

LUIZ FERNANDO COSTA E SILVA

**MINERAL REQUIREMENTS FOR NELLORE CATTLE AND EQUATIONS TO
PREDICT MILK YIELD AND DRY MATTER INTAKE FOR LACTATING NELLORE
COWS AND SUCKLING NELLORE CALVES**

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

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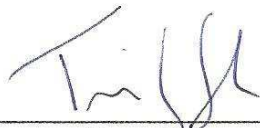
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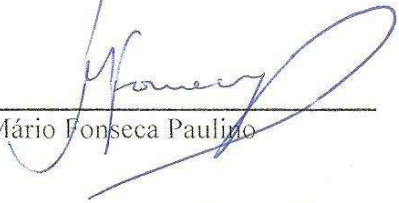
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BIOGRAPHY

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On February 26th of 2015, he submitted his dissertation to the thesis committee to obtain the Doctor Scientiae degree in Animal Science.

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ABSTRACT

SILVA, Luiz Fernando Costa e, D.Sc., Universidade Federal de Viçosa, February of 2015. **Mineral requirements for Nellore cattle and equations to predict milk yield and dry matter intake for lactating Nellore cows and suckling Nellore calves.** Adviser: Sebastião de Campos Valadares Filho. Co-advisers: Marcos Inácio Marcondes and Mário Luiz Chizzotti

This study was developed based on three experiments, and the results are shown in five chapters. The aims of the first and second chapters were to estimate equations that predict the milk yield and dry matter intake of lactating Nellore cows and the dry matter intake of suckling Nellore calves; and to evaluate the effects of days of lactation on intake and nutrient digestibility, performance, milk composition, and microbial efficiency of Nellore cows and their calves. In the experiment I, fifteen multiparous Nellore cows were used, and the diet consisted of corn silage and concentrate. The experiment started on the day of calving, and the lactation period was considered to be from the day of calving until 210 days later. Milking was performed on the 7th and 15th days after calving, and again every 15 days. Additionally, calves were fed corn silage ad libitum and concentrate. The intake of the animals was recorded daily. Digestibility assays were performed every 28-d; fecal samples were collected individually over three consecutive days (6h00, 12h00, and 18h00), while urine samples were collected on the second day of collection. Nutrient intake decreased ($P < 0.01$) for cows while nutrient digestibility increased ($P < 0.001$) during the lactation period. An associative effect was observed between feed and milk intake and nutrient digestibility when calves started to receive concentrate and roughage, and it appears that the apparent digestibility of mixed feeds does not equal the sum of the digestibility of each separately measured component. Cows had little reduction in BW during the first 28-d period; afterwards, there was a quadratic effect on BW gain. The average daily gain of the calves increased ($P < 0.001$) during the lactation period. Microbial efficiency (g CP/kg TDN) did not change ($P > 0.05$) in either Nellore

cows or their calves during the lactation period. Milk composition of Nellore cows did not change ($P > 0.05$) during the lactation period except for protein, which varies from 3.57 (Month 1) to 3.97% (Month 7). DMI of Nellore cows may be estimated by the following equation: $DMI = 24.78 + 0.035 \times \text{week} - 0.009 \times \text{week}^2$, while milk yield (MY) can be estimated by $MY = 8.761 - 0.0664 \times \text{week}$. DMI of the calves may be estimated by the following: $DMI = 0.00289 \times \exp^{(\exp(-0.5672) \times (\exp(-0.0773 \times \text{week}) - 1) / (-0.0773))}$. Therefore, nutrient intake decreases for cows and increases for calves while nutrient digestibility decreases for calves and increases for cows during lactation period. Also, an associated effect between milk and solid feeds increased DM digestibility. Nellore cows produce milk with an average of 4.58% lactose, 5.61% fat, and the milk protein varies from 3.57 to 3.97% according to the lactation period. Also, we recommend the use of the equations proposed here to estimate the DMI of lactating Nellore cows and their calves. Also, the equation to predict MY is recommended since it presented with the best results for Nellore cows. The aim of the third chapter was to evaluate the effects of the reduction of Ca and P levels in diet on nutrient apparent digestibility and intake, performance, microbial efficiency, and the energy and protein requirements for growing Nellore heifers and steers. In the experiment II, fifty animals were used, of which 32 were Nellore heifers and 18 were Nellore steers. Four animals of each sex were used as baseline reference animals and were slaughtered at the beginning of the experiment. Four animals from each sex were fed at MAIN by receiving 11 g/kg BW, whereas 10 steers and 24 heifers were assigned to the ADLIB group. The ADLIB heifers were divided further into 4 groups according to dietary treatment: 1) Ca and P fed at their proposed requirements (CaPR) with a 50:50 roughage:concentrate (R:C) diet, 2) CaPR with a 70:30 R:C diet, 3) 43% of their proposed requirements for Ca and 80% of their requirements for P (CaPL) with a 50:50 R:C diet, and 4) CaPL with a 70:30 R:C diet. The ADLIB and MAIN steers were fed CaPR with a 50:50 R:C diet.

Half of the ADLIB steers and heifers were slaughtered at d 50; the other ADLIB animals were slaughtered after 100 days of the feeding period, whereas all MAIN animals were slaughtered at d 100. Total feces and urine were collected from all animals for 72 h prior to slaughter. After slaughter, EBW was measured. The NEm and MEm requirements were estimated by exponentially relating heat production and metabolizable energy intake; NEg was estimated based on the EBW and EBG. The NPg was estimated based on the EBG and RE. Dry matter digestibility and the apparent absorption and retention of Ca and P were similar across Ca and P treatments. Final body weight, and consequently ADG, was higher ($P < 0.05$) for heifers that were receiving the high compared to the low concentrate diet, but dietary Ca and P concentration did not affect ($P > 0.05$) performance. The NEm and MEm requirements were 70.1 and 118 kcal/kg EBW^{0.75}, respectively. Net protein for maintenance was 1.28 g/kg BW^{0.75} and NEg and NPg were estimated from the following equations: $NEg = 0.053 \times EBW^{0.75} \times EBG^{0.6301}$ and $NPg = 137.85 \times EBG - 0.05 \times RE$, respectively. Under the conditions of this experiment, reducing the dietary concentrations of Ca and P had no significant impact on the intake, digestibility, or performance of growing Nellore heifers and steers. For chapters 4 and 5, data from another experiment (experiment III) was collected and added to the data of the experiment described in the third chapter (experiment II) in order to estimate the net requirements for maintenance and growth as well as the retention coefficients of 13 minerals (macro elements: Ca, P, Mg, K, Na, and S; and trace elements: Co, Cr, Cu, Fe, Mn, Se, and Zn) for Nellore cattle. The description of experiment II can be seen in the third chapter, while 37 Nellore bulls were used in experiment III. Bulls were divided into 3 groups: 1) baseline reference animals (n = 5), 2) animals fed at maintenance (MAIN, n = 4), and 3) animals with ad libitum access to feed (ADLIB, n = 28). The 28 ADLIB plus 4 MAIN animals were slaughtered at 4 different points (42, 84, 126, and 168 d, n = 8 – 7 ADLIB plus one MAIN/time

point). The net requirements for maintenance and the retention coefficient were estimated by using the apparent absorption and retention data, as well as by using body composition. Equations were developed for each mineral as a function of empty BW by using an exponential model. The net requirements for growth for each mineral was determined based on the derivative of these equations. The net requirement for maintenance and the retention coefficients were 17.0 g/kg BW/d and 61% for Ca, 16.4 mg/kg BW/d and 82% for P, 33.0 mg/kg BW/d and 70% for K, 17.2 mg/kg BW/d and 98% for Mg, 8.5 mg/kg BW/d and 58% for Na, 2.5 mg/kg BW/d and 21% for S, 3.00 µg/kg BW/d and 16% for Cr, 1.30 µg/kg BW/d and 50% for Co, 0.03 µg/kg BW/d and 2% for Cu, 1,626 µg/kg BW/d and 43% for Fe, 14.2 µg/kg BW/d and 0.4% for Mn, 0.3 µg/kg BW/d and 15% for Se, and 280 µg/kg BW/d and 6% for Zn. Under the conditions of these experiments, the equations that were generated indicate that for a 300 kg Nellore cattle with 1.00 kg of ADG, the dietary requirements are 23.3 g/d for Ca, 15.4 g/d for P, 16.7 g/d for K, 5.59 g/d for Mg, 6.52 g/d for Na, 25.5 g/d for S, 48.9 mg/d for Cr, 26 mg/d for Co, 385 mg/d for Cu, 1,545 mg/d for Fe, 250 mg/d for Mn, 5.26 mg/d for Se, and 378 mg/d for Zn.

RESUMO

SILVA, Luiz Fernando Costa e, D.Sc., Universidade Federal de Viçosa, Fevereiro de 2015. **Exigências de minerais para bovinos Nelore e equações para predizer produção de leite e consumo de matéria seca para vacas Nelore lactantes e bezerros Nelore lactentes.** Orientador: Sebastião de Campos Valadares Filho. Coorientadores: Marcos Inácio Marcondes e Mário Luiz Chizzotti

Este trabalho foi desenvolvido baseado em três experimentos e os resultados são mostrados em cinco capítulos. Os objetivos do primeiro e segundo capítulos foram estimar equações para predizer produção de leite e consumo de matéria seca de vacas Nelore lactantes e consumo de matéria seca de bezerros Nelore lactentes; e avaliar os efeitos dos dias de lactação no consumo e digestibilidade de nutrientes, desempenho, composição de leite, e eficiência microbiana de vacas Nelore e seus bezerros. No experimento I, quinze vacas Nelore multíparas foram utilizadas e a dieta consistia de silagem de milho e concentrado. O experimento iniciou no dia do parto e o período de lactação foi considerado do dia do parto até 210 dias de lactação. A ordenha foi realizada no sétimo e décimo quinto dia após o parto e novamente a cada 15 dias. Adicionalmente, os bezerros foram alimentados com silagem de milho e concentrado. O consumo dos animais foram medidos diariamente. Ensaios de digestibilidade foram realizados a cada 28 dias; amostras de fezes foram coletadas individualmente durante três dias consecutivos (6h00, 12h00 e 18h00), enquanto que amostras de urina foram coletadas no segundo dia de coleta. O consumo de nutrientes diminuiu ($P < 0,01$) para vacas enquanto que a digestibilidade dos nutrientes aumentou ($P < 0,001$) durante o período de lactação. Efeito associativo foi observado entre alimento, consumo de leite e digestibilidade dos nutrientes quando os bezerros começaram a receber concentrado e forragem e isto acontece quando a digestibilidade aparente da dieta não é igual à soma das digestibilidades de cada componente medido separadamente. Vacas apresentaram redução no PC durante os primeiros 28 dias; depois, houve efeito quadrático para ganho de PC. O ganho médio diário dos bezerros

aumentou ($P < 0,001$) durante o período de lactação. Eficiência microbiana (g PB/kg NDT) não alteram ($P > 0,05$) nem para vacas Nelore nem para bezerros Nelore durante o período de lactação. A composição do leite de vacas Nelore não altera ($P > 0,05$) durante a lactação exceto para proteína que varia de 3,57 (Mês 1) para 3,97% (Mês 7). O consumo de vacas Nelore pode ser estimado pela seguinte equação: $CMS = 24.78 + 0.035 \times SL - 0.009 \times SL^2$, enquanto que a produção de leite (PL) pode ser estimada por $PL = 8.761 - 0.0664 \times SL$. CMS de bezerros pode ser estimado pela seguinte equação: $CMS = 0.00289 \times \exp(\exp(-0.5672) \times (\exp(-0.0773 \times SL)-1) / (-0.0773))$. Portanto, o consumo de nutrientes diminui para vacas e aumenta para bezerros enquanto que a digestibilidade dos nutrientes para bezerros e aumenta para vacas durante a lactação. Também, um efeito associativo entre leite e alimentos sólidos aumentou a digestibilidade da MS. Vacas Nelore produzem leite com média de 4,58% lactose, 5.61% gordura e a proteína varia de 3.57 para 3.97% de acordo com o período de lactação. Também, nós recomendamos o uso das equações aqui propostas para estimar CMS de vacas Nelore e seus bezerros. Também, a equação para prever PL é recomendada uma vez que apresentou os melhores resultados para vacas Nelore. O objetivo do terceiro capítulo foi avaliar os efeitos da redução dos níveis de Ca e P na dieta na digestibilidade aparente dos nutrientes, consumo, desempenho, eficiência microbiana e exigências de energia e proteína para machos castrados e fêmeas Nelore em crescimento. No experimento II, cinquenta animais foram utilizados sendo 32 fêmeas Nelore e 18 machos castrados Nelore. Quatro animais de cada sexo foram usados como referência e foram abatidos no início do experimento. Quatro animais de cada sexo foram alimentados ao nível da manutenção recebendo 11 g/kg PC, enquanto que 10 machos castrados e 24 fêmeas foram designados ao grupo ad libitum. As fêmeas alimentadas ad libitum foram posteriormente divididas em 4 grupos de acordo com os seguintes tratamentos: 1) Ca e P alimentados às exigências propostas (CaPR) e relação

volumoso:concentrado (V:C) de 50:50, 2) CaPR e V:C de 70:30, 3) 43% das exigências para Ca e 80% para P (CaPL) e V:C de 50:50, e 4) CaPL e V:C de 70:30. Os machos castrados alimentados ad libitum e ao nível de manutenção foram alimentados com CaPR e V:C de 50:50. Metade dos animais alimentados ad libitum foram abatidos aos 50 dias enquanto que os restantes foram abatidos após 100 dias do período de alimentação. Todos os animais alimentados ao nível de manutenção foram abatidos aos 100 dias. Coletas totais de fezes e urina foram coletadas de todos os animais por 72 horas antes do abate. Após o abate, o PCVZ foi medido. As exigências de ELM e EMm foram estimadas exponencialmente relacionando produção de calor e consumo de energia metabolizável; ELg foi estimada baseada no PCVZ e GPCVZ. A PLg foi estimada com base no GPCVZ e ER. A digestibilidade da matéria seca e a absorção e retenção aparente de Ca e P foram similares entre os tratamentos. Peso corporal final e conseqüentemente GMD foram maiores ($P < 0.05$) para novilhas recebendo dietas com alto concentrado quando comparado à dieta com mais baixo concentrado, porém a concentração dietética de Ca e P não afetaram ($P > 0.05$) desempenho. A ELM e EMm foram 70.1 e 118 kcal/kg PCVZ^{0.75}, respectivamente. Proteína líquida para manutenção foi 1.28 g/kg PC^{0.75} e ELg e PLg foram estimadas pelas seguintes equações: $ELg = 0.053 \times PCVZ^{0.75} \times GPCVZ^{0.6301}$ e $PLg = 137.85 \times GPCVZ - 0.05 \times ER$, respectivamente. Sob as condições deste experimento, a redução as concentrações de Ca e P não apresentam impacto no consumo, digestibilidade ou desempenho de novilhas e machos castrados Nelore. Para o capítulo 4 e 5, dados de outro experimento (experimento III) foram coletados e adicionados aos dados provenientes do experimento II para estimar as exigências líquidas para manutenção e ganho bem como o coeficiente de retenção de 13 minerais (macro: Ca, P, Mg, K, Na, e S; e micro: Co, Cr, Cu, Fe, Mn, Se, e Zn) para bovinos de corte. A descrição do experimento II pode ser visualizada no terceiro capítulo enquanto que 37 machos Nelore não castrados foram utilizados no experimento

III. Os animais foram divididos em 3 grupos: 1) referência (n = 5), 2) animais alimentados aos nível de manutenção (MAIN, n = 4), and 3) animais com livre acesso à alimentação (ADLIB, n = 28). Os 28 ADLIB mais 4 MAIN foram abatidos em 4 tempos diferentes (42, 84, 126, e 168 dias, n = 8 – 7 ADLIB mais um MAIN por tempo). As exigências líquidas para manutenção e o coeficientes de retenção foram estimados utilizando absorção aparente e dados de retenção. Equações foram desenvolvidas para cada mineral em função PCVZ utilizadno o modelo exponencial. As exigências líquidas para ganho de cada mineral foram determinadas baseada na derivada destas equações. As exigências líquidas para manutenção e os coeficnetes de retenção foram 17.0 mg/kg PC/d e 61% para Ca, 16.4 mg/kg PC/d and 82% para P, 33.0 mg/kg PC/d e 70% para K, 17.2 mg/kg PC/d e 98% para Mg, 8.5 mg/kg PC/d e 58% para Na, 2.5 mg/kg PC/d e 21% para S, 3.00 µg/kg PC/d e 16% para Cr, 1.30 µg/kg PC/d e 50% para Co, 0.03 µg/kg PC/d e 2% para Cu, 1,626 µg/kg PC/d e 43% para Fe, 14.2 µg/kg PC/d e 0.4% para Mn, 0.3 µg/kg PC/d e 15% para Se, and 280 µg/kg PC/d e 6% para Zn. Sob as condições destes experimentos, as equações geradas indicam que para um bovino com 300 kg de PC e 1,0 kg de GMD, as exigências dietéticas são 23.3 g/d para Ca, 15.4 g/d para P, 16.7 g/d para K, 5.59 g/d para Mg, 6.52 g/d para Na, 25.5 g/d para S, 48.9 mg/d para Cr, 26 mg/d para Co, 385 mg/d para Cu, 1,545 mg/d para Fe, 250 mg/d para Mn, 5.26 mg/d para Se, e 378 mg/d para Zn.

INTRODUCTION

Brazil is one of the largest providers of beef cattle in the world; in 2013, Brazil produced the equivalent of 8.25 million of ton of carcasses, thus increasing the production by 3.7% in relation to the previous year (ANUALPEC, 2014). Then, international markets increased their pressure on Brazil to improve meat quality, which can be achieved with the intensification of the production systems, and consequently, this led to a reduction in the slaughter age of the animals. The lactation phase becomes extremely important since 70-75% of all energy required for meat production is used as a vital function involved with maintenance of the cows (Ferrel & Jenkins, 1984).

Ingvartsen (1994) conducted a review regarding the voluntary feed intake of cattle and observed that there are a lot of equations that estimate DMI of dairy cows and growing cattle; the simplest equation was proposed by Journet et al. (1965) who considered just milk yield (MY) and BW, while the most complex system was proposed by Brown et al. (1977) who use season, BW, stage of lactation, MY, milk fat yield, and crude fiber as parameters in the same equation to estimate DMI. Thus, it is important to have knowledge of the DMI of beef cows, but the equation that is proposed has to be the simplest possible so that it is easy for researchers to use and practical for producers.

Body weight at weaning is influenced by MY as well as by milk composition, which provides enough nutrients in terms of quantity and quality for the calf to reach its genetic potential while presenting with an adequate BW gain. Henriques et al. (2011) verified that MY and BW are highly, positively correlated with phenotype at weaning, and they concluded that Nellore cows that produce more milk wean the heaviest calves. Also, MY is an important factor in beef cattle; for the most part, nutrients that are ingested by calves, mainly during the first 2 mo, are supplied by maternal milk. Thus, the MY of Nellore cows is important to predict the BW at weaning (Paulino et al., 2006).

The nutritional level of beef cows is the limiting factor in most production systems when it comes to providing support for high milk yield; this can guarantee good body and physiologic conditions for reproduction. Thus, knowledge of the dry matter intake of beef cows is indispensable in establishing adequate nutritional plans during lactation in order to provide pastures of high quality and quantity. Otherwise, supplementation is an alternative to maintain body condition when pastures do not present with favorable characteristics to achieve the nutritional requirements of beef cows.

Milk dependency decreases while the ability to graze increases as the calf grows. After approximately 3 mo, more than half of the required energy of beef calves is met by sources other than maternal milk. The NRC (1996) relayed that the prediction equations for intake by nursing calves are important; however, no database exists from which we can develop such prediction equations for the wide variety of production situations and feeds that are available to beef cattle producers. Thereunto, knowledge of the DMI of beef calves becomes an indispensable factor to determine the time at which maternal milk is not able to provide all of the nutrients that are required by beef calves.

Focusing on short-term beef cattle production, there is a need to establish several strategies that can be utilized by producers to obtain greater profitability and quality of the product that is offered. The use of feedlot associated animals with higher growth rate potentials can be an alternative to decrease the time spent on the property. Breast-feeding is the phase that most interferes with the time that the animals remain on the property due to the greater stress at weaning and the drought season that they are submitted to, with both providing a reduction in weight gain. This factor is important when more accurate livestock is necessary to increase profit. A reduction

of this period suggests that some alternatives must be adopted. Thus, the determination of the nutrient requirements of animals after weaning becomes indispensable.

Minerals have functions that can only be fulfilled if sufficient amounts of the ingested mineral are absorbed and retained to keep pace with growth, development, and reproduction and to replace minerals that are 'lost' either as products, such as in milk, or insidiously during the process of living (Suttle, 2010). Feedstuffs that are commonly fed to beef cattle can provide these nutrients (Genther and Hansen, 2014); however, their concentrations are variable and/or inadequate (Smart et al., 1981), which contribute to poor growth performance and lower carcass quality (Spears and Kegley, 2002). According to Arthington et al. (2014), supplementation of trace minerals may occur through a variety of means including free-choice loose mineral mixes, trace mineral-fortified salt blocks, injectable trace minerals, and trace mineral-fortified energy/protein supplements. Therefore, knowledge of the necessary amounts of each trace mineral that is supplied to animals is recommended in order to guarantee adequate performance.

The diet formulation in Brazil that is designed for beef cattle has been created based on nutrient requirements suggested by international councils (AFRC, 1991; NRC, 1996) due to the shortage of national data. Although, for most of these councils, the nutrient requirements are based on information obtained from *Bos taurus* cattle, and some differences are assumed for the nutrient requirements of *Bos indicus* cattle.

Then, Valadares Filho et al. (2010) published the second edition of the Nutrient Requirements of Zebu beef cattle – BR CORTE, which is essential in the attempts to optimize animal performance and the economy of formulated diets in Brazil. However, there is a lack of data regarding the nutrient requirements, performance, and feed efficiency of Zebu cattle of different genders during the growing phase.

Therefore, this study was conducted in the Animal Science Department of the Universidade Federal de Viçosa with the following goals:

1 – To estimate equations to predict the milk yield and dry matter intake of lactating Nellore cows and the dry matter intake of suckling Nellore calves;

2 – To evaluate the effects of days of lactation on intake and nutrient digestibility, performance, milk composition, and microbial efficiency of Nellore cows and their calves;

3 – To evaluate the effects of the reduction of Ca and P in diet on nutrient apparent digestibility and intake, performance, microbial efficiency, and energy and protein requirements for growing Nellore heifers and steers; and

4 – To estimate the net requirements for maintenance and growth as well as the retention coefficients of 13 minerals (macro elements: Ca, P, Mg, K, Na, and S; and trace elements: Co, Cr, Cu, Fe, Mn, Se, and Zn) for Nellore cattle.

Chapters 1, 2, 3, and 4 were written according to the guidelines of Tropical Animal Health and Production, Journal of Animal Science, Livestock Science, and Journal of Animal Science, respectively.

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CHAPTER 1

Nellore cows and their calves during the lactation period. Performance, intake, milk composition, and total apparent nutrient digestibility

Abstract An experiment was conducted to evaluate intake and nutrient digestibility, performance, milk composition, and microbial efficiency of Nellore cows and their calves during lactation. Fifteen Nellore cows and their calves were used in this experiment. Cows were fed corn silage and concentrate (85:15) based diet. After calving, calves were kept with their dams for two months, after which they were separated and housed in individual pens adjacent to their dams. Cows were milked every 15 d over seven mo to estimate the milk intake of the calves. After two months post calving, calves were offered ad libitum access to a concentrate (5 g/kg BW) and roughage based diet. Digestibility assays were performed every 28-d; fecal samples were collected individually over three consecutive days (6h00, 12h00, and 18h00), while urine samples were collected on the second day of collection. Nutrient intake decreased ($P < 0.01$) for cows while nutrient digestibility increased ($P < 0.001$) during the lactation period. An associative effect was observed between feed and milk intake and nutrient digestibility when calves started to receive concentrate and roughage and it occurs when the apparent digestibility of mixed feeds does not equal the sum of the digestibility of each separately measured component. Cows had little reduction in BW during the first 28-d period; afterwards, there was a quadratic effect on BW gain. Average daily gain of the calves increased ($P < 0.001$) during the lactation period. Microbial efficiency (g CP/kg TDN) did not change ($P > 0.05$) in either Nellore cows or their calves during the lactation period. Milk composition of Nellore cows did not change ($P > 0.05$) during the lactation period except for

protein that varies from 3.57 (Month 1) to 3.97% (Month 7). Under the conditions of this experiment, nutrient intake decreases for cows and increases for calves during lactation. However, nutrient digestibility decreases for calves and increases for cows during this period. Also, an associated effect between milk and solid feeds increased DM digestibility. Nellore cows produce milk with average of 4.58% lactose, 5.61% fat and the milk protein vary according the lactation period from 3.57 to 3.97%.

Keywords beef cattle • Bos indicus • lactation • milk composition • weight gain

Abbreviations

ADG, average daily gain

AS, ammonium sulfate

BW, body weight

CP, crude protein

DE, digestible energy

DM, dry matter

EE, ether extract

N, nitrogen

NDF, neutral detergent fiber

NFC, non-fiber carbohydrates

TDN, total digestible nutrients

U, urea.

Introduction

Voluntary intake is a complex relationship based on the interactions between the animal, forage, and environment (Freer, 1981). To quantify the intake of animals on pasture is difficult, as consumption cannot be measured directly, and external and internal markers are needed to estimate the forage intake and fecal excretion in order to assess the nutrient digestibility.

Providing adequate nutrition for animals is the greatest operating cost for cow-calf producers (Meyer et al., 2008). Thus, knowledge of the dry matter intake, as well as nutrient intake and digestibility, are important tools to provide the correct amount of feed to the animal so that it can reach its performance potential in terms of body weight gain (males), puberty (heifers), or milk production (cows). Calves begin to consume forage when the amount of milk produced by the cow does not allow the calf to reach its nutritional requirements. Forage intake by nursing calves increases as lactation declines, and subsequently, applies greater pressure on the forage management program (Fox et al., 1988).

Currently, few experiments have been reported (Valadares Filho et al., 2010; Valente et al., 2013) that examine the nutrient intake and digestibility of lactating Nellore cows and their calves. However, Valadares Filho et al. (2010) adopted the constant value of 25g/kg BW as the dry matter intake for nursing Nellore cows. Therefore, an experiment was conducted to evaluate intake and digestibility of the nutrients, performance, milk composition, and microbial efficiency of Nellore cows and their calves during the lactation period.

Material and methods

Animals, design, and treatments

The experiment was conducted at the Experimental Feedlot of the Animal Science Department, Viçosa, Brazil. Laboratory analyses were conducted at the Ruminant Nutrition Laboratory of the Animal Science Department at the Universidade Federal de Viçosa, Viçosa, MG, Brazil. Animal care procedures throughout the study followed protocols approved by the Institutional Animal Care and Use Committee (IACUC) guidelines at the UFV number 10/2013.

Fifteen multiparous Nellore cows and their calves were used in this experiment. Cows were acquired and managed from the beef cattle section to the feedlot when they were 8-months pregnant. The animals were housed in individual pens with concrete floor with total area of 30 m², where 8 m² of the area was under cover, and a concrete feeder and trough for water were provided. The diet consisted of 85% of corn silage on dry matter basis, and 15% of concentrate formulated with ground corn, soybean meal, urea, ammonium sulfate, limestone, common salt, calcium phosphate, and a mineral mix (Table 1). The diet was formulated to simulate high quality pasture.

Feedstuffs were provided twice a day and intake was adjusted to allow minimal orts without restriction DMI. All animal has ad libitum access to clean drinking water throughout the experiment. The amount of feed supplied was recorded daily; additionally, the ingredients in the concentrate were sampled at every concentrate manufacturing time. Corn silage was sampled every day, stored, and a composite sample was created over a 7-d period. After, this sample was stored in the ultra-low temperature freezer (-40°C) to be lyophilized.

The experiment started on the day of calving, and the lactation period was considered to be from the date of calving through 210 d post calving when the calves were weaned. After calving, cows were restrained in a squeeze chute and calves were stimulated to suckle so that the intake of colostrum was guaranteed. At this point in time, a colostrum sample was collected to evaluate the

colostrum composition. Calves were kept in the same pen as their dams during the first two months post calving. Cows were milked manually on day 7, 15, and then every 15 d post calving to quantify milk production. At each milking time point, each cow and calf were weighed.

After two months, the calves were separated from their dams and were housed in individual pens adjacent to their dams. The calves could nurse their dam through the fence, while the dry matter intake of the cows and the calves could be quantified individually. Additionally, calves were fed corn silage ad libitum and concentrate with 30% CP that consisted of soybean meal, ground corn, limestone, calcium phosphate, and a mineral mix (Table 2). The concentrate intake was limited to 0.5% of BW, and every 15 d the intake was adjusted when the calves were weighed. Concentrate and corn silage were provided separately in different buckets to measure the intake of each feed separately. Intake was recorded daily and adjusted to provideorts of approximately 5 to 10% of the intake.

Measurements and analyses

Feces were collected from the cows and the calves during the last 3 d of each 28-d period in order to conduct in order to evaluate the apparent total tract digestibility (ATTD) of nutrients. Fecal collections were performed at 0600, 1200, and 1800 h of days 1, 2, and 3 respectively. Fecal samples were collected by rectum stimulation or as the animal defecated. Fecal samples were weighed, dried in a forced-ventilation oven (55°C) for 72 h, ground through a 2mm screen and then ground through a 1 mm screen (Wiley mill; A. H. Thomas, Philadelphia, PA). A composite sample was obtained per period collection for each cow and each calf by utilizing 15 g of the dried and ground sample per collection time. Indigestible neutral detergent fiber (iNDF) was used as an

internal marker to estimate the fecal excretion (Lippke et al., 1986). Spot urine samples were performed at 1200 h on d 2. Urine samples were taken midstream after manual stimulation, acidified below a pH of 4.0 with concentrated sulfuric acid to prevent ammonia volatilization, and then frozen at -20°C for further analyses of nitrogen (N), urea, allantoin, creatinine, and uric acid.

Allantoin, creatinine, and uric acid were analyzed using HPLC (Agilent 1100 series, Agilent Technologies, Waldbronn, Germany) as previously described by Czauderna and Kowalczyk (2000), with modifications by George et al. (2006). The total excretion of purine derivatives was calculated based on the sum of allantoin and uric acid excreted in the urine, and was expressed in millimoles per day. Absorbed purines (X, in millimoles per day) were calculated based on the excretion of purine derivatives (Y, in millimoles per day) by using the following equation: $Y = (X - (0.30 \times \text{LW}^{0.75})) / 0.80$, where 0.80 was the recovery of purines absorbed as purine derivatives, and $0.30 \times \text{LW}^{0.75}$ was the excretion of endogenous purines per kg of metabolic weight per day (Barbosa et al., 2011). Rumen synthesis of nitrogenous compounds (Y, in grams N per day) was calculated as a function of absorbed purines (X, in millimoles per day) by utilizing the following equation from Barbosa et al. (2011): $Y = (70 \times X) / (0.93 \times 0.14 \times 1,000)$, where 70 was the N content of purines (in milligrams N per mole), 0.14 was the purine N/total N ratio of the bacteria, and 0.93 is the true digestibility of microbial purines.

Samples of corn silage, concentrate ingredients, orts, and feces were quantified as DM, OM, and CP (AOAC, 2012; method number 934.01, 930.05, and 981.10, respectively). Ether extract was analyzed according to AOAC (2006; method number 945.16). Neutral detergent fiber (NDF) content was obtained according to Van Soest et al. (1991). For the analyses of soybean meal, sodium sulfite was added, as described by Undersander et al. (1993). The Ankom® system was

utilized for NDF evaluations; this was achieved by adding thermostable α -amylase. The NDF content was corrected for protein and ash content in all samples.

Non-fiber carbohydrates (NFC) were calculated according to Detmann and Valadares Filho (2010), where $NFC = 100 - [(\%CP - \%CP \text{ derived from urea} + \%urea) - \%apNDF - \%EE - \%ash]$. The content of total digestible nutrients (TDN) in the diets was estimated as the sum of digestible nutrients, where $TDN = CP_{digestible} + 2.25 \times EE_{digestible} + NDF_{digestible} + NFC_{digestible}$ (NRC, 2001). The digestible energy (DE) was obtained by multiplying the digestible nutrients with their respective energy values, as described by the NRC (2001), whereas the concentration of ME was considered to be 82% of the DE (Coelho da Silva and Leão, 1979).

Statistical analysis

Measurements were evaluated in the same animals throughout the experiment; therefore, all data were considered to be repeated measures over time, and were assessed as mixed models through the PROC MIXED feature of SAS (version 9.3). The 28-d periods were considered to be the fixed effect, and the animals accounted for the random effect. Different residual variances were modeled with the repeated feature in SAS (version 9.3). The heterogeneous first-order autoregressive matrix (ARH (1)) was used; this is the variance and covariance matrix of random effects for all of the variables analyzed. For all comparisons, the level of 0.05 was established as the critical level of probability for a type I error.

Results and discussion

Intake and digestibility of the nutrients

Cows

Differences ($P < 0.05$) were observed for all parameters that were evaluated for Nellore cows except for TDN during the lactation period (Table 3). Cow DMI were decreased during the lactation period, while DM digestibility was increased (Figure 1). The decrease in the nutrient intake was offset by the increase in the nutrient digestibility, which resulted in the same TDN availability to the animal during the lactation period.

Valente et al. (2013) evaluated the impact of different nutritional plans of calves on the intake of nursing Nellore cows on pasture. They did not observe differences among treatments. They estimated an average of 12.1 kg/d of dry matter intake for Nellore cows in the last three months of the lactation period. This value is greater than those observed in the current experiment for the same time period. This difference can be explained by the difference in the energy intake, represented by TDN; the value for the present study (7.12 kg/d) was greater than that observed by Valente et al. (2013; 6.56 kg/d). Estermann et al. (2002) evaluated DMI for nursing Simmental and Angus cows on pasture, and estimated a DMI of 14.0 and 12.3 kg/d for Simmental and Angus cows respectively. Valente et al. (2013) estimated lower values for DM digestibility (approximately 10% less) than those observed in this experiment, probably because cows were fed a grass based diet and received only mineral supplementation. Also, greater CP digestibility for this study was observed when compared to results from Valente et al. (2013).

Calves

Contrary to what was observed for the cows, the intake of the nutrients by calves increased ($P < 0.05$) during the lactation period, while the nutrient digestibility decreased (Table 4). However, in the third mo of age, an increase in DM digestibility was observed for the calves (Figure 2). This was the period when the calves started to receive roughage and concentrate. Thus, the increase in DM digestibility during this period likely occurred due to the associative effect between milk, concentrate, and roughage, which increased the DM digestibility without necessarily increasing the DM intake (Preston, 1985; Paulino et al., 2008). The associative effects are verified when the apparent digestibility of mixed feeds does not equal the sum of the digestibility of each separately measured component (Mould, 1988; Van Soest, 1994). Preston (1985) reported that interactions among feeds can rarely be identified by conventional nutrient analyses of feeds. Some studies were developed to identify the associated effect, but this was accomplished through in vitro incubations (Juul-Nielson, 1981), rumen nylon bag techniques (Silva and Orskov, 1985), or the use of ionophores to improve propionic acid production in the rumen (Chalupa, 1980).

Lopes et al. (2014) evaluated the intake and digestibility of suckling calves receiving different concentrations of protein on pasture in 5 different lactation periods. The authors did not observe differences among treatments, and the average of the DMI of the calves was 3.66 kg/d. This value is greater than those observed in the present study, probably because the authors started the evaluation 100 d after calving. Thus, the majority of the intake measurements were seven months after the measurements that were obtained in the current experiment. However, if we consider just the last month (3.24 kg/d), the values were somewhat similar. Also, Lopes et al. (2014) observed the CP intake and digestibility of 0.65 kg/d and 70.8% respectively. These values are similar to those observed in the last three months of the current experiment. This similarity can be explained

by the same source of protein (soybean meal) for the calves used in both studies. Soto-Navarro et al. (2004) evaluated the supplementation, or lack thereof, in suckling beef calves fitted with rumen and duodenal cannulas. They did not observe differences on the intake and digestibility of OM and CP during the three month of evaluation period.

Milk composition

The milk composition of Nellore cows is shown in table 5. Total solids, lactose, and fat were not influenced by lactation period ($P > 0.05$). Thus, we suggest 15.0% total solids, 4.58% lactose, and 5.61% fat as the average milk composition from Nellore cows during all lactation period. However, protein increased ($P < 0.05$) during the lactation period from 3.57% (Month 1) to 3.97% (Month 7). Rodrigues et al. (2014) evaluated four different breeds of cows, and observed milk composition means of 3.90% fat, 3.07% protein, 4.91% lactose, and 12.7% total solids in crossbreed Nellore x Angus cows. Thus, we observed lactose values that were close to those observed in the present study, while total solids, fat, and protein were lower than those observed in this study. Restle et al. (2003) evaluated the milk composition of Nellore cows raised on different pastures (natural or cultivated), and observed similar values for fat (4.90%), total solids (13.8%), and lactose (5.14%). Also, Silva et al. (1995) used data collected from 809 lactations from 376 Nellore cows and estimated 4.50% as the fat percentage. The greatest fat percentage value observed in this study could have occurred because the diet was greater in energy in the present experiment once the cows were maintained in the feedlot.

The concentration of Ca, S, Cr, Fe, and Mn did not change ($P > 0.05$) during the lactation period. Mg concentration increased ($P < 0.01$) during the lactation period, while K, Cu, and Zn

decreased ($P < 0.05$). P, Na, and Co varied ($P < 0.05$) during the lactation period. The authors are not aware of any published experiments where mineral composition was evaluated in the milk for beef cows. Thus, we recommend 1.11% Ca, 0.76% P, 0.20% Na, 0.25% S, 2.29 ppm Co, 3.20 ppm Cr, 29.9 ppm Fe, and 1.40 ppm Mn are the typical mineral concentrations in the milk of Nellore cows.

Performance

Cows

The average body weight, weight gain, and ADG are reported in Table 6. The initial weight for the first 28-d period was measured after calving. Thus, cows lost weight in the first 28-d period. Afterwards, they increased body weight until weaning. Therefore, weight gain had a quadratic effect ($P < 0.01$) during the lactation period, which is shown in Figure 3. Valente et al. (2013) also did not observe differences in final body weight and estimated an average daily gain of 0.13 kg/d. This value is below that observed in this study (0.54 kg/d). This could have occurred because the nutrient supply in terms of protein and energy was greater in the present experiment, which can contribute to greater ADG.

Calves

Lopes et al. (2014) evaluated the performance of suckling Nellore calves and observed an ADG of 880 and 727 g/d for supplemented and non-supplemented calves, respectively. Paulino et al. (2008)

reported that supplementation can lead to an additional gain from 150 to 250 g/d in relation to ADG when obtained in the pasture-mineral mix feed system. The values observed by Lopes et al. (2014) are lower than those observed in the last three months of the evaluation period. This may have occurred because the calves in the present experiment were maintained in the feedlot and they did not have to graze. Thus, by not being required to graze, the cattle would have expended less energy and therefore would have an improved body weight gain. Also, an increase in ADG can be observed when the calves grow. This can be associated with the increase in the DMI when calves switched from consuming only milk to consuming primarily roughage and concentrate.

Microbial efficiency

Differences were not observed in the microbial efficiency (ME) of either cows ($P = 0.89$) or calves ($P = 0.96$) during the lactation period (Figure 4). Lopes et al. (2014) did not observe a significant increase in ME (with values varying from 140 to 161 g_mCP/kg TDN) when Nellore calves were supplemented with increasing levels of crude protein on pasture. Costa e Silva et al. (2013) evaluated the performance of Nellore bulls and observed an average of 141 g_mCP/kg TDN. These authors found values for ME that were greater than those observed for cows and calves in the present experiment. However, Valadares Filho et al. (2010) and NRC (1996) suggested ME values of 120 and 130 g_mCP/kg TDN, respectively, which were close to those calculated in the present experiment.

Under the conditions of this experiment, we concluded that Nellore cows have an increase in the DM digestibility and decrease in DMI intake during the lactation period. An associated effect was observed between milk, concentrate, and roughage when Nellore calves began to receive solid

feed. Also, the performance of Nellore calves increased from calving to weaning. Nellore cows produce milk with average of 4.58% lactose, 5.61% fat and the milk protein vary according the lactation period from 3.57 to 3.97%.

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Table 1

Proportions of feed and its composition on dry matter basis for nursing Nellore cows

	Concentrate	Diet
Proportion (g/kg DM)		
Corn silage	-	850
Ground corn	489	73.3
Soybean meal	340	51.1
Calcium phosphate	59.3	8.9
Urea/Ammonium sulfate (9:1)	57.1	8.6
Limestone	39.0	5.9
Common salt	7.8	1.2
Ammonium sulfate	5.7	0.9
Mineral mix ¹	1.5	0.2
Dry matter (%)	88.5	36.9
Composition (g/kg DM)		
Organic matter	854	931
Crude protein	414	129
Neutral detergent fiber	102	461
Non-fibrous carbohydrates	316	302

¹Mineral mixed composition = 29.2 g/kg of calcium, 0.7 g/kg of phosphorus, 2.1 g/kg of magnesium, 0.9 g/kg of potassium, 0.3 g/kg of sodium, 63.5 g/kg of sulfur, 348 mg/kg of cobalt, 2.6 mg/kg of chromium, 3,296 mg/kg of copper, 2,088 mg/kg of iron, 4,673 mg/kg of manganese, 7,817 mg/kg of zinc, and 318 mg/kg of selenium.

Table 2

Proportions of feed and its composition on dry matter basis for suckling Nellore calves

	Concentrate	Corn Silage
Proportion (g/kg DM)		
Soybean meal	590	
Ground corn	386	
Limestone	14.2	
Calcium phosphate	8.0	
Common salt	1.4	
Mineral mix ¹	1.1	
Dry matter (%)	87.5	27.8
Composition (g/kg DM)		
Organic matter	916	944
Crude protein	396	78.2
Neutral detergent fiber	140	524
Non-fibrous carbohydrates	349	300

¹Mineral mixed composition = 29.2 g/kg of calcium, 0.7 g/kg of phosphorus, 2.1 g/kg of magnesium, 0.9 g/kg of potassium, 0.3 g/kg of sodium, 63.5 g/kg of sulfur, 348 mg/kg of cobalt, 2.6 mg/kg of chromium, 3,296 mg/kg of copper, 2,088 mg/kg of iron, 4,673 mg/kg of manganese, 7,817 mg/kg of zinc, and 318 mg/kg of selenium.

Table 3

Effects of stage of lactation on intake and apparent digestibility of the nutrients for nursing Nellore cows

Items	Stage of Lactation (d)							SEM	P-value
	28	56	84	112	140	168	196		
Organic matter									
Intake (kg/d)	10.5 ^a	10.2 ^a	10.0 ^{ab}	10.3 ^a	9.75 ^{ab}	9.66 ^{ab}	9.33 ^b	0.4	<0.01
Digestibility (%)	65.0 ^b	69.0 ^{ab}	66.2 ^{ab}	70.9 ^{ab}	68.5 ^{ab}	71.2 ^a	71.9 ^a	1.7	<0.01
Crude protein									
Intake (kg/d)	1.5 ^a	1.4 ^{ab}	1.4 ^{ab}	1.5 ^{ab}	1.5 ^{ab}	1.4 ^{ab}	1.4 ^b	0.2	0.03
Digestibility (%)	70.3 ^{ab}	69.4 ^b	67.1 ^b	70.4 ^{ab}	70.9 ^{ab}	73.6 ^a	73.6 ^a	2.3	0.01
Ether extract									
Intake (kg/d)	0.5 ^{ab}	0.5 ^a	0.5 ^{ab}	0.5 ^{ab}	0.4 ^b	0.4 ^c	0.3 ^c	0.1	<0.001
Digestibility (%)	81.4	86.8	84.9	84.2	83.2	83.5	81.7	1.5	0.13
Neutral detergent fiber									
Intake (kg/d)	4.8 ^{ab}	4.5 ^b	4.8 ^{ab}	5.2 ^a	4.9 ^{ab}	4.8 ^{ab}	4.6 ^b	0.2	0.03
Digestibility (%)	47.2 ^c	50.7 ^{bc}	54.6 ^{ab}	55.9 ^{ab}	52.1 ^{abc}	57.1 ^a	59.0 ^a	2.7	<0.001
Non-fibrous carbohydrates									
Intake (kg/d)	3.8 ^a	3.8 ^a	3.3 ^b	3.1 ^b	3.0 ^b	3.1 ^b	3.0 ^b	0.2	<0.001
Digestibility (%)	85.3 ^b	87.3 ^{ab}	86.0 ^b	87.7 ^{ab}	88.3 ^{ab}	90.6 ^a	89.1 ^{ab}	1.4	0.01
Total digestible nutrients	7.2	7.4	6.9	7.4	6.8	7.3	7.0	0.4	0.54

^{a,b,c,d} Within a row, means without a common superscript letter differ (P < 0.05)

Table 4

Effects of age on intake and digestibility of the nutrients for suckling Nellore calves

Items	Age (d)							SEM	P-value
	28	56	84	112	140	168	196		
Organic matter									
Intake (kg/d)	1.1 ^e	1.1 ^e	1.3 ^{de}	1.6 ^{cd}	1.9 ^c	2.5 ^b	3.0 ^a	0.9	<0.001
Digestibility (%)	86.8 ^{ab}	87.0 ^{ab}	91.4 ^a	88.8 ^{ab}	85.1 ^b	84.0 ^b	83.8 ^b	1.9	<0.001
Crude protein									
Intake (kg/d)	0.3 ^d	0.3 ^d	0.3 ^d	0.4 ^{cd}	0.4 ^c	0.5 ^b	0.6 ^a	0.2	<0.001
Digestibility (%)	76.6 ^a	75.3 ^a	74.1 ^a	71.2 ^b	70.9 ^{bc}	69.4 ^{bc}	68.5 ^c	2.1	<0.001
Ether extract									
Intake (kg/d)	0.4	0.4	0.4	0.4	0.4	0.4	0.5	0.03	0.56
Digestibility (%)	94.7 ^b	97.4 ^a	98.6 ^a	98.8 ^a	98.1 ^a	97.8 ^a	97.4 ^a	1.2	<0.001
Neutral detergent fiber									
Intake (kg/d)	nd	nd	0.1 ^c	0.3 ^d	0.5 ^c	0.7 ^b	1.0 ^a	0.3	<0.001
Digestibility (%)	nd	nd	66.5	67.5	59.7	64.1	64.9	1.3	0.16
Non-fibrous carbohydrates									
Intake (kg/d)	0.4 ^e	0.4 ^e	0.5 ^{de}	0.5 ^d	0.6 ^{cd}	0.8 ^b	1.0 ^a	0.5	<0.001
Digestibility (%)	89.0 ^{ab}	87.4 ^b	91.9 ^{ab}	94.6 ^a	92.8 ^{ab}	93.2 ^{ab}	92.8 ^{ab}	0.8	<0.01
Total digestible nutrients	1.5 ^d	1.5 ^d	1.7 ^d	1.9 ^{cd}	2.1 ^c	2.6 ^b	3.0 ^a	1.0	<0.001

^{a,b,c,d,e} Within a row, means without a common superscript letter differ (P < 0.05).

Table 5

Effects of stage of lactation on milk composition of Nellore cows

Items	Stage of lactation (d)							SEM	P-value
	28	56	84	112	140	168	196		
Total solids (%)	14.5	14.7	14.8	14.9	15.1	15.4	15.6	0.4	0.13
Protein (%)	3.6 ^c	3.5 ^c	3.5 ^c	3.6 ^c	3.8 ^b	3.9 ^a	3.9 ^a	0.1	<0.001
Lactose (%)	4.6	4.7	4.6	4.6	4.6	4.5	4.5	0.1	0.05
Fat (%)	5.2	5.4	5.6	5.5	5.7	5.9	6.0	0.4	0.44
Ca (%)	1.1	1.1	1.1	1.1	1.1	1.1	1.1	0.03	0.46
P (%)	0.8 ^a	0.7 ^b	0.7 ^b	0.8 ^{ab}	0.8 ^{ab}	0.8 ^{ab}	0.8 ^{ab}	0.02	0.01
Mg (%)	0.06 ^c	0.07 ^c	0.07 ^c	0.07 ^{bc}	0.08 ^{ab}	0.08 ^a	0.08 ^a	0.01	<0.001
K (%)	0.7 ^{ab}	0.7 ^{ab}	0.7 ^{ab}	0.7 ^a	0.7 ^{ab}	0.7 ^{ab}	0.7 ^b	0.03	0.04
Na (%)	0.2 ^a	0.2 ^b	0.2 ^b	0.2 ^b	0.2 ^b	0.2 ^b	0.2 ^{ab}	0.01	<0.001
S (%)	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.01	0.08
Co (ppm)	2.3 ^{ab}	2.6 ^a	2.0 ^b	2.2 ^{ab}	2.5 ^{ab}	2.2 ^{ab}	2.3 ^{ab}	0.2	0.03
Cr (ppm)	3.2	3.3	3.2	3.0	3.3	3.3	3.1	0.2	0.12
Cu (ppm)	3.0 ^a	2.3 ^b	2.0 ^b	1.8 ^b	1.7 ^b	1.6 ^b	1.5 ^b	0.2	<0.001
Fe (ppm)	27.9	29.9	27.4	29.3	30.1	32.5	32.0	3.1	0.58
Mn (ppm)	1.5	1.3	1.2	1.4	1.5	1.5	1.5	0.2	0.21
Zn (ppm)	41.1 ^a	35.5 ^b	34.1 ^b	33.9 ^b	34.6 ^b	34.7 ^b	33.8 ^b	1.8	<0.001

^{a,b,c} Within a row, means without a common superscript letter differ (P < 0.05).

Table 6

Effects of stage of lactation on performance of nursing Nellore cows and suckling Nellore calves

Items	Stage of Lactation (d)							SEM	P-value
	0-28	28-56	56-84	84-112	112-140	140-168	168-196		
Cows									
Initial body weight (kg)	431.5	430.6	448.4	465.6	486.4	509.6	528.7		
Final body weight (kg)	430.6	448.4	465.6	486.4	509.6	528.7	543.8		
Average body weight (kg)	431 ^d	440 ^{cd}	457 ^{bcd}	476 ^{abcd}	498 ^{ab}	519 ^{ab}	536 ^a	15	<0.001
Weight gain (kg)	-0.90	15.3	17.2	20.8	21.5	17.8	15.1		
Calves									
Initial body weight (kg)	32.5	42.4	65.7	89.0	111	138	166		
Final body weight (kg)	42.4	65.7	89.0	114	138	166	195		
Average body weight (kg)	37.5 ^g	54.1 ^f	77.6 ^e	101 ^d	127 ^c	151 ^b	181 ^a	3.4	<0.001
Average daily gain (kg/d)	0.4 ^c	0.8 ^b	0.8 ^b	0.9 ^{ab}	1.0 ^a	1.0 ^a	1.1 ^a	0.03	<0.001

a,b,c,d,e,f,g Within a row, means without a common superscript letter differ (P < 0.05).

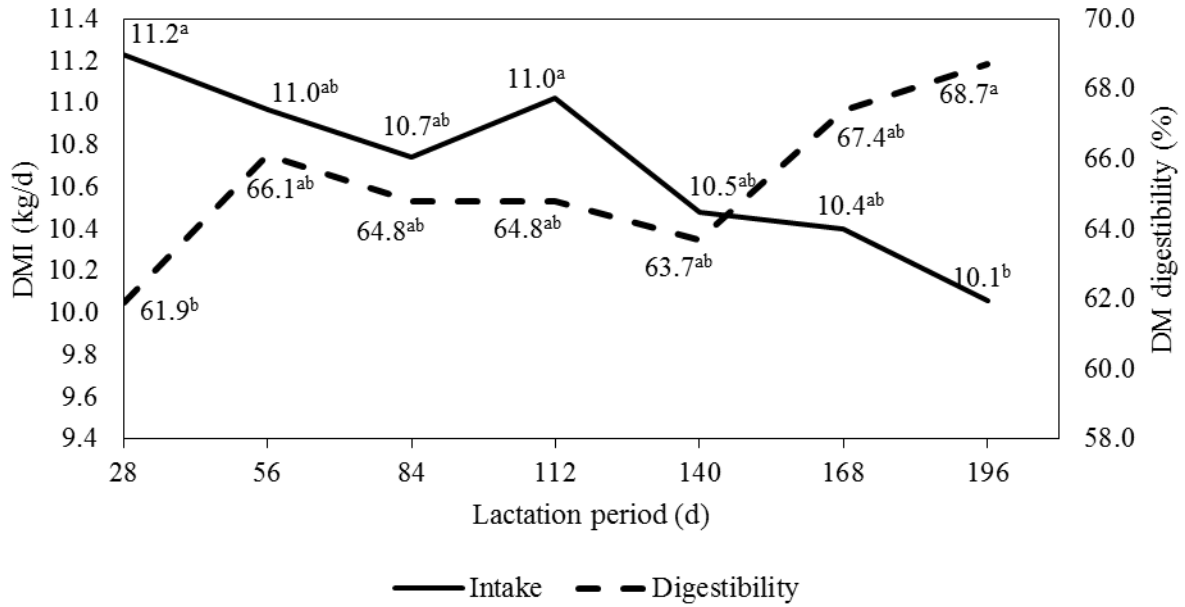


Fig. 1. Relationship between dry matter (DM) intake (DMI, kg/d) and DM digestibility (%) in nursing Nellore cows. ^{a,b} Within a row, means without a common superscript letter differ ($P < 0.05$)

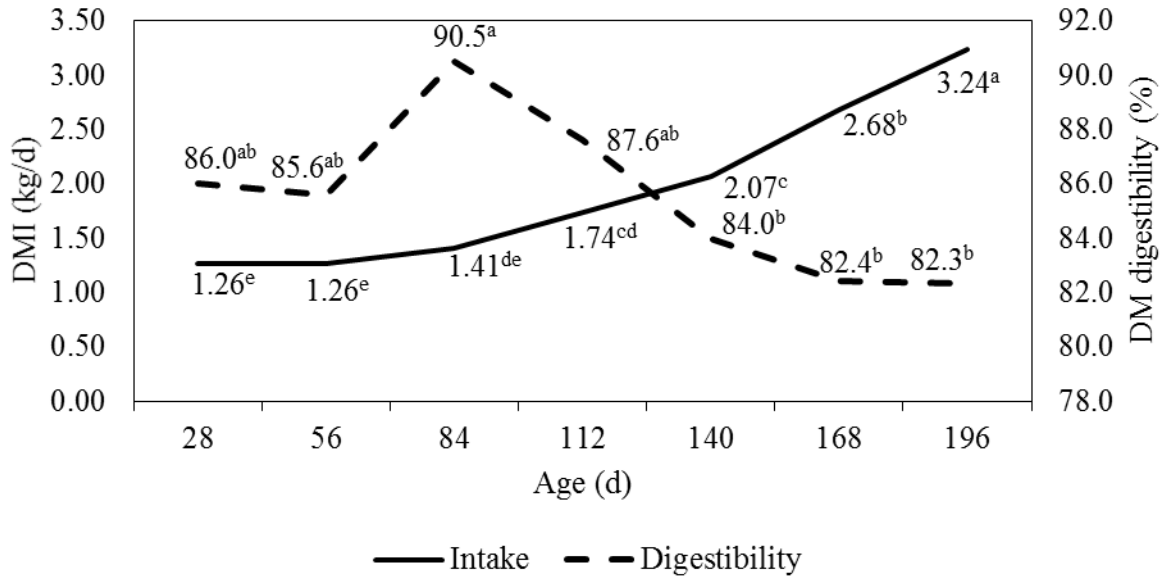


Fig. 2. Relationship between dry matter intake (DMI, kg/d) and DM digestibility (%) in suckling Nellore calves. ^{a,b,c,d,e} Within a row, means without a common superscript letter differ ($P < 0.05$)

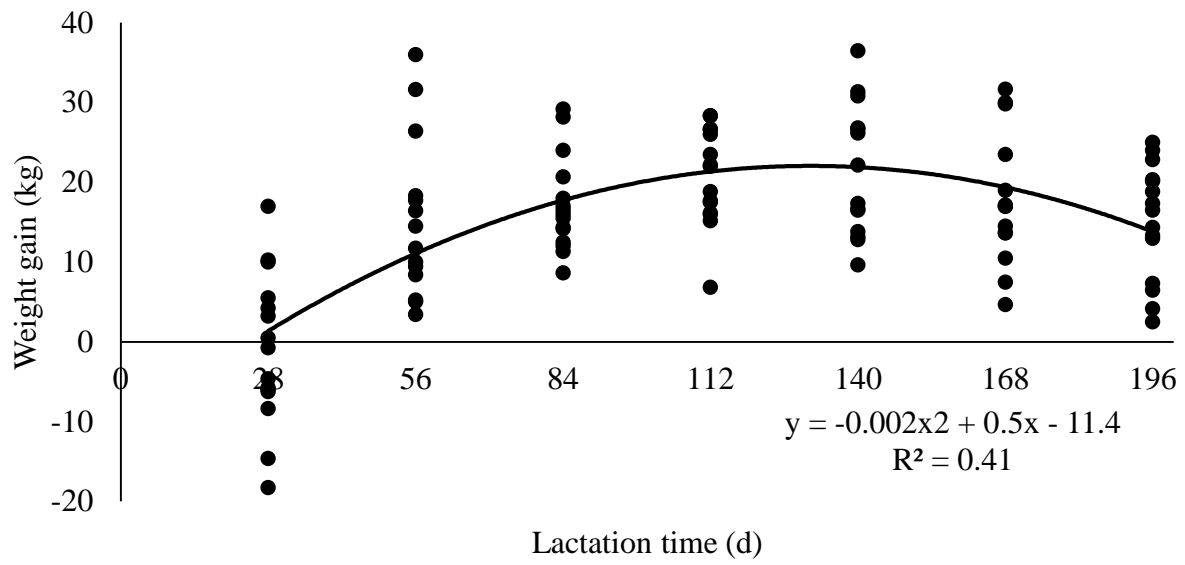


Fig. 3. Effects of stage of lactation on weight gain of nursing Nellore cows

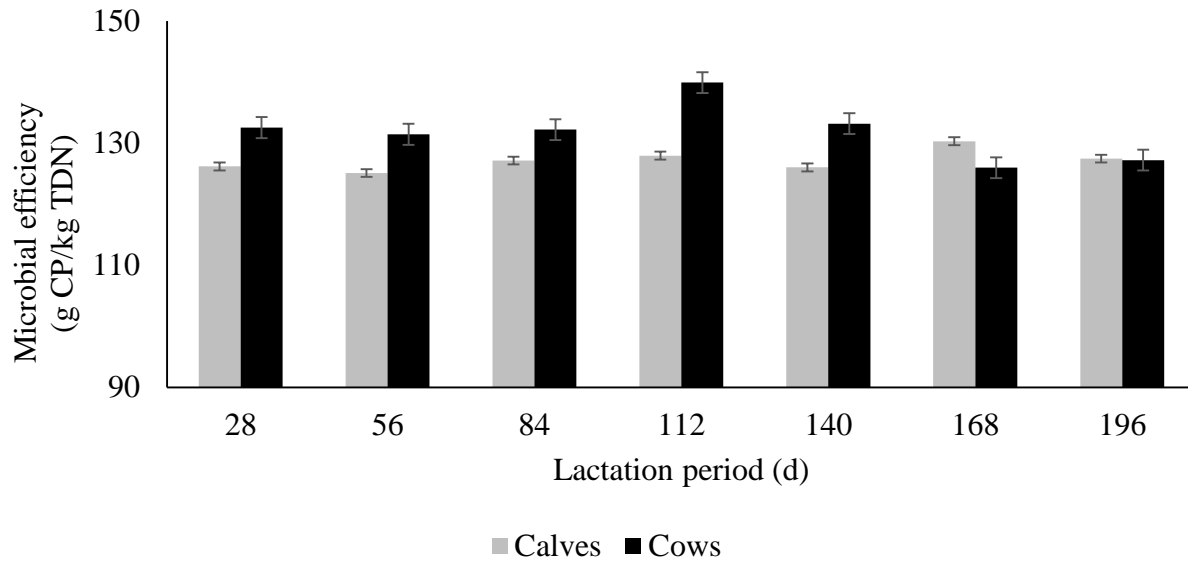


Fig. 4. Microbial efficiency of Nellore cows and calves during the lactation period.

CHAPTER 2

Running Head: Models to predict milk yield and feed intake of beef cattle

Evaluation of equations to predict potential milk yield and dry matter intake of lactating beef cows and suckling beef calves

ABSTRACT

An experiment was conducted to evaluate equations to predict potential milk yield (MY) and potential dry matter intake (DMI) of lactating beef cows and DMI of roughage and concentrate (DMIRC) of suckling beef calves. Fifteen multiparous Nellore cows were used and the diet consisted of corn silage and concentrate. The experiment started on the day of calving and the lactation period was considered to be from the day of calving up to 210 days later. Milking was performed on the 7th and 15th days after calving, and again every 15 days. After two months of age, calves were housed in adjacent pen and additionally fed corn silage ad libitum and concentrate. The intake of the animals was recorded daily. Five models were chosen to predict DMI of lactating beef cows and suckling beef calves while six models were chosen to predict MY. An unrelated database was built to evaluate the precision of MY models. Finally, five models were chosen to predict DMIRC of beef calves. Wilmink (1987) equation added of ADG as additional variable was the best one to predict DMI of cows while those proposed by Cobby and Le Du (1978) was chosen to predict MY. Also, this equation had the best estimate to predict MY from an unrelated database presenting lower CCC and MSE when compared to others on literature. The best equation to predict DMIRC of beef calves was those proposed by Von Bertalanffy (1957).

Due to the lack of information regarding to dry matter intake of lactating beef cows and suckling beef calves, we suggest the use of following equations to predict DMI of beef cows and beef calves, respectively: $DMI \text{ (g/kg BW)} = 27.259 - 13.861 \times e^{(-0.836 \times \text{wk})} - 0.317 \times \text{wk} + 0.606 \times \text{ADG}$ and $DMIRC = 8.224 \times (1 - 1.100 \times e^{(-0.037 \times \text{wk})})^3$ while milk yield of beef cows can be estimated by: $MY = 8.819 - 0.069 \times \text{wk} - 8.819 \times e^{(-3.211 \times \text{wk})}$.

Key words: beef cattle, lactation, milk production, unrelated database, feed intake

INTRODUCTION

The nutritional level of beef cows is the limiting factor for the majority of production systems when it comes to providing support for high milk yield, and to guarantee good body and physiologic conditions for reproduction. Also, DMI and the efficiencies of digestion and metabolism regulates productivity of livestock which is a determinant key of performance (McMeniman et al., 2009). Then, accurate predictions of DMI are needed so diets can be formulated to meet nutrient requirements and, once DMI is predicted, MY can be predicted using equations (NRC, 1996) when the variables are known. Ingvarlsen (1994) conducted a review regarding voluntary feed intake in cattle and observed that there are a lot of equations to estimate DMI of dairy cows and growing cattle; the simplest equation was proposed by Journet et al. (1965) who considered only milk yield (MY) and BW as variables, while the most complex system was proposed by Brown et al. (1977) who used season, BW, stage of lactation, MY, milk fat yield, and crude fiber as parameters in the same equation to estimate DMI.

Milk dependency decreases while the ability to graze increases as the calf grows. After approximately 3 mo, more than half of the required energy of beef calves is met by other sources than maternal milk (Fonseca et al., 2012). The NRC (1996) relayed that the prediction equations

for intake by nursing calves are important; however, no database exists that could develop such prediction equations due to wide variety of production situations and feedstuffs available for beef cattle producers. Therefore, our objective was to evaluate the accuracy and precision of equations to predict dry matter intake and milk yield of lactating beef cows and suckling beef calves.

MATERIALS AND METHODS

The experiment was conducted at the Experimental Feedlot of the Animal Science Department at the Universidade Federal de Viçosa, Viçosa, MG, Brazil. Laboratory analyses were conducted at the Ruminant Nutrition Laboratory of the Animal Science Department at the Universidade Federal de Viçosa. This study was approved by the Institutional Animal Care and Use Committee (IACUC) under process number 10/2013.

Animals, Treatments, and Measurements

Fifteen multiparous Nellore cows and their calves were used in this experiment. Cows were acquired from and managed in the Beef Cattle Section at the feedlot from the time when they were pregnant at 8 mo. The animals were kept in individual pens with concrete floors, a concrete feeder, and a trough for water; the total area was 30 m², with 8 m² of area under cover.

The diet consisted of 85% of corn silage on a DM basis and 15% of concentrate that was formulated with ground corn (7.33%), soybean meal (5.11%), urea (0.86%), ammonium sulfate (0.09%), limestone (0.59%), common salt (0.12%), calcium phosphate (0.89%), and a mineral mix (0.02%). The use of this ratio was chosen to simulate a pasture with good quality and quantity. Feedstuffs were provided twice daily and intake was adjusted to allow the minimumorts without restricting consumption, while water was provided ad libitum. The amount of feed that was

supplied was recorded daily; additionally, the ingredients in the concentrate were sampled directly from the silos of the feed factory every time they were mixed. Feed samples were obtained as described above. Corn silage was sampled every day, stored, and a compound sample was performed every 7-d period. After, this sample was stored in the ultra-freezer (-40°C) to be lyophilized.

The experiment started on the day of calving and the lactation period was considered to be from calving until 210 d when the calves were weaned performing 450 observations for DMI of lactating cows, 240 observations for MY, and 345 observations for DMI of suckling beef calves. After calving, calves were stimulated to suckle to guarantee an adequate colostrum intake. At the same time, a colostrum sample was collected to evaluate colostrum composition. Calves were kept in the same pen as their dams during the first 2 months of age. Milking was performed on the 7th and 15th d after calving, and every subsequent 15 d.

Milking was done performed in the following way: at 15h00 of the previous milking day, the calves were separated from their dams and were taken to the management pen. After 2 h, the calves were again rejoined with their dams. This procedure was necessary to stimulate the calves to suckle, and then the udder could be emptied. After 30 min, the calves were again separated and were taken to the management pen with water provided ad libitum. The time was recorded at the moment of sorting. Cows were milked manually on the following day with 2 mL of oxytocin in the vena jugulars to promote the full milk flow. The calf was presented in front of its dam to reduce the stress during milking. After milking, the time was recorded again to extrapolate the milk yield for a 24 h-period. Each cow and its calf were weighed and managed in their pens until the next milking. The weights were recorded to evaluate the variation in the weight of the cows and ADG of the calves (Gifford, 1953).

After 2 months of age, the calves were separated from their dams and were housed individually in pens adjacent to their dams. Thus, the calves could suckle milk ad libitum by the fence and also, DMI of the cows and the calves could be individually evaluated. Calves were additionally fed corn silage ad libitum and concentrate with 30% crude protein, which consisted of soybean meal (59.0%, ground corn (38.6%), limestone (1.42%), calcium phosphate (0.80%), common salt (0.14%), and mineral mix (0.11%). The concentrate intake was limited to 0.5% body weight, and every 15 d, the intake was adjusted when the calves were weighed. The intake of concentrate was limited to simulate the supply of a supplement provided on pasture condition. Concentrate and corn silage were provided separately in different buckets to measure each intake level. The intake was recorded daily and it was adjusted to provideorts from 5 to 10% of the intake.

Statistical Analyses

Dry matter intake of lactating beef cows

DMI of lactating beef cows were adjusted using five nonlinear functions found on literature with different number of parameters and biological interpretation (Table 1). Due to variation observed on body weight (average daily gain of 0.42 kg/d) presented by lactating beef cows, this variable was added in each model to improve the adjustment of the models.

Milk yield of beef cows

MY of beef cows were adjusted using five nonlinear functions found on literature with different number of parameters and biological interpretation (Table 1) and a linear function was tested to evaluate milk yield. Total milk yield was obtained through the sum of observations of the milk weight, multiplied by 7 for the first 2 milking and by 15 for the following collections. Thus, estimates of lactation at peak (Y_{max}), persistence and milk production accumulated up to weaning (Y_w) were calculated from function's parameters.

Validation of equations to predict milk yield

An independent database was developed to evaluate whether the best equation generated from this study and those suggested by the BR CORTE (Valadares Filho et al., 2010) were accurate when it comes to predicting MY of beef cows. The unrelated database was composed of data from 461 lactations performed in Nellore cows from the beef cattle section of the Animal Science Department at UFV. Thus, the descriptive statistics of the unrelated database can be seen in Table 2. The predicted values were compared to the observed values using the following regression model: $y = \beta_0 + \beta_1 \times x$, where x is the predicted values, y is the observed values, and β_0 and β_1 are intercept and slope, respectively. The regression was evaluated according to the following statistical hypothesis: $H_0: \beta_0 = 0$, $H_0: \beta_1 = 1$, and $H_a: \text{not } H_0$. If the null hypotheses were not rejected, it could be concluded that the equations accurately estimated milk yield of beef cows. The slope and the intercept were evaluated separately in order to observe the equations for possible errors. Estimates were evaluated by using the estimated value of the mean square error of the prediction (MSEP) and its components (Kobayashi and Salam, 2000):

$$\text{MSEP} = \text{SB} + \text{MaF} + \text{MoF} = 1/n \sum_i (x_i - y_i)^2$$

$$SB = (x - y)^2;$$

$$MaF = (s_x - s_y)^2;$$

$$MoF = 2s_x s_y(1 - r),$$

where x is the predicted value; y is the observed value; MSEP is the mean square error of prediction; SB is the square bias; MaF is the component relative to the magnitude of random fluctuation; MoF is the component relative to the model of random fluctuation; s_x and s_y are the standard deviations of the predicted and observed values, respectively; and r is the Pearson linear correlation between the predicted and observed values. For all calculations of variance and covariance, the total number of observations was used as a divisor, because it was an estimate of the prediction error (Kobayashi and Salam, 2000). The prediction of efficiency was determined by estimating the correlation and concordance coefficient or the reproducibility index as described by Tedeschi (2006). The parameters β_0 and β_1 were evaluated separately to determine whether the bias was represented by a constant (it was represented by the intercept with values different from zero) or by a percentage bias (it was represented by the slope with values different from 1.0). The correlation and concordance coefficient indicate the presence of models with good accuracy and precision (when close to 1.0) or models with a problem of reproducibility (when close to 0.0). The smallest mean square error of prediction indicates the best model in the evaluation. In this study, it can indicate that the model error is associated with the squared bias, errors related to the high dispersion of data around the mean, or systematic errors concerning the direction of the predicted curve.

Dry matter intake of suckling beef calves

DMI of roughage and concentrate (DMIRC) of suckling beef calves were modeled using five nonlinear functions found on literature utilized as growth curves (Table 1).

Parameters to evaluate the adjustments of equations

Parameters of adjustment were calculated to be used on model selection for each variable (DMI, MY, and DMIRC): adjusted determination coefficient ($R^2_{adj} = 1 - [(n-1) / (n-p)](R^2)$ where p = number of parameters, n = number of observations), mean square error of prediction ($MSEP = \sum(\text{Observed} - \text{Predicted production})^2 / n$), root MSEP, and Akaike information criteria corrected ($AICC = 2 \times n - 2 \times \log \text{likelihood}$). The parameters of the functions were estimated through the procedure NLMIXED of SAS (SAS Inst. Inc., Cary, NC - version 9.3) where animal was considered as random effect and milk collection, DMI, and DMIRC as fixed effect and were adjusted by the Gauss-Newton method. The level of 0.05 was established as the critical level of probability for a type I error.

RESULTS AND DISCUSSION

Intake, milk yield, and overall animal performance of lactating Nellore cows and their calves during a 7-mo lactation period are shown in Table 3. DMI of lactating Nellore cows varied from 12.3 to 35.2 g/kg body weight, while milk yield varied from 4.55 to 11.4 kg/d. Total milk yield at weaning varied from 1,272 to 2,240 kg with average of $1,728 \pm 285.4$ kg. The selection of the model was simultaneous based on greater value of R^2_{adj} in order to refer to the proportion of total variation on milk yield explained by functions and lower values of MSEP, RMSEP, related to

accuracy of prediction, and lower AICC that measures the adjustment of model by maximum likelihood.

Dry matter intake of lactating beef cows

The equation proposed by Wilmink (1987) with ADG as additional variable (WK) had the best adjustment from database used to predict DMI of lactating beef cows presenting the greatest R^2_{adj} and the lowest AICC and MSE (Table 4). The equation from Dave (1971) modified (DV) had the worst approach to estimate DMI of lactating beef cows with the lowest R^2_{adj} and the greatest AICC and MSE. The Brazilian Table of Nutrient Requirements for Zebu cattle – BR CORTE (Valadares Filho et al., 2010) suggested 23.5 g/kg BW as an average for the dry matter intake of lactating Nellore cows during a 7-mo lactation period. Comparing this recommendation with the best equation proposed here (WK; Figure 1), we can observe that the values are close up to twenty-second week. After this week, the difference varies from 3.40 to 5.76 g/kg BW when the equation of WK estimates lower values than those proposed by BR CORTE (2010).

Shah and Murphy (2006) suggested that in addition to several models that have been developed to predict dry matter intake for lactating cows, the models currently in use indicate that they are inaccurate. In the same way, McMeniman et al. (2009) evaluated the NRC (1996) dry matter intake prediction equations and observed that DMI was overpredicted by the NRC equations suggesting development of new equations. Also, these models did not consider important factors that influence dry matter intake or were developed to apply to a certain type of cow and diet (Mertens, 1996; Ingvarlsen and Andersen, 2000). Due lack of information regarding equations to predict DMI of lactating beef cows, we suggest the use of following equation: $DMI (g/kg BW) = 27.259 - 13.861 \times e^{(-0.836 \times wk)} - 0.317 \times wk + 0.606 \times ADG$ which was the equation with the best adjustment.

However, we highlight that this equation needs to be tested for beef cows raised on pasture and for other breeds to determine whether this equation predicts well for the most common tropical conditions.

Milk Yield

Three equations (LN, WD, and CL) to predict milk yield presented the lowest MSE and AICC (Table 4; Figure 2). The DJ and NRC equations presented the greatest MSE and AICC showing that the model proposed by Dijkstra et al. (1997) and NRC (1996) did not adjust to data of beef cows. Petrie et al. (1984) evaluated colostrum yield in beef cows and verified mean volume of 2.99 kg/d. If LN and WD functions were chosen, colostrum yield might be 8.73 and 8.23 kg/d, respectively, which is biologically unlikely. However, when the model proposed by Cobby and Le Du (1978) was used, colostrum yield estimated might be 3.23 kg/d which close to observed by Petrie et al. (1984). Several equations are currently being evaluated for milk yield of beef cows (Henriques et al., 2011; Rodrigues et al., 2014); however, they used models developed from dairy cattle (Wood, 1967) or were basically theoretical (Nelder, 1966). As previously evaluated, the model proposed by Wood (1967), despite to adjust well to database of this study, did not meet colostrum yield. Thus, we suggest the following equation to predict milk yield of beef cows: $MY = 8.819 - 0.069 \times wk - 8.819 \times e^{(-3.211 \times wk)}$.

The BR CORTE (2010) recommended the following equation: $MY = 5.9579 + 0.4230 \times wk \times \exp^{(-0.1204 \times wk)}$ to estimate milk yield of Nellore cows from study developed by Henriques et al. (2011) which generated based on Jenkins and Ferrell (1984) recommendations. Thus, the best equation in this study (CL) was compared to the equation proposed by BR CORTE (2010) to evaluate which one predicts better milk yield of beef cows. From the unrelated database, the BR

CORTE (2010) equation did not estimate correctly ($P < 0.05$) milk yield because the intercept and slope differed from zero and 1.0, respectively, and its correlation and concordance coefficient (CCC) was not closer to 1 (Table 5; Figure 3). In other hand, Cobby and Le Du (1978) equation estimated correctly ($P > 0.05$) milk yield of beef cows because the intercept and slope did not differ from zero and 1.0, respectively, and presented greater CCC and smaller values for MSE than BR CORTE (2010; Table 5). Also, the main errors are associated with random errors (model of random fluctuation - MoF equal 99.5%; Table 5) which shows that the errors associated to the model are low for the model proposed by Cobby and Le Du (1978) to estimate milk yield. Thus, we recommend the following equation to estimate milk yield (MY): $MY = 8.819 - 0.069 \times wk - 8.819 \times e^{(-3.211 \times wk)}$.

Additionally, the persistency of lactation was estimated by parameter “b” on CL and LN equations as 6.9 and 6.5% which is the decrease rate of production after peak of lactation while for WD equation the persistency was estimated as 1.1% by parameter “c”. As CL equation was chosen as the best equation to predict milk yield of beef cows, we considered as 6.9% the decrease rate of production after peak of lactation.

Dry Matter Intake of Suckling Nellore Calves

The equations proposed here were only used to estimate dry matter intake of roughage and concentrate (DMIRC) and not to predict total dry matter intake. Data of DMIRC of suckling beef calves are shown in Table 3 and Figure 4 and ranged from 0.03 to 2.42 kg/d, and BW ranged from 36.7 to 268 kg.

The model with the best fit to predict DMIRC of suckling beef calves was those proposed by Von Bertalanffy (1957) due to the lowest MSE and AICC (Table 6). The BR CORTE (2010) and

NRC (1996) did not recommend any equation to estimate DMI for nursing calves and also, there is no study in the literature evaluating DMI of roughage and concentrate of calves during breast-feeding period. The BR CORTE (2010) cited only one study (Fonseca et al., 2012) that evaluated nutrient requirements for nursing calves. These authors verified total DMI of suckling Nellore calves presented average values of 18.1 and 25.4 g/kg BW for 0 to 90 and 90 to 180 days of age, respectively, which are below than those recommended by NRC (1996; 35.3 g/kg BW). These authors attributed the lower total DMI to lower milk yield of Nellore cows. Considering the same period in this study and the use of the equation to predict MY to establish DMI from milk, the average total DMI were 21.4 and 14.8 g/kg BW for the same period evaluated by Fonseca et al. (2012). Thus, the calves' BW increased faster than their ability to increase intake.

An important subject that may interfere DMI of beef calves is milk composition (Rodrigues et al., 2014). In this study, the average values for these constituents were 5.61% fat, 3.68% protein, 4.58% lactose, and 15.0% total solids (Costa e Silva et al., 2015). We observed that the greatest variations in milk composition were in terms of fat and total solids contents, and thus, the most important factor that could contribute to lower dry matter intake may be fat content. These values were greater than those presented by the NRC (2001). Rodrigues et al. (2014) evaluated milk composition for 4 different crossbred beef cows and recommended an average 3.59% fat, 3.04% protein, 4.71% lactose, and 12.2% total solids. Therefore, lower dry matter intake observed for nursing Nellore calves by Fonseca et al. (2012) and in the present study are more related to milk composition than milk production. Thus, we suggest the following equation be used to predict DMI of roughage and concentrate of suckling beef calves from the 2nd to 7th mo of age: $DMIRC = 8.224 \times (1 - 1.100 \times e^{(-0.037 \times wk)})^3$.

Conclusions

Dry matter intake and milk yield of beef cows can be estimated by the following equations: $DMI \text{ (g/kg BW)} = 27.259 - 13.861 \times e^{(-0.836 \times \text{wk})} - 0.317 \times \text{wk} + 0.606 \times \text{ADG}$ and $MY = 8.819 - 0.069 \times \text{wk} - 8.819 \times e^{(-3.211 \times \text{wk})}$, respectively. Also, the equation to predict milk yield of beef cows is recommended since it had the best results when compared to the data observed from beef cows raised on pasture. Dry matter intake of roughage and concentrate of suckling beef calves can be estimated by the following equation: $DMIRC = 8.224 \times (1 - 1.100 \times e^{(-0.037 \times \text{wk})})^3$.

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Table 1 Nonlinear functions utilized to modulate DMI and MY of lactating beef cows

Authors	Acronym	Functions	
		Cows	
		Dry matter intake	Milk yield
Wood (1967)	WD	$Y_t = at^b e^{-ct} + d \times \text{ADG}$	$Y_t = at^b e^{-ct}$
Dave (1971)	DV	$Y_t = a + bt + ct^2 + d \times \text{ADG}$	-
Cobby and Le Du (1978)	CL	$Y_t = a - bt - ae^{-ct} + d \times \text{ADG}$	$Y_t = a - bt - ae^{-ct}$
Wilmink (1987)	WK	$Y_t = a - be^{-ct} + dt + f \times \text{ADG}$	$Y_t = a - be^{-ct} + dt$
Dijkstra et al. (1997)	DJ	$Y_t = ae^{b(1-e^{-ct})/c-dt} + f \times \text{ADG}$	$Y_t = ae^{b(1-e^{-ct})/c-dt}$
NRC (1996)	NRC	-	$Y_t = t / (ae^{-ct})$
Linear	LN	-	$Y_t = a + bt$
Calves			
Brody (1945)	BD	$Y_t = a \times (1 - b \times e^{(c \times \text{wk})})$	-
Von Bertalanffy (1957)	VB	$Y_t = a \times (1 - b \times e^{(c \times \text{wk})})^3$	-
Laird (1966)	LR	$Y_t = a \times e^{(b \times e^{(c \times \text{wk})})}$	-
Logistic	LG	$Y_t = a / (1 + b \times e^{(c \times \text{wk})})$	-
Gompertz	GP	$Y_t = a \times e^{(eb \times e^{(c \times \text{wk})} - 1)/c}$	-

Y_t = milk yield (kg/d) or dry matter intake (g/kg BW) for lactating beef cows, or dry matter intake of roughage and concentrate (kg/d) for suckling beef calves, t = time of lactation or age (weeks), e = natural logarithm base, a = theoretical initial production or intake (WK, DV, DJ, LN), b = decrease rate of production after peak of lactation or intake (CL, LN), increase rate of production up to peak of lactation or intake (WD, WK), rate of cell differentiation at calving (DJ), c = increase rate of production up to peak of lactation or intake (CL), decrease rate of production after peak of lactation or intake (WD, DV), time when peak of lactation or intake occurs (WK), speed drop rate of cell differentiation (DJ), d = decrease rate of production after peak of lactation or intake (WK), death rate of differentiated cells (DJ)

Table 2 Descriptive statistics of unrelated database utilized to compare equations to predict milk yield of beef cows

Study	Item	n	Mean	SD	Maximum	Minimum
Cardenas (2012)	Week of lactation	170	28.1	6.38	40.0	12.0
	Milk yield		7.00	1.36	9.87	4.21
Lopes (2012)	Week of lactation	143	26.5	5.45	37.0	12.0
	Milk yield		6.97	1.58	9.99	4.24
Lopes (data no shown)	Week of lactation	37	8.05	2.65	12.0	3.00
	Milk yield		8.47	1.46	10.8	5.79
Contreras (2013)	Week of lactation	61	27.3	8.63	41.0	10.0
	Milk yield		6.49	1.64	9.40	3.37

Table 3 Descriptive statistics of data used in model adjustments

Items	Cows				Calves		
	DMI (kg/d)	BW (kg)	DMI (g/kg BW)	MY ¹ (kg/d)	DMIRC ² (kg/d)	Total DMI (kg/d)	BW (kg)
				mo 1			
Mean	10.4	430	24.3	8.68	0.00	1.19	43.9
SD	1.47	47.4	3.87	1.56	0.00	0.31	5.00
Maximum	12.9	493	31.6	11.4	0.00	1.85	55.4
Minimum	7.46	344	15.4	5.89	0.00	0.60	36.7
				mo 2			
Mean	11.3	449	25.5	8.12	0.00	1.21	65.6
SD	1.27	55.7	4.56	1.46	0.00	0.27	8.70
Maximum	13.9	535	35.2	10.9	0.00	1.74	90.0
Minimum	9.48	354	17.7	6.27	0.00	0.80	56.4
				mo 3			
Mean	11.0	466	24.0	7.99	0.27	1.31	87.4
SD	1.12	57.8	4.47	1.27	0.10	0.24	16.1
Maximum	13.0	557	33.3	11.1	0.39	1.67	120
Minimum	9.00	369	16.5	6.34	0.14	0.70	66.6
				mo 4			
Mean	11.3	488	23.5	7.89	0.59	1.68	111
SD	1.10	57.6	3.87	1.16	0.14	0.30	16.1
Maximum	14.1	584	30.5	10.0	0.82	2.19	149
Minimum	9.18	390	16.9	5.98	0.45	0.94	73.4
				mo 5			
Mean	11.0	511	22.0	7.38	0.97	2.09	138
SD	1.12	65.2	4.35	1.32	0.08	0.33	20.7
Maximum	12.6	629	30.7	9.66	1.09	2.75	185
Minimum	8.50	405	13.7	4.84	0.89	1.41	86.6
				mo 6			
Mean	10.3	532	19.9	6.95	1.46	2.68	169
SD	1.09	70.4	3.93	1.22	0.12	0.39	24.6
Maximum	12.4	668	26.2	9.20	1.64	3.40	218
Minimum	8.47	421	12.9	4.70	1.31	1.94	108
				mo 7			
Mean	10.1	551	18.8	6.97	1.99	3.25	207
SD	1.20	73.3	3.68	1.53	0.11	0.44	28.9
Maximum	12.5	694	25.1	9.66	2.11	3.85	268
Minimum	8.33	437	12.3	4.55	1.85	2.23	138

¹Milk yield.²DMI of roughage and concentrate.

Table 4 Descriptive statistics for equations to predict dry matter intake and milk yield of lactating beef cows

Item	WD	DV	CL	WK	DJ	LN	NRC
Dry matter intake							
a	22.514	24.152	26.780	27.259	14.284	-	-
b	0.135	0.040	0.303	13.861	0.746	-	-
c	0.024	0.010	1.453	0.836	0.098	-	-
d	0.699	0.911	0.561	0.317	0.075	-	-
e	-	-	-	0.606	0.650	-	-
MSE	19.5	20.1	19.5	19.4	20.0	-	-
R ² adj	24.0	22.0	24.2	24.3	23.3	-	-
AICC	1934	1977	1928	1924	1938	-	-
Milk yield							
a	8.606	-	8.819	8.867	7.585	8.737	0.838
b	0.022	-	0.069	1.297	0.169	0.065	0.248
c	0.011	-	3.211	1.111	1.022	-	-
d	-	-	-	0.071	0.009	-	-
MSE	2.76	-	2.76	2.76	5.42	2.76	53.42
R ² adj	11.4	-	11.4	11.5	-4.34	11.2	11.1
AICC	736	-	736	738	847	735	785

WD: Wood (1967); DV: Dave (1971); CL: Cobby and Le Du (1978); WK: Wilmink (1987); DJ: Dijkstra et al. (1997); LN: linear function; NRC: NRC (1996). MSE: mean square error.

Table 5 Mean (kg) and descriptive statistics for relationship among the observed and predicted milk yield

Items	OBS ¹	Cobby and Le Du (1978)	BR CORTE (2010)	NRC (1996)
Mean	7.04	7.05	6.50	3.49
SD	1.57	0.58	0.32	1.98
Maximum	10.8	8.57	7.25	8.00
Minimum	3.37	5.98	6.08	0.83
R	-	0.39	0.15	0.15
CCC ²	-	0.65	0.14	0.13
Regression				
Intercept				
Estimate	-	-0.42	-5.29	5.97
SE	-	0.88	1.45	0.15
P value ³	-	0.64	< 0.001	< 0.001
Inclination				
Estimate	-	1.06	1.90	0.31
SE	-	0.12	0.22	0.04
P value ⁴	-	0.63	< 0.001	< 0.001
MSEP ⁵	-	2.09	2.47	16.6
SA ⁶	-	0.00	0.30	12.6
MaF ⁷	-	0.01	0.08	1.86
MoF ⁸	-	2.08	2.09	3.79
RMSEP ⁹				
kg		1.45	1.57	4.07
%		20.5	24.2	57.8

¹Observed values. Cobby and Le Du (1978) = predicted values from Cobby and Le Du (1978) equation: milk yield = 8.819 - 0.069 x wk - 8.819 x e^(-3.211 x wk); BR CORTE (2010) = predicted values in BR CORTE (Valadares Filho et al., 2010) equation; NRC (1996): milk yield = wk/(0.3911 x e^(0.1176 x wk)). ²Correlation and concordance coefficient. ³H₀: β₀ = 0. ⁴H₀: β₁ = 1. ⁵Mean square error of prediction. ⁶SB = square bias. ⁷MaF = magnitude of random fluctuation. ⁸MoF = model of random fluctuation. ⁹Root mean square error of prediction, in kg or percentage of the average observed value

Table 6 Descriptive statistics for equations to predict dry matter intake of roughage and concentrate of suckling beef calves

Item	GP	BD	VB	LG	LR
a	0.003	-0.883	8.224	3.011	6.279
b	0.604	0.673	1.100	74.758	7.452
c	0.076	0.056	0.037	0.178	0.066
MSE	0.109	0.113	0.109	0.114	0.114
R ² adj	88.2	88.7	89.2	88.3	90.8
AIC	12.3	-113.9	-126.3	-119.7	350.7

GP: Gompertz function; BD: Brody (1945); VB: Von Bertalanffy (1957); LG: logistic function;

LR: Laird (1966). MSE: mean square error.

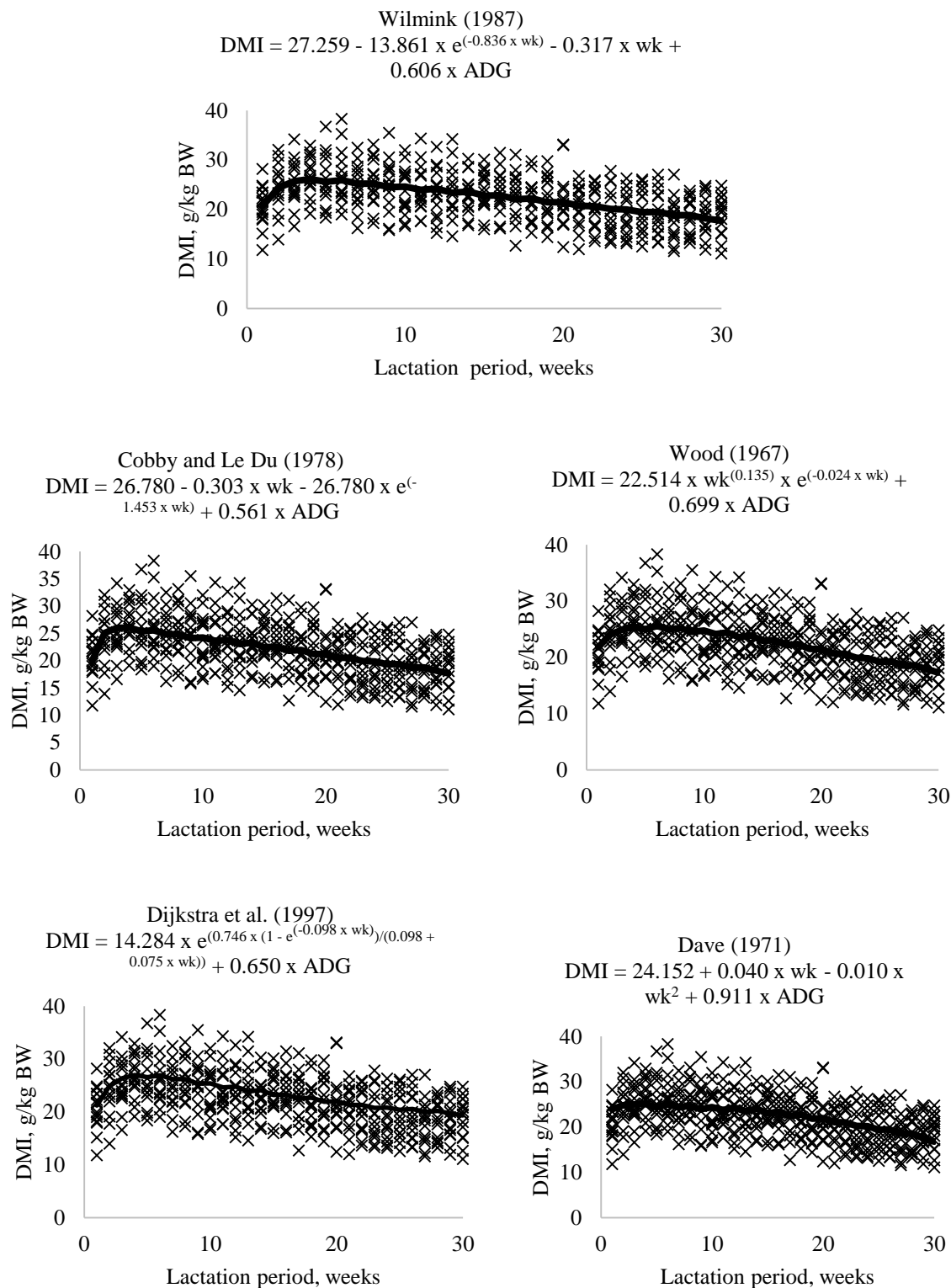


Figure 1 Equations to predict dry matter intake (DMI, g/kg BW) of lactating beef cows.

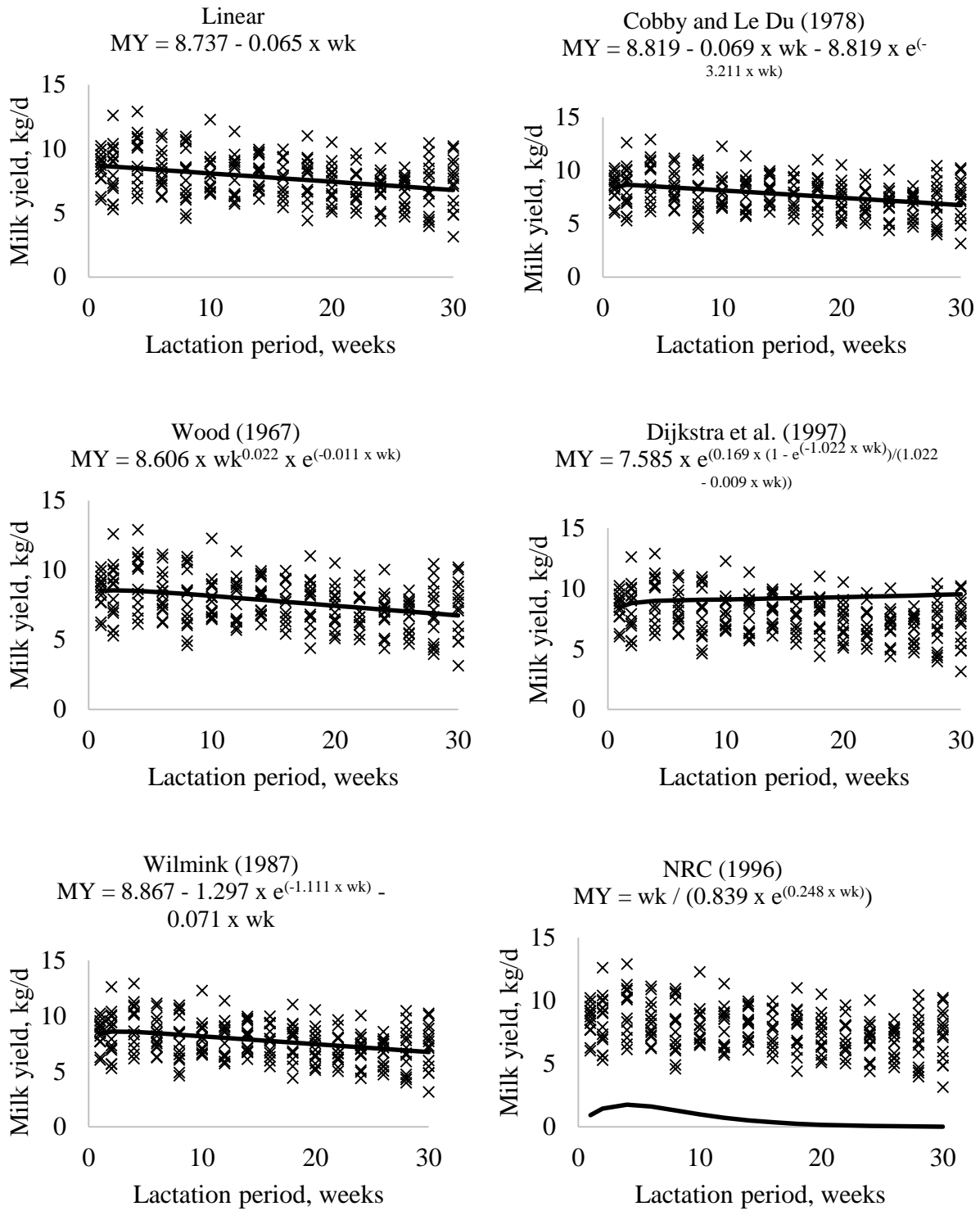


Figure 2 Equations to predict milk yield (MY) of beef cows.

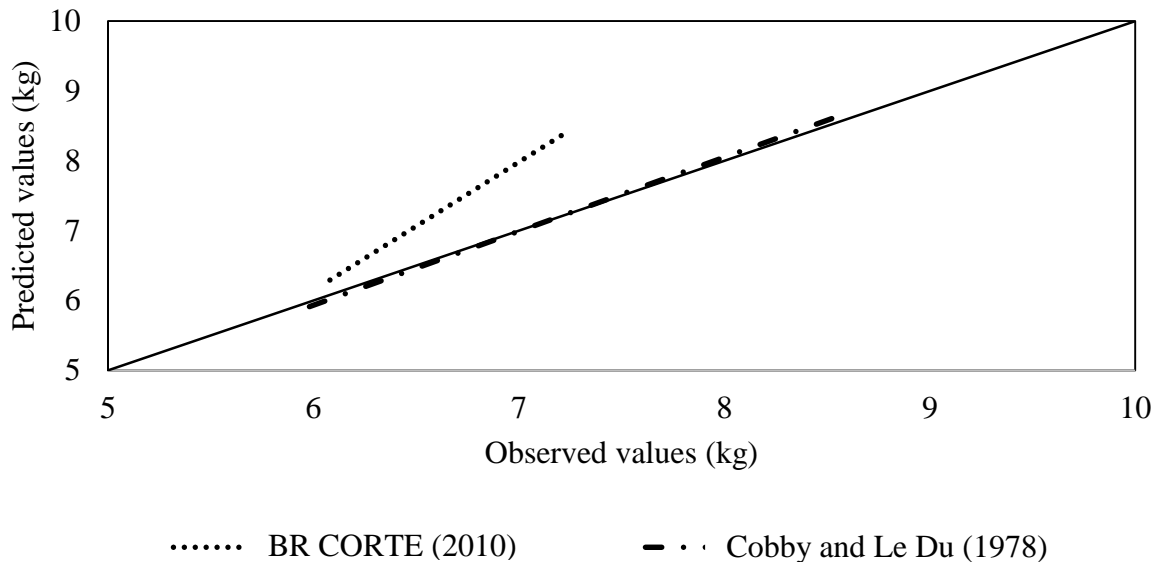


Figure 3 Relationship among the predicted and observed values of estimated MY according to the BR CORTE (Valadares Filho et al., 2010) and (Cobby and Le Du, 1978) equation.

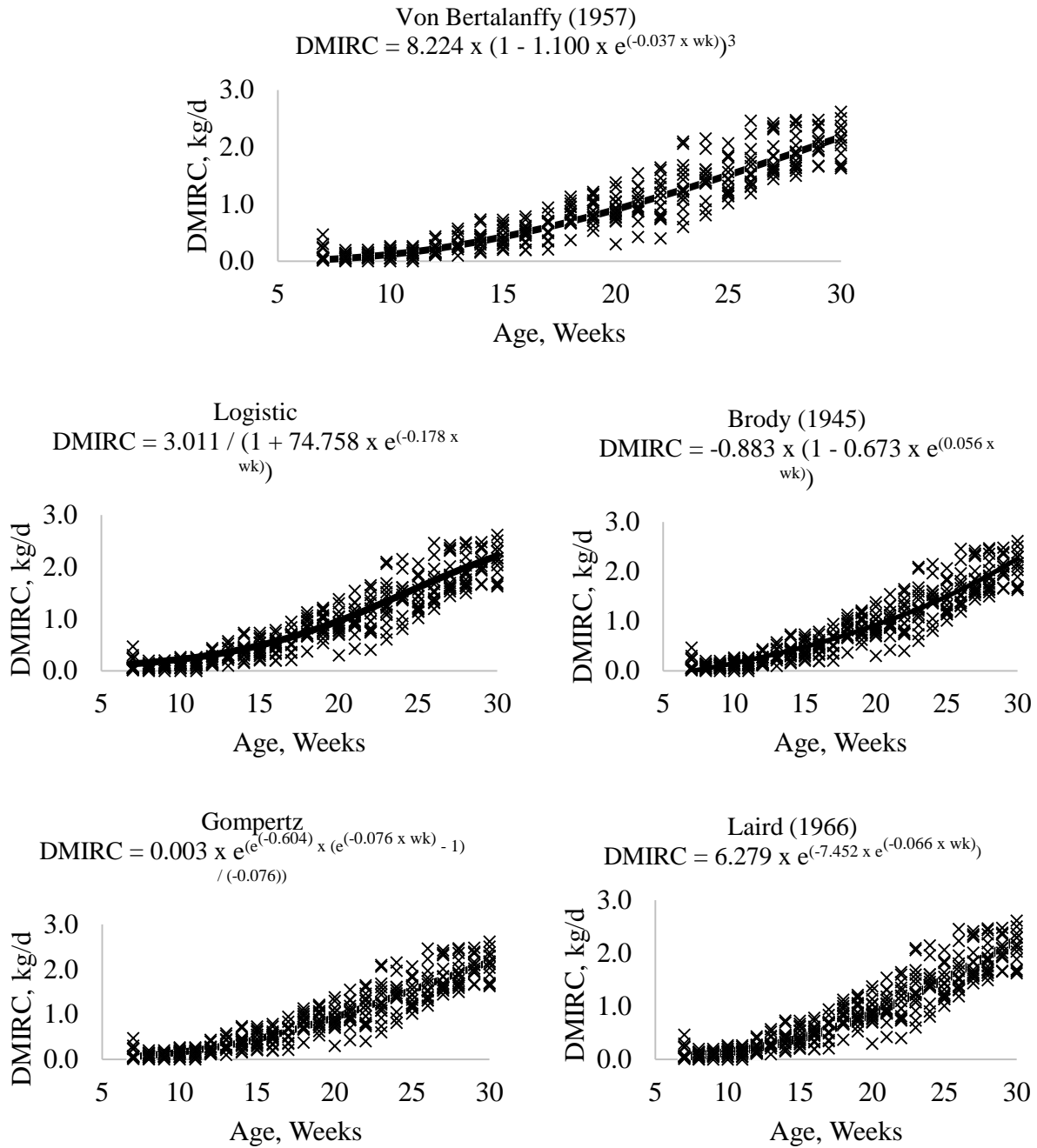


Figure 4 Equations to predict dry matter intake of roughage and concentrate (DMIRC, kg/d) of suckling beef calves.

CHAPTER 3

Intake, apparent digestibility, and nutrient requirements for growing Nellore heifers and steers fed two levels of calcium and phosphorus

Abbreviations: ADG, average daily gain; ADLIB, ad libitum; AS, ammonium sulfate; BW, body weight; CaPR, 100% of Ca and P requirements; CaPL, 43% of Ca requirement and 80% of P requirement; CP, crude protein; DE, digestible energy; DM, dry matter; EBG, empty body weight gain; EBW, empty body weight; EE, ether extract; HP, heat production; KPH, the amount of kidney, pelvic, and heart fat; MAIN, maintenance; MEI, metabolizable energy intake; MEm, metabolizable energy requirement for maintenance; MPm, metabolizable protein requirement for maintenance; N, nitrogen; NDF, neutral detergent fiber; NEm, net energy for maintenance; NEg, net energy for growth; NFC, non-fiber carbohydrates; NPg, net protein requirement for growth; NPm, net protein requirement for maintenance; R:C, roughage:concentrate ratio; RE, retained energy; TDN, total digestible nutrients; U, urea.

ABSTRACT

Nutrient apparent digestibility and intake, performance, microbial efficiency, and energy and protein requirements for growing Nellore heifers and steers were evaluated, fifty animals were used, 32 Nellore heifers and 18 Nellore steers. Four animals of each sex used as baseline reference animals were slaughtered at the beginning of the experiment. Four animals from each sex were fed at MAIN receiving 11 g/kg BW, whereas 10 steers and 24 heifers were assigned to the ADLIB group. The ADLIB heifers were divided further into 4 groups according to dietary treatment: 1)

Ca and P fed at their proposed requirements (CaPR) with a 50:50 roughage:concentrate (R:C) diet, 2) CaPR with a 70:30 R:C diet, 3) 43% of their proposed requirements for Ca and 80% of their requirements for P (CaPL) with a 50:50 R:C diet, and 4) CaPL with a 70:30 R:C diet. The ADLIB and MAIN steers were fed CaPR with a 50:50 R:C diet. Half of the ADLIB steers and heifers were slaughtered at d 50; the other ADLIB animals were slaughtered after 100 days of the feeding period, whereas all MAIN animals were slaughtered at d 100. Total feces and urine were collected from all animals for 72 h prior to slaughter. After slaughter, EBW was measured. The NEm and MEm requirements were estimated by exponentially relating heat production and metabolizable energy intake; NEg was estimated from EBW and EBG. The NPg was estimated from EBG and RE. Dry matter digestibility and the apparent absorption and retention of Ca and P were similar across Ca and P treatments. Final body weight, and consequently ADG, was higher ($P < 0.05$) for heifers receiving the high compared to the low concentrate diet, but dietary Ca and P concentration did not affect ($P > 0.05$) performance. The NEm and MEm requirements were 70.1 and 118 kcal/kg $EBW^{0.75}$, respectively. Net protein for maintenance was 1.28 g/kg $BW^{0.75}$ and NEg and NPg were estimated from the following equations: $NEg = 0.053 \times EBW^{0.75} \times EBG^{0.6301}$ and $NPg = 137.85 \times EBG - 0.05 \times RE$, respectively. Under the conditions of this experiment, reducing the dietary concentrations of Ca and P had no significant impact on intake, digestibility, or performance of growing Nellore heifers and steers.

Keywords: energy; mineral; performance; protein; Nellore

1. Introduction

Beef production in Brazil is primarily a pasture-based system, with Nellore cattle reaching slaughter weight by 24 mo of age. Although this system has a low cost for feed inputs, cattle slaughtered at 24 mo of age typically have low carcass quality. Paulino et al. (2006) suggested that slaughtering Nellore beef cattle at 18 mo of age is a viable method to increase carcass quality. However, in Brazil, weaning of calves coincides with the drought season. Therefore, for slaughter at 18 mo, weaned calves must be fed in a feedlot system to provide sufficient energy to increase their growth rate.

The BR CORTE system (Valadares Filho et al., 2010) provides estimates of the nutrient requirements for Zebu cattle based on experiments conducted in Brazil over the last 2 decades. However, the majority of experiments have used finishing cattle including about 80% bulls (Marcondes et al., 2009; Chizzotti et al., 2007). Thus, there is a lack of information about nutritional requirements and performance in growing Zebu cattle differing in gender and physiological conditions. Furthermore, the number of experiments evaluating Ca and P requirements of Zebu cattle is limited. Factors including age, weight, productivity level, pregnancy, and environment have been reported to influence the Ca and P requirements of cattle.

The aim of this experiment was to evaluate the intake and digestibility of DM and nutrients, performance, microbial efficiency, and energy and protein requirements of growing Nellore heifers and steers fed 2 levels of Ca and P.

2. Materials and methods

2.1. Animals, design, and treatments

This experiment was conducted at the Experimental Feedlot of the Animal Science Department, Viçosa, MG, Brazil. Care and handling of the animals followed guidelines set by the Universidade Federal de Viçosa. Fifty Nellore cattle were used, these included 32 heifers and 18 steers with average initial body weight of 180 ± 25.0 kg and 150 ± 25.1 kg, respectively, and an average age of 8 ± 1.0 mo. Heifers were divided into the following groups: 4 animals were selected randomly as the control group and were slaughtered at the beginning of the experiment to evaluate the initial body composition, 4 were fed at MAIN (11 g/kg BW), whereas 24 heifers were allowed free choice access to feed throughout the experiment. These 24 ADLIB animals were divided into 4 groups and fed with the following treatments relative to the Valadares Filho et al. (2010) recommendations for Ca and P: 1) Ca and P fed at their proposed requirements (CaPR) with a 50:50 roughage:concentrate (R:C) diet, 2) CaPR with a 70:30 R:C diet, 3) 43% of their proposed requirements for Ca and 80% of their requirements for P (CaPL) with a 50:50 R:C diet, and 4) CaPL with a 70:30 R:C diet. Steers were divided into 3 groups: 4 steers served as the control group, 4 steers were fed at MAIN, and 10 steers received the ADLIB treatment. All steers were fed CaPR with a 50:50 R:C diet. Half of the ADLIB heifers and steers were slaughtered at d 50; others were slaughtered after d 100 of the feeding period. All MAIN animals were slaughtered at d 100. Total feces and urine were collected from all animals starting 72 hours prior to slaughter.

This experiment had a completely randomized design with a $2 \times 2 + 1$ factorial arrangement of treatments. Diets were formulated according to BR CORTE system (Valadares Filho et al., 2010) to achieve an ADG of 0.3 kg. This diet consisted of fresh sugarcane and a concentrate supplement formulated from ground corn, soybean meal, limestone, common salt, and mineral mix (Table 1). The DM content of sugarcane was assessed 3 times each week to adjust DM of the amount of sugarcane plus supplement provided to the animals which the amount of U and AS (9:1) supplied

to the animals, where the CP content of diets was adjusted to maintain at 124 g CP/kg DM (19.8 g of N/kg DM).

Fresh feed was provided twice a day for the animals and adjusted daily to maintain orts at approximately 5 to 10 % of the total feed supplied to ADLIB cattle; drinking water was continuously available to all the animals. The amount of feed supplied was recorded daily; additionally, the ingredients in the concentrate were sampled each time concentrate was manufactured. Composite feed samples were obtained for each feeding period in proportion to the amount of each ingredient in the diet mixture. Feed samples (sugarcane and orts) were grouped proportionally for every 7-d period, composited, lyophilized, and ground through a 1-mm mesh sieve. At the end of each 7-d period, a composite sample of orts was performed each 50-d period in proportion to the weight of weekly DM. Sugarcane samples from each week were analyzed.

2.2. Measurements and analyses

Total tract apparent digestibility was assessed during the 72-h immediately before each slaughter date; total excreted feces and urine were collected. Feces were sampled from dropping on concrete floor. At the end of 24-h of sampling, the buckets containing the samples were weighed and homogenized; a sub-sample per day was retained, weighed, dried in a forced-draft oven (55°C), and ground through a knife mill with a 1-mm mesh sieve. Subsequently, one composite sample per animal, based on the DM weight for every collection day was created.

Urine was collected with the aid of collecting funnels attached to the steers with hoses conducting urine to carboys containing 200 mL of 20% H₂SO₄. The carboys were kept in polyethylene boxes with ice to reduce N loss. For heifers, a 2-way Foley catheter (No. 22, Rush

Amber, Kamuting, Malaysia) with a 30-mL balloon was utilized. A polyethylene tube was attached to a free end of the catheter, through which the urine would flow into a plastic container with a lid; each container held 200 mL of 20% H₂SO₄. After collection, the total excreted weight of the urine was determined. Contents of all of the tanks from each heifer and steer were thoroughly mixed, and a 50-mL sample was obtained and was stored at -20°C for further laboratory analyses.

Allantoin, creatinine, and uric acid were analyzed using a HPLC (Agilent 1100 series, Agilent Technologies, Waldbronn, Germany) as described previously by Czauderna and Kowalczyk (2000), with modifications by George et al. (2006). Total excretion of purine derivatives, calculated as the sum of allantoin and uric acid, was expressed in millimoles per day. Absorbed purines (X, in millimoles per day) were calculated based on the excretion of purine derivatives (Y, in millimoles per day) by using the following equation: $Y = (X - (0.30 \times LW^{0.75}))/0.80$, where 0.80 was assumed as the recovery of purines absorbed as purine derivatives while $0.30 \times LW^{0.75}$ was assumed to be daily endogenous purines excretion per kilogram of metabolic weight per day (Barbosa et al., 2011). Ruminal synthesis of nitrogenous compounds (Y, in grams N per day) was calculated as a function of absorbed purines (X, in millimoles per day) by utilizing the following equation from Barbosa et al. (2011): $Y = (70 \times X) / (0.93 \times 0.14 \times 1,000)$, where 70 was the N content of purines (in milligrams N per mole), 0.14 was the presumed purine N/total N ratio of the bacteria, and 0.93 as an estimate of true digestibility of microbial purines.

Samples of fresh sugarcane, concentrate ingredients, orts, and feces were quantified as DM, OM, and CP (AOAC, 2012; method number 934.01, 930.05, and 981.10, respectively). Ether extract was analyzed according to AOAC (2006; method number 945.16). Neutral detergent fiber 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 NDIN and ADIN assays followed the technique described by Licitra et al. (1996). Acid detergent fiber was determined by the method described by Van Soest et al. (1991).

Non-fiber carbohydrates were calculated according to Detmann and Valadares Filho (2010), where $NFC (\% DM) = 1000 - [CP - (CP \text{ derived from } U + U) + NDF + EE + \text{ash}]$. The TDN content of diets was estimated as the sum of digestible nutrients, where $TDN = CP_{\text{digestible}} + 2.25 \times EE_{\text{digestible}} + NDF_{\text{digestible}} + NFC_{\text{digestible}}$ (NRC 2001). The DE was obtained by multiplying the digestible nutrients by their respective energy values (NRC, 2001), whereas the concentration of ME was estimated as 82% of the DE (Coelho da Silva and Leão, 1979).

2.3. Slaughter and samplings

Prior to slaughter, all animals were solid fasted for 16-h to determine shrunk body weight. Animals were slaughtered via captive bolt followed by exsanguination. After bleeding, the contents of the gastrointestinal tract (i.e., the rumen, reticulum, omasum, abomasum, and small and large intestines) were removed by washing. Weights of 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., carcasses, head, hide, limbs, and blood) to determine the 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 and viscera. The head and all of the limbs, after removal of the hide, were ground using a grinding machine to reduce the size of the bones. The hide was sampled in parts, which 2 parts to represent

the shoulder, 3 parts to represent the dorsal line, 2 parts to represent the ventral line, 2 parts to represent the rear, 1 part to represent each foot, and 1 part to represent the head, which represented the entire hide. A composite sample of non-carcass components was constructed, where blood, head, limbs, hide, and organs and viscera were sampled based on the relative proportions of each component after calculating the sum of all components.

After the slaughter, the carcass of each animal was separated into 2 half-carcasses, which were chilled at 4°C for 18-h. After the 18-h period, the half-carcasses were weighed again. The left half-carcass was completely dissected into muscle and fat, and bone. Muscle and fat were ground together while bones were ground separately. A compound sample of the carcass was constructed, where muscle and fat and bone were sampled based on their relative proportions in the carcass. The non-carcass and carcass samples were lyophilized and ground using a ball mill for further laboratory analysis.

Due to the importance of bone characteristics, a bone sample from the 9th and 11th ribs was collected to evaluate bone content and its composition as DM, CP, ash, Ca, and P.

2.4. Procedures used to calculate the energy and protein requirements

Body fat and protein contents were determined as a function of their concentrations in the non-carcass and carcass samples. The determination of body energy was obtained from the body contents of protein and fat and their respective caloric equivalents, according to ARC (1980):

$$EC = 5.6405 X + 9.3929 Y,$$

where EC = energy content (Mcal), X = body protein (kg), and Y = body fat (kg). The net energy requirement for maintenance (Mcal/EBW^{0.75}/d) was estimated to be the intercept (β_0) of the exponential regression between HP and MEI. The model utilized was the following:

$$HP = \beta_0 \times e^{(\beta_1 \times MEI)},$$

where HP = heat production (Mcal/EBW^{0.75}/d), MEI = metabolizable energy intake (Mcal/EBW^{0.75}/d), β_0 and β_1 are regression parameters, and 'e' is the Euler number (2.718281). Also, the metabolizable energy requirement for maintenance (MEM, Mcal/EBW^{0.75}/d) was estimated by the iterative method, with MEM being considered as the value where MEI equals HP.

The efficiency of use of the metabolizable energy for maintenance (km) was calculated as the ratio between the net and metabolizable energy for maintenance. The net energy requirement for growth (NEg) was estimated from the regression between NEg, daily 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 weight gain (kg/d). A linear regression between the retained N and its intake was performed using the following regression model as suggested by Valadares Filho et al. (2010):

$$y = a + b \times x,$$

where x is the N intake, y is the retained N in the body, and a is the intercept and is considered to be the net protein requirement for maintenance (NPM).

The MPM was estimated by dividing the NPM by the slope of the regression between retained and absorbed N. The net protein requirement for growth (NPG) was estimated by a model involving EBG and retained energy in the body:

$$\text{NPg} = \beta_1 \times \text{EBG} + \beta_2 \times \text{RE},$$

where NPg = retained protein or the net protein requirement for growth (g/d), EBG = empty body weight gain (kg/d), RE = retained energy (Mcal/d), and β_1 and β_2 are regression parameters.

The MPg was estimated by dividing the NPg by the utilization efficiency of metabolizable protein for growth (k), according to the equation proposed by Valadares Filho et al. (2010).

2.5. Statistical analyses

Intake and nutrient digestibility, performance, bone characteristics, and microbial efficiency were evaluated as mixed models through the PROC MIXED (SAS Inst. Inc., Cary, NC). The 4 treatments for heifers were evaluated together, data of the steers were evaluated with data of the heifers from the same diet. Data of REF and MAIN animals were only utilized to estimate energy and protein requirements for growing Nellore steers and heifers. Slaughter periods were considered to be the fixed effects while the animals accounted for the random effect. The heterogeneous first-order autoregressive matrix (ARH (1)) was used; this is the variance and covariance matrix of random effects for all of the variables analyzed. Comparisons between treatments were performed with the Tukey–Kramer method.

Therefore, to estimate the net protein requirements for maintenance, data of retained N and its intake were analyzed as a linear model through the feature PROC REG (SAS Inst. Inc., Cary, NC). To estimate the net protein requirements for growth and the net energy requirement for maintenance and for growth, the data were analyzed as non-linear models built by PROC NLIN (SAS Inst. Inc., Cary, NC) and adjusted by the Gauss-Newton method.

For all comparisons, the level of 0.05 was established as the critical level to test the probability of a type I error.

3. Results

3.1. Intake and nutrient digestibility

Effect was observed ($P < 0.05$) only of R:C ratio on nutrient intake except for NDF where heifers fed greater R:C ratio presented greater intake of the nutrients (Table 2). No difference ($P > 0.05$) was observed when these nutrient intakes were expressed in g/kg BW and in the nutrient digestibility (Table 2). When data of the steers were compared to data of the heifers in the same treatment, no significant differences ($P > 0.05$) were detected for any of the parameter evaluated (Table 2).

3.2. Performance and microbial efficiency

The overall animal performance data is shown in Table 3. Performance was only affected ($P < 0.05$) when heifers fed 50% concentrate independent of the mineral level had greater ($P < 0.05$) ADG and EBG than heifers fed 70% roughage. When data from steers were compared to those from heifers receiving the same diet, no differences were detected ($P > 0.05$) for any performance parameters. The efficiency of microbial synthesis also was not affected ($P > 0.05$) by diet or sex (Table 4).

Despite detecting no effects ($P > 0.05$) of decreasing these two minerals simultaneously on animal performance, effects on bone weight and composition, a more sensitive measure of mineral status was evaluated from further analysis of the bone samples that were gathered in this study. Thus, there was no difference ($P > 0.05$) for bone content and its composition of growing Nellore steers and heifers.

3.3. Energy and protein requirements

Due to gender differences being not significant, all data from the animals used in this study were grouped. Data from all animals were used to estimate the energy and protein requirements for growing Nellore steers and heifers. This database was composed of animals with BW between 121 and 300 kg, while ADG varied from 0.00 to 0.84 kg/d; DMI ranged from 1.19 to 6.24 kg/d.

Previous authors (Chizzotti et al., 2008; Tedeschi et al., 2004; Tedeschi et al., 2002) have suggested that to estimate the energy requirements for maintenance, an exponential method is the most adequate. The relationship between HP and MEI was described by the following equation: $HP = 0.0701_{\pm 0.00301} \times e^{(4.4219_{\pm 0.1538} \times MEI)}$, where HP = heat production (Mcal/EBW^{0.75}/d) and MEI = metabolizable energy intake (Mcal/EBW^{0.75}/d). Thus, the net energy requirement for maintenance (NE_m) was estimated to be 70.1 kcal/kg EBW^{0.75}/d for Nellore heifers and steers. The ME_m was 118.2 kcal/EBW^{0.75}/d, the value when HP equals MEI in the above equation.

The NE_g was estimated by the following equation: $NE_g = 0.053_{\pm 0.0012} \times EBW^{0.75} \times EBG^{0.6301}$, where NE_g is the net energy requirement for growth (Mcal/EBW^{0.75}/d), EBW^{0.75} is metabolic empty body weight, and EBG is the empty body weight gain (kg/d).

Most nutritional requirement systems (CSIRO, 2007; NRC, 1996; AFRC, 1993) divide the efficiency of conversion of ME into net energy for maintenance (km) and growth (kg). The km (NEm divided by MEm) was 59.3%. The efficiency of conversion of ME to net energy for growth (kg) was determined based on the slope from the linear regression between RE and MEI (Fig. 1). The kg observed for this study was 17.6%.

The N_{Pm} was estimated as the intercept of the linear regression between retained N and N intake (Fig. 2). The N_{Pm} was 1.28 g/kg BW^{0.75} (0.2042 × 6.25). The NP_g was estimated from the model involving EBG and the ER in the body: NP_g = 137.85_{±15.32} × EBG – 0.05_{±2.34} × RE, where NP_g is the net requirement of protein in g/d, EBG is the empty body weight gain in kg/d, and RE is the retained energy in Mcal/d.

4. Discussion

4.1. Intake and nutrient digestibility

Valadares Filho et al. (2000) evaluated different concentrate levels for dairy cattle and observed that NFC intake increased with concentrate level. Thus, the difference among diets observed in NFC can be explained by the increased amount of grain in the higher concentrate diet. Rotger et al. (2006) evaluated the effect of two sources of NFC and protein on the intake and apparent digestibility of the nutrients and did not detect any significant effects on intake or apparent nutrient digestibility.

However, Huntington et al. (1981) compared hay to an 85% concentrate diet and found a decrease for DM intake in response to higher DM digestibility when the concentrate level was

increased in the diet. These authors also observed reductions in fecal excretions of Ca and P when concentrate was added the diet. However, urinary excretion of Ca and P increased, suggesting that the higher absorption of Ca and P was not reflected in higher retention.

Steen (1995) evaluated different nutritional plans for bulls, steers, and heifers and verified differences in intake, which bulls consumed more than steers, and steers consumed more than heifers. However, this author did not detect differences in the apparent digestibility between sexes. Schwartzkopf-Genswein et al. (2002) varied feeding regimes, and observed that DMI were greater for heifers than steers when animals were fed a barley-based finishing diet. In contrast, Hicks et al. (1990) compared DMI of beef heifers and steers and Holstein steers and observed that beef heifers averaged 2% lower whereas Holstein steers averaged 12% greater than DMI of beef steers.

4.2. Performance and microbial efficiency

Reducing the Ca and P levels in the diet simultaneously did not affect animal performance. Bushman et al. (1968) fed steers an all-concentrate diets that differed in Ca and P concentrations and reported no differences in performance when the amounts of Ca and P were reduced. Hutcheson et al. (1992) evaluated mineral composition of metacarpal bones of feedlot lambs fed 2 dietary levels of Ca and P, they did not observe differences in Ca and P status in these animals.

Schwartzkopf-Genswein et al. (2002) observed that heifers had greater ADG than steers, probably due to their greater DMI. However, Steen (1995) evaluated the performance of bulls, steers, and heifers and detected differences in carcass weight and carcass component gain (lean, bone, and fat); for all parameters, bulls were greater than steers, and steers were greater than heifers. This suggests that differences among gender can occur, when particularly animals are older

than those used in this study. As example, Marcondes et al. (2009) evaluated energy and protein requirements for Nellore of three sexes slaughtered heavier than 400 kg and suggested that steers are 23% more demanding than bulls and 11% less demanding than heifers.

The efficiencies of microbial protein synthesis were lower than those reported in the literature. Costa e Silva et al. (2013) evaluated the efficiency of microbial protein synthesis in Nellore bulls gaining 1.5 kg/d and suggested value of 142 g mCP/kg TDN. The BR CORTE system (Valadares Filho et al., 2010) using data from experiments conducted under Brazilian conditions, assumed the efficiency of microbial protein synthesis as 120 g mCP/kg TDN. The NRC (1996) set the efficiency of microbial protein synthesis as 130 g mCP/kg TDN.

Prados (2012) evaluated the reduction of Ca and P levels in diet of crossbred Zebu x Holstein bulls and verified differences for ash on natural matter basis which the lowest level of Ca and P decreased ash content. This result was different those present in this study once no difference was observed for any parameters.

4.3. Energy and protein requirements

The NRC (1996) and BR CORTE system (Valadares et al., 2010) suggest values of 0.891 and 0.895 as the ratio of EBW to BW respectively. The average of this relationship determined in this study was 0.879 and 0.883 for heifers and steers, respectively. The average ratio between EBG and ADG was 0.998, this is close to the value of 1.013 that Costa e Silva et al. (2012) reported in a study with Nellore bulls, and considerably greater than those suggested by Valadares Filho et al. (2010) and the NRC (1996) of 0.936 and 0.951, respectively.

The value obtained for net requirement for maintenance was close to the basal metabolic rate reported for warm-blooded adult mammals measured in respirometric chambers (69 kcal/kg EBW^{0.75}/d; Poczopko, 1971). Valadares Filho et al. (2010) using the same method reported a value of 74.2 kcal/EBW^{0.75}/d, while Costa e Silva et al. (2012) estimated NEm to be 76.5 kcal/EBW^{0.75}/d. The metabolizable energy needed for maintenance was greater than Valadares Filho et al. (2010) recommendation and those estimated by Costa e Silva et al. (2012) of 112 and 113 kcal/EBW^{0.75}/d, respectively. Valadares Filho et al. (2010) recommended the following equations to estimate NEg: NEg = 0.064 × EBW^{0.75} × EBG^{1.095} for steers and NEg = 0.072 × EBW^{0.75} × EBG^{1.095} for heifers.

The value for km in this study (59.3%) was lower than estimates of 67.0% by both Chizzotti et al. (2008) and Costa e Silva et al. (2012). Several factors can affect km value, such as the level of dietary fiber, gut turnover, the level of metabolizable energy intake, the proportion of fatty acids absorbed, and protein turnover (Garrett, 1980). Marcondes et al. (2013) studied the effect of several factors on km and concluded that the efficiency of the use of ME for growth (kg) and EBG were the most important variables that affected km. This implies that the performance of the animals influences maintenance requirement. The NRC (1996) suggested that *Bos indicus* had 10% less NEm but the equation of km indicates that this difference can be not related to the net energy requirements for maintenance but the efficiency of use of NEm where *Bos indicus* would be more efficient to convert MEI for NEm than *Bos taurus*. Thus, Marcondes et al. (2013) suggested the following model to calculate km: km = 0.513 + 0.173 × kg + 0.100 × EBG for Nellore cattle, where km is the efficiency of conversion of ME to net energy for maintenance, kg is the efficiency of conversion of ME to net energy for growth, and EBG is the empty body weight gain.

The efficiency of use of ME for growth (kg) estimated in this study (17.6%) was lower than the 33% estimated by Costa e Silva et al. (2012). This difference could be due to difference on

performance of his Nellore bulls than our steers and heifers; Costa e Silva et al. (2012) observed an ADG of 1.24 kg/d, while in the present study, the ADG was 0.35 kg/d.

Our NPM value ($1.28 \text{ g/kg BW}^{0.75}$) also was below that estimated by Ezequiel's (1987) of $1.72 \text{ g/kg BW}^{0.75}$ and the value of $2.69 \text{ g/kg BW}^{0.75}$ estimated previously by Valadares Filho et al. (2010). The equation for NPg was different from those by Valadares Filho et al. (2010) and Costa e Silva et al. (2012) of $\text{NPg} = 238.79 \times \text{EBG} - 15.68 \times \text{RE}$ and $\text{NPg} = 263.37 \times \text{EBG} - 23.21 \times \text{RE}$, respectively. This difference may reflect faster growth rates, over 1.0 kg per day in those studies that provided greater NPg compared to this study where ADG of the animals averaged 0.35 kg/d.

The BR CORTE system (Valadares Filho et al., 2010) advocates that for growing Nellore steers and heifers with the same ADG in this experiment, the crude protein requirements are 441, 458, 601, 588, and 549 g/d while the observed crude protein intake were 600, 600, 810, 740, and 650 g/d for the average body weight of 204, 206, 221, 220, and 193 kg respectively from the treatments utilized in this study. Whereas, the TDN requirements for growing Nellore steers and heifers with ADG and body weight similar to the obtained in this study are 2.12, 2.18, 2.93, 2.96, 2.73 kg/d while the observed TDN intake were 2.96, 3.01, 4.07, 3.67, and 3.15 kg/d for the average body weight of 204, 206, 221, 220, and 193 kg respectively from the treatments utilized in this study. Therefore, the TDN and CP intake obtained in this study were greater than those recommended by BR CORTE system (Valadares Filho et al., 2010).

5. Conclusion

Under the conditions of this experiment, the level of dietary Ca and P can be reduced when both are reduced simultaneously in the diet without impact on intake, digestibility, performance, or bone characteristics of growing Nellore heifers and steers; the energy and protein requirements for growth were: $NE_g = 0.053 \times EBW^{0.75} \times EBG^{0.6301}$ and $NP_g = 137.85 \times EBG - 0.05 \times RE$, respectively.

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Table 1

Proportions of feed in each diet, and its composition on DM basis.

Items	70:30	70:30	50:50	50:50	Sugarcane
	CaPR	CaPL	CaPR	CaPL	
Proportion (g/kg DM)					
Fresh sugarcane	700	700	500	500	-
Ground corn	246	246	411	411	-
Soybean meal	45.1	45.1	75.2	75.2	-
Dicalcium phosphate	4.8	2.4	2.5	0.00	-
Salt	0.4	0.4	0.7	0.7	-
Limestone	3.1	0.00	5.3	2.2	-
Mineral mix ¹	0.24	0.24	0.35	0.35	-
Sand	0.00	5.5	5.3	10.8	-
Chemical composition (g/kg DM)					
DM	488	488	599	599	322
CP	162	162	163	162	34
NDF	397	397	326	326	503
Ca	5.7	3.9	5.2	3.4	4.3
P	2.8	2.3	2.7	2.2	1.2

70:30 and 50:50 = roughage:concentrate ratios, CaPR = 100% of Ca and P requirements, CaPL =

43% of Ca requirement and 80% of P requirements

¹Mineral mixed composition = 29.2 g/kg of calcium, 0.70 g/kg of phosphorus, 2.11 g/kg of magnesium, 0.89 g/kg of potassium, 0.31 g/kg of sodium, 63.5 g/kg of sulfur, 348 mg/kg of cobalt, 2.56 mg/kg of chromium, 3,296 mg/kg of copper, 2,088 mg/kg of iron, 4,673 mg/kg of manganese, 7,817 mg/kg of zinc, and 318 mg/kg of selenium

Table 2

Intake and apparent digestibility of the DM and nutrients for Nellore steers (n = 10) and heifers (n = 6 each treatment).

Item	R:C ratio		Mineral		Sex		RSD	P-value			
	70:30	50:50	CaPL	CaPR	Heifers	Steers		Sex	R:C	Min	R:C x Min
Intake (kg/d)											
Dry matter	3.36	4.62	4.06	3.92	4.27	3.72	0.57	0.30	0.004	0.74	0.53
Organic matter	3.21	4.41	3.88	3.74	4.07	3.55	0.56	0.30	0.004	0.73	0.53
Crude protein	0.54	0.73	0.65	0.63	0.68	0.60	0.24	0.37	0.01	0.75	0.49
Ether extract	0.06	0.08	0.07	0.07	0.07	0.06	0.12	0.34	0.002	0.98	0.42
Neutral detergent fiber	1.21	1.37	1.31	1.27	1.38	1.20	0.32	0.27	0.22	0.73	0.62
Non-fiber carbohydrates	1.63	2.44	2.07	2.00	2.17	1.89	0.41	0.30	0.001	0.73	0.50
Total digestible nutrients	2.61	3.61	3.14	3.08	3.37	2.85	0.56	0.30	0.02	0.87	0.38
Intake (g/kg BW)											
DM	17.1	20.3	19.1	18.3	18.8	18.6	1.14	0.91	0.05	0.62	0.94
Crude Protein	2.73	3.19	3.02	2.90	2.96	2.95	0.45	0.98	0.09	0.65	0.84
NDFap	6.11	6.04	6.23	5.92	6.15	5.99	0.66	0.82	0.90	0.57	0.91
Digestibility (%)											
Dry matter	70.8	72.9	70.8	72.4	73.0	70.2	1.58	0.49	0.42	0.61	0.28
Organic matter	71.9	74.3	72.4	73.7	74.3	71.9	1.56	0.53	0.42	0.67	0.29
Crude protein	75.8	73.2	73.8	75.2	75.9	73.1	1.46	0.42	0.33	0.61	0.16
Ether extract	71.0	70.9	69.4	72.5	72.3	69.6	1.89	0.64	0.99	0.50	0.96
Neutral detergent fiber	47.3	48.7	47.3	48.7	48.7	47.3	2.13	0.85	0.81	0.81	0.18
Non-fiber carbohydrates	92.3	91.5	91.7	92.1	93.5	90.3	1.19	0.16	0.63	0.81	0.91

70:30 and 50:50 = roughage:concentrate ratios, CaPR = 100% of Ca and P requirements, CaPL = 43% of Ca requirement and 80% of P requirements

RSD = residual standard deviation

R:C x Min = interaction between roughage:concentrate ratio and mineral level.

Table 3

Performance of Nellore steers (n = 10) and heifers (n = 6 each treatment).

Item	R:C ratio		Mineral		Sex		RSD	P-value			
	70:30	50:50	CaPL	CaPR	Heifers	Steers		Sex	R:C	Min	R:C x Min
Initial empty body weight (kg)	157	160	159	157	171	145	2.92	0.12	0.82	0.88	0.87
Final empty body weight (kg)	176	202	190	189	202	177	3.54	0.21	0.11	0.94	0.90
Initial shrunk body weight (kg)	181	184	184	181	198	168	3.46	0.12	0.82	0.88	0.87
Final shrunk body weight (kg)	202	229	216	215	229	203	3.32	0.23	0.13	0.93	0.97
Average daily gain (kg/d)	0.25	0.56	0.39	0.43	0.37	0.44	0.23	0.46	<0.0001	0.57	0.51
Empty body gain (kg/d)	0.24	0.55	0.38	0.40	0.37	0.41	0.22	0.58	<0.0001	0.67	0.84

70:30 and 50:50 = roughage:concentrate ratios, CaPR = 100% of Ca and P requirements, CaPL = 43% of Ca requirement and 80% of P

requirements

RSD = residual standard deviation

R:C x Min = interaction between roughage:concentrate ratio and mineral level.

Table 4

Mean values of excretion of purine derivatives, microbial protein production, and microbial efficiency for Nellore heifers (n = 6) and steers (n =10).

Item	R:C ratio		Mineral		Sex		RSD	P-value			
	70:30	50:50	CaPL	CaPR	Heifers	Steers		Sex	R:C	Min	R:C x Min
Allantoin (mmol/d)	88.7	93.8	86.0	96.4	89.4	93.0	2.90	0.79	0.63	0.33	0.67
Uric acid (mmol/d)	13.2	12.4	13.3	12.3	11.1	14.5	1.23	0.11	0.69	0.61	0.32
Purine derivative excretion (mmol/d)	104.0	106.0	99.0	111.0	102.0	109.0	2.94	0.61	0.86	0.28	0.42
Microbial nitrogen (g/d)	57.9	56.3	53.2	61.0	52.9	61.3	2.51	0.40	0.85	0.32	0.39
Microbial efficiency (g _m CP/kg TDN)	124	121	126	118	116	133	4.50	0.53	0.91	0.76	0.98

70:30 and 50:50 = roughage:concentrate ratios, CaPR = 100% of Ca and P requirements, CaPL = 43% of Ca requirement and 80% of P requirements

RSD = residual standard deviation

R:C x Min = interaction between roughage:concentrate ratio and mineral level.

Table 5

Bone content and its composition (% natural matter basis) from the section between 9th and 11th ribs for Nellore steers and heifers

Item	R:C ratio		Mineral		Sex		RSD	P-value			
	70:30	50:50	CaPL	CaPR	Heifers	Steers		Sex	R:C	Min	R:C x Min
Bone	26.3	24.7	25.1	25.8	24.5	26.5	0.71	0.15	0.15	0.52	0.96
Dry matter	15.0	13.9	14.2	14.7	14.3	14.6	0.97	0.66	0.07	0.45	0.58
Crude protein	8.94	8.57	8.65	8.86	8.34	9.18	0.63	0.13	0.41	0.63	0.61
Ash	9.56	10.6	9.72	10.4	9.71	10.4	0.60	0.16	0.02	0.09	0.51
Ether extract	6.76	6.56	6.76	6.56	6.46	6.86	0.66	0.51	0.68	0.68	0.27
Ca	2.82	2.6	2.62	2.8	2.57	2.85	0.36	0.13	0.12	0.22	0.34
P	1.43	1.81	1.42	1.81	1.87	1.37	0.43	0.03	0.09	0.09	0.20

70:30 and 50:50 = roughage:concentrate ratios, CaPR = 100% of Ca and P requirements, CaPL = 43% of Ca requirement and 80% of P

requirements

RSD = residual standard deviation

R:C x Min = interaction between roughage:concentrate ratio and mineral level.

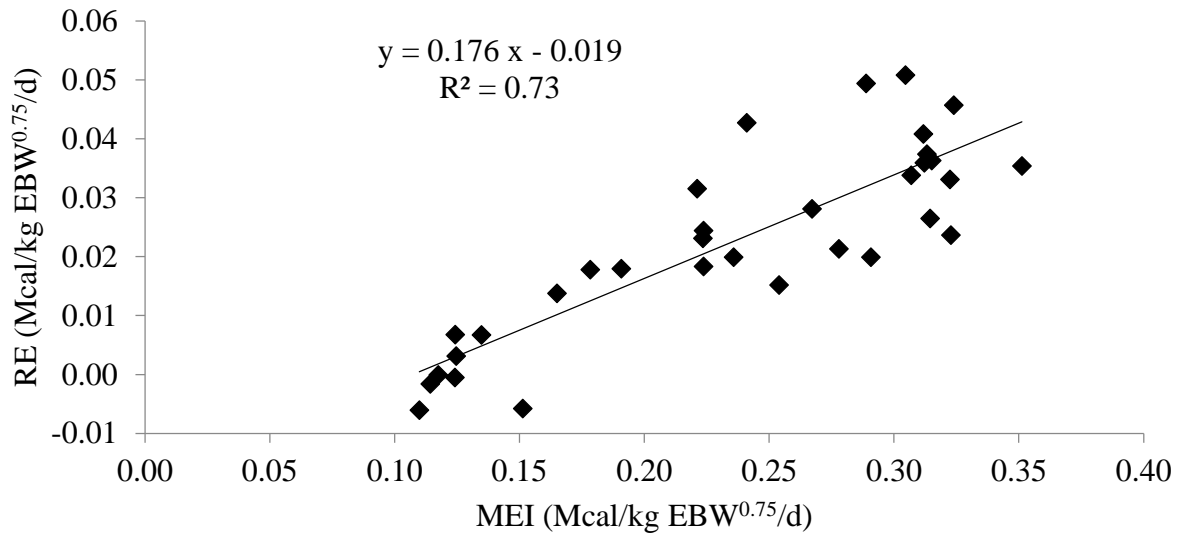


Fig. 1. Relationship between retained energy (RE) and metabolizable energy intake (MEI).

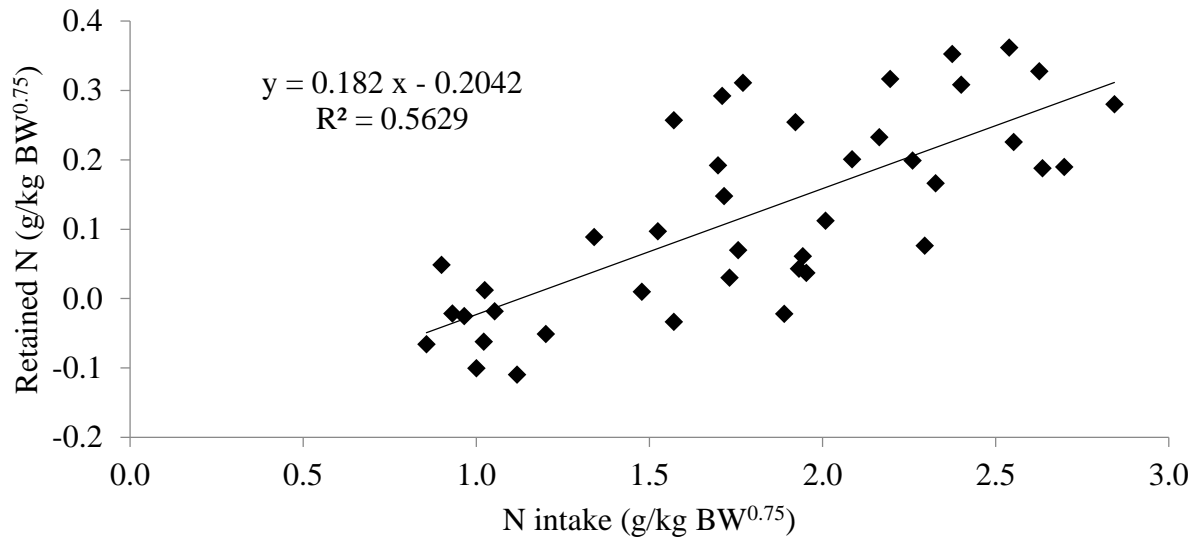


Fig. 2. Relationship between retained nitrogen and nitrogen intake.

1

CHAPTER 4

Can mineral balance method estimate correctly macromineral and trace element requirements of beef cattle?

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19 **ABSTRACT**

20 Two experiments were conducted to estimate the net requirements for maintenance and growth as
21 well as the retention coefficients of 13 minerals: macromineral (Ca, P, Mg, Na, K, and S) and trace
22 element (Co, Cr, Cu, Fe, Mn, Se, and Zn) of beef cattle by mineral balance method. In experiment
23 I, 37 Nellore bulls were divided into 3 groups: 1) baseline reference animals (REF, n = 5), 2)
24 animals fed at maintenance (MAIN, n = 4), and 3) animals with ad libitum access to feed (ADLIB,
25 n = 28). The 28 ADLIB plus 4 MAIN were slaughtered at 4 different points (42, 84, 126, and 168
26 d, n = 7 ADLIB plus one MAIN/time point). In experiment II, 32 Nellore heifers and 18 Nellore
27 steers were used. Heifers were divided into 4 groups. Four animals served as REF, 4 were fed at
28 MAIN, and 24 received ADLIB. The ADLIB animals were further divided into 4 groups and
29 assigned to the following treatments: 1) Ca and P fed at requirement (CaPR) with a 50:50
30 roughage:concentrate (R:C) diet, 2) CaPR with a 70:30 R:C diet, 3) 43% of the Ca and 80% of the
31 P requirements (CaPL) with a 50:50 R:C diet, and 4) CaPL with a 70:30 R:C diet. The ADLIB and
32 MAIN steers were fed CaPR with a 50:50 R:C diet. Half of the ADLIB animals were slaughtered
33 at d 50 and the other half of the animals were slaughtered at d 100 of the feeding period. All MAIN
34 cattle were slaughtered at d 100. The net requirements for maintenance and true retention
35 coefficient were estimated using the regression between apparent retention and intake for each
36 mineral. The net requirements for growth for each mineral was determined based on the derivative
37 of allometric equations relating mineral content in the body and empty body weight. The net
38 requirement for maintenance ($\mu\text{g}/\text{kg BW}/\text{d}$) and retention coefficients (%) were 22.9 and 78 for
39 Cr, 18.4 and 86 for Co, 163 and 85 for Cu, 2,097 and 53 for Fe, 32.3 and 24 for Mn, 3.72 and 48
40 for Se, and 669 and 0.80 for Zn. The dietary requirements of macronutrient minerals (g/kg DMI)
41 were 5.12 for Ca, 2.38 for P, 2.40 for K, 0.79 for Na, 0.96 for Mg, and 1.47 for S. These values

42 were close to those found on literature, except for K. Then, we concluded that the use of the net
43 requirements for maintenance and growth and true retention coefficient for the majority of the
44 trace minerals resulted in different estimates than those obtained in the literature. Also, due to the
45 considerable excretion of Na, K, Mg and S in the urine, the use of true retention coefficient improve
46 the estimate of dietary requirements of these minerals.

47 **Keywords:** growth, maintenance, minerals, retention coefficient

48

49 **Introduction**

50 Minerals can play a role in 4 types of functions in animals: structural, physiological, catalytic,
51 and regulatory (Suttle, 2010). Thus, information on the requirements of minerals for maintenance
52 and growth is essential for herds to express their maximum productive potential (Teixeira et al.,
53 2015). The NRC (1996) suggested that at least 17 minerals are required by beef cattle, however,
54 the requirements of the majority of minerals are suggested as g/kg DMI or mg/kg DMI without
55 discrimination of maintenance or growth neither absorption coefficient for any trace element and
56 sulfur. Nevertheless, for certain minerals, requirements are not listed because research data are
57 inadequate to determine requirements (NRC, 1996).

58 Also, most of councils around the world (NRC, 1996; ARC, 1980; AFRC, 1991) consider
59 mineral requirements based on mineral supplementation disregarding mineral composition from
60 feedstuffs. Although, the knowledge of the amount of each mineral absorbed and, then, retained is
61 important for the achievement of mineral requirement.

62 All councils consider absorption coefficient because the findings of some mineral in the urine
63 are irrelevant (NRC, 1996; ARC, 1980). However, the retention coefficient can represent directly
64 the relationship between intake and retained mineral contemplating also other possible mineral

65 losses as through skin and urine, for example (BR CORTE, 2010). Then, the use of true retention
66 coefficient is more adequate than absorption coefficient to estimate mineral requirements.

67 Then, we hypothesized that the knowledge of the net requirements for maintenance and
68 growth as well as true retention coefficient will improve the requirement precision for all minerals.
69 Therefore, we developed two experiments to estimate macronutrient mineral (Ca, P, Mg, K, Na,
70 and S) and trace element (Co, Cr, Cu, Fe, Mn, Se, and Zn) requirements for maintenance and
71 growth, as well as the true retention coefficient for beef cattle using mineral balance method.

72

73 **Materials and Methods**

74

75 Experiments were conducted at the Experimental Feedlot of Animal Science Department, in
76 Viçosa, Brazil. Laboratory analyses were conducted at the Ruminant Nutrition Laboratory of the
77 Animal Science Department at the Universidade Federal de Viçosa (UFV), Viçosa, MG, Brazil.
78 The procedures for the care and management of the animals followed the guidelines set by UFRV.

79

80 Animals and treatments

81

82 Experiment I – Bulls

83

84 Thirty-seven Nelore bulls, with an average initial BW of 259 ± 25.1 kg and age of 14 ± 1.2
85 mo were used which were randomly divided in five groups as a baseline reference group (REF) and
86 were slaughtered at the beginning of the experiment, 4 bulls were fed at maintenance (MAIN), and

87 the remaining 28 bulls had ad libitum access to feed throughout the experiment (ADLIB). One
88 MAIN bull and 7 ADLIB bulls were slaughtered at 4 different points (42, 84, 126, and 168 d).

89 Sixteen animals were randomly selected and kept in a tie stall system to evaluate the
90 digestibility of the diet. The remaining 12 animals were kept in 2 collective stalls with concrete
91 floors and individual electronic feeders (American Calan Inc., Northwood, NH). The total area of
92 each stall was 50 m², with 9 m² covered, and a collective concrete trough for water. Initially, all
93 animals were weighed, identified with an ear tag, and treated for ectoparasites and endoparasites
94 (Ivomec, Paulina, São Paulo, Brazil).

95 The diet was formulated according to the BR CORTE system (Valadares Filho et al, 2010)
96 diet for a daily body weight gain of 1.30 kg. The diet consisted of 55% corn silage on a DM basis
97 and 45% concentrate, and was formulated with ground corn, soybean meal, urea, ammonium
98 sulfate, limestone, common salt, and a mineral mix (Table 1). Feed was supplied twice daily to the
99 animals and adjusted daily to keep leftovers at approximately 5 to 10% of the total feed supplied.
100 Water was continuously available to the animals. The amount of feed supplied was recorded daily.
101 The MAIN animals were fed at 11 g/kg BW. The feed ingredients in the concentrate were sampled
102 directly from the storage silos each time diets were manufactured. Feed samples were composited
103 weekly, dried in a forced-ventilation oven (55°C) for 72 h, and ground through a 1 mm screen
104 (Wiley mill; A. H. Thomas, Philadelphia, PA). At the end of each 7-d period, a composite sample
105 of leftovers proportional to the weekly DM was constructed for each 42-d period. For the corn
106 silage, the composite samples were made every 21 d and was proportional to the weekly DM.

107

108 Experiment II – Heifers and Steers

109

110 Eighteen Nellore steers (150 ± 44.2 kg) and 32 Nellore heifers (180 ± 41.0 kg) were used.
111 Four animals from each sex were used as REF animals and were slaughtered at the beginning of
112 the experiment. Four animals from each sex were fed MAIN levels, and 10 steers and 24 heifers
113 were assigned to the ADLIB group. The ADLIB and MAIN heifers were further divided into 4
114 groups and assigned to treatments. Treatments were: 1) Ca and P fed at requirement (CaPR) with
115 a 50:50 roughage:concentrate (R:C) diet, 2) CaPR with a 70:30 R:C diet, 3) 43% of the Ca and
116 80% of the P requirements (CaPL) with a 50:50 R:C diet, and 4) CaPL with a 70:30 R:C diet. The
117 ADLIB and MAIN steers were fed CaPR with a 50:50 R:C diet. Half of the ADLIB animals were
118 slaughtered at d 50 and the other half of the animals were slaughtered at d 100 of the feeding
119 period. All of the MAIN-fed cattle were slaughtered at d 100. The values of 43% Ca and 80% P
120 were the lowest values achieved when the sugarcane was analyzed and provided for the animals.
121 Also, low amount of mineral could interfere in the animal's requirements.

122 The animals were kept in individual pens with concrete floors that had a total area of 30 m^2 ,
123 where 8 m^2 of the area was under cover, and a collective concrete feeder and trough for water were
124 provided. This experiment was a completely randomized design with a $2 \times 2 + 1$ factorial
125 arrangement of treatments. The diet was formulated according to the BR CORTE system
126 (Valadares Filho et al, 2010) diet for a daily weight gain of 0.3 kg. The diet consisted of fresh
127 sugarcane and concentrate, which was formulated with ground corn, soybean meal, limestone,
128 common salt, and a mineral mix (12.4% CP; Table 2). The DM from sugarcane was assessed 3
129 times weekly to adjust the amount of urea (U) and ammonium sulfate (AS) (9:1, U/AS) supplied
130 to the animals, and the U/AS mixture was used to adjust the CP content of the diets to 124.0 g/kg
131 DM ($19.8 \text{ g of N/kg DM}$).

132 Feed was supplied twice daily to the animals and adjusted daily to keep leftovers at
133 approximately 5 to 10% of the total feed supplied. Water was continuously available to the
134 animals. The MAIN animals were fed at 11 g/kg BW. The amount of feed supplied was recorded
135 daily. The ingredients in the concentrate were sampled directly from the silos of the feed factory
136 each time they were mixed. Feed samples were obtained as described for the experiment I.

137

138 Measurements

139

140 For both experiments, a digestibility trial was conducted immediately before each slaughter
141 period; total feces and urine were collected for 3 consecutive days from animals that were
142 maintained in tie stalls. At the end of each collection day, feces were weighed and homogenized,
143 and a sample was collected. The samples were weighed, dried in a forced-ventilation oven (55°C)
144 for 72 h, and ground through a 1 mm screen (Wiley mill; A. H. Thomas, Philadelphia, PA). One
145 composite sample per animal, based on the DM weight for every collection day was prepared.

146 Urine collection was performed via collecting funnels attached to the bulls and steers, while
147 hoses carried the urine to tanks kept in polyethylene boxes with ice and 20% H₂SO₄ in order to
148 reduce N loss. For heifers, a 2-way Folley catheter (No. 22, Rush Amber, Kamuting, Malaysia)
149 with a 30-mL balloon was utilized to collect urine. A polyethylene tube was attached to a free end
150 of the catheter, through which the urine flowed into a lidded plastic container that held 200 mL of
151 20% H₂SO₄. After each 24 h collection period, the total excreted weight of the urine was
152 determined. The contents of all tanks were then homogenized. A 50-mL sample was obtained and
153 was stored at -20°C for further laboratory analyses.

154

155 Slaughter and Samplings

156

157 Before slaughter, all animals were fasted for 16 h to obtain the shrunk body weight (SBW).
158 Animals were then slaughtered via captive bolt stunning followed by exsanguination. After
159 exsanguination, the digesta were removed and discarded. The heart, lungs, liver, spleen, kidneys,
160 KPH fat, diaphragm, mesentery, tails, trimmings, and washed gastrointestinal tract were weighed.
161 These values were added to the other parts of the body (i.e., carcasses, head, hide, limbs, and blood)
162 to determine the empty body weight (EBW).

163 The rumen, reticulum, omasum, abomasum, small and large intestines, KPH fat, mesentery,
164 liver, heart, kidneys, lung, tongue, spleen, diaphragm, esophagus, trachea, and reproductive tract
165 were homogenized in an industrial cutter for 20 min. After removing the hide, the head and limbs
166 were also ground in a bone crusher. The hide was sampled in 2 parts to represent the shoulder; 3
167 to represent the dorsal line; 2 to represent the ventral line; 2 to represent the rear; 1 part to represent
168 each foot; and 1 to represent the head, which altogether represented the entire hide. A composited
169 sample of non-carcass components was constructed, in which blood, head, limbs, hide, organs, and
170 viscera were sampled based on the relative proportions of each component after summing all
171 components.

172 After slaughter, the carcass of each animal was separated into 2 half-carcasses which were
173 chilled at 4°C for 18 h. After the 18 h period, the half-carcasses were weighed again. The left half-
174 carcass was completely separated into muscle, fat, and bone. Muscle and fat were ground together,
175 and the bones were ground separately. After, a composite sample of the carcass was constructed
176 using relative individual proportions in the carcass. The non-carcass and carcass samples were
177 lyophilized. Samples from the first experiment were partially defatted through 2 successive washes

178 with petroleum ether by using a Soxhlet extractor in order to determine the partially defatted DM
179 because the high amount of fat in the samples. After this process, all samples were ground in a ball
180 mill for further laboratory analysis.

181

182 Mineral Analyses

183

184 Corn silage (experiment I), sugarcane (experiment II), the feeds in each concentrate, feces,
185 urine, non-carcass, and carcass samples were analyzed as macronutrient minerals (Ca, P, Mg, Na,
186 K, and S) and trace element minerals (Co, Cr, Cu, Fe, Mn, Se, and Zn). Sodium was analyzed only
187 in the experiment I while selenium was analyzed only in experiment II. Ca and Mg were
188 determined by adding up lanthanum, and the readings were performed through atomic absorption
189 spectrometry. For Na and K, the readings were performed through flame emission spectrometry.
190 Inductively coupled plasma-atomic emission spectroscopy with ultrasonic nebulization (Braselton
191 et al., 1997) was used for determination of the following mineral concentration: Co, Cr, Cu, Fe,
192 Mn, Se, and Zn.

193

194 Procedures Used to Calculate the Mineral Requirements

195

196 Net Requirements for Maintenance and True Retention Coefficient

197

198 The true retention coefficient and the net requirement of each mineral were calculated based
199 on regressions between the amounts of each macronutrient mineral (mg/kg EBW/d) and trace
200 element mineral ($\mu\text{g/kg}$ EBW/d) retained in the body. These values were determined from the

201 regression between mineral balance where each retained mineral was evaluated by subtracting the
202 fecal and urinary mineral content from the mineral consumed and the intake of each mineral. The
203 slope of the equation was considered as true retention coefficient. The intercept of the equation
204 was considered the endogenous and metabolic losses of each mineral, and, then, the net
205 requirements for maintenance for each mineral.

206

207 Net Requirements for Growth

208

209 The amounts of each mineral in the body from each animal were estimated as a function of
210 EBW according to the following model:

$$211 \quad M_i = a \times EBW^b,$$

212 where $M_i = "i"$ constituents the animal body mineral content (macronutrient, g or trace
213 element, mg), and "a" and "b" = regression parameters. Based on the regression parameters
214 presented above, the net requirements for growth of each mineral per kg of empty body gain (EBG)
215 were calculated by using the derivative of the equation above:

$$216 \quad Y = a \times b \times EBW^{b-1},$$

217 where $Y =$ net requirement for growth of each mineral (macronutrient, mg/kg EBG or trace
218 element, $\mu\text{g}/\text{kg}$ EBG).

219

220 Dietary Requirements

221

222 After calculating the net requirements for maintenance and growth and the true retention
223 coefficient for each mineral, the dietary requirements were calculated. The sum of the net

224 requirements for maintenance and growth were divided by the true retention coefficient to estimate
225 the dietary requirements.

226

227 Statistical Analyses

228

229 A linear regression between the retained mineral and its intake was performed using the
230 following regression model:

$$231 \quad y = a + b \times x,$$

232 where x is the mineral intake, y is the retained mineral in the body, a is the intercept and is
233 considered the net requirement for maintenance of each mineral, and b is the slope and is
234 considered the true retention coefficient. The intercept was deemed to estimate endogenous and
235 metabolic losses. Thus, to obtain the net requirements for maintenance and the true retention
236 coefficient, data for retained mineral was analyzed as a linear model through the feature PROC
237 REG (SAS Inst. Inc., Cary, NC). To access the net requirements for growth, data for total mineral
238 retained in the body and EBW were analyzed as non-linear models built by the PROC NLIN (SAS
239 Inst. Inc., Cary, NC), and were adjusted by the Gauss-Newton method. For all comparisons, the
240 level of $P < 0.05$ was established as the critical level of probability for a type I error.

241

242 **Results and Discussion**

243

244 The descriptive statistics of the animals used in this study are shown in Table 3 while intake,
245 excretion, and retention of each mineral are shown in Table 4. The study group was composed of
246 animals with SBW between 121.0 and 591.5 kg, ADG varied between 0 and 1.95 kg/d, and DMI

247 varied between 1.18 and 11.1 kg/d. BR CORTE (2010) and NRC (1996) report that the relationship
248 between EBW and SBW is constant. The gastrointestinal tract content was calculated based on the
249 difference between SBW and EBW. Then, an exponential regression between the relative weight
250 of the gastrointestinal tract contents (g/kg of SBW) and SBW (kg) was evaluated (Figure 1).

251 The gastrointestinal content in the whole body decreased as animals grew. Thus, the use of
252 the constant value may be incorrect. The BR CORTE (2010) and NRC (1996) suggest values of
253 0.891 and 0.895 for the relationship between EBW and BW, respectively. Young animals have a
254 greater proportion of the gastrointestinal tract contributing to BW. For example, a 100 kg animal
255 would have 89.1 and 89.5 kg of EBW according to BR CORTE (2010) and NRC (1996),
256 respectively. The same animal would have 84.2 kg of EBW using calculations from the present
257 experiment. A 400 kg animal would have 356.4, 358.0, and 365.5 kg EBW according to BR
258 CORTE (2010), NRC (1996), and our calculations, respectively. The equation proposed here was
259 used to convert BW to EBW.

260

261 Ca

262 Calcium is the most abundant mineral in the body (NRC, 1996). The net Ca requirement for
263 maintenance was 20.0 mg/kg BW/d (Figure 2) which this value close to NRC (1996), NRC (2001)
264 and ARC (1980) recommendations (Table 5). The true absorption coefficient of Ca observed in
265 this study was close to those suggested by NRC (2001) and AFRC (1991; Table 5); although it
266 was greater (72%; Figure 2) than those presented by NRC (1996) and BR CORTE (2010; Table
267 5); however, these councils used the Ca absorption discarding urinary losses. In our study,
268 however, there was considerable mineral excretion in the urine for all of the minerals assayed. Ca
269 excretion in the urine was lower than other minerals. Thus, we considered the retention coefficient

270 to correctly estimate the mineral bioavailability. Fernandes et al. (2013) evaluated mineral
271 requirements in sheep and used fecal excretion of Ca because they did not find Ca in the urine.
272 These authors suggested that the feces are the main route of excretion for unabsorbed dietary and
273 endogenous secretions of Ca from the intestinal mucosa. In turn, urinary excretion is minimal due
274 to the efficiency of renal Ca reabsorption (Underwood and Suttle, 1999). We therefore recommend
275 72% as true retention coefficient for beef cattle.

276 The net Ca requirement for growth can be estimated by the following equation: $Ca = 0.21 \times$
277 $EBW^{-0.94}$, in which the amount of Ca is calculated as g Ca/kg empty body gain (EBG). The negative
278 exponent indicates that deposition of Ca is reduced when the animals grow, and therefore, younger
279 animals require more Ca than older animals. Valadares Filho et al. (2010) recommended the
280 following equation: $Ca = 102 \times EBW^{-0.40}$ per unit of gain.

281 The dietary requirement for Ca was obtained from the sum of the net Ca requirement for
282 maintenance and growth and the subsequent division by the retention coefficient. Therefore, for a
283 300 kg beef cattle with 1.00 kg of ADG, the dietary Ca requirement is 23.6 g/d (Table 6). This
284 value is closer to the Valadares Filho et al. (2010) recommendation of 27.1 g/d for a 300 kg beef
285 cattle.

286

287 P

288 The net P requirement for maintenance is 16.1 mg/kg BW/d (Figure 2) which is close to values
289 recommended by NRC (1996) and ARC (1980; Table 5). The values of true retention coefficient
290 reported in this study (82%; Table 5) are greater than those presented elsewhere (AFRC, 1991;
291 NRC, 1996; NRC, 2001; CSIRO, 2007). The absorption of P can vary based on the P content of
292 forage and concentrate, as well as the mineral sources used to feed animals (AFRC, 1991).

293 The net P requirement for growth can be achieved by the following equation: $P = 6.10 \times$
294 $EBW^{0.04}$, in which the amount of P in the body is calculated as g P/kg EBG. The exponent was
295 close to zero, which indicates the net P requirement for growth is constant. Different behavior was
296 found by Valadares Filho et al. (2010) that recommended the following equation: $P = 29.8 \times EBW^{$
297 0.29 , showing that the net P requirement for growth decreases when animal grows.

298 The dietary P requirement for a 300 kg beef cattle with 1.00 kg of ADG is calculated as 15.4
299 g/d (Table 6) which this value close to those recommended by Valadares Filho et al. (2010).
300 Another way to represent dietary P requirement is based on daily requirement divided by DMI.
301 Erickson et al. (2002) reported that the P requirements are less than 1.60 g/kg DM. In this
302 experiment, the average dietary P requirement, when divided by DMI, is 2.38 g/kg DM (Table 5).
303 ARC (1980) also reported the ratio between Ca and P for ruminant diets is important because both
304 minerals function together in bone formation and recommends the Ca:P ratio is between 1:1 and
305 2:1. However, the NRC (1996) highlighted that the effect of the calcium:phosphorus ration on
306 ruminant performance has been overemphasized in the past (Dowe et al., 1957; Wise et al., 1963)
307 and the dietary calcium to phosphorus ratios of between 1:1 and 7:1 result in similar performance.
308 The Ca:P ratio observed in this study was 2.15:1 being inside of range preconized by these
309 councils.

310

311 K

312 The highest concentrations of potassium in body tissues are found in muscle, and nervous and
313 secretory tissues (ARC, 1980). The ARC (1980) separated endogenous losses as feces (2.6 g/kg
314 DMI), urine (37.5 mg/kg BW), saliva (0.7 g/100 kg BW), and skin (1.1 g/d) reporting different
315 estimates for each loss. Thus, the net K requirements for maintenance can be achieve by the sum

316 of each loss. However, the data used by ARC (1980) was provided by only one study (St. Omer
317 and Roberts, 1967) that utilized only 9 heifers and a 3 x 3 Latin square analysis to evaluate the
318 mineral balance. Then, to standardize the estimate, we calculated the net K requirements for
319 maintenance as 33.0 mg/kg BW/d accounting all losses (Figure 2). This value was close to those
320 recommended by NRC (2001) as 38 mg/kg BW/d; however it was considered an additional of 2.6
321 g/kg DMI as fecal losses.

322 Ward (1966) reported that K is absorbed from the rumen and omasum as well as from the
323 intestine, and this absorption is very high. ARC (1980) used the absorption coefficient to convert
324 net to dietary requirements and assumed 100% for the K absorption coefficient. However, in this
325 study, the retention coefficient was calculated as 69.8% (Figure 2; Table 5). Ward (1966) also
326 indicated that the urine is the major route of K excretion, and body reserves of K are minimal. Our
327 results suggest that this cannot be accurate, as we have shown that the urine excretion represents
328 only 16% of the animal's intake, while the fecal excretion and the amount retained by the cattle are
329 49 and 35%, respectively. The NRC (2001) reports as true absorption coefficient being 90%.

330 The net K requirement for growth can be estimated by the following equation: $K = 1.43 \times$
331 $EBW^{0.03}$, in which the exponent close to zero indicates that the net requirement for growth does
332 not vary when the animal grows. However, Valadares Filho et al. (2010) recommended the
333 following equation to estimate net K requirement for growth: $K = 0.29 \times EBW^{0.38}$ showing that
334 the net K requirement for growth increases when animal grows. For a 300 kg beef cattle with 1.00
335 kg of ADG, the dietary K requirement is 46.5 g/d (Table 6). This value is larger than the value of
336 34.4 g/d recommended for the same animal type by Valadares Filho et al. (2010).

337

338 Mg

339 The net Mg requirement for maintenance is 17.2 mg/kg BW/d for beef cattle in this study.
340 This values is larger than the value suggested by ARC (1980), NRC (2001), and BR CORTE (2010;
341 Table 6). The BR CORTE (2010) utilizing the same procedure of this study used data from only
342 12 growing heifers to estimate this value. The true retention coefficient was calculated as 98.3%
343 (Figure 2). The ARC (1980) presented the overall mean value of 29.4% but for the calculation of
344 allowances which provide a margin of safety, the lower value of 0.17 was recommended which
345 this value also assumed by NRC (1996) and NRC (2001) as absorption coefficient.

346 The net Mg requirement for growth can be estimated by the following equation: $Mg = 0.35$
347 $\times EBW^{-0.02}$. Valadares Filho et al. (2010) suggested the following equation for beef cattle: $Mg =$
348 $333.3 \times EBG$. For a 300 kg beef cattle with 1.00 kg of ADG, the dietary Mg requirement is 5.59
349 g/d. This value is close to the value recommended by Valadares Filho et al. (2010) of 7.27 g/d for
350 a same animal type. When the dietary requirement is represented as g/kg DMI, the value presented
351 in this study was 0.79 while those suggested by NRC (1996) and CSIRO (2007) were 1.00 and
352 1.30 mg/kg DMI respectively.

353

354 Na

355 ARC (1980) suggested that dietary Na can be freely and completely absorbed by cattle, and
356 that the concept of the strict endogenous fecal loss does not apply to Na. The net Na requirements
357 for maintenance was 8.51 mg/kg BW/d (Figure 2) which this value close to those recommended
358 by ARC (1980) and BR CORTE (2010; Table 6). However, NRC (2001) suggested 15 mg/kg
359 BW/d as the net Na requirement for maintenance. NRC (1996) and NRC (2001) recommend 91
360 and 90% as absorption coefficient for Na. However, these recommendations do not consider the
361 amount of Na released in the urine. In this study, the retention coefficients for Na were 57.6%

362 which this value greater than those proposed by BR CORTE (2010) as 19%. Bankir et al. (2010)
363 reported that vasopressin increased Na reabsorption, and from 65 to 80% of the filtrate is
364 reabsorbed.

365 The net Na requirement for growth can be estimated by the following equation: $Na = 0.89$
366 $\times EBW^{0.05}$. Valadares Filho et al. (2010) suggested a linear relationship between Na and EBG: Na
367 $= 1.52 \times EBG$ for bulls and steers and $Na = 1.35 \times EBG$ for heifers. For a 300 kg beef cattle with
368 1.00 kg of ADG, the dietary Na requirement is 6.52 g/d (Table 5). This value is greater than those
369 recommended by Valadares Filho et al. (2010), which is 3.55 g/d for bulls and steers and 3.37 g/d
370 for heifers. When the dietary requirement is represented as g/kg DMI, the value presented in this
371 study was 0.96 while those suggested by NRC (1996) and CSIRO (2007) were both 0.80 mg/kg
372 DMI respectively.

373

374 S

375 The recommendation for S requirement made by NRC (1996) and NRC (2001) as 1.5 and 2.0
376 g/kg DMI reporting that requirements of beef cattle for sulfur are not well defined. Thus, the net
377 S requirement for maintenance has not been evaluated by any previous study. We calculated the
378 values of 9.4 mg/kg BW/d. The true retention coefficient has also not been previously evaluated
379 which the value found in this study as 67%. The net S requirement for growth can be estimated by
380 the following equation: $S = 0.03 \times EBW^{0.89}$. The net S requirement for growth had different
381 characteristics than those of other macronutrient minerals, because most of the macronutrient
382 mineral requirements decline or do not change when the animal grows. For a 300 kg beef cattle
383 with 1.00 kg of ADG, the dietary S requirement is 10.5 g/d (Table 6). When expressed as g/kg

384 DMI, the dietary S requirement 1.47 g/kg DMI which this value close to the recommendations of
385 NRC (1996) and NRC (2001).

386

387 Cr

388

389 Previous studies (Butting et al., 1994; Kegley and Spears, 1995) evaluated Cr supplementation
390 in calves and suggested that the addition of Cr at 0.4 mg/kg DMI increased the glucose clearance
391 rate. Bernhard et al. (2012) evaluated the effects of Cr supplementation on the performance of
392 steers, and observed a difference in ADG between steers without Cr supplementation and those
393 that were supplemented with 0.3 mg Cr per kg DMI basis. Any study was conducted to evaluate
394 the net Cr requirements for maintenance and growth as well as the true retention coefficient. Thus,
395 the net Cr requirement for maintenance was 22.9 µg/kg BW/d while the true retention coefficient
396 was calculated as 78.4%. The net Cr requirement for growth can be estimated by the following
397 equation: $Cr = 0.23 \times EBW^{0.61}$, in which the amount of Cr is calculated as mg Cr/kg EBG. For a
398 300 kg beef cattle with 1.00 kg of ADG, the dietary Cr requirement is 18.6 mg/d (Table 6). This
399 value represents 2.53 mg/kg DMI which is greater than the NRC (1996) recommendation as 0.4
400 mg/kg DMI (Table 5). This difference might be occurred due to in this study the Cr concentration
401 in basal diet was considered while NRC (1996) recommendation is for additional supplementation.

402

403 Co

404

405 Smith (1987) reported that the efficiency with which animals obtain vitamin B₁₂ from dietary
406 Co is very low. Some studies (Monroe et al., 1952; Looney et al., 1976) verified that 84 to 98% of

407 the dietary Co appeared in the feces within 5 to 14 days. Thus, the net Co requirement for
408 maintenance was 18.4 µg/kg BW/d while the true retention coefficient was 85.6% The net Co
409 requirement for growth can be estimated by the following equation: $Co = 0.04 \times EBW^{1.00}$. The net
410 Co requirement for growth increases in the same proportion that animal weight increases.

411 Smith (1987) also suggested that the dietary Co requirement was 0.11 mg/kg DMI. This value
412 was adopted by NRC (1996) and NRC (2001), but they did not consider the absorption coefficient
413 and feed composition. In this study, the average dietary Co requirements were 2.78 mg/kg DMI
414 (Table 5).

415

416 Cu

417

418 ARC (1980) reported that the net Cu requirement for maintenance was 7.1 µg/kg BW;
419 however, this value was generated from an equation with variables such as Cu intake, hepatic Cu
420 loss, and change in live weight. Each of these components in the equation involve certain
421 assumptions. Also, two studies involving cattle yielded estimates of 1.8 and 0.8 µg/kg BW
422 (Clawson et al, 1972; Mills et al, 1976). CSIRO (2007) adopted 4.0 µg/kg BW as the net Cu
423 requirement for maintenance from study developed by Suttle (1974). Thus, the net Cu requirement
424 for maintenance in this study was 163 µg/kg BW/d which this value greater than those observed
425 by ARC (1980) and CSIRO (2007). Also, ARC (1980) estimated the absorption Cu coefficient as
426 6% using hepatic Cu retention technique, but they made some assumptions to achieve this value.
427 In this study, the true retention coefficient was 84.7% showing value greater than those proposed
428 by ARC (1980). The net Cu requirement for growth can be estimated by the following equation:
429 $Cu = 1.25 \times EBW^{0.33}$.

430 Mullis et al. (2003) estimated Cu requirements of Angus and Simmental heifers to be 7 mg/kg
431 DMI. However, these authors did not consider the amount of Cu in the diet. Therefore, the Cu
432 requirement might be lower than the value suggested in this study. A dietary Cu requirement of
433 9.53 mg/kg DMI should be adequate (Table 5).

434

435 Fe

436

437 Thomas (1970) reported that insufficient dietary Fe will reduce body stores as well as plasma
438 Fe and blood hemoglobin levels. Calves can maintain a hemoglobin concentration of 30 mg Fe/d;
439 however, this amount was not enough to allow for a normal increase in hemoglobin or plasma Fe
440 of anemic animals. Thus, the net Fe requirement for maintenance was 2,097 $\mu\text{g}/\text{kg}$ BW while the
441 true retention coefficient was 52.7% (Figure 3). The net Fe requirement for growth can be
442 estimated by the following equation: $\text{Fe} = 15.5 \times \text{EBW}^{0.43}$.

443 Bremner and Dalgarno (1973) evaluated Fe requirements in calves and recommended that a
444 dietary intake of 40 mg soluble Fe/kg DMI is sufficient at preventing the development of anemia.
445 Bernier et al. (1984) recommended an additional Fe supplementation between 30 and 50 mg/kg
446 DMI to prevent anemia. The large difference between the NRC (1996) recommendation and our
447 study for dietary Fe requirements (50 vs 218 mg/kg DMI; Table 5) may have resulted because in
448 the reference studies by ARC (1980), calves received Fe supplementation, but the authors did not
449 verify differences between the lower and higher levels and, thus, they concluded the higher level
450 prevented anemia. It is likely that the basal dietary Fe provided a sufficient Fe level for these
451 calves.

452

453 Mn

454

455 The body of a normal 70 kg animal is estimated to contain a total of 10 to 20 mg Mn (Cotzias,
456 1958). The amount of Mn in the body is distributed widely throughout the tissues and fluids and
457 can vary with age, species, organs, and in relation to some other trace elements. Schroeder et al.
458 (1966) assumes 20 to 25 mg Mn/kg DMI is necessary for the animal to have optimum skeletal
459 development. Approximately 1 to 4% of dietary Mn is absorbed, irrespective of dietary
460 concentration (Sansom et al., 1978; Sullivan et al., 1979; Van Bruwaene et al., 1984). Hurley and
461 Keen (1987) reported that several factors, including a high concentration of Ca and Fe in the diet,
462 can decrease the Mn absorption. Thus, the net Mn requirement for maintenance was 32.3 µg/kg
463 BW and the true retention coefficient was calculated as 23.6% (Figure 3). The NRC (2001)
464 suggested the net Mn requirement for maintenance and true absorption coefficient as 2.00 µg/kg
465 BW and 75% respectively while CSIRO (2007) used recommendation from study developed by
466 Sansom et al., 1978).

467 The net Mn requirement for growth can be estimated by the following equation: $Mn = 0.07 \times$
468 $EBW^{0.80}$. Bentley and Phillips (1951) concluded that 10 mg/kg DMI met the Mn requirements in
469 young heifers for growth. This value was reported by BR CORTE (2010) as the Mn requirement
470 for growth. Hartmans (1974) fed cows for 2.5 to 3.5 years with rations containing 16 to 21 mg of
471 Mn/kg diet DM, and did not find any Mn deficiencies or improvements from Mn supplementation.
472 In this study, the dietary Mn requirement was estimated as 9.59 mg/kg DMI which this value lower
473 to the NRC (1996) recommendation of 20 mg/kg DMI. This difference might be the amount of
474 Mn accounted in the feedstuffs in this study which was not considered by NRC (1996).

475

476 Se

477

478 The Se concentration in the body is dependent upon the amount and chemical form of Se in
479 the diet and upon the tissue evaluated. High concentrations can occur in the liver and kidneys, but
480 the largest amount of Se is sequestered in muscles (Behne and Wolters, 1983). There are no
481 experiments that evaluate net Se requirement for maintenance or the retention coefficient for beef
482 cattle. The net Se requirement for maintenance was estimated as 3.72 µg/kg BW/d. Wright and
483 Bell (1966) evaluated the absorption coefficient in sheep and swine and found that 35% of ingested
484 isotopic Se was absorbed in sheep. The value in this study (48.7%; Figure 3) is greater than values
485 reported by Wright and Bell (1966) and CSIRO (2007) while it was close to the NRC (2001)
486 recommendation (Table 5). The net Se requirement for growth can be estimated by the following
487 equation: $Se = 1.07 \times EBW^{-0.07}$.

488 Oh et al. (1976) fed lambs with milk, increasing Se concentration in the diet from 0.01 to 0.05
489 mg/kg DMI, and observed increases in glutathione peroxidase activity, but the maximal enzyme
490 activity was not obtained until the diet provided 0.1 mg Se/kg DMI. However, these authors did
491 not consider the amount of Se provided by milk. The dietary Se requirement was estimated as
492 0.57 mg/kg DMI which is greater than those observed by Oh et al. (1976) and CSIRO
493 (2007) while it was close to those suggested by the NRC (2001; Table 5).

494

495 Zn

496

497 Some researchers (Delezenne, 1919; Bodansky, 1920; Weitzel et al., 1954) have reported
498 regular presence of Zn in plants and animals in concentrations that are often comparable to those

499 of Fe, and are usually greater than those of most other trace elements (Hambidge et al., 1987). The
500 NRC (1996) used an average of several studies (Miller et al, 1966; Hansard et al., 1968; Schwarz
501 and Kirchgessner, 1975) to estimate the endogenous urinary Zn loss as 12 µg/kg BW (range from
502 4 to 19 µg/kg BW). Weigand and Kirchgessner (1982) evaluated the Zn requirement of lactating
503 dairy cows and estimated that the net Zn requirement for maintenance was 53 µg/kg BW. The
504 NRC (2001) and ARC (1980) estimated the net Zn requirement for maintenance as 55 µg/kg BW
505 while CSIRO (2007) as 45 µg/kg BW. These values are lower than those estimated in this study
506 (669 µg/kg BW/d; Figure 3). ARC (1980) also used an absorption coefficient for Zn of 30% for
507 young growing ruminants, and a value of 20% for mature animals based on data from several
508 studies (Hansard et al., 1968; Miller and Cragle, 1965; Hansard and Mohammed, 1968). The
509 CSIRO (2007) adopted true absorption coefficient as 60% for pre-ruminant calves and 40% for
510 older animals (SCA, 1990). Our estimate based on the retention coefficient was calculated as 80%.

511 The net Zn requirement for growth can be estimated by the following equation: $Zn = 1.16 \times$
512 $EBW^{0.86}$. ARC (1980) suggests that between 16 to 31 mg Zn/kg BW may be incorporated into
513 body tissue for each kg gained. The values for dietary Zn requirements in this study were greater
514 than NRC (1996) recommendations (Table 5), but the data that provided the NRC (1996) estimate
515 was from studies (Perry et al., 1968; Pond and Otjen, 1988) that evaluated the growth response to
516 Zn supplementation.

517

518 **Conclusion**

519 The use of the true retention coefficient improves the estimates of dietary requirements of Na,
520 K, Mg, and S due to the considerable excretion of these minerals in the urine. Also, the use of the
521 net requirements for maintenance and growth and true retention coefficient for the majority of the

522 trace minerals resulted in different estimates than those obtained in the literature. Then, we
523 recommend the retention assay method to get mineral requirements.

524

525 **Conflict of interest**

526

527 We confirm that the manuscript has been read and approved by all named authors and that
528 there are no other person who satisfied the criteria for authorship but are not listed. We further
529 confirm that the order of authors listed in the manuscript has been approved by all of us.

530

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532

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538

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654

655 **Table 1**

656 Proportions of feed in concentrate and diet, and concentrate and diet composition calculated on a
 657 DM basis of the experiment 1

Ingredients	Concentrate	Diet
	Proportion (g/kg DM)	
Corn silage	0.00	550
Corn, ground	816	367
Soybean meal	137	62.0
Urea	18.0	8.00
Ammonium sulfate	2.00	1.00
Salt	10.0	5.00
Limestone	7.00	3.00
Mineral mix ¹	10.0	4.00
	Chemical composition (g/kg DM)	
DM (g/kg)	876	555
CP	195	123
NDF	134	347
Ca	5.90	3.60
P	5.22	3.26
Mg	1.10	1.30
K	4.70	7.70
Na	3.00	1.60
S	1.30	1.01
Co, mg/kg DM	1.90	1.50
Cr, mg/kg DM	3.00	3.10
Cu, mg/kg DM	19.7	11.9
Fe, mg/kg DM	164	388
Mn, mg/kg DM	25.9	34.1
Zn, mg/kg DM	76.0	45.2

658 ¹(266 g/kg calcium (calcium carbonate source); 147 g/kg phosphorus (dicalcium phosphate source); 7 g/kg
 659 magnesium; 3 g/kg potassium; 7 g/kg sulfur (cobalt sulfate and zinc sulfate source); 2 g/kg sodium (sodium chloride
 660 source); 118 mg/kg chromium; 1,191 mg/kg copper (copper chelate source); 5,070 mg/kg iron (iron sulfate source);
 661 1,728 mg/kg manganese (manganese chelate source); 4,198 mg/kg zinc (zinc sulfate source); and 136 mg/kg cobalt
 662 (cobalt sulfate source)).

663 **Table 2**

664 Proportions of feed in each diet, and its composition calculated on DM basis of the experiment 2

Items	70:30	70:30	50:50	50:50	Sugarcane
	CaPR	CaPL	CaPR	CaPL	
	Proportion, g/kg DM				
Sugarcane	700	700	500	500	
Ground corn	246	246	411	411	
Soybean meal	45.1	45.1	75.2	75.2	
Dicalcium phosphate	4.80	2.40	2.50	0.00	
Salt	0.40	0.40	0.70	0.70	
Limestone	3.10	0.00	5.30	2.20	
Mineral mix	0.24	0.24	0.35	0.35	
Sand	0.00	5.50	5.30	10.8	
	Chemical composition, g/kg DM				
DM (g/kg)	488	488	599	599	322
CP	162	162	163	162	34.0
NDF	397	397	326	326	503
Ca	5.65	3.85	5.23	3.44	4.30
P	2.77	2.26	2.66	2.15	1.20
Mg	1.96	1.94	2.50	2.48	1.10
K	4.09	4.09	4.41	4.41	3.60
Na	0.43	0.42	0.57	0.56	0.20
S	1.29	1.26	1.38	1.34	1.10
Co, mg/kg DM	1.64	1.52	1.50	1.37	1.60
Cr, mg/kg DM	3.56	3.10	3.17	2.70	2.60
Cu, mg/kg DM	12.8	14.1	18.8	18.8	3.70
Fe, mg/kg DM	329	341	275	287	422
Mg, mg/kg DM	57.9	59.7	56.7	56.7	59.5
Zn, mg/kg DM	44.8	47.3	60.3	59.8	19.7
Se, mg/kg DM	0.45	0.46	0.41	0.41	0.50

665 ¹(29.2 g/kg calcium; 0.70 g/kg phosphorus; 2.11 g/kg magnesium; 0.89 g/kg potassium (potassium iodate source);

666 63.5 g/kg sulfur (copper sulfate and zinc sulfate source); 0.31 g/kg sodium (sodium chloride source); 2.56 mg/kg

667 chromium; 3,296 mg/kg copper (copper sulfate source); 2,088 mg/kg iron (iron sulfate source); 4,673 mg/kg

668 manganese (manganese sulfate source); 7,817 mg/kg zinc (zinc sulfate source); 318 mg/kg of selenium (sodium

669 selenite source), and 348 mg/kg cobalt (cobalt sulfate source)).

670 **Table 3**

671 Descriptive statistics of data used in the present study (n = 87)

Items	Average	MSE	Minimum	Maximum
Experiment I - Bulls				
SBW, kg	375	104	219	592
EBW ¹ , kg	344	99.2	192	549
ADG, kg/d	1.23	0.45	-0.01	1.95
EBG ² , kg/d	1.24	0.44	-0.01	1.87
DMI ³ , kg/d	7.17	1.74	2.28	11.1
NDT, %	69.8	4.83	59.5	80.6
Experiment II - Heifers and steers				
SBW, kg	207	45.8	121	300
EBW, kg	182	41.4	104	266
ADG, kg/d	0.35	0.24	-0.05	0.84
EBG, kg/d	0.35	0.27	-0.02	0.83
DMI, kg/d	3.50	1.43	1.18	6.24
NDT, %	78.1	6.11	60.6	87.3

672 ¹EBW = empty body weight.673 ²EBG = empty body gain.674 ³DMI = dry matter intake from the animals fed ad libitum and at maintenance in both

675 experiments.

Table 5 - Comparison between the council's recommendations and the values obtained in this study

Items	Ca	P	K	Mg	Na	S	Cr	Co	Cu	Fe	Mn	Se	Zn
Net requirement for maintenance													
	mg/kg BW/d						µg/kg BW/d						
ARC (1965)	16.0	-	-	-	-	-	-	-	-	-	-	-	-
ARC (1980)	-	12.0	-	3.00	6.80	-	-	-	7.1	-	-	-	45.0
NRC (1996)	15.4	16.0	-	-	-	-	-	-	-	-	-	-	12.0
NRC (2001)	15.4	-	38.0	3.00	15.0	-	-	-	-	-	2.00	-	45.0
CSIRO (2007)	-	-	-	-	-	-	-	-	4.0	-	-	-	55.0
BR CORTE (2010)	-	17.6	-	3.30	7.00	-	-	-	-	-	-	-	-
Our result	20.0	16.1	33.0	17.2	8.51	9.40	22.9	18.4	163	2,097	32.3	3.72	669
Absorption or retention coefficient (%)													
ARC (1980)	-	-	100	29.4	-	-	-	-	6.00	-	-	-	30.0
AFRC (1991)	68.0	58.0	-	-	-	-	-	-	-	-	-	-	-
NRC (1996)	50.0	68.0	-	17.0	91.0	-	-	-	-	-	-	-	-
NRC (2001)	70.0	75.0	90.0	17.0	90.0	-	-	-	-	-	75.0	40-50	-
CSIRO (2007)	-	70.0	-	-	-	-	-	-	-	-	1.00	30.0	40.0
BR CORTE (2010)	55.0	-	-	-	19.0	-	-	-	-	-	-	-	-
Our result	72.0	82.0	69.8	98.3	57.6	67.3	78.4	85.6	84.7	52.7	23.6	48.7	80.0
Dietary requirement													
	g/kg DMI						mg/kg DMI						
ARC (1980)	-	-	-	-	-	-	-	-	7.10	-	-	-	-
NRC (1996)	-	-	6.00	1.00	0.80	1.50	0.40	0.11	10.0	50.0	20.0	0.10	30.0
NRC (2001)	-	1.00	-	-	-	2.00	-	0.11	-	-	-	0.30	-
CSIRO (2007)	-	-	-	1.30	0.80	-	-	-	-	-	-	0.05	11.6
Our result	5.12	2.38	2.40	0.79	0.96	1.47	2.53	2.78	9.53	218	9.59	0.57	61.0

Table 6

Dietary mineral requirements of beef cattle using mineral balance method

BW, kg	Dietary Mineral Requirements												
	Ca	P	K	Mg	Na	S	Cr	Co	Cu	Fe	Mn	Se	Zn
				g/d						mg/d			
100	49.1	11.0	7.10	2.09	3.44	3.61	7.37	6.31	25.6	626	37.1	5.35	150
200	28.6	13.3	11.9	3.84	5.00	7.09	13.2	13.0	46.6	1,097	73.4	7.76	294
300	23.6	15.4	16.7	5.59	6.52	10.5	18.6	19.9	67.1	1,545	109	10.2	434
400	22.5	17.6	21.4	7.34	8.04	13.8	23.8	26.7	87.3	1,980	145	12.8	572
500	23.0	19.7	26.2	9.09	9.54	17.1	28.9	33.6	107	2,408	181	15.3	708

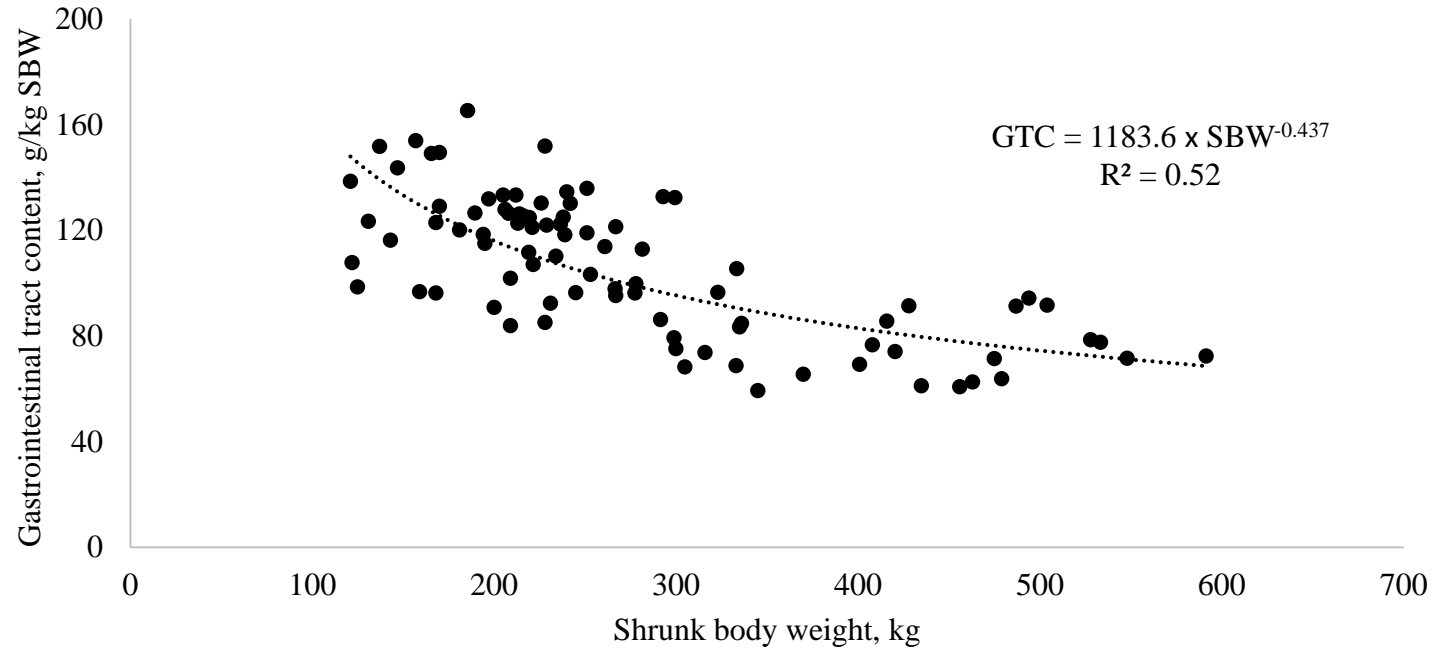


Figure 1 – Relationship between gastrointestinal tract content (GTC, g/kg SBW) and shrunken body weight (SBW, kg)

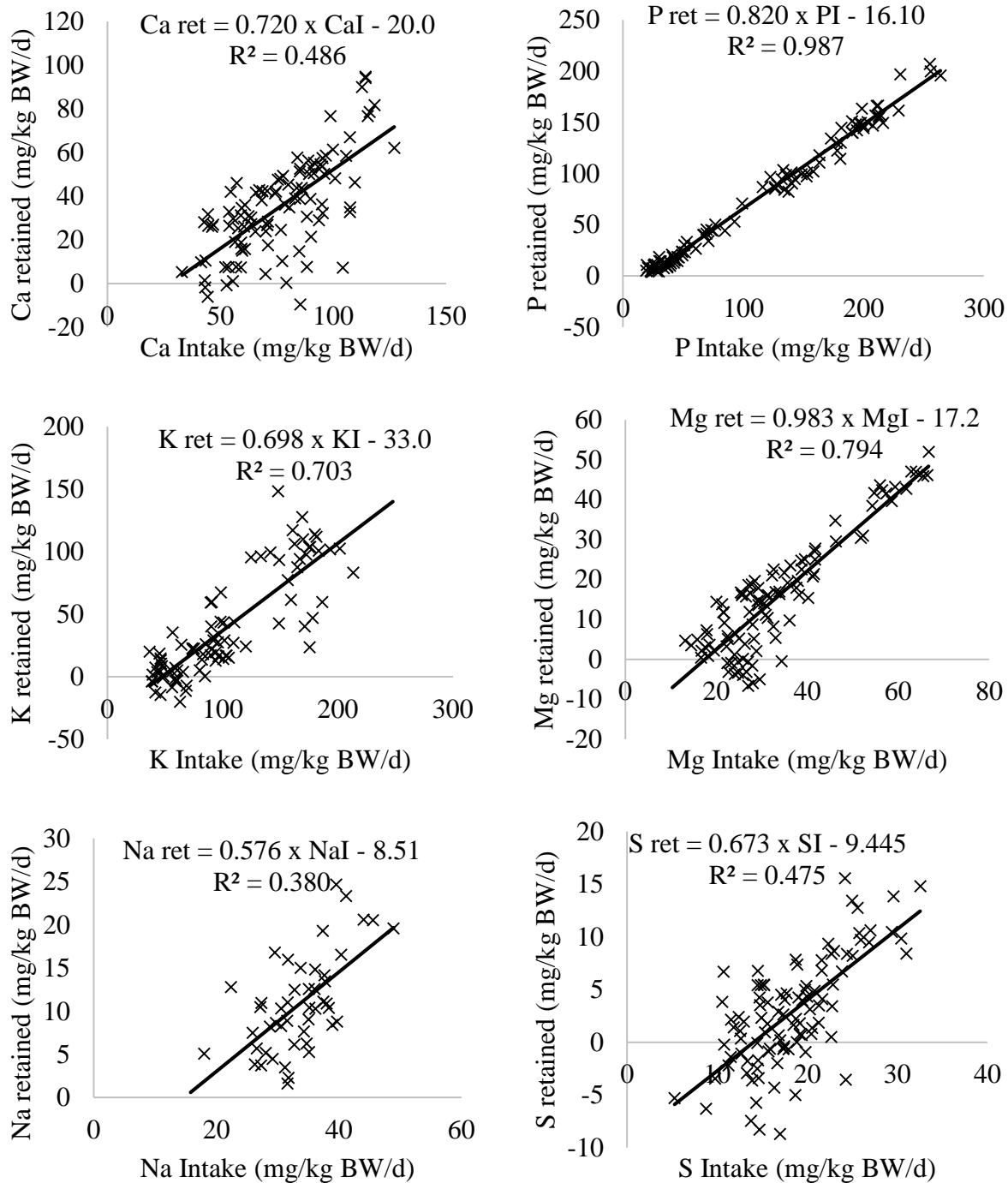


Figure 2 – The net requirements for maintenance and true retention coefficient of macronutrient minerals for beef cattle

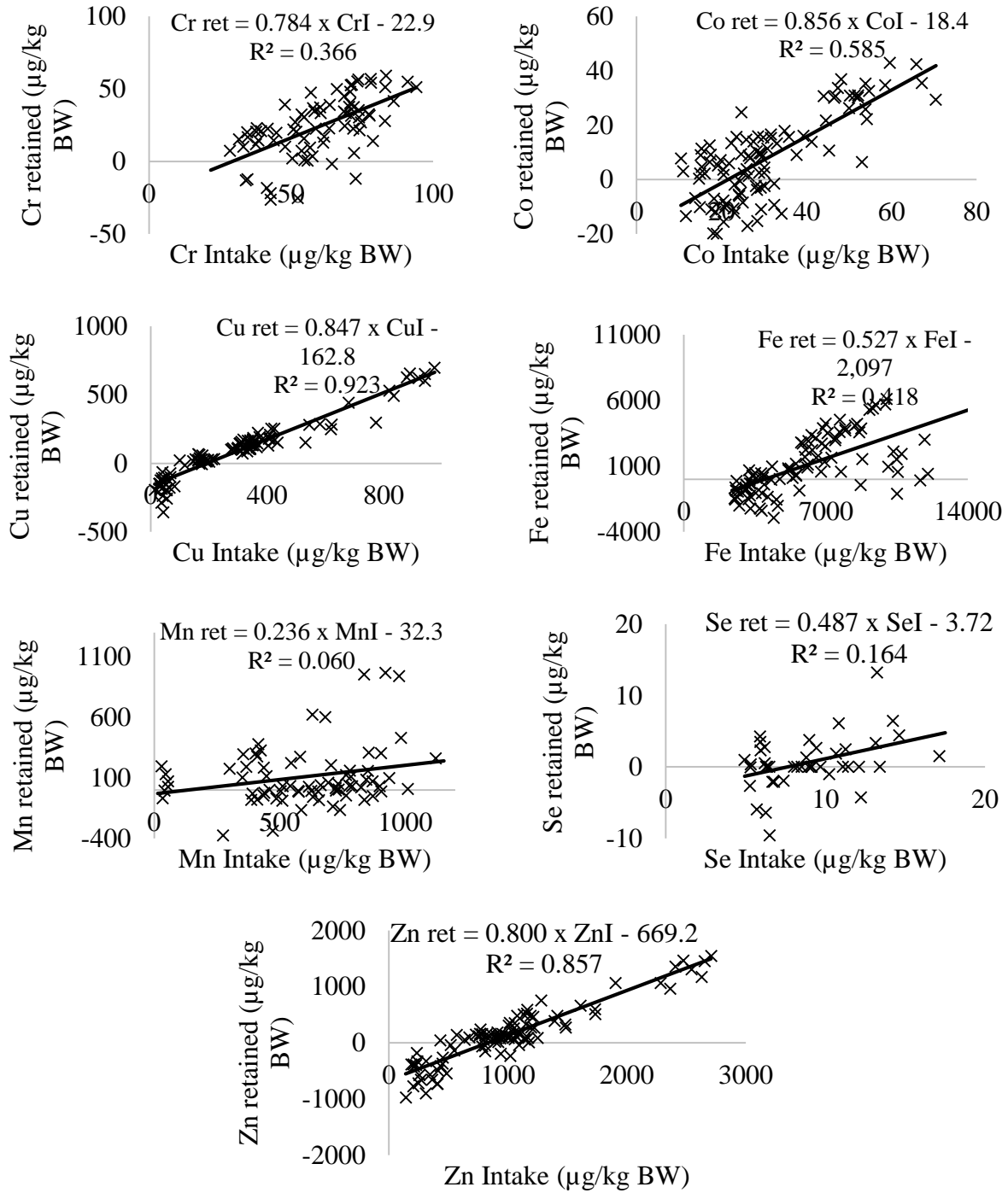


Figure 3 – The net requirement for maintenance and true retention coefficient of trace element minerals for beef cattle