apparent digestibility, g/kg DM | 754.1 | 751.8 | 759.6 | 764.5 | 750.8 | 739.1 | 4.38 | 0.27 | 0.79 | 0.59 | 0.71 | 0.02 |
| | Neutral detergent fiber | | | | | | | | | | | |
| Intake, kg/d | 2.52 | 2.43 | 2.27 | 2.41 | 2.42 | 2.40 | 0.07 | 0.10 | 0.05 | 0.34 | 0.37 | 0.24 |
| Apparent digestibility, g/kg DM | 631.3 | 619.1 | 601.7 | 629.6 | 625.0 | 575.8 | 11.1 | 0.10 | 0.23 | 0.65 | 0.40 | < 0.01 |
| | Non fiber carbohydrates | | | | | | | | | | | |
| Intake, kg/d | 3.67 | 3.60 | 3.41 | 3.56 | 3.56 | 3.59 | 0.10 | 0.19 | 0.18 | 0.44 | 0.61 | 0.55 |
| Apparent digestibility, g/kg DM | 849.8 | 867.0 | 870.5 | 870.5 | 853.2 | 857.0 | 5.35 | 0.59 | < 0.01 | 0.66 | 0.03 | 0.35 |

[1] LO, low (105 g CP/kg DM); MD, medium (125 g CP/kg DM); HI, high (145 g CP/kg DM), LH, oscillating low (LO; 105 g CP/kg DM) to high (HI; 145g CP/kg DM) each 48 h; LM, oscillating low (105 g CP/kg DM) to medium (MD; 125 g CP/kg DM) each 48 h; MH, oscillating medium (125g CP/kg DM) to high (145g CP/kg DM) each 48 h.

[2] SEM = standard error of the mean.

[3] Linear and quadratic contrasts compared 105, 125, and 145 g CP/kg DM; M vs. LH compared 125 versus oscillating 105 to 145 g CP/kg DM each 48 h; M vs. LM compared 125 versus oscillating 105 to 125 g CP/kg DM each 48 h; M vs. MH compared 125 versus oscillating 125 to 145 g CP/kg DM each 48 h.

**Table 3** Nitrogen balance of bulls fed with different crude protein levels.

| Items | Treatments[1] | | | | | | SEM[2] | Contrasts[3] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LO | MD | HI | LH | LM | MH | | Linear | Quadratic | M x LH | Mx LM | M x MH |
| N intake, g/d | 135.2 | 149.7 | 197.6 | 171.9 | 143.8 | 165.9 | 2.38 | <0.01 | <0.01 | 0.02 | <0.01 | <0.01 |
| N digested, % of intake | 67.9 | 70.5 | 73.0 | 71.1 | 67.2 | 69.91 | 0.60 | 0.02 | <0.01 | 0.38 | 0.01 | 0.03 |
| Fecal N, g/d | 43.9 | 44.3 | 52.1 | 49.4 | 47.0 | 50.0 | 1.21 | <0.01 | 0.01 | 0.07 | 0.81 | <0.01 |
| Urine N, g/d | 67.7 | 85.5 | 116.1 | 92.8 | 71.2 | 90.7 | 2.39 | <0.01 | <0.01 | 0.33 | <0.01 | <0.01 |
| Urine N, % of excreted N | 61.1 | 64.0 | 67.7 | 64.9 | 59.4 | 64.2 | 0.71 | <0.01 | <0.01 | 0.09 | <0.01 | <0.01 |
| Retained N, g/d | 23.8 | 29.0 | 29.1 | 29.9 | 26.4 | 27.3 | 1.16 | 0.96 | <0.01 | 0.13 | <0.01 | 0.04 |
| Retained N, % of N intake | 16.2 | 17.9 | 13.8 | 18.8 | 19.1 | 15.4 | 0.39 | <0.01 | 0.58 | <0.01 | 0.01 | 0.23 |
| Blood urea N, mg/dL | 11.2 | 21.5 | 23.8 | 11.8 | 10.7 | 20.9 | 1.72 | 0.49 | <0.01 | 0.84 | <0.01 | <0.01 |

[1] LO, low (105 g CP/kg DM); MD, medium (125 g CP/kg DM); HI, high (145 g CP/kg DM), LH, oscillating low (LO; 105 g CP/kg DM) to high (HI; 145g CP/kg DM) each 48 h; LM, oscillating low (105 g CP/kg DM) to medium (MD; 125 g CP/kg DM) each 48 h; MH, oscillating medium (125g CP/kg DM) to high (145g CP/kg DM) each 48 h.

[2]SEM = standard error of the mean.

[3]Linear and quadratic contrasts compared 105, 125, and 145 g CP/kg DM; M vs. LH compared 125 versus oscillating 105 to 145 g CP/kg DM each 48 h; M vs. LM compared 125 versus oscillating 105 to 125 g CP/kg DM each 48 h; M vs. MH compared 125 versus oscillating 125 to 145 g CP/kg DM each 48 h.

**Table 4.** Animal performance and carcass characteristics of bulls fed with different crude protein levels.

| Items[1] | Treatments[2] | | | | | | SEM[3] | Contrasts[4] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LO | MD | HI | LH | LM | MH | | Linear | Quadratic | M x LH | M x LM | M x MH |
| Initial SBW, kg | 276.1 | 274.8 | 278.9 | 275.1 | 273.7 | 277.2 | 9.09 | 0.75 | 0.95 | 0.85 | 0.92 | 0.93 |
| Final SBW, kg | 439.6 | 447.1 | 432.9 | 455.3 | 444.6 | 448.3 | 14.1 | 0.46 | 0.98 | 0.80 | 0.71 | 0.67 |
| ADG, kg/d | 1.17 | 1.25 | 1.13 | 1.32 | 1.23 | 1.25 | 0.06 | 0.15 | 0.79 | 0.51 | 0.34 | 0.35 |
| G:F | 0.15 | 0.16 | 0.15 | 0.17 | 0.16 | 0.16 | 0.01 | 0.11 | 0.24 | 0.19 | 0.10 | 0.14 |
| Hot carcass weight, kg | 266.8 | 270.4 | 262.9 | 275.9 | 267.4 | 272.9 | 8.44 | 0.53 | 0.99 | 0.96 | 0.77 | 0.61 |
| Cold carcass weight, kg | 261.1 | 265.7 | 258.3 | 271.0 | 262.2 | 268.0 | 8.43 | 0.53 | 0.92 | 0.93 | 0.69 | 0.56 |
| Hot carcass dressing, % | 60.7 | 60.5 | 60.7 | 60.6 | 60.2 | 60.9 | 0.42 | 0.67 | 0.85 | 0.39 | 0.68 | 0.77 |
| Cold carcass dressing, % | 59.4 | 59.4 | 59.7 | 59.5 | 59.0 | 59.8 | 0.43 | 0.73 | 0.79 | 0.53 | 0.96 | 0.53 |
| Carcass length, cm | 127.4 | 131.4 | 129.3 | 129.7 | 129.3 | 128.7 | 1.93 | 0.28 | 0.09 | 0.50 | 0.15 | 0.64 |
| Fat thickness, mm | 4.9 | 3.9 | 3.2 | 4.0 | 3.8 | 5.1 | 0.50 | 0.41 | 0.06 | 0.11 | 0.16 | 0.77 |

[1] SBW, shrunk body weight; ADG, average daily gain; G:F, gain-to-feed ratio

[2] LO, low (105 g CP/kg DM); MD, medium (125 g CP/kg DM); HI, high (145 g CP/kg DM), LH, oscillating low (LO; 105 g CP/kg DM) to high (HI; 145g CP/kg DM) each 48 h; LM, oscillating low (105 g CP/kg DM) to medium (MD; 125 g CP/kg DM) each 48 h; MH, oscillating medium (125g CP/kg DM) to high (145g CP/kg DM) each 48 h.

[3] SEM = standard error of the mean.

[4] Linear and quadratic contrasts compared 105, 125, and 145 g CP/kg DM; M vs. LH compared 125 versus oscillating 105 to 145 g CP/kg DM each 48 h; M vs. LM compared 125 versus oscillating 105 to 125 g CP/kg DM each 48 h; M vs. MH compared 125 versus oscillating 125 to 145 g CP/kg DM each 48 h.

# 3 CHAPTER 2

Running head: Nutrient requirements of young Nellore bulls

## Oscillating and static dietary crude protein supply: II. Energy and protein requirements of young Nellore bulls[1]

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**ABSTRACT:** The objective of this study was to evaluate whole body chemical composition and energy and protein nutrient requirements for maintenance and gain of Nellore bulls. Fifty young bulls, with an average age of 7 ± 1 month and initial body weight (**BW**) of 260.0 ± 8.1 kg, were used in this experiment. Four bulls were used as baseline reference animals and were slaughtered at the beginning of the experiment. Four bulls were fed at maintenance (12 g DM/kg of BW), while 42 bulls were divided into 6 groups (n = 7/group) and were randomly assigned to the following dietary treatments 105 (**LO**), 125 (**MD**), or 145 (**HI**) g crude protein (**CP**) /kg dry matter (**DM**), LO to HI (**LH**), LO to MD (**LM**), or MD to HI (**MH**) oscillating CP at a 48-h interval for 140 d. At the end of the experiment, bulls were slaughtered and samples of the whole body were collected. All samples were lyophilized, ground, and composed as percentage of component of empty body weight (**EBW**) from each bull. A power model was used to estimate carcass, non-carcass components, and gastrointestinal content of the shrunk body weight (**SBW**), and CP and water present in the empty body, while an exponential model was used to estimate adipose tissue and ether extract (**EE**) present in the EBW. Non-linear regression equations were developed to predict heat production from metabolizable energy intake and retained energy (**RE**). The net energy requirements for maintenance and metabolizable energy **(ME)** for maintenance were 77 and 122.75 Kcal/$EBW^{0.75}$/d, respectively. The efficiency of ME utilization for maintenance was 62.7%. The equation obtained for net energy for gain (**NEg**) was: NEg (Mcal/$EBW^{0.75}$/d) = $0.0535 \times EBW^{0.75} \times EBG^{0.7131}$, where EBG is the empty body gain, and the efficiency was 24.25%. Net protein for gain **(NPg)** was: NPg (g/d) = $227.372 \times EBG - 19.479 \times RE$. There was a linear increase for carcass, CP, and water present in the EBW as the animal grew. The EE deposition exponentially increased as EBW increased.

**Key words:** body composition, energy, Nellore, protein, requirements

## INTRODUCTION

Bos indicus represent a large portion of the global cattle herd as more than half of the cattle in the world are maintained in tropical environments (Cundiff et al., 2012). Bos indicus are predominant in the Brazilian herd, the world's largest commercial herd (ANUALPEC, 2017); furthermore, about 40% of the beef cows in the United States are located in relatively hot and humid subtropics of the Southeast or more arid subtropics of the Southwest, where the Bos indicus genetics play a critical role in providing heterosis for beef production (Cundiff et al., 2012). Bos indicus cattle have metabolic and physiologic differences compared with Bos taurus, implying potential differences in nutrient utilization, which is supported by currently available separate requirement systems (the BR-CORTE system, Valadares Filho et al., 2016; NASEM, 2016, respectively).

Appropriate formulation of diets to meet the nutritional requirements of zebu cattle during growing and finishing phases will result in reduced excretion of polluting compounds, like N, without decreasing cattle performance (Menezes et al., 2016). It is known that animal requirements change over time (Robertson et al. 1970), therefore, considering the adoption of feed systems, like oscillation of dietary CP levels, that adjust diets according to the animals' growth stage is an essential tool to improve production systems from both economic and environmental perspectives. A more effective diet formulation to optimize the use of protein can reduce dietary costs, since protein is considered the most expensive nutrient in beef cattle diets (Appuhamy et al., 2014), and the reduction in N excretion by meeting the ruminally degradable protein and metabolizable protein requirements of animals, without decreasing performance, has great potential to reduce the environmental impact of beef cattle production and increase economic returns for producers.

Despite this interest, to the best of our knowledge, there are limited data about metabolizable energy and protein requirements of feedlot young Nellore bulls. Therefore, this paper calls into question the need to improve estimates of metabolizable energy and protein requirements in order to accurately formulate diets to Bos indicus cattle, considering the global importance of this genetic. The objectives of this study were to evaluate the whole body chemical composition and establish the energy and protein requirements for maintenance and gain of young Nellore bulls.

## MATERIALS AND METHODS

### Animals, Experimental Design, and Treatments

The experiment was conducted at the Experimental Feedlot of the Animal Science Department at the Universidade Federal de Viçosa (**UFV**), Viçosa, Minas Gerais, Brazil. Animal care and handling followed guidelines set by the UFV (process 59/2016). Dietary CP levels were determined according to the protein requirements for Nellore bulls suggested by the BR-CORTE system (Valadares Filho et al., 2016), where 125 g CP/kg DM was established as the adequate CP concentration for bulls in this age and weight category. Therefore, we used 125 g CP/kg DM as our medium, or average, treatment.

Fifty weaned Nellore bulls, average 7 ± 1 months of age and with an average initial body weight (**BW**) of 260.0 ± 8.1 kg were used in this trial. The experiment was conducted in a completely randomized design, where the experimental units (bulls) were assigned to treatments at random, allowing every bull equal probability of receiving a treatment. 4 bulls were randomly selected as the baseline reference group, being slaughtered at the beginning of the experiment to evaluate initial body composition. 4 bulls were fed at a maintenance level (12 g DM/kg initial BW), housed in individual pens equipped with concrete feeders and received a 125 g CP/kg DM-based diet. The remaining 42 bulls were fed ad libitum and were randomly assigned to receive one of the six diets (n = 7 bulls per treatment) with different CP concentrations for 140 d, either: 1) Low (**LO**; 105 g CP/kg DM), 2) Medium (**MD**; 125 g CP/kg DM), 3) High (**HI**; 145 g CP/kg DM), 4) Low to High (**LH**; Oscillating CP concentration of 105 to 145 g CP/kg DM at a 48-h interval), 5) Low to Medium (**LM**; oscillating CP concentration of 105 to 125 g CP/kg DM at a 48-h interval), and 6) Medium to High (**MH**; Oscillating CP concentration of 125 to 145 g CP/kg DM at a 48-h interval). The chemical composition of the three diets used in this experiment is presented on Table 1. Briefly, the Low diet (105 g CP/ kg DM), provided 673.2 g RDP /kg CP, and 326.8 g RUP /kg CP; the Medium diet (125 g CP/ kg DM) provided 696.7 g RDP/kg CP, and 303.3 g RUP / kg CP; and the High diet (145 g CP / kg DM) provided 713.7 g RDP/kg CP, and 286.3 g RUP / kg CP.

Each treatment was group-housed in a feedlot pen (48.0 m$^2$) with one electronic feeder (model AF-1000Master; Intergado Ltd., Contagem, Minas Gerais, Brazil) and one electronic waterer per pen (model WD-1000 Master; Intergado Ltd.). Before the experiment, each bull was fitted with an ear tag (left ear) containing a unique radio frequency transponder (FDX- ISO 11784/11785; Allflex, Joinville, Santa Catarina, Brazil). The young bulls were allowed a 21-d acclimation period to the experimental conditions and treated for the control of internal and external parasites by administration of ivermectin (Ivomec; Merial, Paulinia, Brazil). The bulls were weighed at the beginning and end of the experiment after undergoing 16 h of fasting to measure initial and final BW, and weighed every 28 d to evaluate and monitor average daily gain (**ADG**) and BW. The diets (50:50 forage to concentrate ratio) were formulated according to the BR-CORTE system (Valadares Filho et al., 2016) to achieve an ADG of 1.1 kg, and consisted of corn silage and a concentrate that was formulated with ground corn, wheat bran, soybean meal, urea, ammonium sulfate, sodium bicarbonate, salt, and mineral mix (Table 1).

The total mixed rations were provided twice a day, at 0700 and 1600 h. Feed delivery was adjusted daily to maintain minimum refusals the next day and ad libitum intake. The appropriate feed delivery for each group was based on refusal weight every morning. Electronic feeders were evaluated at 0600 h each day to quantify orts and to adjust daily feed delivery to a maximum of 2.5% orts. According to the amount of refusals, the total mixed ration was reduced (more than 2.5% orts at morning evaluation) or increased (less than 2.5% orts at morning evaluation) to reach ad libitum intake. Each treatment was delivered to an electronic feeder that measured daily individual feed intake using the electronic identification tags (model AF-1000 Master; Intergado Ltda., Contagem, Minas Gerais, Brazil; Chizzotti et al.,2015).

### Slaughter and Samplings

The bulls were slaughtered at the end of the experimental trail, after 140 d. Prior to slaughter, all bulls were fasted from feed 16-h to estimate shrunk body weight (**SBW**). Bulls were slaughtered via captive bolt stunning followed by exsanguination. The gastrointestinal tract contents (i.e., rumen, reticulum, omasum, abomasum, and small and large intestines) were removed and washed. The weights of the heart, lungs, liver, spleen, kidneys, KPH fat, diaphragm, mesentery, tails, trimmings, and the washed gastrointestinal tract were added to the other parts of the body (i.e., carcass, head, hide, limbs, and blood) to determine empty body weight (**EBW**). The rumen, reticulum, omasum, abomasum, small and large intestines, KPH fat, mesentery, liver, heart, kidneys, lungs, tongue, spleen, diaphragm, esophagus, trachea, and reproductive tract were ground for 20 min using an industrial cutter to create a homogeneous sample of organs + viscera. The head and all limbs, after removal of the hide, were ground by using a grinding machine (LUNASA, TOL10 model, Araguari, Brazil) to reduce the size of the bones. The hide was manually chopped and sampled. A sample of blood was obtained during the course of bleeding.

After slaughter, the carcass of each bull was separated into two halves that were chilled at 4ºC for 18 h. Next, half-carcasses were removed from the cold chamber for weighing and the hot and cold carcass yields were calculated. Subcutaneous fat thickness was measured using a digital caliper in the region between 11th and 12th rib cut. The section between the 9th and 11th ribs was collected from the left half-carcass according to procedures described by Hankins and Howe (1946). This section was dissected into muscle, fat, and bone, and each portion was weighed separately. The muscle and fat from the section between the 9th and 11th ribs of each bull were homogenized and ground in order to obtain a composite sample of muscle and fat. Bones from the same rib section were sliced with a band saw (Skymsen, model SFL-315HD, Santa Catarina, Brazil) in subsections of 1.5-cm length to obtain a representative sample of the bones. The composite sample of muscle and fat and the sample of rib bones were lyophilized and then were ground in a knife mill (Fortinox, Piracicaba, São Paulo, Brazil) with a 1-mm mesh sieve to evaluate the dry matter (**DM**), organic matter (**OM**), N, and ether extract (**EE**) contents.

## Laboratory Analysis

All samples (blood, organs and viscera, head and limbs, hide, muscle and fat, and bones) were quantified in terms of DM, N, and EE according to the AOAC (AOAC, 2012; methods number 934.01, and 981.10 and AOAC, 2006; method number 945.16, respectively). The moisture content was assessed by drying the samples at 105 °C in a hot-air oven until constant weight was achieved. The crude protein content (N x 6.25) was determined by the Kjeldahl method (Jacobs, 1951), and the assay was comprised of acid digestion and alkali distillation with an auto Kjeldahl System (2200 Kjeltech auto distillation; Foss Tecator, Hoganas Sweden) followed by titration. The ether extract was estimated by the solvent extraction method (Socsplus, SCS-08-As, Pelican equipment, Chennai, India) and the assay was comprised of extraction of lipid with an organic solvent (petroleum ether) at 40-60 °C temperature using a soxhlet apparatus. Digestible energy (**DE**) intake was obtained by multiplying digestible nutrients by their respective energy values (NRC, 2001): $DE = (5.6 \times CPI_{digestible}) + (9.4 \times EEI_{digestible}) + (4.2 \times NDFI_{digestible}) + (4.2 \times NFCI_{digestible})$, where the respective digestibility values can be find in Menezes et al. (2019; submitted). The concentration of metabolizable energy intake (**MEI**) was estimated according to the following equation, $MEI = (0.9455 \times DE) - 0.3032$, as proposed by Detmann et al. (2016).

## Calculations

Empty body chemical composition was estimated using the equations described by Marcondes et al. (2012) for Nellore bulls, which were validated by Costa e Silva et al. (2013a):

$$\text{Crude protein (\%)}: CP_{EBW} = 10.78 + 0.47 \times \%\ CP_{Cor} - 0.21 \times \%\ VF,$$

$$\text{Ether extract (\%)}: EE_{EBW} = 2.75 + 0.33 \times \%\ EE_{Cor} + 1.80 \times \%\ VF, \text{ and}$$

$$\text{Water (\%)}: W_{EBW} = 38.31 + 0.33 \times \%\ W_{Cor} - 1.09 \times \%\ VF + 0.50 \times \%\ OV$$

where $CP_{Cor}$ = % CP in the 9th to 11th rib section; EBW = empty body weight; VF = % visceral fat (renal, pelvic, cardiac, and mesenteric fat) per unit of EBW; $EE_{Cor}$ = % EE in the 9th to 11th rib section; WCor = % water in the 9th to 11th rib section; and OV = % organs and viscera per unit of EBW.

Models were generated from the EBW and body compositions of all animals utilized in this study. For water and CP, the models utilized were as follows: $Ci = a \times EBW^{b}$, where Ci is the i body component of the bull, which is the water or CP content in the empty body weight (kg), and a and b are the regression parameters. The EE content in the EBW was estimated by the exponential model: $Ci = a \times e^{(b \times EBW)}$, in which Ci is the i body component of the bull, which was EE in the empty body weight (kg) and e is the Euler number.

The relationship between SBW and EBW was calculated for all bulls to convert SBW to EBW, while the relationship between the ADG and empty body gain (**EBG**) was calculated to convert ADG to EBG. Body energy content was obtained from body protein and fat contents and their respective caloric equivalents were determined according to the ARC (1980):

$$\text{Energy content (Mcal)} = 5.6405 \times \text{body protein (kg)} + 9.3929 \times \text{body fat (kg)}$$

The heat production was calculated based on the difference between MEI and energy content in the body, by using the equation above. Then, the net energy requirement for maintenance ($Mcal/EBW^{0.75}/d$) was estimated to be the intercept (β0) of the exponential regression between heat production (**HP**) and MEI. The following model was utilized:

$$HP = \beta 0 \times e^{(\beta 1 \times MEI)},$$

where HP = heat production (Mcal/EBW$^{0.75}$/d), MEI = metabolizable energy intake (MJ/EBW$^{0.75}$/d), β0 and β1 are regression parameters, and 'e' is the Euler number (2.718281). The ME requirement for maintenance (**MEm**, Mcal/EBW$^{0.75}$/d) was estimated by the iterative method, with MEm considered to be the value where MEI equals HP.

The efficiency of ME utilization for maintenance was calculated as the ratio between the net energy and ME for maintenance. The net energy requirement for growth (**NEg**) was estimated from the regression between NEg, EBG, and metabolic EBW by using the following model:

$$NEg = a \times EBW^{0.75} \times EBG^{b},$$

where NEg = the net energy for growth represented as the energy retained in the body (Mcal/d), EBW$^{0.75}$ = metabolic empty body weight, and EBG = empty body gain (kg/d). Metabolizable protein for maintenance was obtained based on the linear regression between metabolizable protein intake and empty body gain (EBG) divided by average EBW$^{0.75}$, while the net protein requirement for growth (**NPg**) was estimated by a model involving EBG and retained energy in the body:

$$NPg = \beta_1 \times EBG - \beta_2 \times RE,$$

where NPg = retained protein or the net protein requirement for growth (g/d), EBG = empty body gain (kg/d), RE = retained energy (Mcal/d), and b1 and b2 are regression parameters. The metabolizable protein for gain was estimated by dividing the NPg by the efficiency of metabolizable protein utilization for growth, according to the equation proposed by Valadares Filho et al. (2016).

**Statistical Analyses**

Statistical procedures were performed using SAS (SAS Inst. Inc., Cary, NC, USA). Data were analyzed in a completely randomized design, with the bull being the experimental unit. Data of reference and maintenance animals were utilized to estimate energy and protein requirements for young Nellore bulls. A linear regression model between metabolizable protein intake and EBG was analyzed to estimate the net protein requirements for maintenance by using PROC REG (SAS Inst. Inc., Cary, NC). To estimate the net protein requirements for gain and the net energy requirement for maintenance and gain, data were analyzed using non-linear models through PROC NLIN (SAS Inst. Inc., Cary, NC) and were adjusted by the Gauss–Newton method.

## RESULTS AND DISCUSSION

### Body composition

During the course of this study, the bulls had changes in their body composition (Fig. 1). Thus, it became necessary to evaluate growth composition throughout the experiment. The growth composition data were used to develop equations to estimate the chemical body composition of young Nellore bulls from their EBW (Table 2).

Crude protein and water content increased with increasing EBW (Fig. 1). This is evidenced by the respective equations coefficient, close to 1, as reported in Table 2. On the other hand, EE content increased as the animal reached maturity, indicating that the animal starts to deposit more adipose tissue in proportion to the other tissues thereby resulting in an exponential equation of EE content in the EBW. Costa e Silva et al (2013b) reported that the chemical composition of growing and finishing Nellore bulls specifically the CP, EE, and mineral content, presented a similar pattern of deposition as that of muscle, adipose, and bone tissues, respectively. This similarity in deposition can be explained because these are the prevailing components of each corresponding tissue. Additionally, according to Robelin and Geay (1984), the chemical composition of skeletal muscle tissue varies during animal growth, in which, after birth, protein content increases greatly and then remains constant, and afterward fat increases. The data of the current study agree with the results of Costa e Silva et al. (2013b) and Robelin and Geay (1984).

### Relationship Between EBW and SBW, and EBG and ADG

Empty body weight is the most accurate index of energy and nutrient content in the body (Owens et al., 1995). According to Gionbelli et al. (2016), there is a gain in precision and accuracy with the use of a variable EBG/ADG ratio, obtained from the nonlinear model. The equation obtained for the ratio between EBW and SBW was EBW (kg) = $1.3620 \times \mathrm{SBW}^{0.9365}$ (kg). For conversion of ADG in EBG, this study found: EBG = $1.0119 \times \mathrm{ADG}^{0.8315}$. Pacheco (2018) described the relationship between EBG and ADG of young Nellore bulls as EBG = $1.0120 \times \mathrm{ADG}^{0.8299}$, where 1.020 is the EBG/ADG ratio, value similar to 1.0119 found in this study.

Considering a 450 kg Nellore bull gaining 1.2 kg/d raised in a feedlot, the EBW and EBG estimated by these equations were 415.83 kg and 1.18 kg/d, respectively. While the values estimated by the following equations proposed by Valadares Filho et al. (2016), EBW = $0.8126 \times \mathrm{SBW}^{1.0134}$ and EBG = $0.963 \times \mathrm{ADG}^{1.0151}$ were 396.86 kg and 1.16 kg/d. These data suggest a reduction in the contribution of intestinal fill (gastrointestinal tract contents) in this study compared to Valadares Filho et al. (2016). This reduction can be explained by dietary characteristics, or the proportion of concentrate, which reduces the dry matter intake and consequently increases the EBW:SBW ratio. Considering the following equations proposed by NASEM (2016), EBW = 0.891

× SBW and EBG = 0.956 × ADG and the same animal used in the previous example, the estimated EBW and EBG were 400.95 kg and 1.14 kg/d, respectively.

For the current study, the quantity of digesta (g/kg SBW) was compared with SBW at the time of slaughter (Fig. 2), and the carcass and non-carcass components (blood, organs and viscera, head and limbs, and hide) were compared with the SBW for each bull (Fig. 3). The equations obtained are reported in Table 3. As SBW increases, there is a decrease in the digesta content that is represented by the negative exponent linked to SBW, which results from a reduction in the contribution of intestinal fill, as suggested in the current study by the ratio obtained between EBG and ADG. The correlation coefficient among carcass and SBW was greater than 1.00 and the regression equation had high determination coefficient ($R^2$ = 98.95); thus, with increases in SBW, there is a greater proportion of the carcass component. The equation derived between non-carcass components and SBW additionally shows a positive relationship between these variables (correlation coefficient = 0.9539 and $R^2$ = 94.98). At this point, these correlations have been scarcely studied, so no comparisons could be made with previous studies.

## Energy Requirements

Energy requirements can be estimated by long-term feeding experiments, respirometric techniques, and comparative slaughter. This trial used the comparative slaughter method where we measured directly the MEI and RE, and heat production (HP) was determined as the difference between the other variables. The NEm can be understood as total heat production of the animal in a state of absolute fasting (Valadares Filho et al., 2016). For this experiment, the relationship between HP and MEI was described by the following equation: $HP = 0.077 \times e^{(3.7992 \times MEI)}$ (Fig.

4) where NEm was estimated as 77 Kcal/kg EBW$^{0.75}$/d for young Nellore bulls. This estimated value was similar to the 74.9 Kcal/kg EBW$^{0.75}$/d proposed by Valadares Filho et al. (2016) for Nellore bulls. Maintenance energy expenditures vary with BW, breed or genotype, sex, age, season, temperature, physiological state, and previous nutrition (NASEM, 2016).

The use of the value found for NEm is limited and has no practical application in diet formulation because producing animals are not found in a fasting state. Therefore, the maintenance requirement was calculated in a more applicable form, as metabolizable energy. The metabolizable energy requirement for maintenance (**MEm**) was 122.75 Kcal/EBW$^{0.75}$/d, this value was considered the point where heat production and metabolizable energy intake are equal, and was obtained by applying an iterative process to the exponential model of heat production as a function of the metabolizable energy intake. This value was superior to the 107 and 106.79 kcal/EBW$^{0.75}$/d reported by Prados (2016) and Pacheco (2018) who also worked with growing Nellore bulls. A possible explanation for the high MEm value in the present study is that the average final SBW of the animals (444.64 kg) was superior than the values reported by Prados (2016) and Pacheco (2018), 397.85 and 440.0, respectively. Additionally, Prados (2016) worked with a 40:60 sugar-cane:concentrate ratio, and Pacheco with 28:72 corn silage:concentrate ratio, while in this study the forage:concentrate ratio was 50:50, thus, dietary composition and proportion of concentrate can contributed to the high MEm of the Nellore bulls in the present study.

The efficiency of utilization of metabolizable energy for maintenance was estimated from the NEm and MEm ratio, resulting in 62.7%. The efficiency can be affected by several factors, for instance sex, genetic group, age, environment and the metabolizable energy concentration in the diet (AFRC, 1993; NRC, 2000; CSIRO, 2007). In addition, there is strong evidence that the efficiency of utilization of metabolizable energy for maintenance is also affected by characteristics linked to animal performance, such as rate of weight gain and feed intake (Williams and Jenkins, 2003; Marcondes et al., 2010).

The net energy requirement for gain is defined as all the energy that is retained in the empty body weight of the animals in the form of protein or fat (Garrett et al., 1959). Therefore, what determines the composition of the empty body gain is the weight relative to the weight at maturity of the animal (NASEM, 2016). In this experiment, the NEg (daily Mcal/$EBW^{0.75}$) was estimated by the following equation: $RE = 0.0535 \times EBW^{0.75} \times EBG^{0.7131}$, where RE = retained energy or net energy requirement for weight gain (Mcal/d), EBW = empty body weight (kg), EBG = empty body gain (kg/d).

The value of the efficiency of the use of ME for gain was obtained based on the linear regression between RE and MEI, and this value was 24.25% (Fig. 5). The efficiency for gain depends on the proportions of energy retained in form of protein and fat (Costa e Silva et al., 2012). In this study, the proportion of energy retained as protein (**REp**) was 0.2265, and this value was calculated according to the following model proposed by Marcondes et al. (2013): $REp = 1.140 \times (RE/EBG)^{-1,137}$. According to the same author, the efficiency for gain and EBG were the most important variables that affected km.

## Protein Requirements

Metabolizable protein for maintenance was 3.83 g/$SBW^{0.75}$. This value was obtained based on a linear regression between metabolizable protein intake and EBG divided by the average metabolic EBW (Fig. 6). This value is similar to the 3.6 and 3.8g/$SBW^{0.75}$ suggested by the BR-CORTE system (Valadares Filho et al., 2016) and NASEM (2016), respectively.

Net protein for gain (g/d) was estimated by the following equation: NPg = 227.372 × EBG – 19.479 × RE, while the model proposed by the BR-CORTE system (Valadares Filho et al., 2016) is NPg = 210.09 × EBG – 10.01 × RE. The NASEM (2016) adopted the following equation to estimate net protein for gain: NPg = SWG × {268 – [29.4 × (RE/SWG)]}. Considering a 400 kg Nellore bull with SWG of 1 kg/d, EBG of 0.963 kg/d, and RE of 4.69 Mcal/d in a feedlot system, the net protein requirements for gain are 128, 155, and 130 g/d according to this study, Valadares Filho et al. (2016), and NASEM (2016), respectively. The protein required for animal growth depends on body composition (Boin, 1995), thus, protein requirements vary based on mature size, sex, and nutrition. The net protein requirements for gain are lower for bulls that are late maturing rather than early maturing because bulls deposit more lean tissue than steers (Vanderwert et al., 1985). The efficiency of the use of metabolizable protein for gain was 24.43%, which was obtained based on the linear regression between RP and MPI (Fig. 7). Several factors such as age, body composition, and feeding condition can affect the efficiency of the use of protein for growth (Marcondes et al., 2013).

There is a constant need for updating nutrient requirements aiming to reduce nutrient excretion, decrease production costs, and improve performance at the same time. So, improving the nutritional requirements of the Brazilian national herd is best accomplished by offering Brazilian producers technology that is generated under Brazilian conditions. Additionally, the present study provides useful information about nutritional requirements to help diet formulation in tropical countries, where predominantly Bos indicus cattle are used for meat production. The current data partially agrees with previously published nutrient requirements; however, improvements in estimates of energy and protein requirements for Nellore bulls contained herein should be consider in production settings using similar animals and considered by the next committee assessing energy and protein requirements for Nellore bulls in Brazilian production scenarios.

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**Table 1.** Proportion of ingredients and nutrient composition of the experimental diets

| Item | Experimental Diets[1] | | |
|---|---|---|---|
| | Low | Medium | High |
| Proportion | | | |
| Corn Silage | 50.0 | 50.0 | 50.0 |
| Ground corn | 39.4 | 39.4 | 39.4 |
| Soybean meal | 2.38 | 4.92 | 7.46 |
| Wheat bran | 6.09 | 3.05 | 0.00 |
| Urea | 0.47 | 0.98 | 1.49 |
| Salt | 0.30 | 0.30 | 0.30 |
| Limestone | 0.06 | 0.06 | 0.06 |
| Mineral mix[2] | 0.29 | 0.29 | 0.29 |
| Sodium bicarbonate | 0.75 | 0.75 | 0.75 |
| Magnesium oxide | 0.25 | 0.25 | 0.25 |
| Total | 100 | 100 | 100 |
| Chemical composition | | | |
| Dry matter, g/kg as-fed | 406.0 | 406.1 | 406.2 |
| Organic matter, g/kg DM | 944.1 | 943.8 | 943.5 |
| Crude protein, g/kg DM | 102.7 | 122.3 | 141.9 |
| Rumen degradable protein, g/kg CP | 673.2 | 696.7 | 713.7 |
| Rumen undegradable protein, g/kg CP | 326.8 | 303.3 | 286.3 |
| Ether extract, g/kg DM | 42.8 | 42.1 | 41.4 |
| Neutral detergent fiber, g/kg DM | 321.3 | 314.2 | 307.1 |
| Indigestible neutral detergent fiber, g/kg DM | 98.09 | 95.7 | 93.4 |
| Non-fiber carbohydrates, g/kg DM | 480.6 | 476.2 | 471.8 |

[1] Low = 105 g CP/kg DM; Medium = 125 g CP/kg DM; High =145 g CP/kg DM [2] Mineral mix = 7.83 g S/kg; 5,950 mg Co/kg; 10,790 mg Cu/kg; 1,000 mg Mn/kg; 1,940 mg Se/kg; 1,767.4 mg Zn/kg.

**Table 2.** Equations to estimate chemical body composition from empty body weight (EBW)

| Item | Equation | $R^2$ |
|---|---|---|
| Crude Protein, kg | $0.3413 \times EBW^{0.8882}$ | 95.56 |
| Ether Extract, kg | $16.2886 \times e^{(0.0041 \times EBW)}$ | 90.44 |
| Water, kg | $1.0058 \times EBW^{0.9017}$ | 98.86 |

**Table 3.** Equations to estimate the contribution of the carcass, non-carcass and gastrointestinal content from shrunk body weight (SBW)

| Item[1] | Equation | $R^2$ |
|---|---|---|
| Carcass, kg | $0.3821 \times SBW^{1.0753}$ | 98.95 |
| Non-carcass, kg | $0.4213 \times SBW^{0.9539}$ | 94.98 |
| Gastrointestinal content, g/kg SBW | $2228.4 \times SBW^{-0.5537}$ | 35.65 |

[1] Carcass = muscle, fat, and bones; Non-carcass = blood, organs and viscera, head, limbs, and hide

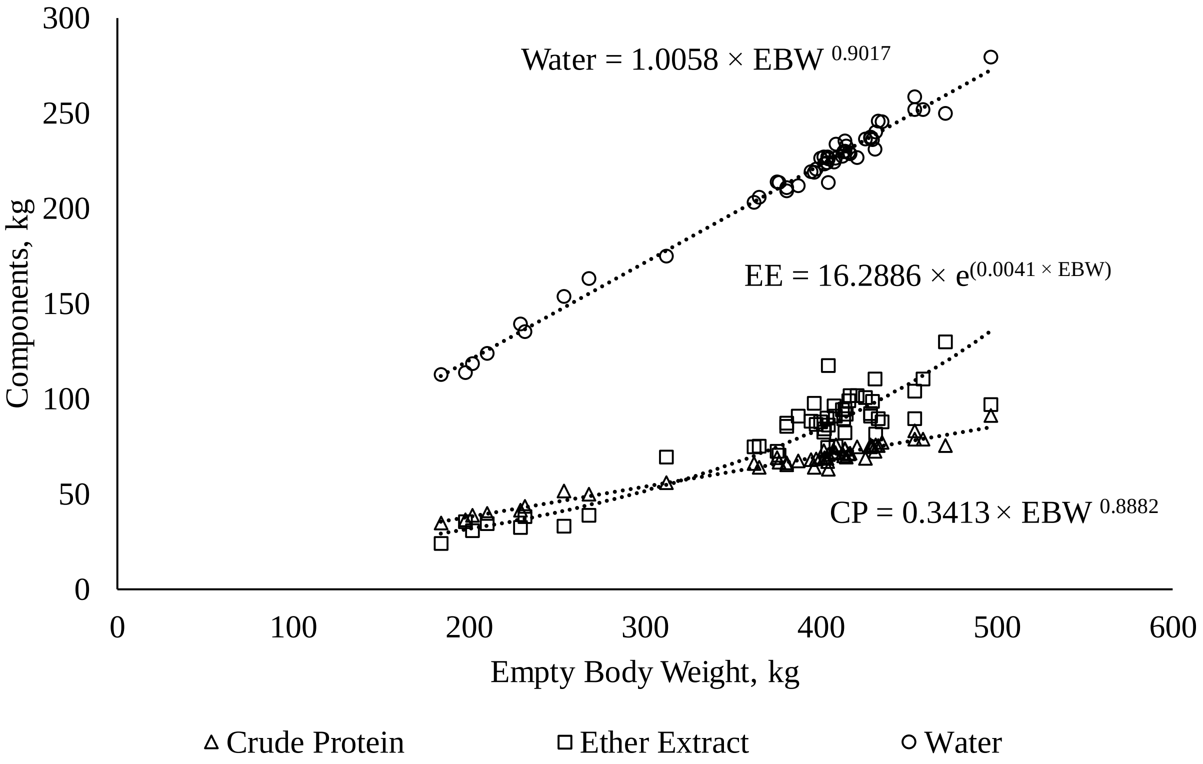

**Figure 1.** Relationship between the amount of crude protein (CP), ether extract (EE), and water and empty body weight (EBW) of young Nellore bulls.

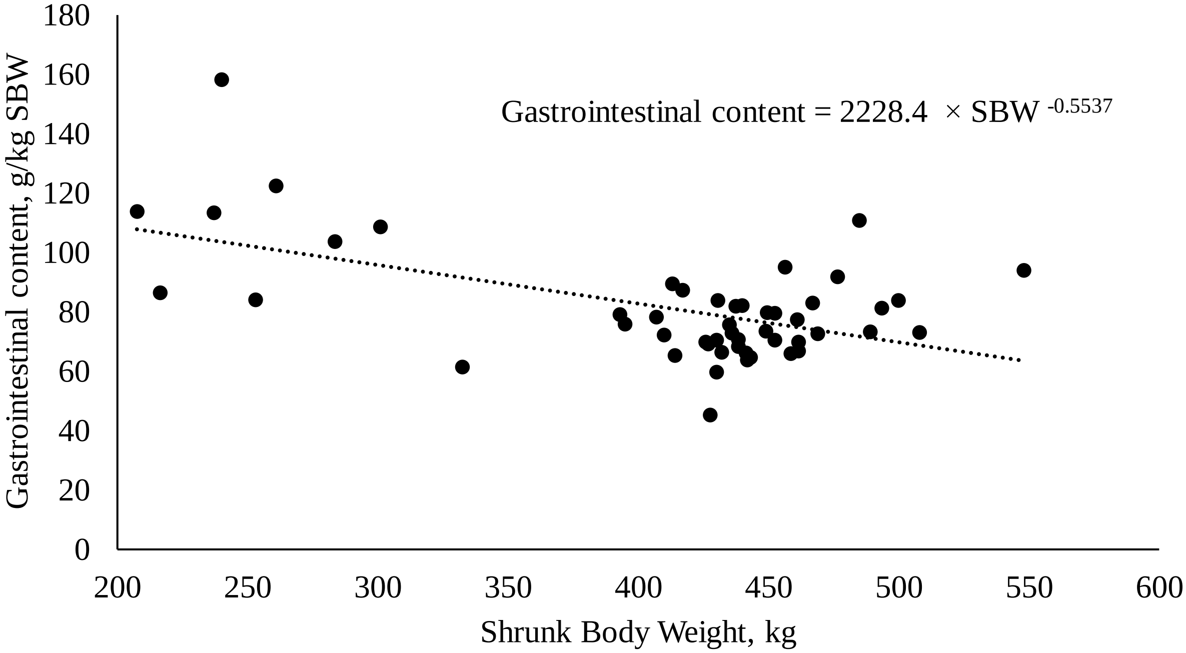

**Figure 2.** Relationship between the gastrointestinal content and shrunk body weight (SBW) of young Nellore bulls.

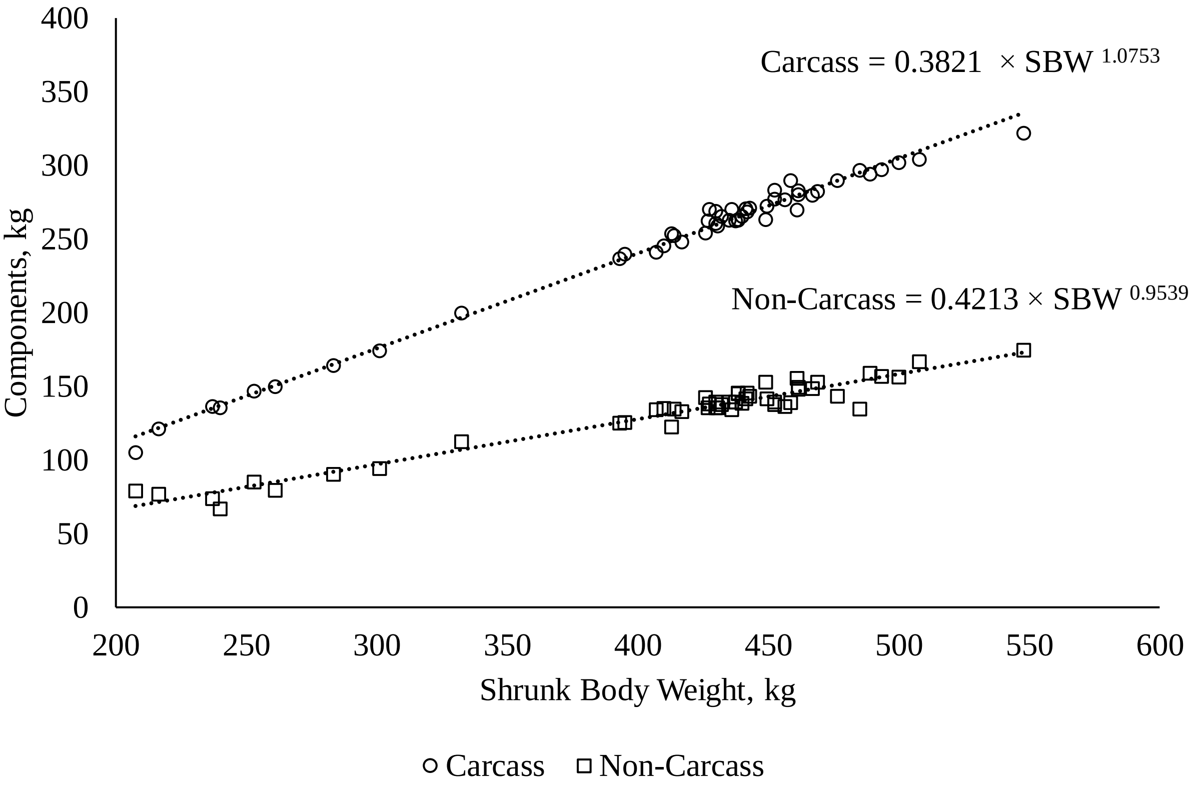

**Figure 3.** Relationship between the carcass and non-carcass components and shrunk body weight (SBW) of young Nellore bulls.

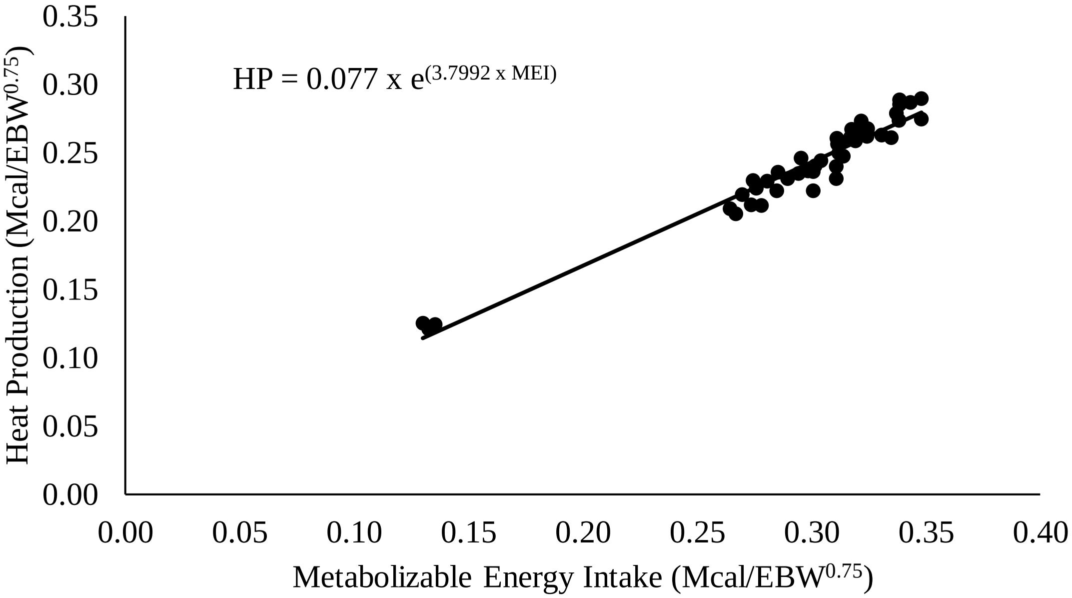

**Figure 4.** Relationship between heat production (HP) and metabolizable energy intake (MEI) of young Nellore bulls.

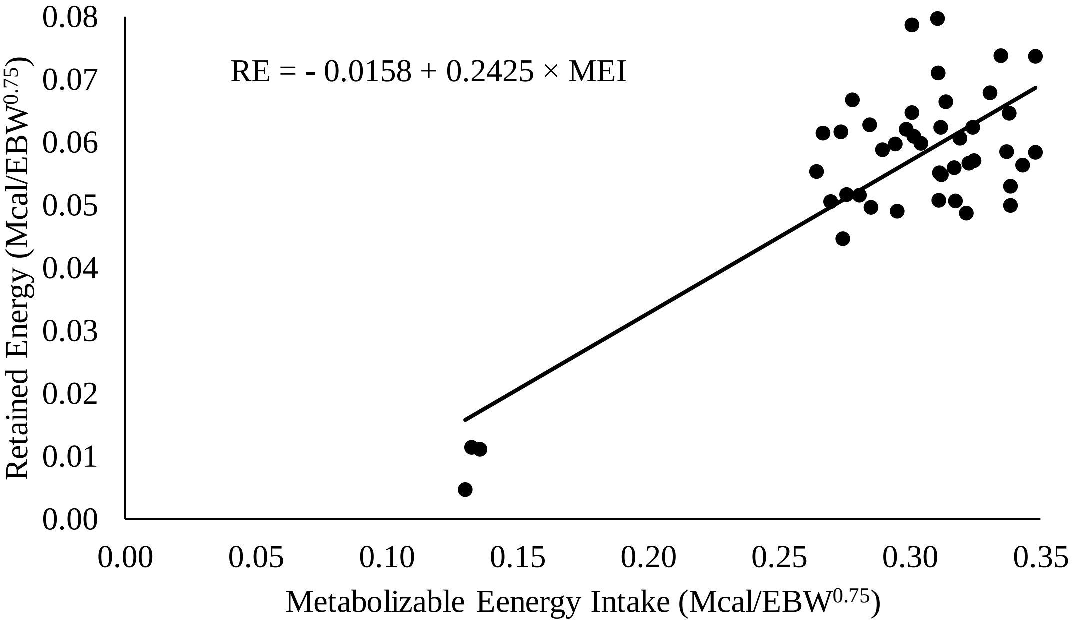

**Figure 5.** Relationship between retained energy (RE) and metabolizable energy intake (MEI) of young Nellore bulls.

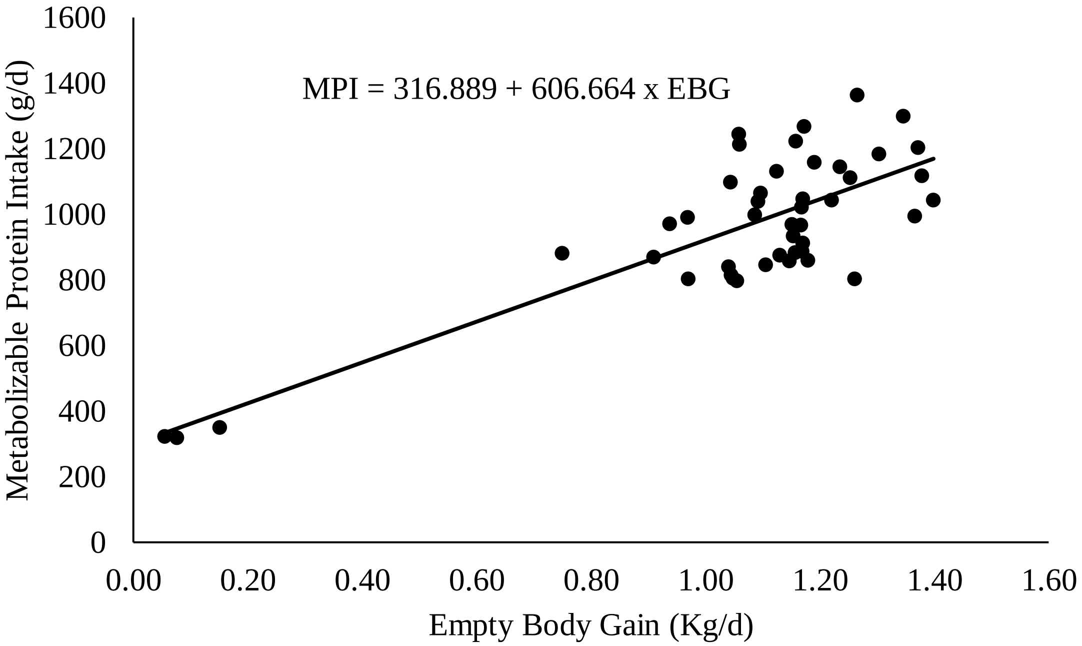

**Figure 6.** Relationship between metabolizable protein intake (MPI) and empty body gain (EBG) of young Nellore bulls.

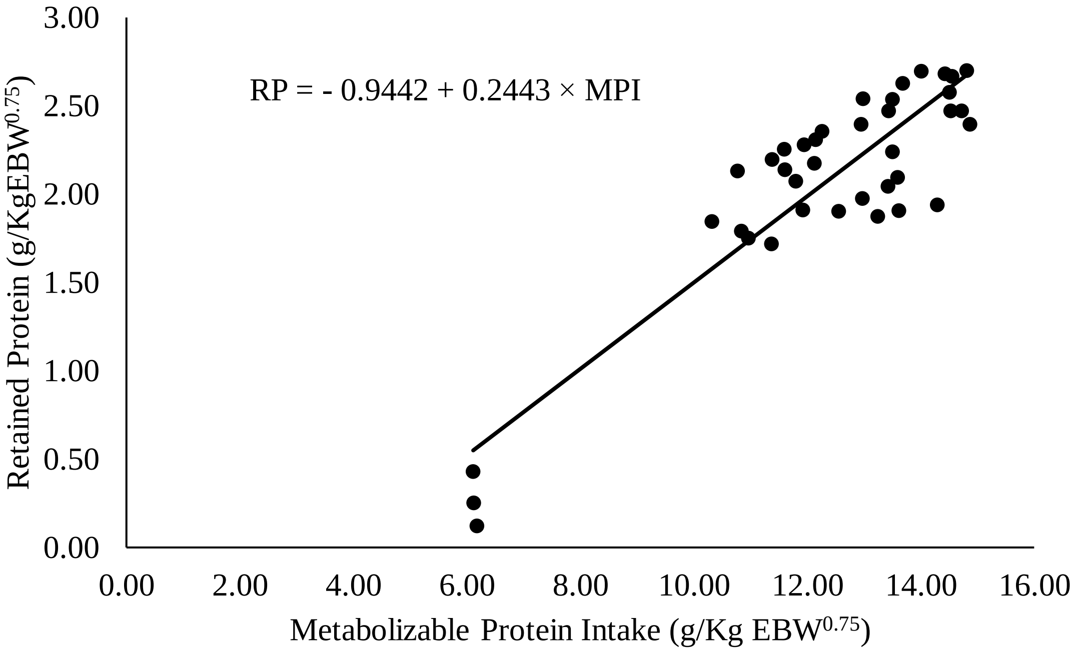

**Figure 7.** Relationship between retained protein (RP) and metabolizable protein intake (MPI) of young Nellore bulls.

# 4 CHAPTER 3

Running head: Feeding behavior and maintenance of RFI bulls

## Feeding behavior, water intake, and energy and protein requirements of young Nellore bulls with different residual feed intakes [1]

[1] This study was made possible by grants from CNPq-INCT/Ciência Animal, FAPEMIG and Capes.

[2] Corresponding author: ana.menezes@ufv.br

**ABSTRACT:** This study aimed to determine feeding behavior, water intake, and energy requirements of high and low residual feed intake (**RFI**) Nellore bulls. Data were collected from forty-two weaned Nellore bulls (initial BW 260 ± 8.1 kg; age 7 ± 1.0 mo) housed in a feedlot in group pens that contained electronic feeders, waterers, and a scale connected to the waterers. The individual dry matter intake (**DMI**), water intake (**WI**) and body weight (**BW**) were recorded daily. The indexes of average daily gain (**ADG**), feed efficiency (gain to feed ratio), and residual feed intake (**RFI**) were calculated based on data collected. The number of feeder and waterer visits, and the time spent feeding or drinking water per animal per day were recorded as feeding behavior measures. Energy requirements for maintenance and gain were calculated according to the BR-CORTE system. Low RFI bulls had lower DMI ($P < 0.01$) than high RFI bulls, and no differences ($P > 0.05$) were observed between the two groups regarding water intake, performance and feeding behavior measurements. The net energy requirements for maintenance, metabolizable energy for maintenance, and efficiency of metabolizable energy utilization were 63.4, 98.6 kcal/EBW$^{0.75}$/d, and 64.3%, respectively for low RFI bulls, and 78.1, 123.9 kcal/EBW$^{0.75}$/d, and 63.0% for high RFI bulls. The equations obtained for net energy for gain (**NEg**) were: NEg (Mcal/EBW$^{0.75}$/d) = $0.0528 \times EBW^{0.75} \times EBG^{0.5459}$ for low RFI, and $0.054 \times EBW^{0.75} \times EBG^{0.8618}$ for high RFI bulls, where EBG is the empty body gain. We did not observe any difference ($P > 0.05$) regarding the composition of gain in terms of protein or fat deposition between the two groups. Both groups presented also similar ($P > 0.05$) carcass and non-carcass traits. Therefore, our study shows that low RFI Nellore bulls eat less, grow at a similar rate, and have lower maintenance energy requirements than high RFI bulls. We also suggest that the lower feed intake did not compromises the carcass traits of more efficient animals, which would reduce production costs and increase the competitiveness of the Brazilian beef sector on the world market.

**Key words:** feeding behavior, maintenance, Nellore, residual feed intake, water intake

# INTRODUCTION

The residual feed intake (**RFI**) has increasingly become the measure of choice for studying physiological mechanisms underlying variation in feed efficiency in beef cattle (Berry and Crowley, 2013), since it is conceptually independent of growth and body size (Kenny et al., 2018). Cantalapiedra-Hijar et al. (2018) suggestthat the main physiological mechanisms identified and related to RFI are feeding behavior, feed digestibility, tissue metabolism, and heat increment. Feeding and digestive-related mechanisms could be associated with RFI mainly because they co-vary with feed intake, while metabolic-related mechanisms such as protein turnover affect heat production leading to the belief that efficient animals have a significantly lower energy metabolic rate. Regarding body composition, even though this is an important economic trait, the limited published literature examining the protein and fat deposition to variation in RFI is equivocal.

In addition to its economic importance, the body content of muscle and fat tissues make a significant contribution to overall energy status. Recent published data of Fitzsimons et al. (2017) and Kenny et al. (2018), highlight the potential contribution of differences in energy utilization relating to composition, maintenance and metabolic processes within muscle and adipose tissue depots to variations for the RFI trait. However, the meta-analysis conducted by Kenny et al. (2018) found no statistically significant differences in ultrasonically measured back fat nor carcass measures between growing beef cattle of high- or low-RFI status. In contrast, Berry and Crowley (2013) reported a genetically based tendency for RFI status to be negatively correlated with muscularity and positively associated with body fat in the live animal or carcass, while a literature review conducted by Cantalapiedra-Hijar et al. (2018) show that low-RFI steers often have leaner carcasses. Thus, the linkage between body composition and RFI status has not been conclusively determined.

Another relevant question for the beef industry is the beef cattle water demand, and if it could be associated with RFI since water intake (**WI**) is strongly correlated with dry matter intake. Studies (Ahlberg et al., 2018 and Zanetti et al., 2019) show a positive relationship between WI and DMI, which suggests that those two variables can co-vary. However, to the best of our knowledge, there are no studies evaluating the possible relation between water intake and RFI classes. Therefore, this study aimed at understanding the feeding behavior, water intake, energy and protein requirements of high and low residual feed intake Nellore bulls. We hypothesized that low RFI bulls 1) visit less the feeder and spent less time eating; 2) drink less water; 3) have lower maintenance and gain requirements; and 4) deposit more fat than high RFI bulls.

## MATERIALS AND METHODS

Data were collected from an experiment (Menezes et al., 2019a) conducted at the Experimental Feedlot of the Animal Science Department at Federal University of Viçosa (**UFV**), Viçosa, Minas Gerais, Brazil, and followed the recommendations of the Ethics Committee for Animal Use and Care of UFV (protocol number 59/2016). The experiment was performed using 42 weaned Nellore bulls (initial BW 260 ± 8.1 kg; age 7 ± 1.0 mo) group-housed in a feedlot pen (48.0 $m^2$). The bulls were fed ad libitum and randomly assigned to receive one of six diets with different CP concentrations for 140 d: 105 (**LO**), 125 (**MD**), or 145 g CP /kg DM (**HI**), and LO to

HI (**LH**), LO to MD (**LM**), or MD to HI (**MH**) oscillating CP at a 48-h interval for each feed. The diets were made up by corn silage with inclusion of 500 g/kg concentrate on DM basis. The concentrate composition and dietary nutrients of the diets are given in Table 1. As presented by Menezes et al. (2019a) the dietary treatments did not affect the DM intake or the performance of the animals, therefore in this study there was no possible confounding effects regarding the dietary component. All the animals were slaughtered at the end of the experimental period, and the slaughter procedures are described by (Menezes et al., 2019b).

### Feeding Behavior and Water Intake Measurement

To determine feed and water intake the bulls were housed in a feedlot with 6 group pens, with 7 bulls per pen. Each pen was equipped with one electronic feeder (Model AF-1000 Master, Intergado Ltd., Contagem, Minas Gerais, BRA) and one electronic waterer (Model WD-1000 Master, Intergado Ltd., Contagem, Minas Gerais, BRA). Before the experiment, each bull was fitted with an ear tag in the left ear containing a unique radio frequency transponder (FDX- ISO 11784/11785; Allflex, Joinville, Santa Catarina, Brazil). The bulls were allowed a 21-d adaptation period to the experimental conditions and treated against internal and external parasites by administration of injectable ivermectin (Ivomec; Merial, Paulinia, Brazil).

The Intergado® system was validated by Oliveira et al. (2017) as a useful tool for monitoring feeding and drinking behavior as well as water and feed intakes in young cattle housed in groups. Therefore, feeding behavior was monitored automatically using the electronic feeders, which recorded every time each animal entered the feeder providing the number and the duration of feeding events per bull per day. Feeding events were then refined by eliminating visits in which no feed was consumed. The feeders measured the weight of feed consumed during each visit. The daily feed intake was multiplied-by the percentage of DM of the diet to calculate the DMI. The water intake was measured daily using electronic waterers, which also recorded the time, number and duration of waterers visits. All the waterers were connected to a scale, allowing the voluntary weighing of the animals at each drinking event, thus the electronic system generated the average BW per animal per day, and the average daily gain of each bull.

## Residual feed intake

The residual feed intake (**RFI**) was calculated as the difference between the observed intake measured during the experiment and the intake predicted by the regression using the following model:

$$DMI = a + b \times ADG + c \times BW^{0.75} + \varepsilon$$

Where ADG is the average of daily gain, $BW^{0.75}$ is the average of metabolic body weight, a, b and c are parameters of the regression, and $\varepsilon$ is the error term defined as RFI. From the 42 animals, we obtained initially 27 negative values for RFI, and 15 positive values. Then, to classify half of the animals in each category, we adopted the following criteria: Low RFI from -2.585 to -0.093 kg/d, representing efficient animals, and high RFI (-0.075 a 1.013) representing inefficient animals.

## Energy and Protein Requirements

Net energy requirements for maintenance (**NEm**) were obtained using a non-linear exponential model between heat production (**HP**) and metabolizable energy intake (**MEI**). The model used was $HP = \beta 0 \times e^{(\beta 1 \times MEI)}$, where $\beta 0$ and $\beta 1$ are regression parameters, and e is Euler's number. Under this model, $\beta 0$ represents the NEm (Mcal/$EBW^{0.75}$/d). The metabolizable energy for maintenance (**MEm**, in Mcal/$EBW^{0.75}$/d) was determined by the iterative method, when MEI equaled HP. The efficiency of utilization of metabolizable energy for maintenance (**km**) was obtained from the relation between the net and metabolizable energies for maintenance. The net energy requirement for growth (**NEg**) was estimated from the regression between NEg, empty body gain (**EBG**), and metabolic empty body weight EBW (**$EBW^{0.75}$**) by using the following model: NEg (Mcal/d) = $a \times EBW^{0.75} \times EBG^{b}$. While the net protein requirement for growth (**NPg**) was estimated by a model involving EBG and retained energy (**RE**) in the body: $NPg = \beta1 \times EBG - \beta2 \times RE$.

## Chemical body composition

To estimate the body composition, equations were generated from EBW and the body chemical composition of the bulls. For crude protein (**CP**), the model utilized was as follows: $Ci = a \times EBW^{b}$, where Ci is the i body component of the bull, which is the CP content in the empty body weight (kg), and a and b are the regression parameters. The EE content in the EBW was estimated by the exponential model: $Ci = a \times e^{(b \times EBW)}$, in which Ci is the i body component of the bull, which was EE in the empty body weight (kg) and e is the Euler number. Those models were adopted considering the fact that the body deposition of fat increases exponentially as an animal matures, whereas body deposition of protein tends to plateau (Tedeschi., 2019).

## Statistical Analyses

Statistical procedures were performed using SAS (SAS Inst. Inc., Cary, NC, USA). Data were analyzed in a completely randomized design, with the bull being the experimental unit. The MIXED procedure of SAS was used to perform the statistical analyses of performance, intake, and feeding behavior variables. The ANOVA was weighted by the inverse of treatment variances in order to adjust for possible heterogeneity of variances. Tukey multiple comparison test was applied to compare RFI groups. To estimate the net energy requirement for maintenance and gain, and the net protein requirements for gain, data were analyzed using non-linear models through PROC NLIN (SAS). These models were fitted by the Gauss–Newton method. All analysis were performed under a significance level of 0.05.

## RESULTS AND DISCUSSION

### Feeding Behavior, Water Intake and Performance

Performance, dry matter and water intake, and feeding behavior data are presented in Table 2. No differences were observed between the two RFI groups regarding ADG ($P = 0.89$), G:F ratio ($P = 0.18$), and final body weight ($P = 0.86$). Low RFI bulls had lower DMI ($P < 0.01$) than high RFI bulls, expressed in kg/d or as a percentage of BW, but both groups had similar water intake ($P = 0.95$). No differences in feeding behavior were observed between the two groups ($P > 0.06$).

By definition, residual feed intake is the difference between observed and predicted feed intake, where the conventional basic multiple regression model used to predict DMI includes metabolic body weight and ADG (Koch et al., 1963), therefore animals with lower RFI are deemed to be more efficient since they eat less than expected. Indeed, as expected, low RFI Nellore bulls had lower DMI, eating on average 0.40 kg/d less feed than high RFI bulls. Thus, if we consider the 21 more efficient animals used in this study, it represents 1,176 kg less feed throughout the 140-d feedlot period. Considering that a typical Brazilian feedlot diet costs on average $ 0.15 / kg DM (Valadares Filho et al., 2016), it represents an economy of $ 8.40 per animal during a feedlot period of 140 days. Additionally, the lack of difference in ADG and G:F observed in this study can be explained by the lack of phenotypic correlation between RFI, body weight gain and animal size (Cantalapiedra-Hijar et al., 2018). This mathematical independence of the traits used to predict

DMI resulted in a gain of popularity of RFI among geneticists as a selection trait in breeding programs (Cantalapiedra-Hijar et al., 2018).

Water is a key nutrient that aids in temperature regulation, growth, digestion, metabolism, and excretion (NRC, 2000). Also, water intake is strongly correlated with dry matter intake. Studies of Ahlberg et al. (2018) and Zanetti et al. (2019) showed a positive relationship between WI and DMI in the water intake prediction equations proposed by them. Thus, we decided to evaluate possible differences in water intake between Nellore bulls phenotyped for RFI. Initially, we thought that low RFI bulls would have a lower WI (kg/d or %BW) than high RFI bulls, which could be indirectly linked to RFI through their association with feed intake, however our results did not show any differences in water intake between the two groups. We believe that the lack of differences in water intake may be explained by the body composition of the animals in this study. At the next session we discuss in detail that the water, protein, and fat deposition between the two RFI groups was similar ($P > 0.13$) which may explain the absence of differences regarding water intake. Due to its molecular structure and biochemical composition protein molecules have high attractiveness to water molecules (Listrat et al., 2016), thus muscular tissue has higher capacity to retain water, but since the protein deposition of the animals in this study was similar, the water demanded for this was also similar.

We also speculated if the differences observed in DMI would reflect in differences in feeding behavior between low and high RFI bulls. However, our results showed no differences in the number of visits nor time spent eating between the two RFI groups (Table 2). According to Cantalapiedra-Hijar et al. (2018) the role of feeding and digestive-related mechanisms as a true determinant of animal variability in feed efficiency could be minor, additionally, the diet type offered to the animals can influence the association between RFI status and daily feeding events.

A meta-analysis (Kenny et al., 2018) of nine published studies with growing beef cattle offered high-concentrate energy-dense diets found that high RFI cattle spent on average, 10.3 min longer eating, out of an average of 93 min within a 24-h period, than their low RFI contemporaries. On the other hand, Corvino et al. (2009) observed that low RFI Nellore bulls, fed a diet with 18.6% inclusion of Brachiaria hay, spent greater time on feeding than high RFI bulls. Thus, the forage inclusion of 500 g/kg DM in this study may also explain the lack of differences in feeding behavior between low and high RFI Nellore bulls.

We suggest that other mechanisms, such as differences is rumen microbiome or biochemical mechanisms in the rumen epithelium of low and high RFI bulls may influence more RFI than feeding behavior, which can explain differences in DMI. Results of Carberry et al. (2012) may support this, since the authors observed an association between RFI ranking and bacterial profiles in beef heifers fed grass silage, where Prevotella was the bacterial genera more abundant in inefficient cattle. Additionally, the interplay between the production and consumption of ATP that takes place at the rumen epithelium, such as the cell process of ion pumping, protein turnover, thermogenesis, and volatile fatty acids uptake may significantly contribute to energy requirements of the animal (Johnson, 2013), and explain possible differences regarding animals feed efficiency. As an example, Benedeti et al. (2018) reported that high RFI Nellore bulls had increased expression of UQCR10 and NDUFB4, genes involved in oxidative phosphorylation in rumen epithelium. The authors also reported a lack of differences in mRNA expression of UCP2, a uncoupling protein, between efficient and inefficient animals, which lead Benedeti et al. (2018) to speculate that more efficient bulls may have lower mitochondrial activity and, consequently, a decreased production and expenditure of energy in their rumen epithelium.

## Energy and Protein Requirements and Chemical Body Composition

There is still a scarcity of data on energy expenditure and its underlying components of divergent RFI lines. Literature (Richardson and Herd., 2004, Herd and Arthur, 2009, and Cantalapiedra-Hijar et al., 2018) suggests a reduced maintenance energy requirements for low RFI lines, however, as highlighted by Cantalapiedra-Hijar et al. (2018) only two studies (Nkrumah et al., 2006; Chaves et al., 2015) evaluated heat production from oxygen consumption measurements in beef cattle, but the results are equivocal. Chaves et al. (2015) estimated the HP of 18 Nellore steers using the oxygen pulse methodology, and observed no differences between the divergent RFI animals, while Nkrumah et al. (2006) conducted a calorimetry trial with 27 Angus cross steers, and suggested a positive correlation between RFI and HP. Our trial used the comparative slaughter method where we measured directly the MEI and RE, and HP was determined as the difference between the other variables, and we did observe differences regarding HP ($P = 0.01$), MEI ($P = 0.01$), and maintenance energy requirements between the two RFI groups.

The average HP (Mcal/ $EBW^{0.75}$) was 0.2404 for low RFI bulls, and 0.2587 for high RFI bulls, while the MEI (Mcal/ $EBW^{0.75}$) was 0.2967 and 0.3205 for low and high RFI bulls respectively. The relationship between HP and MEI was described by the following equations: $HP = 0.0634 \times e^{(4.4797 \times MEI)}$ and $HP = 0.0781 \times e^{(3.7277 \times MEI)}$ where NEm was estimated as 63.4 and 78.1 Kcal/kg $EBW^{0.75}$/d for low and high RFI Nellore bulls respectively (Fig. 1). According to the NASEM (2016), maintenance energy expenditures vary with BW, breed or genotype, sex, age, season, temperature, and physiological state. Thus, NEm is influenced by characteristics that affect the basal metabolism and is independent of diet (Garrett et al., 1959), which justifies having different values for low and high RFI bulls reared in the same system and receiving the same basal diet.

The use of the value found for NEm is limited and has no practical application in diet formulation because producing animals are not found in a fasting state. Therefore, the maintenance energy requirements were calculated in a more applicable form, as metabolizable energy. The metabolizable energy requirements for maintenance were 98.6 and 123.9 Kcal/kg $EBW^{0.75}$/d for low and high RFI Nellore bulls respectively. These values were considered the point where heat production and metabolizable energy intake are equal, being obtained by applying an iterative process to the exponential model of heat production as a function of the metabolizable energy intake. The efficiency of utilization of metabolizable energy for maintenance was estimated from the NEm and MEm ratio, resulting in 64.3% for low RFI and 63.0% for high RFI Nellore bulls. Literature data (Garrett, 1980 and Marcondes et al., 2010) suggests that the km would be affected by body composition, thus, to test this hypothesis we evaluated the contents of water, protein, and fat in the EBW of low and high RFI Nellore bulls (Table 3).

We did not observe any differences regarding body composition ($P > 0.13$) between the two RFI classes. Since the composition of the EBW gain, which can be understood as all the energy that is retained in the empty body weight of the animals in the form of protein or fat (Garrett et al., 1959), is the main determinant of the energy requirements for weight gain (**NEg**), we estimated the following NEg (Mcal/$EBW^{0.75}$/d) equations in our study: $0.0528 \times EBW^{0.75} \times EBG^{0.5459}$ , and $0.054 \times EBW^{0.75} \times EBG^{0.8618}$ for low and high RFI bulls respectively. The parameters of these equations were similar ($P > 0.05$), thus we suggest that the efficiency of use of ME for growth ($\mathbf{k_g}$) would not be different between the two RFI classes. Literature data regarding $\mathbf{k_g}$ is controversial and scarce. A meta-analysis of five studies conducted by Cantalapiedra-Hijar et al. (2018) suggests that the kg may vary between RFI divergent animals, with a higher partial efficiency of ME use for growth in low-RFI steers, however the authors emphasize that their suggestion of reduced maintenance requirements and increased partial growth efficiency is largely the result of mathematical conventions, experimental conditions and limited numbers of simultaneous measurements, and does not help in identifying the underlying biological mechanisms.

We observed that low and high RFI Nellore bulls presented not only a similar fat deposition (P = 0.13) in the empty body (Table 3), but also a similar visceral fat (P = 0.43), backfat thickness (P = 0.43), carcass weight (P = 0.55), and carcass dressing (P = 0.19); data presented on Table 4. These findings demonstrate that, although consuming less feed, the carcass traits of more efficient animals were similar to those of animals that consumed more feed, suggesting that the lower feed intake did not compromise carcass traits. Literature data is still inconsistent, some studies with Nellore cattle suggest that low RFI animals would present greater subcutaneous fat deposition (Santana et al., 2012; Gomes et al., 2012), while others observed no differences (Fidelis et al., 2017) or a negative impact on carcass traits (Pereira et al., 2016). A recent meta-analysis conducted by Kenny et al. (2018) involving studies with growing beef cattle offered energy-dense diet found no statistically significant differences in either live animal or carcass measures between high and low RFI steers. Similarly, they failed to observe a statistically significant difference in ultrasonically measured back fat depth between cattle divergent in RFI. It was concluded that RFI rank in growing cattle was not obviously associated with final muscle area, carcass muscle area and change in back fat depth during the linear phase of the growth curve, typical of RFI test periods in many studies (Cantalapiedra-Hijar et al., 2018).

Furthermore, the independence of RFI with respect to body size could be observed in this study not only through the similarity between RFI classes for carcass traits, but also to the absence of differences regarding non-carcass traits (Table 4). Even though organ and tissue growth patterns, and the high metabolic cost associated with organs such as the gastro-intestinal tract or liver, may influence energy requirements (Fitzsimons et al., 2017 and Meale et al., 2017), our results show no differences between low and high RFI Nellore bulls regarding organs weight and non-carcass traits ($P > 0.28$). The limited published literature that has examined variation in visceral organ size amongst animals of divergent feed efficiency status is inconsistent (Kenny et al., 2018, Cantalapiedra-Hijar et al., 2018). For instance, Renand and Krauss (2002) observed a positive genetic relationship between RFI and the empty digestive tract in pure Charolais young bulls. Bonilha et al. (2009) observed that low RFI Nellore bulls had smaller important internal organs, like liver, kidneys, and gastrointestinal tract than high RFI bulls, and no differences were observed in KPH fat between low and high RFI groups. Whereas other studies have failed to establish an effect of RFI status on the weight of these organs (Bonilha et al., 2013, Fitzsimons et al., 2014, Kenny et al., 2018, Meale et al., 2017).

Therefore, our study shows that low RFI Nellore bulls eat less, grow at a similar rate, and have lower maintenance energy requirements than high RFI bulls. We also suggest that the lower feed intake did not compromises the carcass traits of more efficient animals, which would reduce production costs and increase the competitiveness of the Brazilian beef sector on the world market.

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**Table 1.** Proportion of ingredients and nutrient composition of the experimental diets

| Item | Experimental Diets[1] | | |
|---|---|---|---|
| | Low | Medium | High |
| Proportion | | | |
| Corn Silage | 50.0 | 50.0 | 50.0 |
| Ground corn | 39.4 | 39.4 | 39.4 |
| Soybean meal | 2.38 | 4.92 | 7.46 |
| Wheat bran | 6.09 | 3.05 | 0.00 |
| Urea | 0.47 | 0.98 | 1.49 |
| Salt | 0.30 | 0.30 | 0.30 |
| Limestone | 0.06 | 0.06 | 0.06 |
| Mineral mix[2] | 0.29 | 0.29 | 0.29 |
| Sodium bicarbonate | 0.75 | 0.75 | 0.75 |
| Magnesium oxide | 0.25 | 0.25 | 0.25 |
| Total | 100 | 100 | 100 |
| Chemical composition | | | |
| Dry matter, g/kg as-fed | 406.0 | 406.1 | 406.2 |
| Organic matter, g/kg DM | 944.1 | 943.8 | 943.5 |
| Crude protein, g/kg DM | 102.7 | 122.3 | 141.9 |
| Rumen degradable protein, g/kg CP | 673.2 | 696.7 | 713.7 |
| Rumen undegradable protein, g/kg CP | 326.8 | 303.3 | 286.3 |
| Ether extract, g/kg DM | 42.8 | 42.1 | 41.4 |
| Neutral detergent fiber, g/kg DM | 321.3 | 314.2 | 307.1 |
| Indigestible neutral detergent fiber, g/kg DM | 98.09 | 95.7 | 93.4 |
| Non-fiber carbohydrates, g/kg DM | 480.6 | 476.2 | 471.8 |

[1] Low = 105 g CP/kg DM; Medium = 125 g CP/kg DM; High =145 g CP/kg DM [2] Mineral mix = 7.83 g S/kg; 5,950 mg Co/kg; 10,790 mg Cu/kg; 1,000 mg Mn/kg; 1,940 mg Se/kg; 1,767.4 mg Zn/kg.

**Tabela 2** Performance, intake, and feeding behavior of Nellore bulls with high and low residual feed intakes

| Itens | Treatments | | SEM | P-value |
|---|---|---|---|---|
| | High | Low | | |
| Performance | | | | |
| Average Daily Gain, kg/d | 1.28 | 1.27 | 0.04 | 0.89 |
| Gain:Feed | 0.17 | 0.17 | 0.01 | 0.18 |
| Final Body Weight, kg | 472.9 | 475.1 | 8.67 | 0.86 |
| Intake | | | | |
| Dry Matter Intake, kg/d | 7.77 | 7.37 | 0.11 | 0.02 |
| Dry Matter Intake, %BW | 2.02 | 1.91 | 0.03 | <0.01 |
| Water Intake, kg/d | 17.09 | 17.04 | 0.60 | 0.95 |
| Water Intake, %BW | 3.62 | 3.61 | 0.13 | 0.96 |
| Feeding Behavior | | | | |
| Duration of feeding events, min | 101.4 | 101.9 | 3.42 | 0.91 |
| Number of feeder visits | 62.38 | 66.82 | 2.97 | 0.29 |
| Duration of water intake events, min | 25.01 | 24.66 | 1.94 | 0.89 |
| Number of waterer visits | 4.88 | 5.50 | 0.23 | 0.06 |

**Table 3** Energy intake, heat production, and body composition of Nellore bulls with high and low residual feed intakes

| | Treatments | | SEM | P-value |
|---|---|---|---|---|
| Itens | High | Low | | |
| Metabolizable Energy Intake, Mcal $EBW^{0.75}$ | 0.32 | 0.29 | 0.005 | <0.01 |
| Heat Production, Mcal $EBW^{0.75}$ | 0.26 | 0.24 | 0.005 | 0.01 |
| Protein, kg | 70.9 | 71.9 | 1.31 | 0.57 |
| Fat, kg | 93.9 | 88.1 | 2.68 | 0.13 |
| Water, kg | 228.1 | 228.9 | 3.79 | 0.89 |

**Table 4** Non-carcass components and carcass traits of Nellore bulls with high and low residual feed intakes

| | Treatments | | SEM[1] | P-value |
|---|---|---|---|---|
| Itens, kg | High | Low | | |
| Visceral Fat, kg | 24.2 | 23.2 | 0.91 | 0.43 |
| Rumen-reticulum, kg | 5.56 | 5.38 | 0.16 | 0.40 |
| Liver, kg | 5.11 | 5.30 | 0.13 | 0.28 |
| Small Intestine, kg | 4.23 | 4.40 | 0.18 | 0.49 |
| Large Intestine, kg | 2.17 | 2.15 | 0.11 | 0.89 |
| Organs and Viscera[2], kg | 37.5 | 37.4 | 0.6 | 0.99 |
| Blood, kg | 14.7 | 14.4 | 0.44 | 0.58 |
| Hide, kg | 46.7 | 48.2 | 1.22 | 0.38 |
| Head and limbs, kg | 18.5 | 18.6 | 0.31 | 0.78 |
| Non-Carcass[3], kg | 141.5 | 141.8 | 2.54 | 0.94 |
| Hot Carcass Weight, kg | 271.4 | 267.4 | 4.69 | 0.55 |
| Hot Carcass Dressing, % | 60.8 | 60.4 | 0.23 | 0.19 |
| Back Fat Thickness, mm | 4.05 | 3.59 | 0.34 | 0.35 |

[1] Standard error of mean

[2] Organs and viscera (empty, free of removable fat) included gastro-intestinal tract, tongue, lungs, diaphragm, trachea, spleen, kidneys, heart, pancreas, bladder, esophagus, liver, and reproductive tract.

[3] Non-carcass components included visceral fat, organs, viscera, blood, hide, head, and limbs.

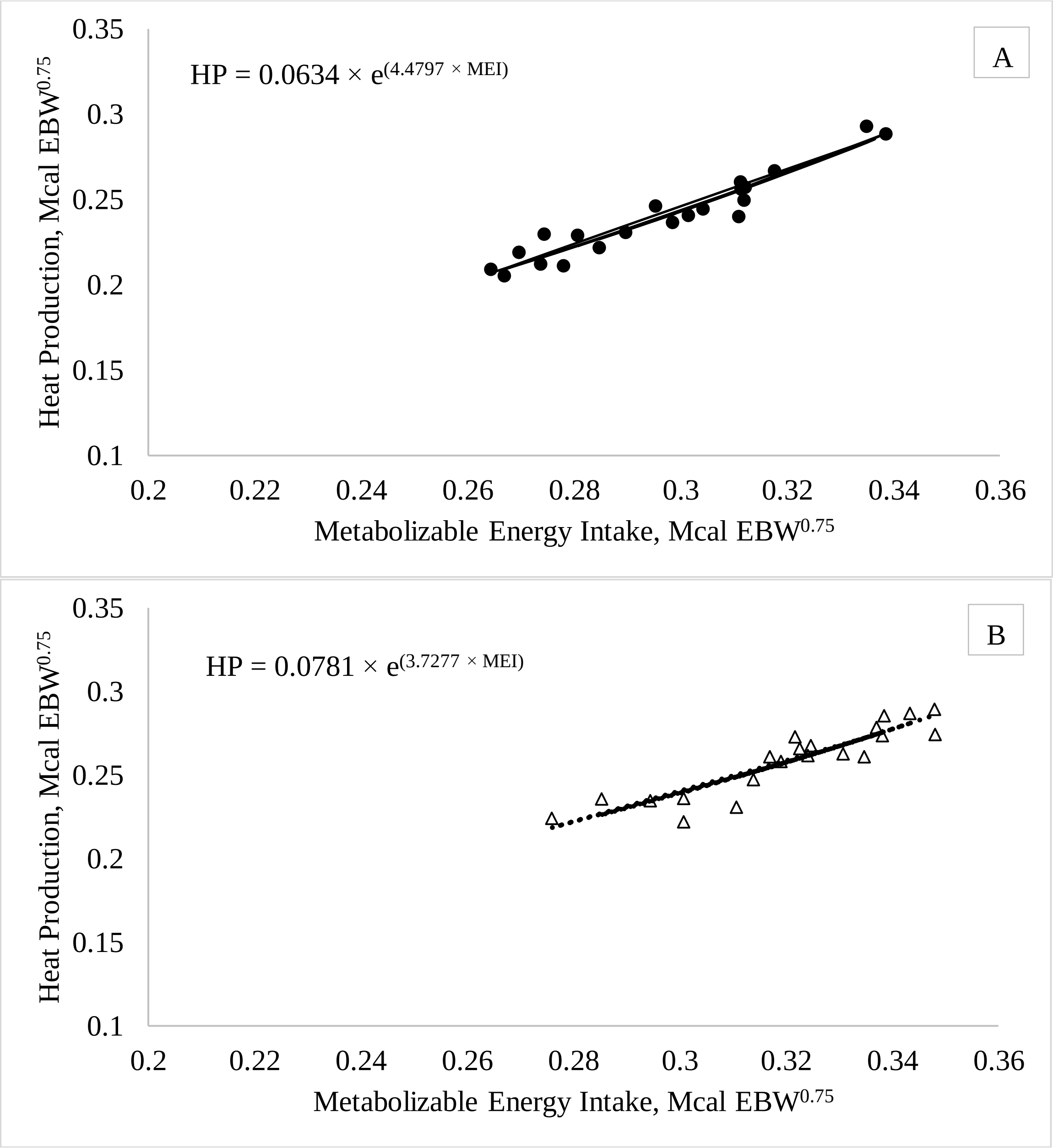

**Figure 1** Relationship between heat production (HP) and metabolizable energy intake (MEI) of young low (A) and high (B) RFI Nellore bulls