LAURA FRANCO PRADOS

REDUCTION OF MINERALS IN FEEDLOT DIETS OF NELLORE CATTLE: IMPACTS ON INTAKE, PERFORMANCE, AND NUTRIENT REQUIREMENTS; AND PREDICTION OF CHEMICAL RIB SECTION COMPOSITION BY DUAL ENERGY X-RAY ABSORPTIOMETRY IN ZEBU CATTLE

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

VIÇOSA
MINAS GERAIS – BRAZIL
2016

Ficha catalográfica preparada pela Biblioteca Central da Universidade Federal de Viçosa - Câmpus Viçosa

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P896r
2016

Prados, Laura Franco, 1987-
Reduction of minerals in feedlot diets of nellore cattle : impacts on intake, performance, and nutrient requirements; and prediction of chemical rib section composition by dual energy x-ray absorptiometry in zebu cattle / Laura Franco Prados. – Vicosa, MG, 2016.
xiv, 77f. : il. ; 29 cm.

Orientador: Sebastião de Campos Valadares Filho.
Tese (doutorado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. Nelore (Zebu). 2. Bovino - Alimentação e rações. 3. Bovino - Nutrição. 4. Minerais na nutrição animal. 5. Densitometria óssea. I. Universidade Federal de Viçosa. Departamento de Zootecnia. Programa de Pós-gradução em Zootecnia. II. Título.

CDD 22 ed. 636.291

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APPROVED: July 29, 2016

Mario Fonseca Paulino

Mário Luiz Chizzotti
(Co-adviser)

Stefanie Alvarenga Santos

Ana Luiza Costa Cruz Borges

Sebastião de Campos Valadares Filho
(Adviser)

To my parents, by their LOVE, work and encouraging examples.

To my siblings, by their love and friendship.

To Helena, my support and my strength!

To Andre, by his love.

To my grandfather, by his passion for cattle.

To my relatives, for always being by my side, giving me strength and praying!

In memory of my grandmother Silvia and my brother in law Leandro!

I dedicate.

ACKNOWLEDGEMENT

I would like to begin by thanking God for the strength to keep going and never give up, and to put awesome people in my way!

My thesis would not have been possible without the help of a large number of people.

I cannot express how thankful I am for the love and support I received from my parents, siblings and fiancée. My parents and siblings for their love, encouragement and their support in the most difficult hours. My dad that passed this passion on to me. My mom, the rock of the family. My relatives that have supported me throughout my academic career as well as with everything I have pursued in life and without their love and support I would not be the person I am today. To my grandpa, my example of life.

To André, my love, for your patience and encouragement in all these years of academic training. Thank you for everything and especially for always believing in me! Thank you for staying by my side through the ups and downs and for always being there to cheer me up! You truly are my best person.

To the Animal Science Department at the Universidade Federal de Viçosa, for the 10 years of training.

To CNPq, INCT-CA, CAPES and FAPEMIG for providing the financial support of this research and granting scholarships.

A big thank also needs to be given to my adviser Dr. Sebastião de Campos Valadares Filho, you have all been so instrumental in my growth in graduate school and I thank you for all the opportunities and the excellent guidance and for being an example of professional. Thank you for providing me direction to my studies and research.

To Dr. Mario Chizzotti, my co-adviser, for your patience and indispensable help in the completion of this work. You have believed in me and I have thus been able to do things I never thought possible of myself.

Members of my committee, Dr. Edenio Detmann, Dr. Mario Paulino, Dra. Stefanie Alvarenga, Dra Ana Luiza, Dra Luciana Rennó. You provided invaluable assistance in the completion of my thesis and I am especially thankful for the great deal of help by Dr. Detmann.

Dr. Erickson for helped me to become a more confident in a strange university and in USA. I am very grateful for all opportunities you gave me. Dr. Jim MacDonald for the friendship and teachings in Nebraska. Dr. Terry Klopfenstein for all teachings and trust.

Friends, interns and undergraduate research fellows, which were essential and allowed the realization and completion of this work: Ana Clara, Ana Clara Lopes, Brenimar, Danillo, Diego, Dino, Flavia, Herlon, Hugo, Jarbinha, Jessikinha, Leandrao, Leticia, Lorenzo, Lucas,

Luiz doente, Lyvian, Marcelo, Markin, Raphael, Tiago, Valdir, Zanetti, Sidnei, thanks for all dedication and commitment, not only assisted with data collection and lab analysis, but also helped me to stay positive and have a sense of humor when things did not go exactly as planned! Especially to Zana, Danillo, Breno, Herlon, Jessikinha and Markin not only for the help, but the friendship built, you have become some of my closest friends. Doente, thanks for all your help and company.

Danillo, you made the most pleasant experiment! Thanks for your patience and friendship.

Jarbinha, thanks for your friendship, help in the cutter and for always listen to me in lunch time.

Paloma (Piri) and Lays, my friends and companionship experiment. Thanks for the friendship. The fights and discussions made our friendship even more increased!

My dear friends: Stefs, Luciana, Fabi Lana, Carol Filardi, Aline (Sinha), Palominha, Laysão, Luizão, Erick, Jarbas, Leandrão, thanks for the friendship, help, encouragement, studies and special moments.

Friends of SIMCORTE: Sinha, Luiz, Zana, Pedrão, Peao, Markin, Cajuru, Jessikinha, Ranyeri, and Thiago.

Employees of DZO, especially Vanor, Zeze, Pum, Ti Jojo, Marcelo Cardoso, Niel, Seu Jorge, Venancio, Edinho and Monteiro, for their support, cooperation, friendship. Plinio, Seu Mario and Fernando, for help in the analysis. Fernandinha, for your patience and help!

To my Viçosa friends and ZOO6 friends.

To my Nebraska friends, Camilinha, Dri, Felipin, Felipe, Carlinha, Leo, Ulmas, Antonio, Fede, Curt, Juliano, Gilberto, Tasha, Bri, Zac, Kristen, Cody, Jordan, Colton, Aline, Lia, Rodrigo, Leilinha, Lucas, Koff, Jilmar, Meredith, Robby, Henry, Taylor, Jim, Liana, Wal, Flavia e Rodrigo, Nirosh…

Finally, I would like to THANK ALL who somehow contributed to the realization of this work and cheers for me.

I appreciate all that each one of you have done for me.

"Success consists of going from failure to failure without loss of enthusiasm."
Winston Churchill

BIOGRAPHY

Laura Franco Prados, daughter of Marcus Vinicius Franco Prados and Vera Lucia da Silva Prados, was born in São Gotardo/MG-Brazil on May 20, 1987.

She started the undergraduate in Animal Science at Universidade Federal de Viçosa in 2006 and became a Bachelor in Animal Science in 2010. At the same year, she started the Magister Scientiae program in Ruminant Nutrition with major emphasis in Beef cattle.

In August of 2010, she became a Master in Animal Science. At the same year, she started her PhD program in Animal science in the same area of Master.

From August of 2014 to July of 2015, she was a visiting research at University of Nebraska – Lincoln/NE-USA, where part of her research was developed under supervision of Dr. Galen Erickson.

On July of 2016, Laura F. Prados submitted her thesis to the committee to obtain the Doctor Scientiae degree in Animal Science under supervision of Dr. Sebastião de Campos Valadares Filho.

LIST OF CONTENTS

ABSTRACT

PRADOS, Laura Franco, D.Sc., Universidade Federal de Viçosa, July, 2016. **Reduction of minerals in feedlot diets of Nellore cattle: impacts on intake, performance, and nutrient requirements; and prediction of chemical rib section composition by dual energy x-ray absorptiometry in Zebu cattle.** Adviser: Sebastião de Campos Valadares Filho. Co-advisers: Mário Luiz Chizzotti and Edenio Detmann.

The present work was developed based on three studies. The objective of the first study was to evaluate a method to predict the 9th to 11th rib section (rib$_{9-11}$) composition through empirical equations using Dual Energy X-ray Absorptiometry (DXA). DXA is a validated method used to describe tissue composition in humans and other animals, but few studies have evaluated this technique in beef cattle, and especially in the Zebu genotype. A total of 116 rib$_{9-11}$ sections were used to evaluate published prediction equations for rib$_{9-11}$ composition and to develop new regression models using a cross-validation procedure. For the proposed models, 93 ribs were randomly selected to calculate the new regression equations, and 23 different ribs were randomly selected to validate the regressions. The rib$_{9-11}$ sections from left carcasses were taken from Nellore and Nellore × Angus bulls from three different studies and scanned using DXA equipment (GE Healthcare, Madison, Wisconsin, USA) in the Health Division at Universidade Federal de Viçosa. The outputs of the DXA report were DXA lean (g), DXA fat free mass (g), DXA fat mass (g), and DXA BMC (bone mineral content; g). After being scanned, the rib$_{9-11}$ sections were dissected, grounded, and chemically analyzed for total EE, CP, water, and ash content. The predictions of rib fat and protein from previous published equations were different ($P < 0.01$) from the observed composition. New equations were established through leave-one-out cross-validation using REG procedure using SAS. The equations were as follows: Lean (g) = 37.082 + 0.907× DXA lean ($R^2 = 0.95$); Fat free mass (g) = 103.224 + 0.869 × DXA fat free mass ($R^2 = 0.93$); EE mass (g) = 122.404 + 1.119 × DXA fat mass ($R^2 = 0.86$); Ash mass (g) = 18.722 + 1.016 × DXA BMC ($R^2 = 0.39$). The equations were validated using Mayer's test, the concordance correlation coefficient (CCC), and the mean square error of prediction (MSEP) for decomposition. Comparing observed and predicted values using the new equations, Mayer's test was not significant for lean mass ($P = 0.26$), fat free mass ($P = 0.67$), EE mass ($P = 0.054$), and ash mass ($P = 0.14$). We concluded that the rib$_{9-11}$ composition of Nellore and Nellore × Angus bulls can be estimated from DXA using the proposed equations. The second study was developed using weaned Nellore bulls (n = 36; 274 ± 34 kg) in a randomized complete block 2 × 2 factorial design experiment to evaluate intake, fecal excretion and performance with different levels of minerals. The design included two

levels of Ca and P (macro mineral factor; CaP+ or CaP-) and two levels of micro minerals (micro mineral factor; ZnMnCu+ or ZnMnCu-). The factor CaP- was without supplementation of limestone and dicalcium phosphate and the factor ZnMnCu- was without inorganic supplementation of micro minerals. The diets were isonitrogenous (13.3% CP). Intake was individually monitored every day. Indigestible NDF was used as an internal marker for fecal excretion measurements. The animals were slaughtered (84 and 147 days on feed; DOF), carcass characteristics were measured and liver and rib samples were collected. Feed, feces, rib bones and liver samples were analyzed for DM, ash, CP, EE, Ca, P, and micro minerals (Zn, Mn, and Cu). There were no significant interactions ($P \geq 0.06$) between macro and micro minerals supplementation for any variables in the study. Calcium, P and micro minerals concentrations did not affect ($P \geq 0.20$) intakes of DM, OM, NDF, EE, CP, TDN and NFC. Calcium and P intake were affected ($P < 0.01$) by macro mineral factor. Animals fed without Ca and P supplementation consumed lower levels of these minerals. Dry matter and nutrient fecal excretion were similar ($P \geq 0.23$) among factors. Performance and carcass characteristics were similar ($P \geq 0.09$) among diets. The content of ash in rib bones was not affected ($P \geq 0.06$) by diets. Phosphorus and phosphatase alkaline plasma concentration were similar ($P \geq 0.52$) among diets. Calcium plasma concentration was affected ($P < 0.01$) by micro mineral factor; nevertheless, all blood metabolites were within the reference values. Fecal excretion of Ca and P was different ($P < 0.01$) among macro mineral factor. These results indicate that supplementation of minerals (Ca, P, Zn, Mn, and Cu) is not necessary in conventional feedlot diets for finishing Nellore. Dietary reductions in these minerals would represent a decrease in the costs of feedlot diets. Dietary reductions in Ca and P decrease fecal excretion of these minerals. Decreasing the P fecal excretion through decreasing content minerals is an opportunity to reduce environmental impact of feedlot operations. The third study aimed to evaluate the water intake, the chemical body composition, the residual feed intake and gain, and the nutritional requirements of energy, protein for maintenance and gain, and calcium and phosphorus for maintenance of Nellore bulls, as well as their efficiencies. Weaned Nellore bulls ($n = 44$; 273 ± 34 kg) were fed in a randomized complete block design 2×2 factorial arrangement to evaluate the nutritional requirements with absence or presence of mineral supplementation. The design included two levels of Ca and P (macro mineral factor; CaP+ or CaP-) and two levels of micro minerals (micro mineral factor; ZnMnCu+ or ZnMnCu-). The diets were isonitrogenous (13.3% CP). Intake was individually monitored every day. Indigestible NDF was used as an internal marker for digestibility measurements. Four animals were used in the reference group (harvested d 0); four bulls were fed at the maintenance level

(1.1% of BW); and the remaining 36 bulls were fed ad libitum. Bulls are blocked by days on fed, they were slaughtered on d 84 and 147, after slaughter, samples of the whole body were taken. All samples were lyophilized, ground with liquid nitrogen and grouped as percentage of component in empty BW from each bull. Samples were analyzed for DM, ash, CP, EE, Ca, and P. The water intake was similar ($P \geq 0.07$) among treatments. The average of free water intake was 17 L/d for each bull. High residual feed intake and gain (RFIG) bulls had lower DMI ($P < 0.01$) than low RFIG bulls, but similar ADG ($P = 0.82$). The CP, EE and water present in the EBW increased as the animal grew, the ash growth is lower than the EBW. Non-linear regression equations were developed to predict heat production (HP) from metabolizable energy (ME) intake and retained energy (RE). The net energy requirements for maintenance (NE_m) and metabolizable energy for maintenance (ME_m) were 66.5 and 107 kcal/$EBW^{0.75}$/d, respectively. The efficiency (k_m) was 62%. The equation obtained for net energy for gain (NE_g) was: NEg (Mcal/day) = $0.0388 \times EBW^{0.75} \times EBWG^{1.095}$ and the efficiency was 25%. For net protein for gain was: NP_g (g/day) = $179.74 \times EBWG - 5.43 \times RE$. The net maintenance requirement for Ca was 2.33 mg/EBW and for P was 9.10 mg/EBW. The coefficient of absorption for Ca was 54% and P was 64%. In conclusion, the requirement of net energy for maintenance for Nellore feedlot cattle is 66.5 kcal/$EBW^{0.75}$/day. Requirements of net energy for gain and net protein for gain can be obtained by the following equations: NE_g (Mcal/day) = $0.0388 \times EBW^{0.75} \times EBWG^{1.095}$ and NP_g (g/day) = $179.74 \times EBWG - 5.43 \times RE$. Net maintenance requirement for Ca is 2.33 mg/EBW and for P is 9.10 mg/EBW. The coefficient of absorption for Ca is 54% and P is 64%. The water intake is not influenced by supplementation of Ca, P, Zn, Mn, and Cu. High residual feed intake and gain bulls has lower DMI than low RFIG bulls, with similar ADG. The CP, EE and water present in the EBW increased as the animal grew, the ash growth is lower than the EBW. Overall, intake and performance was not affect by minerals (Ca, P, Zn, Mn, and Cu) diet reduction. It was concluded that the reduction of minerals contents in feedlot diets for Nellore finishing bulls appears not influence in intake, performance, chemical body composition, and nutrient requirements of Nellore finishing bulls in feedlot.

RESUMO

PRADOS, Laura Franco, D.Sc., Universidade Federal de Viçosa, julho de 2016. **Redução de minerais na dieta de Nelore confinados: impactos no consumo, desempenho e exigências nutricionais; e predição da composição química da seção entre as costelas usando a densitometria óssea em zebuínos.** Orientador: Sebastião de Campos Valadares Filho. Coorientadores: Mário Luiz Chizzotti e Edenio Detmann.

O presente trabalho foi desenvolvido baseado em três estudos. O objetivo do primeiro estudo foi avaliar um novo método para estimar a composição química entre a 9 e 11ª seção das costelas (costela$_{9-11}$) através de equações usando um aparelho de absorptometria radiológica de dupla energia (DXA). O DXA é um método validado usado para caracterizar a composição de tecidos em seres humanos e outros animais, mas poucos estudos avaliaram esta técnica em bovinos de corte e, especialmente, em animais Zebus. Um total de 116 costela$_{9-11}$ foram utilizadas para desenvolver novos modelos de regressão usando o procedimento de validação cruzada (cross-validation). Para os modelos propostos, 93 costela$_{9-11}$ foram selecionadas aleatoriamente para gerar as novas equações de regressão, e 23 costela$_{9-11}$ diferentes foram selecionados aleatoriamente para validar as equações geradas. As seções da costela$_{9-11}$ de carcaças foram retiradas de animais Nelore e Nelore × Angus de três diferentes experimentos e escaneadas usando o equipamento DXA (GE Healthcare, Madison, Wisconsin, EUA) na Divisão de Saúde da Universidade Federal de Viçosa. Os outputs do relatório do DXA foram DXA massa magra (g), DXA massa livre de gordura (água, proteína e matéria mineral), DXA massa gorda (g), e DXA BMC (conteúdo mineral ósseo; g). Depois de serem escaneadas, as seções das costela$_{9-11}$ foram dissecadas, liofilizadas, e analisadas para EE, PB, MS e teor de cinzas. Novas equações foram estabelecidas através do procedimento de validação cruzada usando o procedimento REG do SAS. As equações foram como se segue: magro (g) = 37,082 + 0,907 × DXA magra (R^2 = 0,95); massa livre de gordura (g) = 103,224 + 0,869 × DXA massa livre de gordura (R^2 = 0,93); EE (g) = 122,404 + 1.119 × DXA massa gorda (R^2 = 0,86); cinzas (g) = 18,722 + 1,016 × DXA BMC (R^2 = 0,39). As equações foram validadas pelo teste de Mayer (teste conjunto do intercepto e inclinação), o coeficiente de correlação e concordância (CCC), e a decomposição do quadrado médio do erro predição (MSEP). Comparando valores observados e preditos usando as novas equações propostas, o teste de Mayer foi não significativo para a massa magra (P = 0,26), massa livre de gordura (P = 0,67), EE (P = 0,054), e cinzas (P = 0,14). Concluiu-se que a composição da costela$_{9-11}$ de novilhos Nelore e Nelore × Angus pode ser estimada a partir do DXA usando as equações propostas. O segundo estudo foi desenvolvido utilizando novilhos Nelore desmamados (n = 36; 274 ± 34 kg) em

delineamento em blocos casualizados em arranjo fatorial 2 × 2, para avaliar o consumo, a excreção fecal e desempenho com diferentes níveis de minerais na dieta de terminação. O experimento incluiu dois níveis de Ca e P (fator macro mineral; CaP+ ou CaP-) e dois níveis de micro minerais (fator micro mineral; ZnMnCu + ou ZnMnCu-). O fator CaP- foi sem suplementação de calcário e fosfato bicálcico e o fator ZnMnCu- foi sem suplementação inorgânica de micro minerais (premix). As dietas foram isoprotéicas (13,3% PB). A ingestão foi monitorada individualmente todo dia. A fibra em detergente neutro indigestível foi usada como indicador interno para calcular a digestibilidade. Os animais foram abatidos em diferentes períodos (84 e 147 dias de confinamento), características de carcaça foram medidas e amostras de fígado e ossos da costela foram retirados. Amostras de alimentos, fezes, costelas e fígado foram analisadas para MS, cinzas, PB, EE, Ca, P, e micro minerais (Zn, Mn e Cu). Não houve interação significativa ($P \geq 0,06$) entre a suplementação de macro e micro minerais para todas as variáveis estudadas. Concentração de Ca, P e micro minerais na dieta não afetou ($P \geq 0,20$) os consumos de MS, MO, FDN, EE, PB e CNF. Ingestão de Ca e P foram influenciados ($P < 0,01$) pelo fator macro mineral. Animais alimentados sem suplementação de Ca e P consumiram menores quantidades destes minerais. A excreção de matéria seca e de nutrientes foram similares ($P \geq 0,23$) entre os fatores estudados. Desempenho e características de carcaça foram similares ($P \geq 0,09$) entre as dietas. O teor de cinzas nos ossos das costelas não foi afetado ($P \geq 0,06$) pelas dietas. O fósforo e a concentração de fosfatase alcalina no plasma foram semelhantes ($P \geq 0,52$) entre as dietas. A concentração plasmática de cálcio foi afetada ($P < 0,01$) pelo fator micro mineral; no entanto, todos os metabólitos do sangue analisados estavam dentro dos valores de referência. A excreção fecal de Ca e P foi diferente ($P < 0,01$) entre o fator macro mineral. Estes resultados indicam que a suplementação de minerais (Ca, P, Zn, Mn e Cu) não é necessária em dietas convencionais de confinamento para animais Nelore em terminação. A redução nas dietas destes minerais representaria uma diminuição nos custos de dietas de confinamento. Reduções nas concentrações de Ca e P na dieta pode diminuir a excreção fecal destes minerais. Diminuir a excreção fecal de P através da diminuição deste mineral na dieta é uma oportunidade para reduzir o impacto ambiental dos confinamentos. O terceiro estudo teve como objetivo avaliar o consumo de água, a composição química corporal, o consumo alimentar residual e ganho residual conjunto, e as exigências nutricionais de energia, proteína para mantença e ganho, e cálcio e fósforo para a mantença de novilhos da raça Nelore, bem como suas eficiências. Foram utilizados animais Nelore desmamados (n = 44; 273 ± 34 kg), os novilhos foram alimentados em um delineamento em blocos casualizados em arranjo fatorial 2 × 2 com ausência ou presença de suplementação

mineral (Ca, P, Zn, Mn e Cu). O experimento incluiu dois níveis de Ca e P (fator macro mineral; CaP+ ou CaP-) e dois níveis de micro minerais (fator micro mineral; ZnMnCu+ ou ZnMnCu-). As dietas foram isoprotéicas (13,3% PB). A ingestão foi monitorada individualmente todo dia. Fibra em detergente neutro indigestível foi usada como indicador interno para mensurar a digestibilidade. Quatro animais foram utilizados no grupo de referência (abatidos no dia 0); quatro novilhos foram alimentados ao nível de mantença (1,3% do peso corporal); e os 36 animais restantes foram alimentados ad libitum. Após o abate, as amostras de todo o corpo foram amostradas. Todas as amostras foram liofilizadas, moídas com nitrogênio líquido e agrupadas como percentagem do peso de corpo vazio (PCVZ) de cada animal. As amostras foram analisadas para MS, cinzas, PB, EE, Ca e P. O consumo de água foi semelhante ($P \geq 0,07$) entre os tratamentos. A média de ingestão de água livre foi de 17 L/d. O consumo alimentar residual e ganho residual conjunto (GCAR) alto de touros tiveram menor CMS ($P < 0,01$) do que touros de baixo GCAR, mas com GMD semelhante ($P = 0,82$). A PB, EE e água presente no PCVZ aumentou à medida que o animal cresceu, o crescimento de cinzas foi menor do que o PCVZ. Equações de regressão não-lineares foram desenvolvidos para predizer a produção de calor, o consumo de energia metabolizável (CEM) e a energia retida (ER). As exigências líquidas de energia para mantença (EL_m) e energia metabolizável para mantença (EM_m) foram 66,5 e 107 kcal/$PCVZ^{0,75}$/d, respectivamente. A eficiência (k_m) foi de 62%. A equação obtida para energia líquida para ganho (EL_g) foi: EL_g (Mcal/dia) = 0,0388 × $PCVZ^{0,75}$ × $GPCVZ^{1.095}$ e a eficiência foi de 25%. Para a proteína líquida para ganho obteve-se: PL_g (g/dia) = 179,74 × GPCVZ - 5,43 × ER. A exigência de mantença líquida do Ca foi de 2,33 mg/PCVZ e de P foi de 9,10 mg/PCVZ. O coeficiente de absorção de Ca foi de 54% e do P de 64%. Em conclusão, a exigência de energia líquida para mantença de bovinos Nelore confinados é 66,5 kcal/$PCVZ^{0,75}$/dia. Exigências de energia líquida para ganho e de proteína líquida para ganho podem ser obtidas através das seguintes equações: EL_g (Mcal/dia) = 0,0388 × $PCVZ^{0,75}$ × $GPCVZ^{1.095}$ e PL_g (g/dia) = 179,74 × GPCVZ - 5,43 × ER. Exigência de mantença líquida do Ca é 2,33 mg/PCVZ e para o P é 9,10 mg/PCVZ. O coeficiente de absorção do Ca é de 54% e do P é de 64%. O consumo de água não é influenciado pela suplementação de Ca, P, Zn, Mn e Cu. Animais que possuem alto consumo alimentar residual e ganho residual conjunto tem menor CMS que animais que apresentam baixo GCAR, e apresentando o mesmo ganho de peso. A PB, EE e água presente no PCVZ aumenta à medida que o animal cresce, o crescimento de cinzas não segue o mesmo padrão que o PCVZ. No geral, o consumo e o desempenho de animais Nelore em terminação confinados não foram afetados pela redução de minerais (Ca, P, Zn, Mn e Cu) na dieta. Concluiu-se que a redução dos teores de minerais em

dietas de confinamento para Nelore em terminação não influencia no consumo, desempenho, composição química corporal e exigências nutricionais de bovinos Nelore terminados em confinamento.

GENERAL INTRODUCTION

Non-renewable resource and food production are popular and polemic topics in the current sustainability discussion (Odegard and Van der Voet, 2013). It has been estimated that the world population will exceed 9 billion people by 2050 (FAO, 2011). Sustainable production of adequate quantities of food to support a growing population in the world is an international challenge. This will increase the demand for food, majority for animal products, which will be challenging to meet without intensification because very little room for land expansion exists (Kebreab, 2016).

It is expected that the largest growth will be in developing countries and will overtake developed countries in their intake of livestock products (FAO, 2011). Due to this increasing global demand for livestock products, there are concerns over sustainable animal agriculture practices and particularly environmental impacts of livestock production (Kebreab, 2013). So, the challenges of meat supply chain are sustainable production and reduce costs, thereby increasing profitability. These challenges can be overcome by rational feed management, feeding animals with just required amounts, according to their categories and gain.

Brazil has the biggest commercial cattle herd of the world in continuous growth; however Brazilian productivity rates is low. Subsequently, Brazil may be the country available to improve the food production. In order to improve the efficiency of meat production, some strategies need to be adopted. This production efficiency is critical and must be accompanied by sustainability. It is eminent concern of the world to develop production systems that preserve the environment and concern about the use of non-renewable resource.

Beef cattle production that purposes at sustainability requires an understanding of multiple factors. In any production system, feeding cost is the highest cost component and is one of the main factors that affect animal performance. Thus, knowledge and adoption of

rational measures in the food management of beef cattle have the ability to generate a large economic impact and quality of meat production system.

Animal growth depends on diets being formulated with adequate amounts and proportions of energy and essential nutrients (BCNR, 2016). Additional improvements may be achievable determining the nutrient requirements and may be an important strategy to increase the economic and environmental sustainability of beef cattle system.

Minerals are essential nutrient in many biochemical processes, including skeletal and muscular development (Suttle, 2010). Minerals in ruminant nutrition and metabolism are not understood completely (BCNR, 2016). However, research examine mineral requirements to Zebu cattle are scarce (Valadares Filho et al., 2005). This way, mineral nutritional studies on Zebu cattle are necessary to avoid wastes and improve economics indexes.

Growing demand for mineral resources on agriculture and livestock has increased researches on the use of mineral. Minerals, particularly phosphorus, are expensive. Moreover, waste of these nutrients can cause environmental pollution, such as phosphorus buildup in soil and eutrophication.

Frequently, micro minerals such as Zn, Mn, and Cu are included in feedlot diets at concentrations in excess (Vasconcelos and Galyean, 2007). However, some experiments have shown no improvements resulting from the supplementation of micro minerals (Galyean et al., 1999) and phosphorus (Erickson et al., 2002) using Bos taurus cattle.

Phosphorus is an expensive supplement in the diets of feedlot cattle (Spears, 1996). Furthermore, existing rock phosphate reserves currently used for supplementation could be exhausted within 50 - 100 years (Herring and Fantel, 1993; Gunther, 2005). Erickson et al. (2002) suggested that the P requirements for finishing cattle was less than 1.6 g/kg of the diet DM, whereas nutritional models have suggested higher diet contents. In evaluating previous Ca and P requirements in feeding trials, Prados et al. (2015) concluded that NRC (2000) and

BR-CORTE (Valadares Filho et al. 2010) overestimated Ca and P requirements. Many of essential minerals are usually found in sufficient concentrations in feedstuffs (NRC, 2000) used in feedlots. Supplementing diets at concentrations in excess of requirements greatly increases mineral loss in cattle waste (NRC, 2000). Over supplementation of minerals should be avoided to prevent possible environmental problems associated with runoff from waste or application of cattle waste to soil (NRC, 2000).

Animals products are considered to be the highest consumers of water (Mekonnen and Hoekstra, 2012). Water is an essential nutrient for cattle. Water has important play in the animal body as: transport of nutrients, maintaining body temperature, digestion and metabolism (BCNR, 2016). The animal body is composed of two-thirds water. The water requirement of cattle could be met by: free water, water present in feedstuffs, and water formed in the body (metabolic water). And the water requirement is influenced by several factors: environmental conditions, feed intake, composition of gain, and physiological state of animals, as well as others factors. Water requirements can increase when a diet is high in protein, salt, and minerals (NRC, 2000).

Evaluate the nutrient requirements of all categories of cattle and genetic is important due to the reduction in the cost of feed nutrients and waste and therefore environmental pollution. Nutrient requirements can be defined as the amount of nutrients necessary for the normal healthy and performance of cattle. For ruminants, the primary nutrients of interest are protein, energy (a property of nutrients but functionally treated like other nutrients in terms of requirements), vitamins, minerals and water (Galyean, 2014).

Establish a good nutrient requirements program can improve performance without nutrient excess or deficiencies and this may improve the cattle efficiency. So, improve the nutritional requirements of the national herd means offering to Brazilian producer's technology generated under Brazilian conditions.

The first step to determining the nutritional requirements for the growth of cattle is to measure their body composition (BR-CORTE, 2010), and this can be done using direct or indirect methods. A direct method requires the whole dissection of the carcass and body, which is expensive and arduous. Indirect methods involve easily obtained parameters and cheaper methods. Methods that can estimate body composition deprived of sacrificing the whole carcass or animal body are important because they save time, labor, and costs. The most common and classic indirect method is the rib section proposed by Hankins and Howe (1946), using equations to estimate the chemical and physical composition of the carcass from the composition of the 9^{th} to 11^{th} rib section. Nevertheless, the evaluation of rib section composition demands dissection and laboratory analyses, which might limit its large-scale use. Therefore, a faster method would be helpful.

Dual energy x-ray absorptiometry (DXA) is a validated method used to assess body composition. This method is faster and cheaper (there is just the initial cost of the equipment) than direct evaluation (Mercier et al., 2005). Its application is very common for predicting the mineral composition of bone as well as fat and muscle mass in humans, but scarce evaluations have been conducted in beef cattle (Mitchell et al., 1997 and Ribeiro et al., 2011).

On other hand, feed efficiency has a major influence on the unit cost of production (Basarab et al., 2003). Residual feed intake (RFI) is defined as the difference between an animal actual feed intake and its expected (estimated by an equation). It is expected differences in efficiencies of growth due to differences in composition of live weight gain (Ferrell and Jenkins, 1998).

Thus, there is a constant need for updating the nutrient requirements aiming a reducing of nutrient excretion and decreasing the production cost.

The hypothesis of these studies is that supplementation of Ca, P, Cu, Mn, and Zn is not necessary in conventional feedlot diets for finishing Nellore bulls. Thus, the objective of these

studies were to evaluate the effects of decreasing calcium, phosphorus and micro minerals contents on intake, digestibility, performance, and carcass characteristics of feedlot Nellore bulls, to developed empirical equations to estimate the composition of rib sections for Nellore and Nellore × Angus cattle, and finally to evaluate their accuracy and precision, and to evaluate the nutritional requirements of energy, protein, and calcium and phosphorus for maintenance of Nellore bulls, as well as their efficiencies, the residual feed intake and to evaluate the water intake of animals fed with or without supplementation of Ca, P and micro minerals.

This thesis was written in scientific paper format. The papers were written according to the Journal of Animal Science.

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CHAPTER 1

Technical note: Prediction of chemical rib section composition by dual energy x-ray absorptiometry in Zebu beef cattle[1]

L. F. Prados,[*2] D. Zanetti,[*] P. M. Amaral,[*] L. D. S. Mariz,[*] D. F. T. Sathler,[*] S. C. Valadares Filho,[*] F. F. Silva,[*] B. C. Silva,[*] M. C. Pacheco,[*] H. A. Meneguelli,[*] M. L. Chizzotti [*]

* Universidade Federal de Viçosa, Viçosa-MG; Brazil. 36570-000

[1]This study was made possible by grants from CNPq, CAPES, FAPEMIG and INCT-CA.

[2]Corresponding author: Laura Prados; laurafrancoprados@hotmail.com

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Published at Journal of Animal Science

Submitted: December 28, 2015
Accepted: April 1, 2016
Published: June 3, 2016

J. Anim. Sci. 2016.94:2479–2484

doi:10.2527/jas2015-0257

ABSTRACT: It is expensive and laborious to evaluate carcass composition in beef cattle. The objective of this study was to evaluate a method to predict the 9^{th} to 11^{th} rib section (rib_{9-11}) composition through empirical equations using Dual Energy X-ray absorptiometry (DXA). DXA is a validated method used to describe tissue composition in humans and other animals, but few studies have evaluated this technique in beef cattle, and especially in the Zebu genotype. A total of 116 rib_{9-11} sections were used to evaluate published prediction equations for rib_{9-11} composition and to develop new regression models using a cross-validation procedure. For the proposed models, 93 ribs were randomly selected to calculate the new regression equations, and 23 different ribs were randomly selected to validate the regressions. The rib_{9-11} sections from left carcasses were taken from Nellore and Nellore × Angus bulls from three different studies and scanned using DXA equipment (GE Healthcare, Madison, Wisconsin, USA) in the Health Division at Universidade Federal de Viçosa. The outputs of the DXA report were DXA lean (g), DXA fat free mass (g), DXA fat mass (g), and DXA BMC (bone mineral content; g). After being scanned, the rib_{9-11} sections were dissected, grounded, and chemically analyzed for total EE, CP, water, and ash content. The predictions of rib fat and protein from previous published equations were different ($P < 0.01$) from the observed composition. New equations were established through leave-one-out cross-validation using REG procedure. The equations were as follows: Lean (g) = 37.082 + 0.907× DXA lean (R^2 = 0.95); Fat free mass (g) = 103.224 + 0.869 × DXA fat free mass (R^2 = 0.93); EE mass (g) = 122.404 + 1.119 × DXA fat mass (R^2 = 0.86); Ash mass (g) = 18.722 + 1.016 × DXA BMC (R^2 = 0.39). The equations were validated using Mayer's test, the concordance correlation coefficient (CCC), and the mean square error of prediction (MSEP) for decomposition. For both equations, Mayer's test indicated that if the intercept and the slope were equal to zero and one ($P > 0.05$), respectively, then the equation correctly estimated the rib composition. Comparing observed and predicted values using the new equations, Mayer's test was not

significant for lean mass (P = 0.26), fat free mass (P = 0.67), EE mass (P = 0.054), and ash mass (P = 0.14). We concluded that the rib_{9-11} composition of Nellore and Nellore × Angus bulls can be estimated from DXA using the proposed equations.

Key words: ash, dual energy X-ray, ether-extract-lipid, fat free, lean, rib section

INTRODUCTION

The first step to determining the nutritional requirements for the growth of cattle is to measure their body composition (BR-CORTE, 2010), and this can be done using direct or indirect methods. A direct method requires the whole dissection of the carcass and body, which is expensive and arduous. Indirect methods involve easily obtained parameters and cheaper methods. The most common indirect method is the rib section proposed by Hankins and Howe (1946), using equations to estimate the chemical and physical composition of the carcass from the composition of the 9^{th} to 11^{th} rib section. Nevertheless, the evaluation of rib section composition demands dissection and laboratory analyses, which might limit its large-scale use. Therefore, a faster method would be helpful.

Dual energy x-ray absorptiometry (DXA) is a validated method used to assess body composition. This method is faster and cheaper (there is just the initial cost of the equipment) than direct evaluation (Mercier et al., 2005). Its application is very common for predicting the mineral composition of bone as well as fat and muscle mass in humans, but scarce evaluations have been conducted in beef cattle (Mitchell et al., 1997 and Ribeiro et al., 2011).

Ribeiro et al. (2011) have proposed regression equations to predict the physical and chemical composition of the rib in Angus cattle using DXA, but their accuracy has to be evaluated for the Zebu genotype, since a variation in rib composition is expected.

This study first tested the Ribeiro et al. (2011) equations to predict the crude protein and fat in the rib section in percentage. Next, we developed empirical equations to estimate the

composition of rib sections for Nellore and Nellore × Angus cattle, and finally we evaluated their accuracy and precision.

MATERIALS AND METHODS

The trials were conducted at the Universidade Federal de Viçosa, Brazil. The institutional ethics committee approved all procedures involving animals (protocols #05/2013, #20/2013, and #17/2015).

Database

One hundred sixteen Nellore and Nellore × Angus cattle (initial BW of 251 ± 42 kg, and age of 9 ± 0.6 mo) from three different studies were used in this trial. Study 1 consisted of 36 Nellore bulls. Study 2 consisted of 20 Nellore and 20 Nellore × Angus bulls. Study 3 consisted of 40 Nellore bulls. Descriptive statistics of cattle performance and carcass characteristics are presented in Table 1.

Rib_{9-11} sampling, DXA scanning, and chemical analyses

Before slaughter, feed was restricted for 14 h. The slaughter process used a captive bolt stunning followed by exsanguination from the jugular vein, evisceration, and hide removal. After slaughter, the carcasses were divided into two halves and were chilled at 4°C for 24 h. Once chilled, the one half carcasses were cut and the sections were collected between the 9th and 11th ribs (rib_{9-11}) were collected according to Hankins and Howe (1946). The rib sections were identified, vacuum-packed in plastic bags, and conducted to the Health Division at the Universidade Federal de Viçosa. The rib_{9-11} sections were placed on the DXA equipment (GE Lunar Prodigy Advance® DXA System, GE Healthcare, Madison, Wisconsin, USA), and scanning was performed using small animal composition software at small mode to obtain the DXA lean, DXA fat free (DXA lean + DXA BMC), DXA fat, and DXA BMC masses. The rib sections were always positioned on the DXA table the same way (bone side down – horizontal position). After scanning, the rib_{9-11} sections were dissected, ground separately into meat and

bone tissues, sampled, lyophilized, ground again with liquid nitrogen in a rotary mill with a 1-mm screen, and stored at -15°C for subsequent chemical analysis.

The $rib_{9\text{-}11}$ samples were analyzed for moisture (method 934.01), ash (method 942.05), CP (method 954.01), and EE content (method 920.39) in accordance with the Association of Official Analytical Chemists (AOAC, 1990) to determine the observed chemical composition.

Ribeiro et al. (2011) equations

From the observed DXA values, the following equations, proposed by Ribeiro et al. (2011), were used: chemical EE (%) = 5.9267 + 0.8944 × DXA fat (%) and chemical protein (%) = -2.7676 + 0.2736 × DXA lean (%). The predictive equations were tested using the model evaluation system (MES, v. 3. 1. 15, http://nutritionmodels.com/mes.html), as proposed by Tedeschi (2006).

Cross-validation and prediction evaluation

Statistical procedures were performed using SAS® (SAS 9.4 Inst. Inc., Cary, NC) with the animal being the experimental unit. The training data set had 93 animals from three studies, and the validation data set had 23 animals (a random sampling of 20% from each study). In order to develop new prediction equations based on the input variables (DXA fat free mass, DXA EE mass, and DXA BMC), a leave-one-out cross-validation method was proposed. Without loss of generality for each variable, the original training data set with 93 individuals was divided into 93 new data sets (D_k) with 92 individuals, D_1, D_2,…, D_{93}. Each index k in this data set notation indicates that the k^{th} observation was removed. Linear regression models were fitted separately for each D_k, which represents the estimated intercept and slope from each fit. Thus, at the end of this process, empirical distributions and coefficients of determination containing 93 values were obtained for these parameters. The means of the parameter distributions were assumed as the coefficients of the prediction equations. The mentioned linear regression analyses were performed using PROC REG in SAS® (SAS 9.4 Inst. Inc., Cary, NC) software.

For comparisons between the DXA-predicted and observed chemical composition we used MES, as proposed by Tedeschi (2006). To verify the effectiveness of the generated models, the observed values were regressed in predicted values, and the hypothesis that intercept = 0 and slope = 1 was tested (Mayer et al., 1994). The accuracy between the DXA-predicted and observed values was evaluated on the basis of their adjusted coefficients of determination (r^2) and the mean square error of prediction (MSEP; Bibby and Toutenburg, 1977) for decomposition. The concordance correlation coefficient (CCC) was used to verify precision and accuracy, and CCC is a gold standard (Lin, 1989).

RESULTS AND DISCUSSION

Descriptive statistics of database

Summary descriptive statistics of training and validation data are presented in Table 2. The average weight of the rib sections was 3.46 kg. The average of observed EE mass was 965 g compared with 753 g of EE tissue in the DXA output. This shows an underestimation for EE content in the ribs by using the EE tissue mass estimated by DXA, probably because the lipid content of the muscles and bones were not detected by DXA. The average of observed fat free mass was 2495 g compared with 2753 g in the DXA output. The average of observed lean mass was 2355 g compared with 2556 g in the DXA output. The average of observed ash was 219 g compared with 197 g of bone mineral content in the DXA output.

Figure 1 (A, B, C and D) shows the relation between the rib sections of cattle measured by DXA outputs and those determined by a direct chemical composition method. Fat mass was underestimated and fat free was overestimated by DXA. Thus, new prediction equations were proposed.

Ribeiro equations

Ribeiro et al. (2011) propounded two regression equations to predict chemical fat and protein on rib sections. Therefore, we tested these predictions for the data set (116 animals).

The predictions of EE and protein in percentage were not predicted correctly, according to Mayer's test (Table 3). There was a significant result ($P < 0.01$) for fat and protein ($P < 0.01$). The results showed that Ribeiro's equations were not adequate to measure fat and protein in ribs from Nellore and Nellore × Angus bulls. The averages of the observed values were 26.7% and 21.5%, and the averages of the predicted values were 24.5% and 17.7% for EE and protein, respectively. Probably these differences are due to differences between the genotypes used in the two studies. Protein and fat content were underestimated. Therefore, new equations were developed for the Zebu genotype.

Equations based on DXA

Chemical composition was considered to be the standard measurement of rib composition. The relationship between the chemical composition of the whole $rib_{9\text{-}11}$ section and the DXA lean, DXA fat free, DXA EE, and DXA BMC masses was analyzed using cross-validation method. The best models were evaluated (Table 4). A comparison of the observed and predicted chemical composition from the DXA equations for lean, EE, fat free, and ash content is presented in Table 5.

The average of observed EE was 938 g compared with prediction of 964 g. A plot of the relationship between the scaled weight of the components and the DXA equation is shown in Figure 1. Mitchell et al. (1997) observed significantly more fat in DXA than was measured by dissection. The average of observed fat free was 2480 g compared with the predicted 2453 g. The average of observed lean content was 2404 g compared with the predicted 2355 g. The average of observed ash content was 210 g compared with the predicted 221 g. The highest predictive accuracy was shown for fat free composition. Figure 1 presents the relation between rib section composition predicted from DXA equations and that determined by chemical composition. The data dispersion around the identity line was homogenous and shows the good accuracy of the equations to estimate EE, fat free, lean and ash contents.

Table 5 depicts the results from statistical evaluations of the proposed models. The DXA equations predicted the chemical composition of the rib_{9-11} sections with good accuracy and precision. The lean, fat free and EE variables had a very high correlation (CCC = 0.975, CCC = 0.968 for fat free and CCC = 0.945 for EE), and the ash variable had a high correlation (CCC = 0.784), according to Hinkle et al. (2003), suggesting satisfactory precision and accuracy.

The DXA equations are an accurate, easy, and fast tool for assessing the chemical composition of rib_{9-11} sections. The equations developed are recommended to be used for Nellore and Nellore × Angus cattle. The DXA measurements (GE Lunar Prodigy Advance® Dxa System, GE Healthcare, Madison, Wisconsin, USA), and scanning performed using small animal composition software were correlated with the composition of the beef carcass sections, but future work should evaluate its accuracy when estimating the composition of the whole carcass.

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Table 1. Descriptive statistics of cattle performance used in this study.

	Training data set				Validation data set			
Variable	**Mean**	**SD[1]**	**Minimum**	**Maximum**	**Mean**	**SD[1]**	**Minimum**	**Maximum**
Initial BW, kg	250.55	41.15	167.00	390.00	255.52	49.13	188.00	321.00
Final BW, kg	397.85	84.13	150.00	551.50	393.67	93.58	202.00	543.00
ADG, kg	0.94	0.38	-0.14	1.43	1.00	0.35	0.12	1.36
12th rib fat, cm	4.26	2.38	0.38	12.96	3.87	1.68	0.84	7.38
Dressing, %	58.9	2.20	52.6	63.6	58.7	1.68	54.7	62.2

[1] SD = standard deviation

Table 2. Means, standard deviation, and maximum as well minimum values for rib section and chemical composition (g), which were estimated through dual energy X-ray absorptiometry (DXA).

	Direct observations				DXA observations			
Variable	**Mean**	**SD[1]**	**Minimum**	**Maximum**	**Mean**	**SD[1]**	**Minimum**	**Maximum**
Training data set (93 observations)								
Section, kg	3.46	0.95	0.90	5.58	3.50	0.96	0.91	5.72
EE[2], g	965.87	418.62	142.15	2284.24	753.49	346.27	82.00	2011.00
Fat free[3], g	2495.09	597.84	724.25	3761.17	2753.79	664.58	831.10	4068.00
Lean, g	2355.70	588.98	611.18	3545.12	2556.57	633.22	733.00	3760.00
Ash, g	219.37	70.75	113.07	577.11	197.48	43.21	98.10	307.70
Validation data set (23 observations)								
Section, kg	3.42	1.02	1.45	5.36	3.45	1.01	1.52	5.47
EE[2], g	938.36	456.19	180.04	2044.01	752.57	337.41	162.00	1532.00
Fat free[3], g	2479.86	677.35	1269.96	3951.00	2704.46	737.94	1358.00	4144.00
Lean, g	2404.39	675.89	1171.87	3660.00	2556.00	724.58	1236.00	3869.00
Ash, g	210.24	81.38	98.09	453.33	199.33	53.77	122.60	353.00

[1] SD = standard deviation
[2] EE = ether extract
[3] Fat free = Lean + BMC

Table 3. Adequacy of predictions of chemical ether extract (EE, %) and protein (%) using Ribeiro et al. (2011) equations.

						MSEP decomposition		
Variable	**R^2**	**Mayer`s test**	**CCC**	**Cb**	**RMSEP**	**MB,%**	**SB,%**	**RE,%**
Ether extract	0.59	<0.01	0.68	0.88	4.68	23.85	0.09	76.06
Protein	0.23	<0.01	0.17	0.35	5.28	53.16	1.02	45.82

Mayer`s test = H0: a = 0 and b = 1; CCC = concordance correlation coefficient, varies from 0 to 1; Cb = bias correction, varies from 0 to 1, one indicates no deviation from Y = X; RMSEP = root mean square error; MSEP = mean square error of prediction; MB = mean bias, % of MSEP; SB = systematic bias, %MSEP; RE = random errors, % of MSEP.

Table 4. Models for the prediction of chemical composition (g) for rib section using dual energy X-ray absorptiometry (DXA).

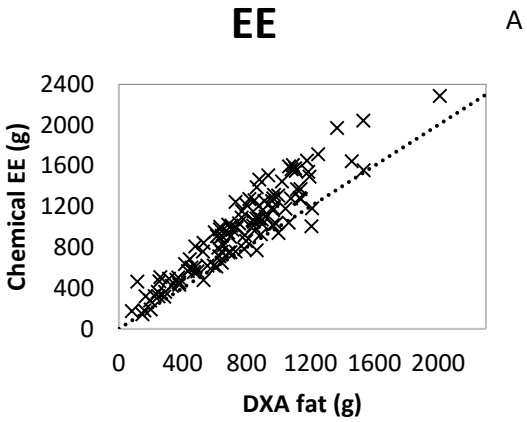
	Estimated[1]		
Variable	**Intercept**	**Slope**	**R^2**
Ether extract	122.404±3.593	1.119±0.005	0.857±0.0017
Fat free	103.224±6.154	0.869±0.002	0.933±0.0007
Lean	37.082±4.523	0.907±0.001	0.950±0.0005
Ash	18.722±2.274	1.016±0.011	0.385±0.0149

[1] Value ± standard error

Table 5. Adequacy of predictions of chemical ether extract (g), fat free (g), and ash (g) using dual energy X-ray absorptiometry (DXA).

						MSEP decomposition		
Variable	**R^2**	**Mayer`s test**	**CCC**	**Cb**	**RMSEP**	**MB,%**	**SB,%**	**RE,%**
Ether extract	0.930	0.054	0.945	0.980	135.20	3.75	20.41	75.84
Fat free	0.942	0.679	0.968	0.997	162.29	2.66	0.95	96.39
Lean	0.956	0.261	0.975	0.996	145.87	11.41	0.07	88.52
Ash	0.736	0.140	0.784	0.914	44.85	6.03	11.00	82.97

Mayer`s test = H0: a = 0 and b = 1; CCC = concordance correlation coefficient, varies from 0 to 1; Cb = bias correction, varies from 0 to 1. One indicates no deviation from Y = X; RMSEP = root mean square error; MSEP = mean square error of prediction; MB = mean bias, % of MSEP; SB = systematic bias, %MSEP; RE = random errors, % of MSEP.


EE
A
Chemical EE (g)
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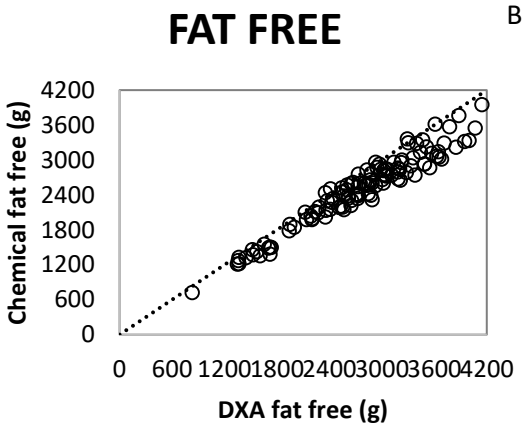
DXA fat (g)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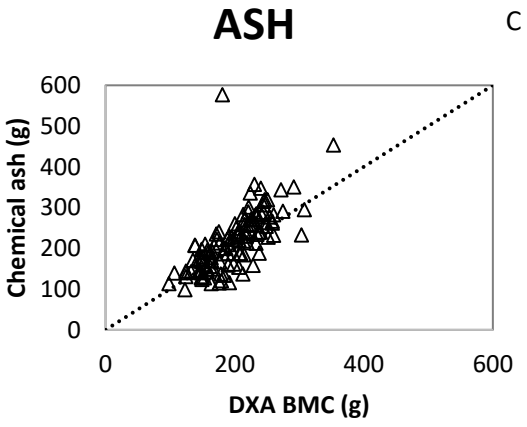
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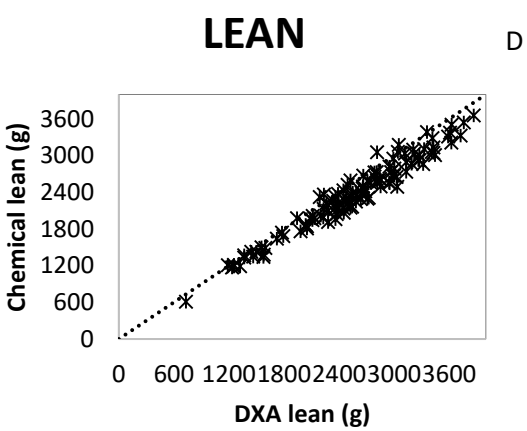
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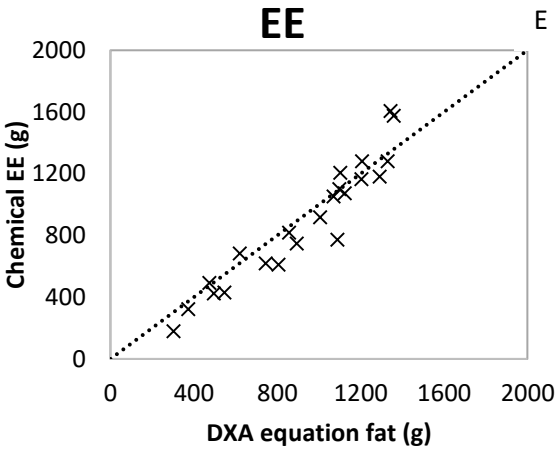
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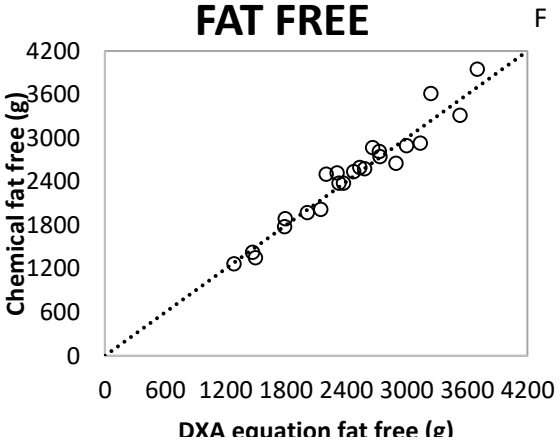
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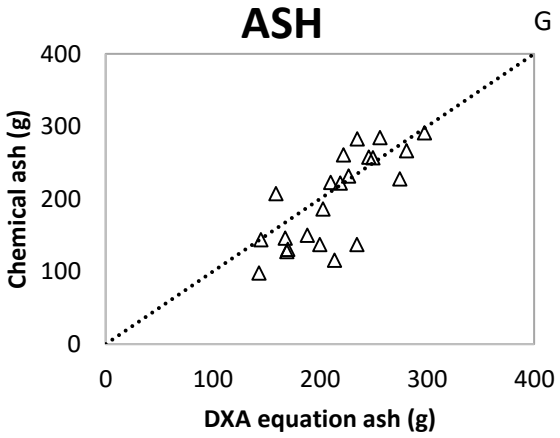
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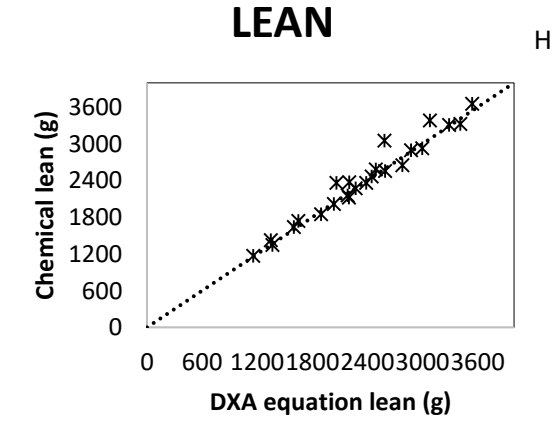
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Figure 1. Relation between rib section of cattle measured by DXA outputs and that determined by chemical composition (A, B, C and D). Relation between rib section of cattle measured by DXA equations and that determined by chemical composition (E, F, G and H). **EE = ether extract; Fat free = Lean + BMC**. Model predicted values are plotted in the X-axis; observed values are plotted in Y-axis. The dotted line represents the ideal line (Y = X); intercept = 0 and slope = 1.

CHAPTER 2

Reducing minerals usage in feedlot diets for Nellore cattle: impacts of calcium, phosphorus, zinc, manganese, and copper content on intake, performance, and liver and bone status

ABSTRACT: Weaned Nellore bulls (n = 36; 274±34 kg) were used in a randomized block 2 × 2 factorial design experiment to evaluate intake, fecal excretion and performance with different levels of minerals. Experimental diets were formulated with two levels of Ca and P (macro mineral factor; CaP+ or CaP-) and two levels of micro minerals (micro mineral factor; CuMnZn+ or CuMnZn-). The factor CaP- was formulated without addition of limestone and dicalcium phosphate, and the factor CuMnZn- was formulated without inorganic supplementation of micro minerals (premix). The diets were isonitrogenous (13.3% CP). Intake was individually monitored every day. Indigestible NDF was used as an internal marker for digestibility estimates. The bulls were slaughtered (84 or 147 days on feed), then carcass characteristics were measured and liver and rib samples were collected. Feed, feces, rib bones and liver samples were analyzed for DM, ash, CP, EE, Ca, P, and micro minerals (Zn, Mn, and Cu). There were no significant interactions ($P \geq 0.06$) between macro and micro minerals supplementation for any variables in the study. Calcium, P and micro minerals concentrations did not affect ($P \geq 0.20$) intake of DM, OM, NDF, EE, CP, TDN and NFC. Calcium and P intake were affected ($P < 0.01$) by macro mineral factor. Animals fed without Ca and P supplementation have consumed lower levels of these minerals. Dry matter and nutrient fecal excretion (OM, NDF, EE, CP, and NFC) were similar ($P \geq 0.23$) among factors. Performance and carcass characteristics were similar ($P \geq 0.09$) among diets. The content of ash in rib bones was not affected ($P \geq 0.06$) by diets. Phosphorus and phosphatase alkaline plasma

concentration were similar ($P \geq 0.52$) among diets. Calcium plasma concentration was affected ($P < 0.01$) by micro mineral factor; nevertheless, all analyzed blood metabolites were within the reference values. Fecal excretion of Ca and P was different ($P < 0.01$) among macro mineral factor. These results indicate that supplementation of minerals (Ca, P, Zn, Mn, and Cu) is not necessary in conventional feedlot diets for Nellore bulls. Dietary reductions in these minerals would represent a decrease in the costs of feedlot diets. Dietary reduction in Ca and P content cause decrease in fecal excretion of these minerals. Decreasing the P fecal excretion through decreasing dietary P is an opportunity to reduce environmental impact of feedlot operations.

Key words: bone mobilization, calcium, mineral supplementation, Nellore, phosphorus

INTRODUCTION

Minerals are essential nutrients for many biochemical processes, including skeletal and muscular development (Suttle, 2010). Minerals in ruminant nutrition and metabolism are not understood completely (BCNRM, 2016). Mineral requirement researches are poorly defined (Spears and Weiss, 2014), mainly in Zebu cattle (Valadares Filho et al., 2005). So, mineral nutritional studies in Zebu cattle are necessary. Besides that, growing demands for mineral resources on agriculture and livestock production has increased research on the efficient use of minerals in cattle to avoid wastes and improve economic indexes.

Frequently, micro minerals such as Cu, Mn, and Zn are included in feedlot diets at concentrations in excess (Vasconcelos and Galyean, 2007). However, some trials have shown no improvements resulting from the supplementation of micro minerals (Galyean et al., 1999) and phosphorus (Erickson et al., 2002) using Bos taurus cattle.

Phosphorus is an expensive supplement in cattle feedlot diets (Spears, 1996). Moreover, waste of this nutrient can cause environmental pollution, such as phosphorus buildup in soil and water eutrophication. Furthermore, existing rock phosphate reserves currently used for

supplementation could be exhausted within 50–100 years (Herring and Fantel, 1993; Gunther, 2005). Erickson et al. (2002) suggested that P requirements for finishing Bos taurus cattle was less than 1.6 g/kg of the diet DM, whereas nutritional models have suggested higher diet contents. Prados et al. (2015) suggested that Ca and P requirements for finishing crossbred cattle was less than 1.8 g/kg and 2.4 g/kg of the diet DM, respectively.

We hypothesized that the decreased of Ca, P, Cu, Mn, and Zn in diets could not decrease the performance of finishing Nellore bulls in feedlot. Therefore, the objective of this study was to evaluate the effects of reducing Ca, P, Cu, Mn, and Zn content on intake, performance, blood parameters, and liver and bone status of feedlot Nellore bulls.

MATERIALS AND METHODS

The feeding and performance trial was conducted at the Animal Science Department of the Universidade Federal de Viçosa, Brazil. The institutional animal care and use committee approved all procedures involving animals (protocol number 20/2013).

Animals, diets and experimental design

Thirty-six weaned Nellore bulls (initial BW of 274±34 kg, and age of 9±0.6 mo) were used in this trial. Cattle were weighed and treated for the control of internal and external parasites by administration of ivermectin (Ivomec, Merial, Paulinea, BRA) prior to entering the feedlot. Animals were adapted during 21 d prior the experiment period.

Four animals were slaughtered on d 0, as a reference group to quantify the daily carcass gain (**DCG**) and empty body weight gain (**EBG**). Bulls were blocked by feedlot periods (84 or 147 d) and they were used in a randomized complete block 2 × 2 factorial design experiment. The factors have consisted of two levels of Ca and P (macro mineral factor; **CaP+** or **CaP-**) and two levels of micro minerals (micro mineral factor; **CuMnZn+** or **CuMnZn-**). All treatments encompassed an ad libitum conventional diet that contained 40% of sugar cane and 60% of concentrate (DM basis), and components are described on Table 1. The factors were: Supplying 100% of Ca and P requirements (CaP+); without using Ca and P supplements (CaP-

84); supplying micro minerals (CuMnZn+); without supplementation of micro minerals
85 (CuMnZn-) via premix.

86 The CaP+ contained 96.3 and 97.9% and CaP- contained 47.8 and 66.8% of Ca and P
87 required (BR-CORTE, 2010), respectively. Decreased Ca and P was achieved by not
88 supplementing diet with limestone and dicalcium phosphate (Table 1).

89 The diets were isonitrogenous (13.3% CP), formulated to ensure meeting BR-CORTE
90 (Valadares Filho et al., 2010; http://www.brcorte.com.br) nutrient requirements except for Ca,
91 P, Cu, Mn, and Zn, for a target ADG of 1.25 kg/d (considering an average BW of 350 kg).

92 Total mixed rations was delivered to pens twice daily at 0700 and 1500 h. Animals
93 received ad libitum access to water. Bulls were fitted with unique electronic identification tags
94 and daily individual animal intake was measured using an electronic system equipment for
95 monitoring individual feed intake (INTERGADO®, Contagem, MG, BRA) using electronic
96 tags with radio frequency identification (Chizzotti et al., 2015), in each pen.

97 Samples of concentrate ingredients were collected directly at the feed mill. Sugar cane
98 samples were collected every day, oven-dried (60˚C) and ground using a Willey mill (TE-650,
99 Tecnal, Piracicaba, SP, Brazil) to pass through 1 and 2-mm (for indigestible neutral detergent
100 fiber (**iNDF**) analyses) screen, and pooled based on DM basis for laboratory chemical analysis.

101 After a fasting period (14 h without feed), the cattle were weighed to measure initial
102 and final BW.

103 **Fecal and urine collection**

104 Spot fecal samples were collected on d 75-77 and 129-131. Fecal samples were
105 collected from the cattle at 0600, 1200 and 1800 h each day of the collection period. Samples
106 (feed and fecal samples of the collection period) were ground using a Willey mill (TE-650,
107 Tecnal, Piracicaba, SP, BRA) to pass through a 2-mm screen sieve for indigestible neutral
108 detergent fiber analyses. Fecal samples were pooled on a DM basis by period for each bull.

For analyses of iNDF, samples were ruminally incubated in F57 filter bags (ANKOM, Macedon, NY, USA) at two cannulated Nellore bulls for 288 h (Valente et al., 2011). This time interval is required to account for iNDF in tropical C4 forages such as sugarcane. When all bags were removed from each rumen, they were soaked in water for 30 min and washed by hand under running water until the wash water ran clear. Contents of iNDF were then evaluated using an ANKOM^{200} fiber analyzer. The iNDF was obtained by weighing the filter bags after drying them in an oven, first at 60˚C for 72 h followed by 105˚C for 12 h. The residue was considered the iNDF. Fecal iNDF concentration was determined and was then used to calculate the estimated fecal output per day. Indigestible neutral detergent fiber was used as an internal marker to estimate the fecal excretion.

Spot urine sample was collected on d 76 and 130 at 1200 h. After collection, the urine was homogenized, a sample of 50 mL were taken and stored at -20˚C until further laboratory analyses were performed. Urine was analyzed for inorganic Ca and P by an automated biochemistry analyzer (Autoanalyser, Mindray, model BS-200E Chemistry Analyzer).

Blood sampling

Jugular blood samples were taken on d 0, 54 and before slaughter (before morning feed delivery) into evacuated tubes (LABOR IMPORT, Osasco, SP, BRA) and immediately cooled in ice. Blood samples were transported to the laboratory on ice, and centrifuged to separate plasma (1200 × g for 10 min at 4˚C). Once separated, plasma was removed by pipetting and immediately frozen at −40°C until analysis could be completed. Plasma was analyzed for inorganic Ca and P and total alkaline phosphatase using an automated biochemistry analyzer (Autoanalyser, Mindray, model BS-200E Chemistry Analyzer).

Slaughter, rib bones and liver sampling and processing

At the end of the experiment period (d 84 or 147), bulls were weighted and harvested. The slaughter process used a captive bolt stunning followed by exsanguination from jugular vein, evisceration, and hide removal. The gastrointestinal tract was washed. The heart, lungs,

liver, spleen, kidneys, internal fat, diaphragm, mesentery, tail, trachea, esophagus, reproductive system, gastrointestinal tract, head, hide, hoofs, blood and carcass were weighed to quantify empty body weight (**EBW**). Dressing percentage was estimated from hot carcass weight (**HCW**) and BW.

Liver samples were collected after slaughter for determination of micro minerals status. The liver usually is the main storage site for micro mineral reserves (Vitti and Kebreab, 2010).

After slaughter, carcasses were divided into 2 halves and were chilled at 4°C for 24 h. Once chilled, the left half carcass was cut to measure LM area and 12th rib fat thickness. Rib bones were collected between 9th to 11th rib. This study evaluated rib bones because bone stores body Ca and P and bone mineral content is a critical assessment of P status of animals (Crenshaw et al., 1981). Rib bones and liver samples were ground separately, lyophilized, ground again with dry ice through a 1-mm screen and stored at -15°C for subsequent chemical analysis.

Chemical analysis

Before use during the collection period, all plastic containers were acid washed in 10% HCl (Pogge et al., 2014). For chemical analysis, sample of diet ingredients and fecal samples were oven-dried (60˚C) and ground through a 1-mm screen sieve (method 950.02; AOAC, 1990).

Feed, feces, rib bones, and liver samples were analyzed for DM (method 934.01), organic matter (method 942.05), CP (method 954.01), EE (method 920.39), and mineral solution (method 968.08) in accordance with Association of Official Analytical Chemists (AOAC, 1990). Minerals were analyzed for Ca, P, Na, K, Mg, Zn, Mn, and Cu using inductively coupled plasma atomic and optical emission spectroscopy.

Neutral detergent fiber was determined using thermostable α-amylase using Ankom^{200} Fiber Analyzer (ANKOM, Fairport, NY). Neutral detergent fiber was expressed in function of residual ash and protein.

Calculations

The non-fiber carbohydrates (NFC) were calculated as proposed by Detmann and Valadares Filho (2010).

The average daily gain was calculated as the difference between final BW and initial BW divided by the number of days on feed (84 or 147 d). Dry matter intake was calculated as the average of the DM consumed for all days on feed.

Statistical analysis

The experimental design was a completely randomized block in a 2 × 2 factorial arrangements (CaP+ or CaP-, CuMnZn+ or CuMnZn-). Main effects of macro and micro minerals were tested as well as their interactions. Data were analyzed as a mixed model with the fixed effects of macro and micro minerals and their interactions and the random effect of days on fed (block). Statistical analysis was performed using the MIXED procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC) with bull being the experimental unit. There were eight replications per treatment. Significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

There were no significant effect of CaP × CuMnZn interaction ($P \geq 0.06$) for any of the variables measured. Thus, only the main effects of concentration of CaP and CuMnZn were presented and discussed.

Nutrient intake and fecal excretion

The concentration of Ca, P, and micro minerals did not affect ($P \geq 0.20$) DM, OM, NDF, EE, CP, TDN and NFC intake (Table 2). Dry matter intake as a percentage of BW was similar ($P \geq 0.16$) between treatments. Erickson et al. (1999) obtained similar results to this study when five P levels (1.4, 1.9, 2.4, 2.9 and 3.4 g/kg of DM) and two Ca levels (3.5 and 7.0 g/kg of DM) were fed to finish Bos taurus cattle and did not observe any effects ($P > 0.05$) on DMI in cattle.

As these minerals are related with regulation of appetite, they did not influence DMI (Table 2) suggesting no deficiency in the finishing diet. In spite of phosphorus and Zn are involved in the control of appetite (O`Dell and Reeves, 1989; Suttle, 2010).

Fecal excretions were similar ($P \geq 0.23$) among treatments (Table 2). Intake of TDN was similar ($P = 0.28$) by CaP level (Table 2). Suggesting that Ca and P did not influence on TDN intake. Micro minerals have critical roles in the key interrelated systems of immune function, oxidative metabolism, and energy metabolism in ruminants (Overton and Yasui, 2014). In this experiment, reduction in micro minerals did not influence intake and excretion, suggesting that Ca, P, Cu, Zn, and Mn requirements may be overestimated for finishing Nellore cattle in feedlot.

Mineral intake

The concentration of Ca and P in CaP- diet was lower than CaP+ (Table 1). Because of dietary inclusion of limestone and dicalcium phosphate, Ca and P intake (g/d and mg/kg BW) was decreased ($P < 0.01$) for cattle fed CaP- (Table 3). These differences were due to differences in Ca and P concentrations in diet and not due to changes in DMI. According to AFRC (1991), an excess of P intake does not increase absorption of this mineral. Consequently, all mineral fed above the requirement is excreted in feces and urine. Thus, the concentration of these minerals in finishing diets is above the requirements of Nellore bulls.

Magnesium intake was affected ($P = 0.01$) by CaP level. Bulls fed CaP+ had higher intake of Mg in g/d (Table 3). It is noteworthy that Mg intake in mg/kg of BW was similar among diets ($P \geq 0.30$). All mineral intakes (mg/kg of BW), except Ca and P, were similar ($P \geq 0.29$) among diets.

Excretion of calcium and phosphorus

Calcium and P fecal excretion were different ($P < 0.01$) among macro factor (Table 4). Bulls fed without Ca and P supplementation had lower ($P < 0.01$) excretion of these minerals in feces compared to cattle supplemented. The reduction of fecal excretion for Ca was 58%

and for P was 26% comparing factors with or without supplementation. According to Vitti and Kebreab (2010), feces are the principal path of P excretion in herbivores; in ruminants, urinary P output can be considered almost negligible, the surplus is excreted in feces. Fecal P increases as dietary P supplementation increases and can this fecal P loading to bodies of surface water, resulting in eutrophication.

This reduction in fecal excretion is most important for P, which has a potential for pollution of surface water when overfed. In recent years, there is a growing interest in improving the utilization of dietary P for animals due to excess P excretion, depletion of non-renewable inorganic phosphate reserves, and increased prices of inorganic phosphate feed supplements (Selle and Ravindran, 2007). The reduction of fecal excretion of Ca and P can show that these minerals are overfeeding in the finishing diets for finishing cattle.

Calcium and P urine excretion were similar ($P \geq 0.43$) among factors (Table 4). Nellore bulls fed CaP+ or CaP- had the same excretion of these minerals in urine. The factor CuMnZn did not influence ($P \geq 0.56$) on fecal and urinary excretion of Ca and P in feces and urine.

The Figure 1 indicates that total Ca and P losses were a function of Ca and P intake. As dietary Ca and P increased, Ca and P excretion also increased. Phosphorus losses from cattle are primarily in the feces (Table 4). Increasing dietary P levels above the animal requirement leads to greater concentration of total P excretion (Figure 1).

Performance and carcass characteristics

Final BW, EBW, ADG, and empty BW gain (EBG) were similar ($P \geq 0.09$) among main factors (Table 5). This show that CaP and CuMnZn levels do not influence on final cattle weight and gain. Call et al. (1978) studied beef calves over a two-year period, they did not find difference in the ADG of animals supplied with P levels below the levels recommended by the NRC. Erickson et al. (1999), who studied calves treated with varying dietary levels of Ca and P (3.5 and 7.0 g/kg of DM for Ca and 1.4, 1.9, 2.4, 2.9 and 3.4 g/kg of DM for P), observed a

lower daily gain in animals fed high levels of Ca (7.0 g/kg of DM). Thus, CaP and CuMnZn did not influence performance of Nellore bulls.

No differences were found ($P \geq 0.11$) in daily carcass gain (DCG) among diets. Erickson et al. (1999) reported no differences in performance and carcass characteristics when finishing cattle were fed diets containing 0.14 to 0.34% P. Prados et al. (2015) in an experiment with three levels of Ca and P (1.8, 3.0, and 4.2 g/kg for Ca; 2.2, 2.4, and 2.6 g/kg for P in DM basis) in diets found that Ca and P supplementation did not improve performance.

Phosphorus is involved in the efficiency of feed utilization (BCNRM, 2016). Feed efficiency was similar ($P = 0.58$) among CaP supplementation (Table 5). Nellore bulls fed without limestone and dicalcium phosphate had the same feed efficiency compared to animals fed CaP+. CuMnZn supplementation did not influence ($P = 0.09$) efficiency of animals (Table 5). Some research (Erickson et al., 1999; Geisert et al., 2010) did not find differences in the efficiency.

Dressing (HCW as a percentage of BW) did not differ ($P \geq 0.79$) across treatments (Table 5). Carcass characteristics (LM area and fat in 12th rib) were not influenced ($P \geq 0.31$) by treatments. Thus, Ca, P and micro minerals supplementation did not impact carcass characteristics of finishing Nellore bulls.

It is plausible suggest that there may be opportunities to reduce 48.5% of Ca and 31.1% of P beef cattle requirements recommended within BR-CORTE (2010) or 41 and 26% recommended by NRC (2000) for finishing cattle. Erickson et al. (2002) reported similar results in feedlot steers with 1.6 (non-supplemented), 2.2, 2.8, 3.4 or 4.0 g/kg of P in DM and suggested that the P requirements for finishing cattle was less than 1.6 g/kg of the diet DM. In this trial we recommend 1.57 g/kg of P in DM basis. Prados et al. (2015) suggested 1.8 and 2.2, respectively, for Ca and P in DM basis.

Liver, rib bones and blood

The liver and the bones have proved especially useful because they are storage organs for certain minerals (Underwood, 1999). The liver is the primary organ for the storage and metabolism of micro minerals within the body, and therefore, liver micro mineral concentrations give the best indication of the true micro mineral status of an animal (Suttle, 2010). Liver ash in percentage was not influenced ($P = 0.07$) by micro mineral factor (Table 6). Liver DM (kg) and liver ash (g) were not influenced ($P \geq 0.29$) among diets (Table 6).

The present study shown that Mn liver concentration was not affected ($P \geq 0.06$) among diets (Table 6). Liver manganese is frequently measured because the liver stores Mn (Vitti and Kebreab, 2010). The concentration of Mn was on average 6.64 mg/kg. According to Vitti and Kebreab (2010), below 4.2 mg/kg is considered deficient, suggesting the bulls in this trial were not deficient in Mn.

Zinc liver concentration was affected ($P = 0.01$) by CaP. Bulls fed CaP- had a higher Zn liver concentration compared to animal fed CaP+. Significant depression in zinc absorption has been demonstrated when the Ca content of the diet was raised (Heth and Hoekstra, 1965). Zinc is stored in lower amount in liver (Vitti and Kebreab, 2010), with reference values of 101 to 200 mg/kg. On average, the cattle had 125 mg/kg of Zn in liver, value within the reference.

Copper liver concentration was greater ($P < 0.01$) for CuMnZn+ diet compared to cattle fed CuMnZn- (Table 6). The liver is well developed as a storage organ, if the cattle were deficient in Cu, the first change will be decreased liver Cu (Vitti and Kebreab, 2010). The normal concentration of liver Cu is 100 to 400 mg/kg (Chapman Jr et al., 1963), and the bulls were within this range (average was 216 mg/kg). The literature for liver micro mineral concentrations are somewhat varied (Wilson et al., 2016).

According to Ellenberger et al. (1950), 98.5% of Ca and 87% of P are found in bones. Bones can supply short-term dietary deficiencies of Ca and P by the animal removing a portion of Ca and P to meet then requirements (Weiss, 2012). The content of ash in the rib bones was

not affected ($P \geq 0.06$) by levels of Ca, P, and micro minerals in the diet (Table 6). According to Ternouth (1990), calcium and phosphorus are more easily mobilized from vertebrae and rib bones than structured bones. The content of ash in the fat free rib bones was influenced ($P = 0.01$) by CaP (Table 6). Bulls fed CaP- had lower concentration of fat free ash in rib bone compared to bulls fed CaP+.

Subnormal concentrations of Ca and P in the bones suggest deficiencies of Ca, P, or vitamin D (Underwood, 1999). Bone characteristics, whether expressed as ash (%) or Ca or P concentration, were unaffected ($P \geq 0.06$) by macro and micro mineral factors (Table 6). The average of Ca in the rib bones was 13.4% and average of P was 5.7%. In this study, there is no indication these minerals were mobilized from rib bone. Erickson et al. (1999) conducted a trial with two levels of Ca (3.5 and 7.0 g/kg of DM) and five levels of P (1.4, 1.9, 2.4, 2.9, and 3.4 g/kg of DM). The authors concluded that the levels did not influence on bone ash. A reduction in the P content of rib bones has been observed with cows offered low P diets (Wu et al., 2001; Ferris et al., 2010), when the concentration in diet is lower than the requirements. Mobilization of Ca and P become weak and fragile bones, constraining intake and performance of cattle. In this study, bone Ca and P contents were not different among treatments, so the animals did not pull minerals from bone.

Clinical signs of P deficiencies are depressed ADG, DMI and reproductive failures, but these manifest over time (Underwood, 1981). According to Nicodemo et al. (2000), identification of deficiencies requires the use as well as of biochemistry analyzes (blood metabolites). Calcium plasma was influenced ($P < 0.01$) by CuMnZn. Bulls fed CaP- had higher Ca concentration compared to cattle fed CaP+. We noted an antagonism of calcium and micro minerals. Perhaps, this may have relationship with zinc, due the antagonism observed between Ca and Zn. However, Ca concentration average was 9.55 mg/dL. This average is within reference values (Kaneko et al., 1997). Plasma P concentration across all diets was not

different ($P \geq 0.52$) and averaged 7.46 mg/dL. Plasma P concentration below 4.5 mg/dL is indicative of a P deficiency (Kaneko et al., 1997).

Total alkaline phosphatase is an enzyme associated with bone formation. Elevated levels of alkaline phosphatase are observed in situations where there are disturbances in mineralization, such as deficiency of P (Radostitis et al., 1994; Scott et al., 1997). Alkaline phosphatase was similar among factors ($P \geq 0.85$), averaging 316 IU/L. All blood metabolites analyzed in this study are within the reference values (Kaneko et al., 1997; Payne and Payne, 1987).

It is unlikely that only one criterion can be used for mineral status, the combination of multiple parameters, including biochemical, appears the most logical way to identify animals with subclinical deficiency of P (Nicodemo et al., 2000), Ca, and micro minerals.

Blood metabolites, liver and bone mineral contents are within reference values, suggesting that it is possible to reduce Ca, P and micro minerals (Zn, Mn, and Cu) for cattle without affecting intake and final BW, dressing, LM area, and fat. This suggest that the councils are overestimating the mineral requirements for finishing cattle in feedlot diets.

Mineral supplementation did not affect performance of bulls. So, reductions in concentration of Ca, P and micro minerals in finishing diets did not affect intake, performance, carcass characteristics, bone and liver status. Thus, it can infer that requirements of BR-CORTE (2010) and NRC (2000) are overestimated for Ca, P, Zn, Mn and Cu for finishing cattle in feedlot. The mineral concentration can be reduced to 50 and 32% of Ca and P respectively recommended by BR-CORTE (2010) or 41 and 26% recommended by NRC (2000). The results of this experiment indicate 1.84 and 1.57 g/kg of Ca and P on dry matter basis supply adequate levels of these minerals for growing and finishing Nellore cattle, so Ca, P and microminerals supplementations are unnecessary with a conventional finishing diet. The reduction of phosphorus in the cattle diets decreases P fecal excretion, and consequently the

excretion of P on environment. Feeding to minimum mineral requirements in feedlot could represent a significant decrease in the cost of production and benefit the environment due to lower mineral excretion.

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460 Table 1. Components and chemical composition of experimental diets (% DM).

Components[1]	CaP+		CaP-	
	CuMnZn+	CuMnZn-	CuMnZn+	CuMnZn-
Ingredients				
Sugar cane	40.0	40.0	40.0	40.0
Ground corn	30.3	30.3	30.3	30.3
Soybeans meal	7.00	7.00	7.00	7.00
Soybeans hulls	19.5	19.5	19.5	19.5
Salt	0.20	0.20	0.20	0.20
Urea	1.17	1.17	1.17	1.17
Ammonium sulfate	0.13	0.13	0.13	0.13
Limestone	0.31	0.31	-	-
Dicalcium phosphate	0.37	0.37	-	-
Bicarbonate/Mg oxide	1	1	1	1
Micromineral premix[2]	0.03	-	0.03	-
Sand	-	0.03	0.68	0.71
Chemical Composition				
DM	65.5	65.5	65.5	65.5
OM	94.9	94.9	94.9	94.9
CP	13.3	13.3	13.3	13.3
EE	2.3	2.3	2.3	2.3
NDF	31.5	31.5	31.5	31.5
NFC	50.0	50.0	50.0	50.0
Calcium[3]	0.395	0.395	0.182	0.182
Phosphorus[3]	0.222	0.222	0.155	0.155
Zinc, mg/kg	116.1	31.72	116.1	31.72
Manganese, mg/kg	104.8	49.05	104.8	49.05
Copper, mg/kg	36.22	7.25	36.22	7.25

461 [1] CaP+ = supplying 100% of Ca and P according to BR-CORTE (2010); CaP- = diet without limestone and
462 dicalcium phosphate; CuMnZn+ = diet with supplementation of trace minerals (Zn, Mn, and Cu);
463 CuMnZn- = diet without supplementation of trace minerals (Zn, Mn, and Cu).
464 [2]The premix micromineral was composed of 56.3% of zinc sulfate, 26.2% of manganese sulfate, 16.8% of
465 copper sulfate, 0.4% of potassium iodine, 0.2% of cobalt sulfate, and 0.1% of sodium selenite.
466 [3] Ration Ca:P = 1.78:1 (CaP+) e 1.17:1 (CaP-); CaP- corresponded to 50 and 68% of requirements,
467 respectively of Ca and P (BR-CORTE, 2010); and according NRC (2000) 59 and 74% of requirements,
468 respectively of Ca and P.

Table 2. Effect of calcium, phosphorus, and trace minerals (zinc, manganese, and copper) on intake and fecal excretion of Nellore cattle.

Item	CaP+		CaP-		SEM	P-value		
	CuMnZn+	CuMnZn-	CuMnZn+	CuMnZn-		CaP	CuMnZn	CaP×CuMnZn
DM								
Intake, % of BW	2.06	2.17	2.10	2.16	0.08	0.87	0.16	0.72
Intake, kg/d	6.90	7.30	6.76	6.90	0.33	0.20	0.21	0.53
Fecal excretion, kg/d	2.15	2.22	2.24	2.22	0.12	0.61	0.83	0.64
OM								
Intake, kg/d	6.55	6.93	6.42	6.55	0.31	0.20	0.21	0.54
Fecal excretion, kg/d	1.97	2.05	2.06	2.01	0.11	0.79	0.88	0.50
NDF								
Intake, kg/d	2.17	2.30	2.13	2.17	0.10	0.21	0.21	0.54
Fecal excretion, kg/d	1.10	1.14	1.09	1.08	0.04	0.36	0.75	0.52
EE								
Intake, kg/d	0.16	0.17	0.16	0.16	0.008	0.21	0.21	0.54
Fecal excretion, kg/d	0.06	0.07	0.07	0.06	0.006	0.76	0.38	0.11
CP								
Intake, kg/d	0.92	0.97	0.90	0.92	0.04	0.21	0.21	0.54
Fecal excretion, kg/d	0.34	0.34	0.33	0.32	0.02	0.43	0.82	0.89
NFC								
Intake, kg/d	3.45	3.65	3.38	3.45	0.17	0.20	0.21	0.54
Fecal excretion, kg/d	0.47	0.50	0.55	0.55	0.05	0.12	0.64	0.76
TDN, kg/d	4.39	4.82	4.45	4.40	0.20	0.28	0.26	0.15

[1] CaP+ = supplying 100% of Ca and P according to BR-CORTE (2010); CaP- = diet without limestone and dicalcium phosphate; CuMnZn+ = diet with supplementation of trace minerals (Zn, Mn, and Cu); CuMnZn- = diet without supplementation of trace minerals (Zn, Mn, and Cu).

Table 3. Effect of calcium, phosphorus, and trace minerals (zinc, manganese, and copper) on intake of macro minerals in Nellore cattle.

Item	CaP+		CaP-		SEM	P-value		
	CuMnZn+	CuMnZn-	CuMnZn+	CuMnZn-		CaP	CuMnZn	CaP×CuMnZn
Ca								
Intake, g/d	25.67	27.16	12.51	12.76	1.20	<0.01	0.18	0.34
Intake, mg/kg BW	75.60	77.41	38.36	39.52	2.12	<0.01	0.38	0.85
P								
Intake, g/d	15.90	16.83	10.61	10.83	0.75	<0.01	0.19	0.41
Intake, mg/kg BW	46.84	47.96	32.56	33.55	1.58	<0.01	0.35	0.95
Na								
Intake, g/d	26.77	28.33	26.16	26.70	1.28	0.18	0.21	0.54
Intake, mg/kg BW	78.86	80.74	80.24	82.68	3.50	0.46	0.34	0.90
K								
Intake, g/d	36.36	38.48	35.62	36.35	1.74	0.21	0.21	0.54
Intake, mg/kg BW	107.10	109.66	109.27	112.59	4.76	0.41	0.34	0.90
Mg								
Intake, g/d	20.98	22.20	19.67	20.07	1.00	0.01	0.21	0.52
Intake, mg/kg BW	61.78	63.26	60.34	62.17	2.65	0.46	0.34	0.91

[1] CaP+ = supplying 100% of Ca and P according to BR-CORTE (2010); CaP- = diet without limestone and dicalcium phosphate; CuMnZn+ = diet with supplementation of trace minerals (Zn, Mn, and Cu); CuMnZn- = diet without supplementation of trace minerals (Zn, Mn, and Cu).

Table 4. Effect of calcium, phosphorus, and trace minerals (zinc, manganese, and copper) on fecal and urine excretion of Ca and P (g/d) in Nellore cattle.

Item	CaP+		CaP-		SEM	P-value		
	CuMnZn+	CuMnZn-	CuMnZn+	CuMnZn-		CaP	CuMnZn	CaP×CuMnZn
Ca								
feces, g/d	15.37	13.95	5.57	6.72	0.67	<0.01	0.84	0.07
urine, g/d	1.75	2.12	1.53	1.67	0.50	0.39	0.52	0.77
P								
feces, g/d	7.59	8.23	5.80	6.01	0.47	<0.01	0.31	0.60
urine, g/d	2.50	2.89	2.17	2.04	0.65	0.28	0.81	0.64

[1] CaP+ = supplying 100% of Ca and P according to BR-CORTE (2010); CaP- = diet without limestone and dicalcium phosphate; CuMnZn+ = diet with supplementation of trace minerals (Zn, Mn, and Cu); CuMnZn- = diet without supplementation of trace minerals (Zn, Mn, and Cu).

Table 5. Effect of calcium, phosphorus, and trace minerals (zinc, manganese, and copper) on performance and carcass characteristics in Nellore cattle.

Item[1,2]	CaP+		CaP-		SEM	P-value		
	CuMnZn+	CuMnZn-	CuMnZn+	CuMnZn-		CaP	CuMnZn	CaP×CuMnZn
Final BW, kg	410.00	423.63	394.19	390.19	42.94	0.09	0.73	0.53
Final EBW, kg	373.80	388.05	361.39	360.04	42.86	0.13	0.63	0.56
ADG, kg/d	1.14	1.21	1.11	1.11	0.05	0.13	0.37	0.42
EBG, kg/d	1.11	1.21	1.07	1.10	0.04	0.10	0.18	0.42
DCG, kg	0.66	0.72	0.63	0.64	0.04	0.11	0.33	0.52
Dressing, %	57.95	57.89	57.73	58.07	1.30	0.97	0.79	0.70
LM area, cm^2	61.67	57.19	63.92	58.85	9.38	0.68	0.31	0.94
Fat, mm	3.38	3.16	3.39	3.46	0.72	0.68	0.85	0.71
Efficiency, kg/kg	0.165	0.154	0.163	0.161	0.007	0.58	0.09	0.25

[1] CaP+ = supplying 100% of Ca and P according to BR-CORTE (2010); CaP- = diet without limestone and dicalcium phosphate; CuMnZn+ = diet with supplementation of trace minerals (Zn, Mn, and Cu); CuMnZn- = diet without supplementation of trace minerals (Zn, Mn, and Cu).
[2] EBG = empty body weight gain; Dressing = hot carcass weight as a percentage of body weight; Fat = fat in 12th.

Table 6. Effect of calcium, phosphorus, and trace minerals (zinc, manganese, and copper) on liver and rib bones in Nellore cattle.

Item	CaP+		CaP-		SEM	P-value		
	CuMnZn+	CuMnZn-	CuMnZn+	CuMnZn-		CaP	CuMnZn	CaP×CuMnZn
Liver								
DM, kg	1.36	1.41	1.41	1.26	0.15	0.29	0.30	0.06
Ash, %	5.44	6.37	5.87	6.35	0.35	0.61	0.07	0.55
Ash, g	74.15	87.87	83.33	80.04	8.42	0.90	0.37	0.15
Zn, mg/kg	117	116	137	131	6.72	0.01	0.55	0.69
Mn, mg/kg	4.85	5.19	7.10	9.43	1.67	0.06	0.42	0.54
Cu, mg/kg	309	157	219	177	34.39	0.23	<0.01	0.07
Rib bone								
Fat free ash, %	59.61	60.08	57.68	57.57	0.84	0.01	0.83	0.73
Ash, %	44.75	46.15	42.75	43.48	1.23	0.06	0.39	0.78
Ca, %	14.05	13.21	13.08	13.09	0.69	0.39	0.62	0.60
Ca, % of bone ash	31.81	28.89	30.75	30.11	1.81	0.96	0.33	0.53
P, %	5.69	5.95	5.63	5.57	0.36	0.55	0.78	0.66
P, % of bone ash	12.86	12.89	13.20	12.74	0.86	0.90	0.80	0.77

[1] CaP+ = supplying 100% of Ca and P according to BR-CORTE (2010); CaP- = diet without limestone and dicalcium phosphate; CuMnZn+ = diet with supplementation of trace minerals (Zn, Mn, and Cu); CuMnZn- = diet without supplementation of trace minerals (Zn, Mn, and Cu).

Table 7. Effect of calcium, phosphorus, and trace minerals (zinc, manganese, and copper) on plasma blood in Nellore cattle.

Item	CaP+		CaP-		SEM	P-value		
	CuMnZn+	CuMnZn-	CuMnZn+	CuMnZn-		CaP	CuMnZn	CaP×CuMnZn
Ca, mg/dl	9.24	9.82	9.33	9.81	0.15	0.80	<0.01	0.73
P, mg/dl	7.65	7.44	7.43	7.31	0.28	0.52	0.55	0.88
Phosphatase, IU/L	318.14	315.86	308.94	322.00	29.84	0.95	0.85	0.79

[1] CaP+ = supplying 100% of Ca and P according to BR-CORTE (2010); CaP- = diet without limestone and dicalcium phosphate; CuMnZn+ = diet with supplementation of trace minerals (Zn, Mn, and Cu); CuMnZn- = diet without supplementation of trace minerals (Zn, Mn, and Cu).
[2] Reference values: Ca = 9.48 to 12.4 mg/dL (Kaneko et al., 1997; Payne and Payne, 1987); P = 4.3 to 7.7 mg/dL (Kaneko et al., 1997); Phosphatase = 0 to 488 IU/L (Kaneko et al., 1997);

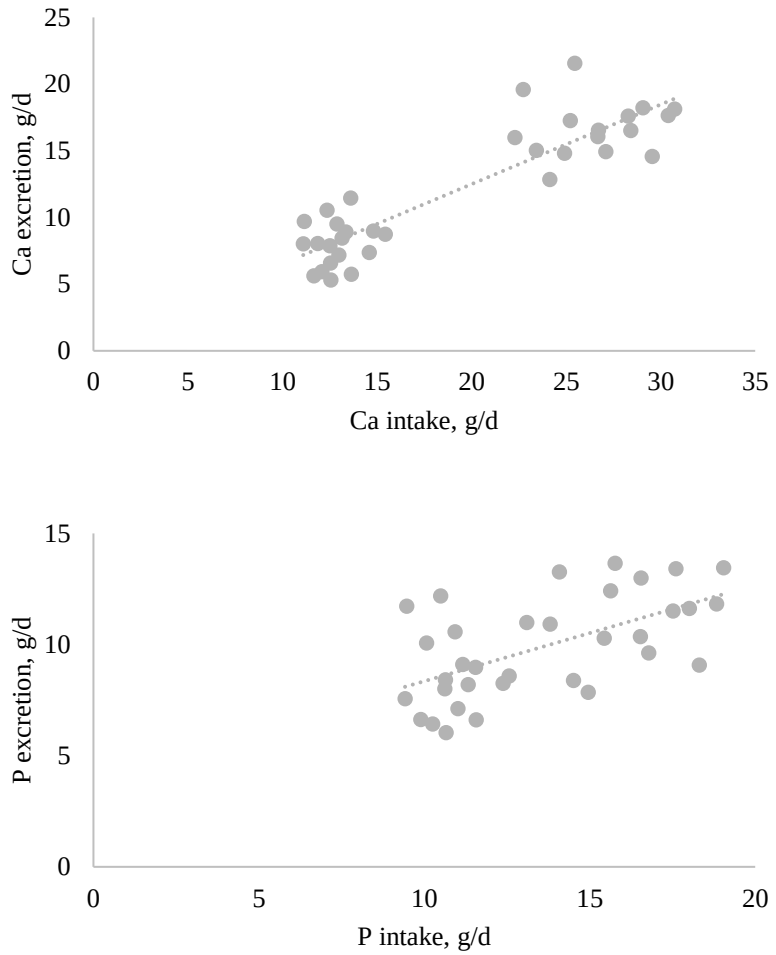

Figure 1. Relationship between Ca and P intake and Ca and P total excretion for Nellore bulls fed varying concentrations of dietary Ca and P.

CHAPTER 3

Growth, water intake, residual feed intake and nutrient requirements of Nellore feedlot bulls

ABSTRACT: The objective of this study was to evaluate the water intake, the chemical body composition, the residual feed intake and gain, and the nutritional requirements of energy and protein for maintenance and gain, and calcium and phosphorus requirements for maintenance as well as their efficiencies of Nellore bulls. Weaned Nellore bulls (n = 44; 273 ± 34 kg) were fed in a randomized complete block design 2 × 2 factorial arrangement to evaluate the nutritional requirements and water intake with absence or presence of mineral supplementation. The design included two levels of Ca and P (macro mineral factor; CaP+ or CaP-) and two levels of micro minerals (micro mineral factor; ZnMnCu+ or ZnMnCu-). The factor CaP- was without supplementation of limestone and dicalcium phosphate and the factor ZnMnCu- was without inorganic supplementation of micro minerals. The diets were isonitrogenous (13.3% CP). Intake was individually monitored every day. Indigestible NDF was used as an internal marker for digestibility measurements. A total of 44 Nellore bulls was used in this trial, where four animals were used as the reference group (harvested d 0); another four were fed at the maintenance level (1.1% of BW); and the remaining 36 were fed ad libitum. Bulls were blocked by days on fed, they were slaughtered on d 84 or 147, and samples of the whole body were taken. All samples were lyophilized, ground with liquid nitrogen and grouped as percentage of component in empty BW from each bull. Samples were analyzed for DM, ash, CP, EE, Ca, and P. The water intake was similar ($P \geq 0.07$) among treatments. The average of free water intake was 17 L/d for each bull. High residual feed intake and gain (RFIG) bulls had lower DMI ($P < 0.01$) than low RFIG bulls, but similar ADG ($P = 0.82$). The CP, EE and water content in the EBW increased as the animal grew; the ash growth was lower than the EBW. Non-linear

regression equations were developed to predict heat production (HP) from metabolizable energy (ME) intake and retained energy (RE). The net energy requirements for maintenance (NE_m) and metabolizable energy for maintenance (ME_m) were 66.5 and 107 kcal/$EBW^{0.75}$/d, respectively. The efficiency (k_m) was 62%. The equation obtained for net energy for gain (NE_g) was: NE_g (Mcal/d) = 0.0388 × $EBW^{0.75}$ × $EBWG^{1.095}$ and the efficiency was 25%. Net protein for gain was: NP_g (g/d) = 179.74 × EBWG – 5.43 × RE. The net maintenance requirement for Ca was 2.33 mg/EBW and for P was 9.10 mg/EBW. The true coefficient of absorption for Ca was 54% and P was 64%. In conclusion, the requirement of net energy for maintenance for Nellore feedlot cattle is 66.5 kcal/$EBW^{0.75}$/day. Requirements of net energy for gain and net protein for gain can be obtained by the following equations: NE_g (Mcal/d) = 0.0388 × $EBW^{0.75}$ × $EBG^{1.095}$ and NP_g (g/d) = 179.74 × EBG – 5.43 × RE. Net maintenance requirement for Ca is 2.33 mg/EBW and for P is 9.10 mg/EBW. The true coefficient of absorption for Ca is 54% and P is 64%. The water intake was not influenced by supplementation of Ca, P, Zn, Mn, and Cu. High residual feed intake and gain bulls has lower DMI than low RFIG bulls, however with similar ADG. The CP, EE and water present in the EBW increased as the animal grew, the ash growth was lower than the EBW.

Key Words: calcium, coefficient of absorption, efficiency, nutritional requirements, phosphorus, water intake

INTRODUCTION

Non-renewable resource and food production are popular and polemic topics in the current sustainability discussion (Odegard and van der Voet, 2013). The challenges of meat supply chain are sustainable production and reduce costs, thereby increasing profitability. These challenges can be overcome by rational feed management, feeding animals with just required amount, according to their categories and gain. Animal growth depends on diets being

formulated with adequate amounts and proportions of energy and essential nutrients (BCNR, 2016).

Nutrient requirements can be defined as the amount of nutrients necessary for the normal healthy and performance of cattle. For ruminants, the primary nutrients of interest are protein, energy (a property of nutrients but functionally treated like other nutrients in terms of requirements), vitamins, minerals and water (Galyean, 2014).

Establish a good nutrient requirements program can improve performance without nutrient excess or deficiency and this may improve the cattle efficiency. In evaluating previous Ca and P requirements in feeding trials, Prados et al. (2015) concluded that NRC (2000) and BR-CORTE (Valadares Filho et al. 2010) overestimated Ca and P requirements both for Zebu cattle. Supplementing diets at concentrations in excess of requirements greatly increases mineral loss in cattle waste (NRC, 2000). Over supplementation of minerals should be avoided to prevent possible environmental problems associated with runoff from waste or application of cattle waste to soil (NRC, 2000).

Animal products are considered to be the highest consumers of water (Mekonnen and Hoekstra, 2012). Water is an essential nutrient for cattle. Water has important play in the animal body as: transport of nutrients, maintaining body temperature, digestion and metabolism (BCNR, 2016). The animal body is composed around of two-thirds water. The water requirement of cattle could be met by: free water, water present in feedstuffs, and water formed in the body (metabolic water; NRC, 1981). And the water requirement is influenced by several factors: environmental conditions, feed and mineral intake, and physiological state of animals, as well as others factors.

The objectives of this study were to conduct a comparative slaughter aiming to evaluate the chemical body composition, residual feed intake and gain, and nutritional requirements of energy and protein for maintenance and gain, and calcium and phosphorus for maintenance of

Nellore bulls, as well as their efficiencies and to evaluate the water intake of animals fed with or without supplementation of Ca, P and micro minerals.

MATERIALS AND METHODS

The feeding and performance trial was conducted at the Animal Science Department of the Universidade Federal de Viçosa, Brazil. The institutional ethics committee approved all procedures involving animals (protocol number 20/2013).

Animals, diets and experimental design

A total of 44 weaned Nellore bulls (initial BW of 273±34 kg, and age of 9±0.6 mo) were used in this trial. Initially, cattle were weighed, identified and treated for the control of internal and external parasites by administration of ivermectin (Ivomec, Merial, Paulinea, BRA) prior to entering in the feedlot. Bulls were adapted to a common diet (sugar cane and concentrate) during 21 d prior the experiment.

The bulls were subdivided into 3 groups (reference, maintenance, and ad libitum). Four bulls were designated to slaughter on day zero as a reference group to estimate the initial body weight (EBW) and initial body composition. Four bulls were fed to maintenance level (1.1% of BW) and 36 bulls were fed ad libitum. Thirty-six bulls were blocked by feedlot periods (84 and 147 d) and they were used in a randomized complete block 2 × 2 factorial design experiment. The factors consisting of two Ca and P levels (macro mineral factor; **CaP+** or **CaP-**) and two levels of micro minerals (micro mineral factor; **ZnMnCu+** or **ZnMnCu-**). All treatments were ad libitum fed a conventional diet that contained 40% of sugar cane and 60% of concentrate (DM basis), described on Table 1.

The CaP+ contained 96.3 and 97.9% and CaP- contained 47.8 and 66.8% of Ca and P required (BR-CORTE, 2010), respectively. Decreased Ca and P was achieved by not supplementing diet with limestone and dicalcium phosphate. Treatments ZnMnCu+ contained 56.8 mg/kg of Zn, 49.2 mg/kg of Mn, and 15.4 mg/kg of Cu and ZnMnCu- contained 31.2 mg/kg of Zn, 42.3 mg/kg of Mn, and 5.8 mg/kg of Cu (Table 1).

The diets were isonitrogenous (13.3% CP), formulated to ensure meeting BR-CORTE (Valadares Filho et al., 2010; http://www.brcorte.com.br) nutrient requirements except for Ca, P, Cu, Mn, and Zn, for a target ADG of 1.25 kg/d (considering an average BW of 350 kg).

The restricted feeding was 1.1% of BW and the four bulls were fed once daily (0700 h). Feed was delivered to pens twice daily at 0700 and 1500 h. Animals had ad libitum access to water. The pens were collective, two pens per treatment, with area of 11.1 m^2 / animal. Pens were with a concrete floor and 15 m^2 of covered area. Bulls were tagged with electronic identification tags, which allowed for feed intake by a single bull to be monitored. Each pen had a trough with an electronic system equipment for monitoring individual feed intake (INTERGADO®, Contagem, MG, Brazil) using electronic tags with radio frequency identification, where intake of animals was recorded daily (Chizzotti et al., 2015).

Samples of concentrate ingredients were collected directly at the feed mill. Sugar cane samples were collected every day, oven-dried (60˚C) and ground using a Willey mill (TE-650, Tecnal, Piracicaba, SP, Brazil) to pass through 1 and 2-mm (for indigestible neutral detergent fiber (**iNDF**) analyses) screen, and pooled based on DM basis for laboratory chemical analysis.

After a fasting period (14 h without feed), the cattle were weighed to measure initial and final BW on a scale (COIMMA, model 9024, Dracena, SP, Brazil). The animals were weighed every 28 d just for monitoring ADG and BW.

Water intake

For water intake measurements were made in a group pen that contained electronic drinking fountains (INTERGADO®, Contagem, MG, Brazil). The Intergado monitoring systems was used for determine individual water intake using electronic tags with radio frequency identification (Chizzotti et al., 2015).

Digestibility

Spot fecal samples were collected on d 75-77 and 129-131. Fecal samples were collected from the cattle at 0600, 1200 and 1800 h each day of the collection period. Samples

(feed and fecal samples of the collection period) were ground using a Willey mill (TE-650, Tecnal, Piracicaba, SP, BRA) to pass through a 2-mm screen sieve for indigestible neutral detergent fiber analyses. Fecal samples were pooled on a DM basis by period for each bull.

For analyses of iNDF, samples were ruminally incubated in F57 filter bags (ANKOM, Macedon, NY, USA) at two cannulated Nellore bulls for 288 h (Valente et al., 2011). This time interval is required to account for iNDF in tropical C4 forages such as sugarcane. When all bags were removed from each rumen, they were soaked in water for 30 min and washed by hand under running water until the wash water ran clear. Contents of iNDF were then evaluated using an ANKOM^{200} fiber analyzer. The iNDF was obtained by weighing the filter bags after drying them in an oven, first at 60˚C for 72 h followed by 105˚C for 12 h. The residue was considered the iNDF. Fecal iNDF concentration was determined and was then used to calculate the estimated fecal output per day. Indigestible neutral detergent fiber was used as an internal marker to estimate the fecal excretion.

Residual feed intake and gain

The residual feed intake (RFI) was calculated as the difference between the observed intake measured during the experiment and the intake predicted by the regression using the following model: $DMI = a + b \times BW^{0.75} + c \times ADG$, where $BW^{0.75}$ is the average of metabolic body weight, ADG is the average of daily gain and a, b and c are parameters of the regression. Similarly, the residual gain (RG) was calculated as the difference between the observed gain measured during the experiment and the gain predicted by the regression using the following model: $ADG = a + b \times BW^{0.75} + c \times DMI$, where $BW^{0.75}$ is the average of metabolic body weight, DMI is the average of dry matter intake and a, b and c are parameters of the regression.

The RFI and RG were standardized to have equal variances. The residual intake and gain (RFIG) sets was calculated according to the equation (Berry and Crowley, 2012): RFIG =

-RFI + RG, where RFIG is the residual feed intake and gain sets, RFI is the residual feed intake and RG is the residual gain.

Slaughter

Before slaughter, feed was restricted for 14 h. The slaughter process used a captive bolt stunning followed by exsanguination from jugular vein, evisceration, and hide removal. The whole bull body was segregated into head, hoofs, hide, organs and viscera, blood and carcass. The blood was sampled at the moment of bleeding. After bleeding, the gastrointestinal tract was removed and washed. The heart, lungs, liver, spleen, kidneys, internal fat, diaphragm, mesentery, tail, trachea, esophagus, reproductive system, cleaned gastrointestinal tract, head, hide, hoofs, blood and carcass were weighed to quantify EBW of each bull.

All of non-carcass compounds (head, hoofs, blood, hide, organs and viscera) were ground and homogenized using a bowl cutter and a sample was taken (organs and viscera). The head and hoofs were ground initially in an industrial bone grinder, and after in a bowl cutter and sample were taken (head and hoofs). The hide of each animal was sampled and minced in small pieces. The carcasses were weighed and chilled in a cold chamber (4 ºC) for 24 h. After chilled, the left half-carcasses were ground in an industrial grinder, and after the ground carcass was homogenized in a bowl cutter. A sample was taken.

All samples (blood, organs and viscera, head and hoofs, hide, and carcass) from each animal were lyophilized, ground with liquid nitrogen using a Willey mill. After ground, a composite sample of the whole bull was made by using the percentage of each component in the empty body of each bull. All samples were stored frozen (-80 ºC) until chemical analyses could be completed.

Chemical analysis

For chemical analysis, the samples (diet ingredients and feces) were oven-dried (60˚C) and ground to pass through a 1-mm screen sieve. All samples (feed, feces, and animals) were analyzed for DM, CP, EE, Ca, and P.

Dry matter was collected by oven-dried (100˚C) for 18 hours (Method 934.01; AOAC, 2000). Crude protein was calculated based on nitrogen content multiplied by 6.25 factor. Nitrogen content was calculated by Kjeldahl procedure (Method 934.87; AOAC, 2000). Ether extract was determined by the Ankom® technique (Ankom, Macedon, NY). Neutral detergent fiber was determined by the Ankom technique. Neutral detergent fiber was with amylase and expressed inclusive of residual ash and protein.

Calculations

Intake of DM and nutrients were calculated on the basis of the amounts ingested daily. An average was calculated for total experimental period.

To estimate the body composition, equations were generated from EBW and the body chemical composition of the bulls. For CP, EE, ash, and water content in the EBW, the models utilized were as follow: $C_i = a \times EBW^b$, where C_i is the i body component of the bull, which can be CP, EE, ash, or water content in the empty body weight (kg), and a and b are the regression parameters.

The quantity of digestible energy and nitrogen of the diet was estimated as the coefficient of the total tract disappearance (intake minus fecal excretion) and intake of these nutrients. The digestible energy (DE) intake by bulls was obtained from the digestible nutrients multiplied by their respective energy values. Metabolizable energy (ME) was determined by multiplying DE by 82%.

The metabolizable protein (MP) intake was calculated as the sum of the digestible true microbial protein and the digestible ruminal undegradable protein (RUP) intakes. The values of microbial protein synthesis were obtained from 120 g/kg TDN (BR-CORTE, 2010).

Nutrient requirements were estimated using a factorial approach (ARC, 1965) for maintenance and gain. In the factorial approach it has been assumed that the needs for maintenance and production are independent (AFRC, 1991).

For conversion of shrunk BW (SBW) into empty BW (EBW), a linear regression was performed. The same procedure was done for ADG and empty body weight gain (EBG).

Heat production (HP; Mcal/$EBW^{0.75}$/d) was calculated as the difference between ME intake (MEI) and retained energy (RE; Mcal/$EBW^{0.75}$/d). The RE was determined as the difference between the final energy content and initial energy content in the EBW.

Net energy requirements for maintenance (NE_m) were obtained using a non-linear exponential model between heat production (HP) and ME intake. The model used was $HP = \beta_0 \times e^{\beta 1 \times MEI}$, 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 Euler's number. Under this model, β_0 represents the NE_m (Mcal/$EBW^{0.75}$/d).

The metabolizable energy for maintenance (ME_m, in Mcal/$EBW^{0.75}$/day) was determined by the iterative method, when MEI equaled HP.

The efficiency utilization of metabolizable energy for maintenance (K_m) was obtained from the relation between the net and metabolizable energies for maintenance.

Regression equations for retained energy (RE) versus EBG were fitted for a given metabolic EBW ($EBW^{0.75}$), using the following model: $RE = a \times EBW^{0.75} \times EBG^b$, where RE = retained energy (Mcal/$EBW^{0.75}$/day); $EBW^{0.75}$ = metabolic empty body weight (kg); EBG = empty body weight gain (kg/day); and a and b are regression parameters.

To obtain the partial utilization efficiencies of metabolizable energy for fat and protein synthesis, we used the equation $MEI = \beta_0 + \beta_1 \times RE_p + \beta_2 \times RE_f$, where MEI = metabolizable energy intake (Mcal/$EBW^{0.75}$/day); RE_p = body energy retained in the form of protein (Mcal/$EBW^{0.75}$); RE_f = body energy retained in the form of fat (Mcal/$EBW^{0.75}$); β_0 = the metabolizable energy requirement for maintenance; and β_1 and β_2 = the efficiencies of deposition of energy as protein and fat, respectively.

The metabolizable protein requirement for maintenance (MP_m) was estimated as the intercept from a linear regression, where MPI was contrasted with the empty body weight gain: $MPI = \beta_0 + \beta_1 \times EBG$, where MPI = metabolizable protein intake (g/d) and EBG = empty body weight gain (kg/d). The division of the intercept of the regression mentioned above by the average metabolic weight of the animals yields an estimate of the requirements of metabolizable protein for maintenance: $MP_m = \beta_0/EBW^{0.75}$, where MP_m is the metabolizable protein requirement for maintenance ($g/BW^{0.75}/d$); β_0 is the intercept of the regression presented above; and $EBW^{0.75}$ is the average metabolic empty body weight (kg).

To obtain the net requirements of protein for weight gain (NP_g), we adjusted a model according to the energy retained: $RP = \beta_0 \times EBG + \beta_1 \times RE$, where RP = retained protein (g/d); EBG = empty body weight gain (kg/d); RE = retained energy (Mcal/d); and β_0 and β_1 are regression parameters.

The utilization efficiency of metabolizable protein for gain was calculated from a regression model for retained protein as a function of metabolizable protein intake, according to the model described in BR-CORTE (2010): $RP = \beta_0 + \beta_1 \times MPI$, where RP is retained protein ($g/EBW^{0.75}/d$); MPI is metabolizable protein intake ($g/EBW^{0.75}/d$); and β_1 is efficiency of the use of metabolizable protein for gain (k).

The metabolizable protein requirement for gain (MP_g) was calculated by dividing the net protein requirement for gain by the utilization efficiency of metabolizable protein for gain.

The mineral requirements for maintenance was calculated by a regression between retained mineral (Ca or P) and mineral intake in mg/ kg EBW.

Statistical analysis

The experimental design was a completely randomized block in a 2 × 2 factorial arrangements (CaP+ or CaP-, ZnMnCu+ or ZnMnCu-). Main effects of macro and micro minerals were tested as well as their interactions. Data were analyzed as a mixed model with

the fixed effects of macro and micro minerals and their interactions and the random effect of days on fed (block) to analyze water intake.

Chemical composition, residual feed intake and requirements were analyzed deprived of design or arrangement.

All statistical procedures were executed using SAS (SAS Inst. Inc., Cary, NC) with animal being the experimental unit. The linear models were built with PROC REG statement and non-linear models with PROC NLIN. Non-linear models were adjusted by the Gauss-Newton iterative method. For all comparisons and tests were used 0.05 as critical level of probability.

RESULTS AND DISCUSSION

Water intake

The pH of water was in average 6.68. The average of environment temperature was 19.2˚C with maximum temperature of 24.8˚C. There were no significant effects of CaP × ZnMnCu interactions ($P \geq 0.07$) for water intake. Thus, main effects of concentration of CaP and ZnMnCu were discussed (Table 2). The concentration of Ca, P, and micro minerals did not affect ($P \geq 0.21$) water intake (Table 2). Cattle fed CaP+ had 17.5 l/d of water intake and cattle fed CaP- had 16.9 l/d.

Winchester and Morris (1956) suggested a constant relationship between water intake and DMI for cattle at thermal neutral conditions. The DMI of cattle was similar among treatments (Prados, 2016). The similarity can be explained due the dietary and environmental characteristics. The diet had the same concentration of moisture, salt, and ash (Table 1) and the bulls were in the same environment.

Further studies will be necessary to evaluate the water requirements since water is an essential nutrient.

Residual feed intake and gain

The equations obtained for the prediction of RFI and RG from the experiment data are in Table 3. Ranking the animals for RFIG (Berry and Crowley, 2012), there are 15 animals with high RFIG and 20 animals with low RFI. The variation observed between values of RFI was -0.75 to +0.56 kg/d (Table 4). This represented a difference in actual feed intake of 1.31 kg/d between the most and least efficient bulls. Costa e Silva et al. (2012) observed 2.0 kg/d of variation using Nellore bulls. This variation in DMI represents a difference in feed cost for the producers.

Residual gain ranged from -0.61 to +0.81 kg/d (Table 4). The low RFIG animals had 5% higher DMI with lower ($P = 0.04$) ADG and similar chemical composition ($P > 0.05$). The high RFIG bulls had greater ADG ($P = 0.04$).

We highlight that RFIG are important parameters to evaluate the individual and not the group. This is an important tool for genetics.

Body composition

Diverse genotypes, changing environmental conditions, energy intake, hormonal status, and tissue turnover also affect rate and composition of tissue accretion (Owens et al., 1995). The chemical components were associated with the EBW (Figure 1). The animals had changes in their body composition during the experiment (Figure 1). So, we propose some equations to estimate the chemical body composition of Nellore bulls, based on kilograms' values (Table 5). The equations (Table 5) estimated the chemical body composition of Nellore bulls.

Crude protein and water increases with increases of EBW (Figure 1). Ash in the EBW presents behavior similar through increase of EBW. The equations (Table 5) shown that the increase in CP is similar to the EBW. This is evidenced by the coefficient (1.09) close to 1. The chemical composition of muscle tissues varies during the animal growth. After birth, the protein content increases greatly, then remain constant, and after, fat increases (Robelin and Geay, 1984).

Minerals (ash) growth is lower than the EBW (0.85), which can be consequence of bone tissue have reduced relative growth as the bull increases the weight.

The amount of body fat increases exponentially when the animal reaches maturity. This increase in EE was not evidenced in this experiment (Figure 1), possibly because the animals had not reached maturity. Trenkle and Marple (1983) suggested that it is possible to estimate weight at maturity when animal reaches 22% EE in EBW, in this trial the animals reached only 17% of EE in EBW, this can be explaining due the animals used in this experiment were young.

Relationship between EBW and SBW and EBG and ADG

According to Owens et al. (1995), EBW is the most accurate index of energy and nutrient content in the body. The equation obtained for the ratio between EBW and SBW was EBW (kg) = $0.904_{\pm 0.0013}$ × SBW (kg). The ratio obtained was close that equation used by BR-CORTE (2010) of 0.895 and by the NRC (2000), 0.891. The difference between this trial and NRC values can be explained due the breeds used, NRC used Bos taurus and this trial used Bos indicus. Costa e Silva et al. (2012) reported value equal 0.914, using Nellore bulls' cattle. The equation proposed in this study is within the range reported by NRC (2000) in which can vary from 0.85 to 0.95. The BR-CORTE (2016) used a new model to estimate the EBW for bulls as follow: EBW (kg) = $0.8126 \times SBW^{1.0134}$.

For conversion of ADG in EBG, this study found: EBG (kg) = $0.979_{\pm 0.0061}$ × ADG (kg). The ratio obtained is close to the 0.956 recommended by NRC (2000). For Nellore, BR-CORTE recommended 0.936, value lower than the value obtained in this study. This lower value can be explained due the used of younger animals because the deposition of protein is more efficiently. The BR-CORTE (2016) used a new model to estimate the EBG for bulls as follow: EBW (kg) = $0.9630 \times SBW^{1.0151}$.

Energy requirements

Three methods can be used to determine energy requirements, as follow: feed long trials, calorimetry and comparative slaughter. This trial used the comparative slaughter method,

where we measured directly the MEI and RE and heat production (**HP**) was determined for difference between the others variables.

The maintenance requirement for energy has been defined as the amount of feed energy intake that will result in no net loss or gain of energy from the tissues of the animal body (NRC, 2000). Heat production correspond to NE_m, the equation for HP was $HP = 0.0665_{\pm 0.0035} \times e^{(4.4531 \pm 0.2082 \times MEI)}$, where HP = heat production in Mcal · $(EBW^{0.75}/d)^{-1}$ and MEI = metabolizable energy intake in Mcal · $(EBW^{0.75}/d)^{-1}$. The parameter in this equation is the NE_m that was equal 66.5 kcal · $(EBW^{0.75} \cdot d)^{-1}$. This value is below that found in literature. BR-CORTE (2010) indicates that the requirement for maintenance is 74.2 kcal · $(EBW^{0.75} \cdot day)^{-1}$ and NRC (2000) recommends 77 kcal · $(EBW^{0.75} \cdot day)^{-1}$. Therefore, NRC (2000) did not use Nellore cattle (neither Bos indicus) in its database, they used the study of Lofgreen and Garrett (1968) that used growing steers and heifers of British ancestry. The NRC (2000) reviewed several studies with Zebu breeds, and concluded that a 10% discount should be applied, which would result in NE_m of 69 kcal · $(EBW^{0.75} \cdot day)^{-1}$, value comparable to this study. The BCNR (2016) uses 77 kcal · $(SBW^{0.75} \cdot day)^{-1}$ as NE_m requirements.

The NE_m should be converted to metabolizable energy for maintenance (ME_m). Metabolizable energy for maintenance was calculated by iterative process. This method makes the HP equal to the MEI (HP = MEI), there will be not energy retention, and this MEI is equivalent to the ME_m requirement (BR-CORTE, 2010). By using this procedure, the k_m was obtained when NE_m was divided by ME_m. The value obtained was 107 kcal · $(EBW^{0.75} \cdot day)^{-1}$. Value below the 112.4 kcal · $(EBW^{0.75} \cdot day)^{-1}$ suggested by BR-CORTE (2010). The ME_m value was 5% inferior to that suggested by BR-CORTE (2010).

The K_m is the efficiency of utilization of metabolizable energy for maintenance (**K_m**), this value was obtained dividing NE_m by ME_m (66.5 / 107), resulting in 62.15%. Several factors

can affect K_m, such as level of dietary fiber, level of metabolizable energy intake, protein turnover, age, gender, and others (Garrett, 1980; CSIRO, 2007).

Net energy for gain is defined as the energy content of the tissue deposited, which is a function of the proportion of fat and protein in the empty body tissue gain (Garrett et al., 1959). The composition of empty body gain is the main driver of energy requirements for weight gain, which are estimated from retained energy in the body (BR-CORTE, 2010) and the composition of gain depends the EBW, because of the animal maturity. An equation for retained energy (RE) or net energy for gain as function of EBW and EBWG was obtained to estimate the net energy requirements for gain for any weights and weight gain. The equation was $RE = 0.0388_{\pm 0.00104} \times EBW^{0.75} \times EBG^{1.095}$. The intercept for determining NE_g in this trial (0.0388) was lower than that reported by BR-CORTE (2010; 0.053). This lower intercept may suggest that the animals used in this study had lower fat concentration in the gain.

For conversion of NE_g requirement in ME_g, it is necessary the efficiency for gain (**K_g**). The K_g can be estimated as the slope coefficient of the regression between RE and MEI (Figure 2). $RE = -0.01751_{\pm 0.00887} + 0.25086_{\pm 0.03582} \times MEI$. The value of kg was 25.09%. According Costa e Silva et al. (2012), the efficiency for gain depends on the proportions of energy retained in form of protein and fat.

Geay (1984) reported that the efficiencies of fat and protein deposition are different and the efficiencies are functions of the proportions of gain of each one in the animal body. The efficiency of energy deposition in the form of fat is greater than protein form (Owens et al., 1995). The equation for obtained these efficiencies was MEI in function of RE as fat and protein. The k_{fat} was 56.64% and k_{ptn} was 18.14%. The ratio between k_{fat} and k_{ptn} was 3.12, similar that found in Rattray and Joyce (1976) in a trial with sheep. Costa e Silva et al. (2012), in a trial with Nellore, found k_{fat} equal 71% and k_{ptn} equal 18%. The deposition efficiency for protein was very similar to this trial, different for the k_{fat}.

Protein requirements

The metabolizable protein requirement was determined by factorial method. The NRC (2000) proposed that metabolic fecal, urinary, and scurf losses represent the requirement needed for maintenance.

The equation for protein requirements for maintenance was in function of EBG, where the intercept divided by the average of $EBW^{0.75}$ is the metabolizable protein requirement for maintenance. The equation obtained was $MEI = 195.02_{\pm 26.15} + 446.09_{\pm 24.33} \times EBG$. Dividing the intercept (195.02) by the average of $EBW^{0.75}$ (72.33 kg) was obtained 2.70 $g/EBW^{0.75}$. This value for metabolizable protein maintenance requirements was lower than 3.8 $g/BW^{0.75}$ value recommended by NRC (2000) according to Wilkerson et al. (1993), and suggested by BCNR (2016) also. However, NRC (2000) suggests if actual bacterial crude protein synthesis efficiency was less than 0.13 (we used 0.12 based in BR-CORTE, 2010), the estimate of maintenance would be less than 3.8 g MP/kg $BW^{0.75}$. Nevertheless, the AFRC (1993) assumes that daily metabolizable protein requirement for maintenance is 2.30 $g/BW^{0.75}$, based on the sum of basal endogenous nitrogen requirements and other losses by scurf and skin.

The BCNR (2016) suggest two equations to estimate the microbial crude protein (MCP) synthesis: MCP (g/d) = 0.087 TDNI + 42.73 (to diets with EE < 3.9%); and MCP (g/d) = 0.096 FFTDNI + 53.33, where TDNI is the TDN intake (g/d) and FFTDNI is the fat free TDN intake (g/d).

The NRC (2000) suggests a model to estimate the net protein requirements for gain (**NP_g**) based in ADG. In this study was used a similar model, however replaced ADG to EBWG. The equation was $NP_g = 179.74_{\pm 32.22} \times EBG - 5.43_{\pm 10.02} \times RE$.

The net protein requirements for gain need to be replaced to metabolizable protein requirement for gain. For this it is necessary to obtain the efficiency of net protein requirement in metabolizable for gain. The slope of the regression equation for retained protein (**RP**) in function of MPI is the efficiency for protein. The equation for this experiment was RP = -

$1.46_{\pm 0.60} + 0.4644_{\pm 0.065} \times$ MPI. Therefore, the efficiency for protein was 46.44%. This value was lower than the 49.2% found in NRC (2000) and similar the 46.91% found in BR-CORTE (2010).

Mineral maintenance requirements (calcium and phosphorus)

Calcium and phosphorus are the most abundant mineral in the cattle body. They are often discussed together due the function together in bone formation. According to BCNR (2016), research to update the Ca and P requirements would be beneficial.

The predominant constituent of maintenance requirements for both Ca and P is the obligatory endogenous fecal loss (AFRC, 1991). Maintenance requirement was calculated using the regression between retained mineral in function of mineral intake. The daily net maintenance requirements were obtained from the intercept in axis "x", endogenous fecal loss were obtained from intercept in axis "y" and the slope of regression represents the true coefficient of absorption (Figures 3 and 4). The daily maintenance requirements of Ca and P were lower than the amount recommended by BCNR (2016).

The equation for calcium was $Ca = 0.492x - 2.33$ ($R^2 = 0.87$); thus for Ca, 49.2% was the true retention coefficient and 2.33 mg/EBW was endogenous loss. For dietetic maintenance requirement was 4.74 mg/EBW.

The equation for phosphorus was $P = 0.6425x - 9.10$ ($R^2 = 0.42$); thus for P, 64.25% was the true retention coefficient and 9.10 mg/EBW was endogenous loss. Dietetic maintenance requirement of P was 14.16 mg/EBW.

Equations, coefficients, and ultimate predictions of requirements must be tested to determine how well they fit observed data (Galyean, 2014). Nutrient requirements can be an especially challenge for ruminant nutrition (Galyean, 2014).

The net energy requirements for maintenance of Nellore bulls are 67 kcal/$EBW^{0.75}$/d. The requirements for NE_g and NP_g can be obtained using the following equations: NE_g (Mcal/day) $= 0.039 \times EBW^{0.75} \times EBG^{1.095}$ and NP_g (g/day) $= 179.74 \times EBG - 5.43 \times RE$. The utilization

efficiencies of metabolizable energy for maintenance and gain of growing and finishing Nellore bulls are 62.2 and 25.1%, respectively. The utilization efficiency of metabolizable protein for gain in Nellore bulls is 46.4%. The true retention coefficients for Ca and P were respectively 49.2 and 64.2%. The calcium, phosphorus and micro minerals supplementations did not affect water intake in feedlot bulls. High residual feed intake and gain bulls have lower dry matter intake than low RFIG bulls with similar ADG. The CP, EE and water present in the EBW increased as the animal grew, the ash growth is lower than the EBW.

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Table 1. Components and chemical composition of experimental diets (% DM).

Components[1]	CaP+		CaP-	
	ZnMnCu+	ZnMnCu-	ZnMnCu+	ZnMnCu-
Ingredients				
Sugar cane	40	40	40	40
Corn	30.3	30.3	30.3	30.3
Soybeans	7	7	7	7
Soybeans hulls	19.5	19.5	19.5	19.5
Salt	0.2	0.2	0.2	0.2
Urea	1.17	1.17	1.17	1.17
Ammonium sulfate	0.13	0.13	0.13	0.13
Limestone	0.31	0.31	-	-
Dicalcium phosphate	0.37	0.37	-	-
Bicarbonate/Mg oxide	1	1	1	1
Trace mineral premix	0.03	-	0.03	-
Sand	-	0.03	0.68	0.71
Chemical Composition				
DM	65.5	65.5	65.5	65.5
OM	94.9	94.9	94.9	94.9
CP	13.3	13.3	13.3	13.3
EE	2.3	2.3	2.3	2.3
NDF	31.5	31.5	31.5	31.5
NFC	50.0	50.0	50.0	50.0
Calcium[2]	0.371	0.371	0.184	0.184
Phosphorus[2]	0.230	0.230	0.157	0.157
Zinc, mg/kg	57.54	31.25	56.15	31.15
Manganese, mg/kg	48.19	41.19	50.22	43.56
Copper, mg/kg	15.77	5.85	15.17	5.74

[1] CaP+ = supplying 100% of Ca and P according to BR-CORTE (2010); CaP- = diet without limestone and dicalcium phosphate; ZnMnCu+ = diet with supplementation of trace minerals (Zn, Mn, and Cu); ZnMnCu- = diet without supplementation of trace minerals (Zn, Mn, and Cu).

[2] Ration Ca:P = 1.61:1 (CaP+) e 1.17:1 (CaP-); CaP- corresponded to 50 and 68% of requirements, respectively of Ca and P (BR-CORTE, 2010); and according NRC (2000) 59 and 74% of requirements, respectively of Ca and P.

Table 2. Effect of calcium, phosphorus, and trace minerals (zinc, manganese, and copper) on free water intake of Nellore cattle.

Item	CaP+		CaP-		SEM	P-value		
	ZnMnCu+	ZnMnCu-	ZnMnCu+	ZnMnCu-		CaP	ZnMnCu	CaP×ZnMnCu
Water intake, l/d	18.7	16.3	16.7	17.1	1.5	0.45	0.21	0.07

[1] CaP+ = supplying 100% of Ca and P; CaP- = diet without limestone and dicalcium phosphate; ZnMnCu+ = diet with supplementation of trace minerals (Zn, Mn, and Cu); ZnMnCu- = diet without supplementation of trace minerals (Zn, Mn, and Cu).

Table 3. Equations to estimate the residual feed intake.

Item[1]	Equation	R^2
RFI	$0.34494_{\pm 0.709} + 0.05361_{\pm 0.009} \times BW^{0.75} + 2.10876_{\pm 0.584} \times ADG$	73.7
RG	$0.33957_{\pm 0.171} - 0.00193_{\pm 0.003} \times BW^{0.75} + 0.13704_{\pm 0.038} \times DMI$	45.8

[1] RFI = residual feed intake; RG = residual gain.

Table 4. Composition of body chemical components (kg) DMI, ADG in bulls with high and low gain and RFI.

Item[1]	Low RFIG	High RFIG	SEM	P-value
n	20	15	-	-
DMI, kg/d	7.17	6.83	0.17	0.15
$BW^{0.75}$, kg	79.8	78.9	2.06	0.73
ADG, kg/d	1.11	1.20	0.03	0.04
MEI, Mcal/$EBW^{0.75}$/d	0.258	0.255	0.005	0.66
HP, Mcal/$EBW^{0.75}$/d	0.209	0.210	0.005	0.93
RE, Mcal/$EBW^{0.75}$/d	0.049	0.045	0.002	0.19
EBW, kg	384	368	14.9	0.40
EE, kg EBW	66.3	57.1	4.30	0.11
CP, kg EBW	79.6	73.6	3.53	0.20
Water, kg EBW	228	226	7.57	0.90
RFI	0.563	-0.749	0.20	<0.01
Residual gain	-0.612	0.815	0.18	<0.01
RFIG	-1.17	1.56	0.29	<0.01

[1] n = number of bulls; DMI = dry matter intake; BW0.75 = metabolic body weight; ADG = average daily gain; MEI = metabolizable energy intake; HP = heat production; RE = retained energy; EBW = empty body weight; EE = ether extract in EBW; CP = crupe protein in EBW; RFI = residual feed intake; RFIG = residual feed intake and gain.

Table 5. Equations to estimate the chemical body composition (in kilograms) from EBW (in kilograms).

Item	Equation	R^2
CP_{EBW}	$0.1158_{\pm 0.0373} \times EBW^{1.0953 \pm 0.0542}$	99.6
EE_{EBW}	$0.00543_{\pm 0.00355} \times EBW^{1.5750 \pm 0.1096}$	98.7
Ash_{EBW}	$0.0697_{\pm 0.0526} \times EBW^{0.8522 \pm 0.1275}$	97.6
$Water_{EBW}$	$1.5734_{\pm 0.2432} \times EBW^{0.8379 \pm 0.0261}$	99.9

Table 6. Summary of equations for nutritional requirements for Nellore in feedlot.

Item	Equation	Unit
EBW	$0.90 \times SBW$	Kg
EBWG	$0.97 \times ADG$	Kg/d
NE_m	$0.067 \times EBW^{0.75}$	Mcal/d
K_m	62.2	%
NEg	$0.039 \times EBW^{0.75} \times EBWG^{1.095}$	Mcal/d
K_g	25.1	%
ME_g	NE_g/K_g	Mcal/d
ME_{total}	$ME_m + ME_g$	Mcal/d
TDN	$ME_{total}/0.82/4.409$	Kg/d
MP_m	$2.70 \times EBW^{0.75}$	g/d
NP_g	$179.74 \times EBG - 5.43 \times RE$	g/d
K_{ptn}	46.4	%
MP_g	NP_g/ K_{ptn}	g/d
MP_{total}	$MP_m + MP_g$	g/d
MicP	$120 \times TDN$	g/d
RDP	$1.11 \times MicP$	g/d
RUP	$[MP_{total} - (MicP \times 0.64)]/0.80$	g/d
CP	RDP + RUP	g/d
NCa_m	2.33	mg/EBW
$NPho_m$	9.10	mg/EBW
Retention Ca	49.2	%
Retention Pho	64.2	%

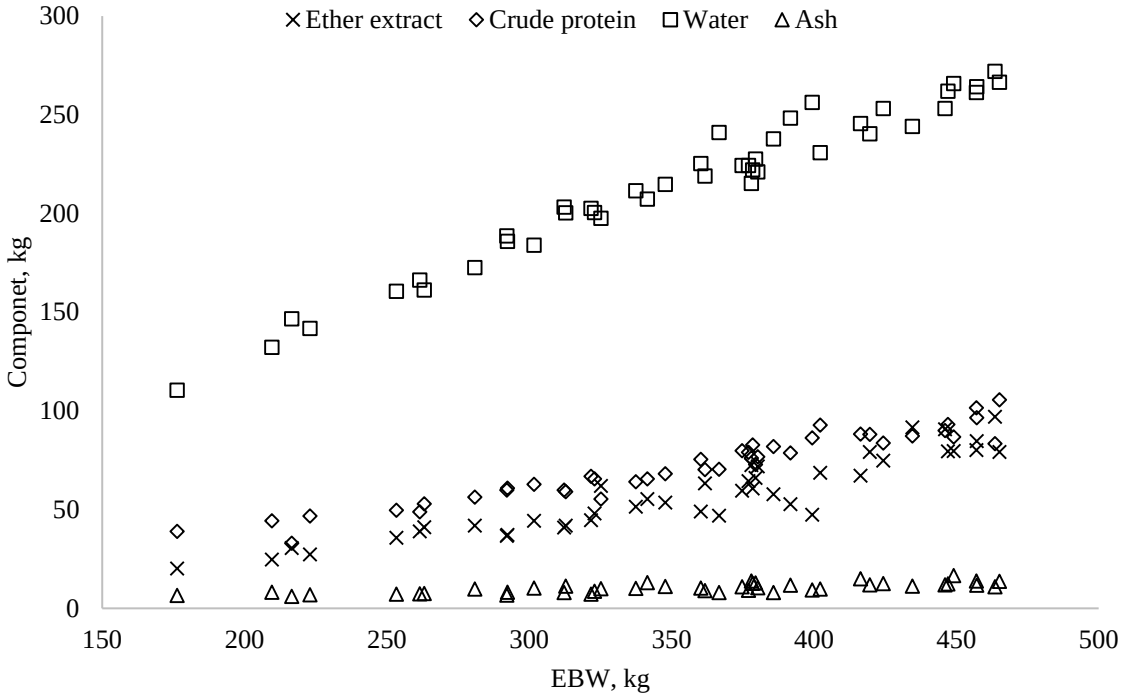

Figure 1. Relationship between the amount of crude protein, ether extract, ash, and water and empty body weight of Nellore bulls.

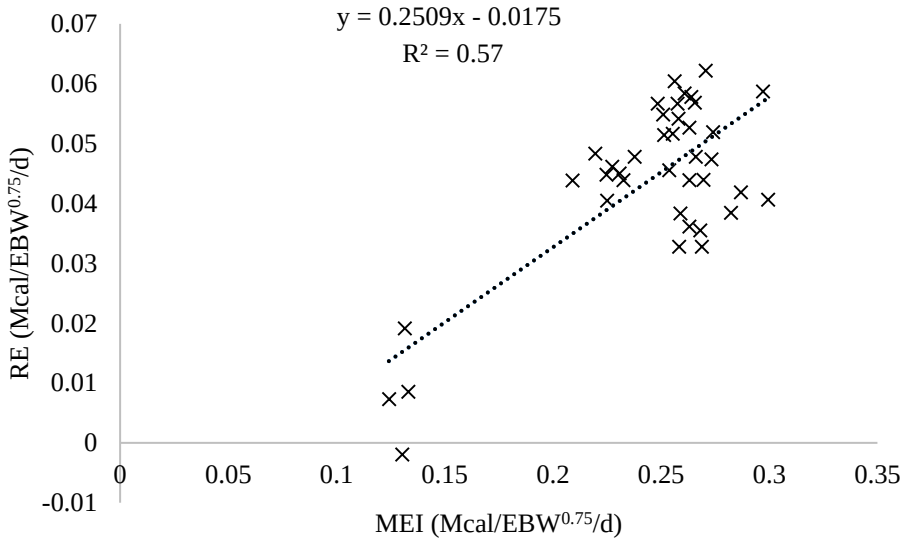

Figure 2. Retained energy (RE) as a function of metabolizable energy intake (MEI) of Nellore bulls.

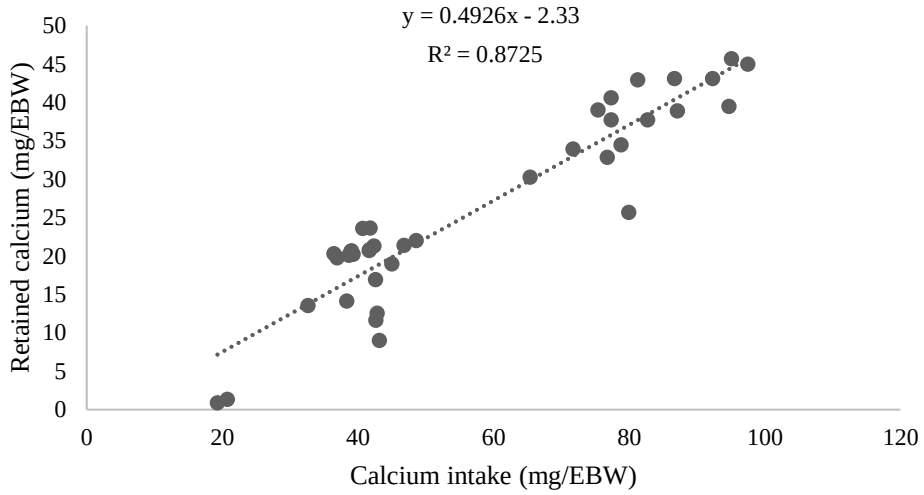

Figure 3. Retained calcium as a function of calcium intake of Nellore bulls.

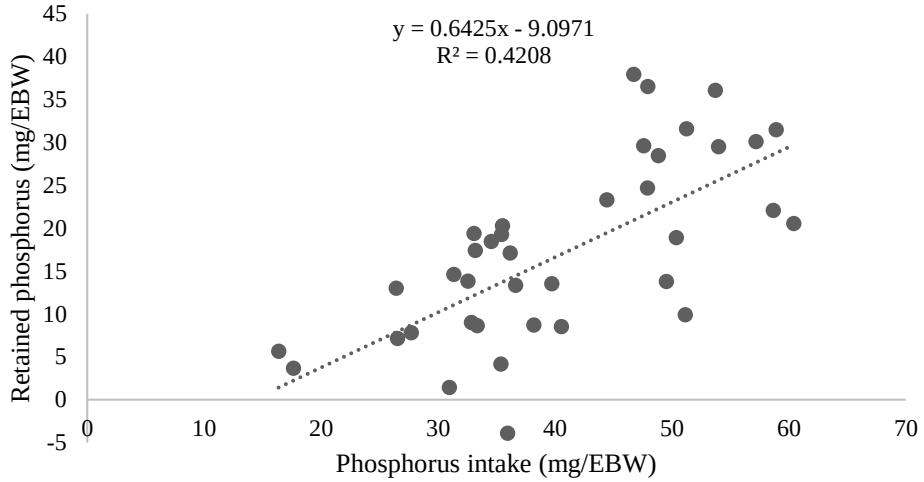

Figure 4. Retained phosphorus as a function of phosphorus intake of Nellore bulls.