

PALOMA DE MELO AMARAL

**PERFORMANCE AND AMINO ACIDS REQUIREMENTS OF NELLORE AND
CROSSBRED ANGUS×NELLORE FED DIETS WITH DIFFERENT CRUDE
PROTEIN CONTENT DURING GROWING AND FINISHING STAGES**

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

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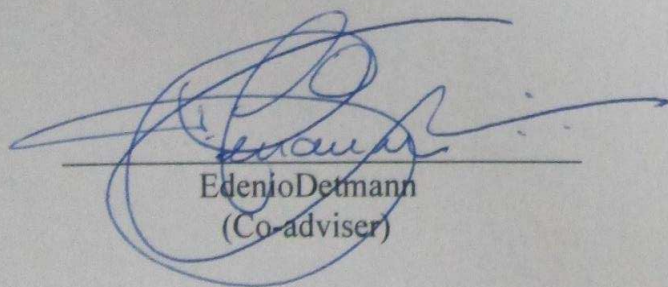
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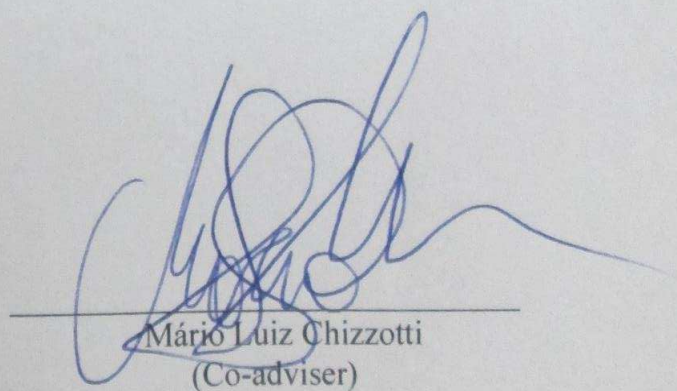
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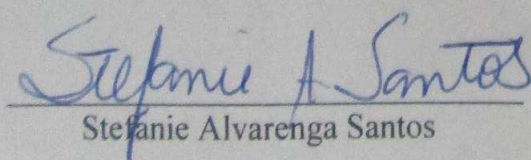
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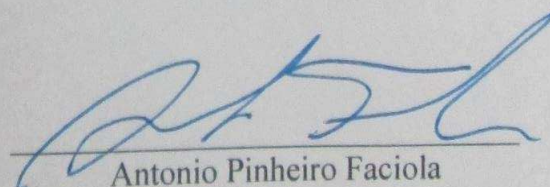
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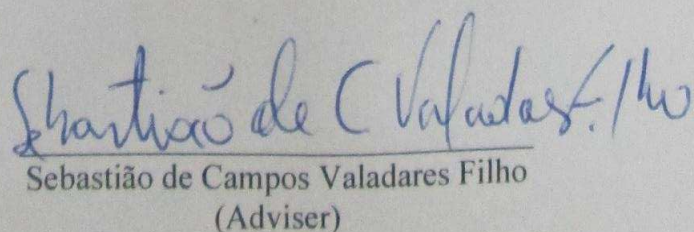
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To my parents, for dedication, love and encouragement. You are my safe haven.

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To Guilherme and Duda, my support, happiness and strength!

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I dedicate.

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“Strength doesn’t come from what you can do. It comes from overcoming the things you once
thought you couldn’t.”
Rikki Rogers

BIOGRAPHY

Paloma de Melo Amaral, daughter of José Abílio Lopes Amaral and Maria José de Melo Amaral, was born in Rio de Janeiro/RJ-Brazil on Feb 24, 1986.

Started the undergraduate in Animal Science at Universidade Federal de Viçosa in 2005 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 February 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 Novembre of 2014 to August of 2015, she was a visiting research at University of Nevada – Reno/NV-USA, where part of her research was developed. On September of 2016, Paloma de Melo Amaral submitted her thesis to the committee to obtain the Doctor Scientiae degree in Animal Science.

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ABSTRACT

AMARAL, Paloma de Melo, D.Sc., Universidade Federal de Viçosa, September, 2016. **Performance and amino acids requirements of Nellore and Crossbred Angus×Nellore fed diets with different crude protein content during growing and finishing stages.** Adviser: Sebastião de Campos Valadares Filho. Co-Advisers: Edenio Detmann and Mário Luiz Chizzotti.

An experiment was conducted to evaluate whether a reduction in dietary crude protein and genetic group (GG) affects animal performance, feed efficiency and carcass traits of beef cattle at different stages of feedlot. Forty-four animals (22 Nellore and 22 crossbred F1 Angus x Nellore), with 8 months and initial shrunk BW (SBW) = 218.6 ± 2.15 kg (Nellore = 211.5 kg; Angus x Nellore = 225.7 kg) were used in this experiment. Eight animals (4 from each genetic group) slaughtered at the beginning of the experiment were used as baseline reference to estimate initial chemical body composition. The 36 remaining bulls were randomly assigned to one of three dietary treatments. The experiment was conducted in a completely randomized design with six replicates, in a 2x3 factorial scheme. The factors were two genetic groups (Nellore and crossbred F1 Angus x Nellore - A×N) and three crude protein contents (CP) (100, 120 and 140 g/kg DM). The experimental period lasted 224 days, being divided in 2 growing stages (GS; Growing (GR) = 112 days, and Finishing (FS) = 112 days). At the end of the experiment, all animals were slaughtered to evaluate their chemical body composition, and carcass characteristics. Crossbred A×N had a higher DMI (%BW) (19.28) compared to Nellore (18.26), and also higher ADG (A×N = 1.18; Nellore = 0.88 kg/d), regardless of growth stage or CP. Crossbred A×N had a higher intake of all nutrients regardless the CP or growth stage. Crossbred A×N ended up the experiment heavier than Nellore, since they showed a greater ADG and were more efficient when compared to Nellore animals. DMI did not differ ($P > 0.05$) among CP contents within growth stages, however it increased over time. Animals fed 14% CP had a lower DMI (g/BW) during growing stage, however differences were vanished on finishing stage.

During growing stage, animals fed 10% CP had a lower ADG compared with animals fed 12 and 14% CP, which did not differ between them. However, during finishing stage there was no difference among CP on ADG. Differences among CP regarding efficiency were also eliminated during finishing stage. Our study found that crossbred animals have, not only greater performance, but also show better carcass traits compared to Nellore, representing an option to increase productivity. We also found that it is possible to adjust diets according to feedlot stage. During the growing stage, 12% of CP should be used, being reduced to 10% during finishing stage of feedlot, without adversely affects performance or carcass characteristics. A second trial aimed to evaluate amino acids requirements for beef cattle. It is expected to work with AA instead of CP requirements in order to provide the correct amount of essential AA to meet maintenance and production requirements. Despite the advances in determining microbial protein synthesis and AA digestibility in small intestine (Rutherford et al., 2016), lesser advances were done in intermediate metabolism of AA and especially in net requirements of AAs (Tedeschi et al., 2015). Furthermore, the use of crossbred animals has been increasingly, however, there is no data in the literature comparing AAs requirements between GG, or correlating efficiency with amino acids requirements. Therefore, the objective of this study was to evaluate the retention of AA in the empty body weight and also to determine net requirements of AA in purebred (Nellore) and crossbred (A×N) bulls, fed different crude protein content. Fifty-two bulls, (26 Nellore and 26 crossbred F1 Angus x Nellore) with 8 months and initial shrunk BW (SBW) = 215.0 ± 15.0 kg (Nellore = 208.0 ± 12.78 kg; Angus x Nellore = 221.9 ± 14.16 kg) were used in this experiment. Eight animals (4 from each genetic group) slaughtered at the beginning of the experiment were used as baseline reference to estimate initial chemical body composition. The 36 remaining bulls were randomly assigned to one of three

dietary treatments. The experiment was conducted in a completely randomized design with six replicates, in a 2x3 factorial scheme. The factors were two genetic groups (Nellore and crossbred F1 Angus x Nellore - A×N) and three crude protein contents (CP) (100,120 and 140 g/kg DM).The experimental period lasted 224 days. At the end of the experiment, all animals were slaughtered to evaluate their amino acid composition in the body and also the net requirements of amino acids for gain. In the present study it is observed that some AA as lysine and phenylalanine have high correlation with muscle, however, other amino acids (e.g. methionine) have a stronger correlation with body's composition. It is worth mentioning that this behavior is not constant among different weights, showing that more studies like this need to be conducted to determine more precisely the net requirements of amino acids.A third experiment was divided in two trials. The objective of the first trial was to the nutritional requirements of protein and energy for maintenance and weight gain and the efficiencies of use of metabolizable energy for maintenance and weight gain using the comparative slaughter technique. In the second trial, the net energy requirements for maintenance were also estimated using the heat production measured in a respirometric chamber. In trial 1, 52 animals (26 Nellore and 25 Angus × Nellore), with 8 months and initial body weight = 215.0 ± 15.08 kg (Nellore = 208.0 ± 12.78 kg; F1 Angus x Nellore = 221.9 ± 14.16 kg). The experiment was conducted in a completely randomized design with six replicates, in a 2×3 factorial scheme. The factors were two genetic groups (Nellore and cross-breed F1 Angus × Nellore - A × N) and three crude protein contents (CP, 100, 120 and 140 g / kg DM). The animals selected for voluntary consumption were randomly assigned to one of three dietary treatments. The animals in maintenance were fed with 12g DM/kg BW, with the diet of 12% CP on the DM basis. The animals assigned to the reference group were slaughtered for evaluation of initial empty body weight (EBW) and initial chemical composition of EBW. At

the end of 225 days the animals were slaughtered. Trial 2 was carried out at the Federal University of Minas Gerais, where the production of heat (PC) and methane from eight animals (4 Nellore and 4 Crusaders Angus × Nellore) were measured in a respirometric chamber. Soon after, these same animals returned to the UFV, where a digestibility experiment was conducted using a 4 × 4 Latin Square. The relationship between SBW and EBW found in this study was 0.925. There was a difference ($P < 0.05$) between GG for the ADG x EBWG ratio, with an average ratio of 0.966 for Nellore animals and 0.947 for crossbred animals. The value found for NEm was 74.6 Kcal / PCVZ $^{0.75}$ / day, with no difference between the GGs. The MEm found for Nellore animals was 122 kcal / EBW $^{0.75}$ / day and 119 kcal / EBW $^{0.75}$ / day for crossbred animals. There was no difference between GG ($P > 0.05$) for NEg, and the equation was: RE = 0.0643 x EBW $^{0.75}$ x EBWG $^{0.6191}$. The conversion efficiencies of NEm to MEm of Nellore and crossbred animals in this experiment were, respectively, 61.1 and 62.7%. The equation obtained to estimate the net protein requirement for gain was: RP = 188.37 x EBWG - 9.39 x RE. The efficiency of use of the metabolizable protein for gain (k) was 0.3302. In trial 2, apparent digestibilities of nutrients did not differ ($P > 0.05$) among GG. Likewise, there was no difference for apparent digestibilities of nutrients ($P > 0.05$) when comparing restricted and voluntary consumption. CPI increased linearly ($P < 0.05$) with CP levels. There was a linear effect of dietary levels of CP ($P < 0.05$) on the apparent digestibilities of DM and OM. An NEm of 85.2 kcal / EBW $^{0.75}$ / day and an MEm of 114 kcal / EBW $^{0.75}$ / day were obtained using the respirometric chamber, these values being higher and lower, respectively, than those obtained by the comparative slaughter.

RESUMO

AMARAL, Paloma de Melo, D.Sc., Universidade Federal de Viçosa, setembro de 2016. **Desempenho e exigências de aminoácidos de bovinos Nelore e cruzados Angus × Nelore alimentados com dietas contendo diferentes teores de proteína bruta durante os estágios de crescimento e terminação.** Orientador: Sebastião de Campos Valadares Filho. Coorientadores: Edenio Detmann e Mário Luiz Chizzotti.

Um experimento foi realizado para avaliar se uma redução na proteína bruta na dieta e grupo genético (GG) afetam o desempenho animal, a eficiência alimentar e as características de carcaça de bovinos de corte em diferentes fases de confinamento. Quarenta e quatro animais (22 Nelore e 22 mestiças F1 Angus x Nelore), com 8 meses de idade e peso corporal em jejum (PCj) inicial = $218,6 \pm 2,15$ kg (Nelore = 211,5 kg; Angus x Nelore = 225,7 kg) foram utilizados neste experimento. Oito animais (4 de cada grupo genético) foram abatidos no início do experimento, tendo sido utilizados como base de referência para estimar a composição química corporal inicial. Os 36 touros restantes foram distribuídos aleatoriamente a um dos três tratamentos. O experimento foi conduzido em delineamento inteiramente casualizado, com seis repetições, em esquema fatorial 2x3. Os fatores foram dois grupos genéticos (Nelore e cruzados F1 Angus x Nelore - A × N) e três teores de proteína bruta (PB) (100, 120 e 140 g / kg MS). O período experimental foi de 224 dias, sendo dividido em 2 fases (Crescimento = 112 dias, e Terminação = 112 dias). No final do período experimental, os animais foram sacrificados para avaliar a sua composição química corporal, e as características da carcaça. Mestiços A × N apresentaram um CMS (%PV) superior (19,28) em relação ao Nelore (18,26), e também maior GMD (A × N = 1,18; Nelore = 0,88 kg / d), independentemente do estágio de crescimento ou PB. Mestiços A × N tinham uma maior ingestão de todos os nutrientes, independentemente do PB ou da fase de crescimento. Mestiços A × N terminaram o experimento mais pesados do que Nelore, uma vez que apresentaram maior GMD e foram mais eficientes quando comparados com animais da raça

Nelore. CMS não diferiu ($P>0,05$) entre os PB dentro de estágios de crescimento, no entanto, aumentou ao longo do tempo. Animais alimentados com 14% PB tiveram um CMS (%PV) inferior durante a fase de crescimento, no entanto, diferenças foram desaparecendo durante a terminação. Durante fase de crescimento, os animais alimentados com 10% PB tiveram um GMD menor em comparação com os animais alimentados com 12 e 14% PC, que não diferiram entre si. No entanto, durante a terminação, não houve diferença entre PB em GMD. As diferenças entre PB em matéria de eficiência também foram eliminadas durante a fase de terminação. Nosso estudo mostra que os animais mestiços têm, não só um maior desempenho, mas também apresentam melhores características de carcaça em relação ao Nelore, representando uma opção para aumentar a produtividade. Nós também acreditamos que seja possível ajustar as dietas de acordo com a fase de confinamento. Durante a fase de crescimento, 12% da PB deve ser usado, sendo reduzido para 10% durante a fase de terminação do confinamento, sem afetar o desempenho ou as características de carcaça. Um segundo experimento teve como objetivo avaliar as exigências de aminoácidos para bovinos de corte. Espera-se poder trabalhar com exigência de AA e não de PB, a fim de proporcionar a quantidade correta de AA essenciais para satisfazer os requisitos de manutenção e de produção. Apesar dos avanços na determinação de síntese de proteína microbiana edigestibilidade dos AA no intestino delgado (Rutherford et al., 2016), os avanços menores foram feitas no metabolismo intermediário de AA e especialmente em exigências líquidas de AAs (Tedeschi et al., 2015). Além disso, a utilização de animais cruzados tem sido cada vez mais, no entanto, não há dados na literatura comparando requisitos AAs entre GG, ou correlacionando a eficiência com as exigências de aminoácidos. Portanto, o objetivo deste estudo foi avaliar a proporção e a retenção dos AA na carcaça, bem como determinar as exigências líquidas de AA em touros Nelore e

cruzados A × N, alimentado com dietas contendo diferentes níveis de proteína. Cinquenta e dois touros, (26 Nelore e 26 mestiças F1 Angus x Nelore), com 8 meses e peso corporal em jejum (PCj) inicial = $215,0 \pm 15,0$ kg (Nelore = $208,0 \pm 12,78$ kg; Angus x Nelore = $221,9 \pm 14,16$ kg) foram utilizados neste experimento. Oito animais (4 de cada grupo genético) foram abatidos no início do experimento, e utilizados como base de referência para estimar a composição química corporal inicial. Os 36 touros restantes foram distribuídos aleatoriamente a um dos três tratamentos. O experimento foi conduzido em delineamento inteiramente casualizado, com seis repetições, em esquema fatorial 2x3. Os fatores foram dois grupos genéticos (Nelore e cruzados F1 Angus x Nelore - A × N) e três teores de proteína bruta (PB) (100, 120 e 140 g / kg MS). O período experimental foi de 224 dias. Ao final do experimento, todos os animais foram abatidos para avaliar sua composição corporal de aminoácidos e as exigências líquidas de aminoácidos para ganho. No presente estudo, observa-se que alguns AA como lisina e fenilalanina tem alta correlação com o músculo, no entanto, outros aminoácidos (por exemplo, metionina) têm uma forte correlação com a composição do corpo. Vale ressaltar que este comportamento não é constante entre os diferentes pesos, mostrando que mais estudos como este precisam ser realizados para determinar com maior precisão as necessidades líquidas de aminoácidos para bovinos de corte. Um terceiro experimento foi conduzido com o objetivo de avaliar as exigências nutricionais de proteína e energia para manutenção e ganho de peso e as eficiências de utilização da energia metabolizável para manutenção e ganho de peso, usando a técnica do abate comparativo, e, ainda, estimar as exigências líquidas de energia para manutenção utilizando-se a produção de calor mensurada em câmara respirométrica. Para avaliação da exigência pelo método do abate comparativo, foram utilizados 52 bovinos machos, não castrados, com idade de média de 8 meses e peso médio inicial de $215,0 \pm 15,08$ kg (Nelore = $208,0 \pm 12,78$ kg; F1 Angus x Nelore =

221,9±14,16 kg). O experimento foi conduzido em delineamento inteiramente casualizado com seis repetições, em esquema fatorial 2x3. Os fatores foram dois grupos genéticos (Nelore e cruzado F1 Angus × Nelore - A × N) e três conteúdos de proteína bruta (PB) (100, 120 e 140 g/kg MS). Os animais selecionados para consumo voluntário foram redistribuídos aleatoriamente em três grupos que receberam uma das 3 dietas experimentais. Os animais em manutenção foram alimentados com 12g de MS por kg de peso corporal, com a dieta de 12% de PB na base da MS. Os animais designados ao grupo referência foram abatidos para avaliação do peso PCVZ inicial e da composição química inicial do PCVZ. Ao final de 225 dias os animais foram abatidos. Para a avaliação em câmara respirométrica, oito animais (4 Nelores e 4 cruzados Angus × Nelore) foram enviados para a Universidade Federal de Minas Gerais, onde, a produção de calor (PC) e de metano foram mensuradas. Logo após, esses mesmos 8 animais voltaram para a UFV, onde um experimento de digestibilidade foi conduzido utilizando um QL 4 × 4. A relação entre PCJ e o PCVZ encontrada neste estudo foi de 0,925. Houve diferença (P < 0,05) entre GG para a relação GMD x GPCVZ, sendo obtida a relação média de 0,966 para animais Nelore, e 0,947 para animais cruzados. O valor encontrado para Elm foi de 74,6 Kcal/PCVZ^{0,75}/dia, não havendo diferença entre os GG. A EMm encontrada para animais Nelore foi de 122 kcal/PCVZ^{0,75}/dia e de 119 kcal/PCVZ^{0,75}/dia para animais cruzados. Não houve diferença entre os GG (P > 0,05) para ELg, sendo obtida a equação: ER = 0,0643 x PCVZ^{0,75} x GPCVZ^{0,6191}. As eficiências de conversão da ELm para EMm de animais Nelore e cruzados neste experimento foram, respectivamente, 61,1 e 62,7%. A equação obtida para estimar a exigência líquida de proteína para ganho foi: PR = 188,37 x GPCVZ – 9,39 x ER. A eficiência de utilização da proteína metabolizável para ganho (k) foi de 0,3302. No experimento 2, as digestibilidades aparentes dos nutrientes não diferiram (P > 0,05) entre os GG. Da mesma forma, não houve diferença para as

digestibilidades aparentes dos nutrientes ($P > 0,05$) quando se comparou consumo restrito e voluntário. O CPB aumentou linearmente ($P < 0,05$) com os níveis de PB. Houve efeito linear dos níveis dietéticos de PB ($P < 0,05$) sobre as digestibilidades aparentes da MS e MO. Uma Elmde $85,2 \text{ kcal/PCVZ}^{0,75}/\text{dia}$ e uma EMm de $114 \text{ kcal/PCVZ}^{0,75}/\text{dia}$ foram obtidas com a utilização da câmara respirométrica, sendo esses valores superior e inferior, respectivamente, aos obtidos pelo abate comparativo.

GENERAL INTRODUCTION

Beef cattle production is an important activity occupying a prominent position in the Brazilian agribusiness. It creates direct and indirect jobs, playing an important role in the country's economy. Production costs are rising, forcing the producer to become more competitive and sustainable. Furthermore, it is known that nutrition is a costly item in livestock production, explaining the attempt to improve the system's efficiency.

Protein is an expensive nutrient of beef cattle nutrition, therefore, attention should be paid to crude protein requirements. In general, ruminant animals convert about 20-30% of their dietary nitrogen (N) into animal protein, and about 70-80% is excreted in the urine and feces (Doranali et al., 2011). Excessive use of N is quite common in practice (Millen et al., 2009) due to positive responses in animal performance associated with increased dietary N (Cole, 2003; Luden et al., 2003), and to the risk of losing production. This practice leads to economic and environmental implications.

Balanced diets that meet protein requirements during the different growth stages, is a way to ensure that N excess will not be excreted into the environment. Nitrogen losses in urine were 24-50 % greater in animals receiving diet with 13% rather than 11.5 % CP (Cole et al., 2005; Todd et al., 2006). Furthermore, it is expected that the CP intake reduction can be compensated by recycling N into the rumen (Cole et al., 2012).

Furthermore, Nutritional models for feeding protein have evolved from basic CP (NRC, 1978; ARC, 1980) to more complex systems based on rumen-degradable and rumen-undegradable protein (INRA, 1988; NRC, 1985, 1989; AFRC, 1992; NRC, 2001). However, amino acids (AA), and not protein per se, are the required nutrient. Thus, to ensure a more

accurate nutrition, it is expected to work with AA instead of CP requirements in order to provide the correct amount of essential AA to meet maintenance and production requirements.

Microbial crude protein synthesis in the rumen provides the majority of protein supplied to the small intestine of ruminants, accounting for 50 to 80% of total metabolizable protein (Bach et al., 2005). The more similar is the profile of essential AA available for absorption in the small intestine to the animal's requirement, the higher will be the AA usage efficiency for protein synthesis (NRC, 2001). However, the unique features of the intermediate metabolism, in addition to the complex feed transformation during ruminal fermentation, and the difficulties to determine the AA available for absorption in the duodenum, pose a challenge to formulate diets based on AA requirements for cattle (Silva et al., 2002).

Due to these challenges, there is a deficiency of AA requirement data in the literature, especially for beef cattle. However, with the increased use of protected AA, there is a growth in this research field, and, consequently, a greater understanding of the microbial protein modeling (NRC, 2016) and the amino acids digestibility. Thus, with this advances in research, it is expected to associate the absorption of amino acids in the small intestine with the retention of amino acids on the carcass. However, despite the advances in evaluating microbial protein synthesis, and AA digestibility in small intestine (Rutherford et al., 2016), lesser advances were obtained in understanding the intermediate metabolism of AA and, especially, net requirements of AAs (Tedeschi et al., 2015).

The Brazilian herd consists mostly by Zebu animals, however the use of crossbred animals has been increasingly common, aiming to optimize performance and productivity of beef production system. European breeds with greater growth potential, could be an alternative to reduce feedlot period and increase profits.

Studies in tropical conditions have shown that there are differences in the use of feed and productive responses between genetic groups (GG), and greater performances have been observed in crossed animals compared to zebu (Marcondes et al., 2011). However, there is no data in the literature comparing AAs requirements between GG, or correlating efficiency with amino acids requirements.

Thus, we consider the possibility of adopting feeding systems that adjust diets according to the animal's growth stage, since it is known that the animal requirements change over time. Working with different dietary CP content (CP), according to the growth stage, could reduce CP content of beef cattle diets, without adversely affecting animal performance, resulting in reduction of CP intake and excreted N compounds to the environment. We also aimed to evaluate the proportion and retention of AA in carcass in order to determine net requirements of AA in purebred (Nellore) and crossbred (Angus × Nellore) bulls, fed different protein content. The responses obtained from this study will provide valuable data, aiming to reducing costs and minimizing environmental damage.

The industrial crossing is an alternative to optimize the performance and to improve beef cattle performance. This breeding management leads to animals with high performance potential and a greater resistance to parasites, compared with animals of European races. However, there is a lack of understanding on crossbred requirements. The importance in determining the nutritional requirements of beef cattle in Brazil is to obtain more information about the reality of our herd and provide appropriate information to its conditions, especially those present in temperate countries. Working with appropriate information leads to economic and quality impact on meat production system.

Considering the need for a nutritional requirements system for bovines under such conditions (Valadares Filho et al., 2010) published a second edition of the Brazilian Tables of Nutritional Requirements of Zebuínos (BR-CORTE). Its database uses animals with similar characteristics to those that compose the Brazilian her.

However, few studies were carried out to determine the nutritional and energy requirements for animals coming from industrial crossings (F1 Angus x Nellore). According to the BCNRM (2016), protein requirements decrease at the feedlot period, corroborating with Menezes et al. (2016) and Amaral et al. (2014). However, commercial feedlots are using high crude protein levels in order to reduce the confinement period. Millen et al. (2014) reported that an average crude protein level (on DM basis) recommended for finishing diets is 13.4%. BR-CORTE (2010) recommends for cattle with 450kg of body weight, approximately 1300 g of CP for one kg of body weight gain. Considering the average DM intake of 10 kg per day, it represents a diet with 13% CP. However, analyzing the CP intake data, of experiments performed under tropical conditions, it can be observed that in general there is an excess of dietary CP. This can be verified by comparing the CP intake observed in the experiments with the CP intakes predicted by the BR-CORTE system. Thus, it can be inferred that the values predicted by BR-CORTE (2010) need to be adjusted, and it is considered the possibility of adopting food systems that reduce dietary crude protein contents without compromising animal performance. This, would result in a reduction in the amount of crude protein ingested and nitrogen compounds excreted into the environment, representing a technological option to achieve the desired performance, with lower production costs and minimizing environmental damage.

For energy requirements calculations for beef cattle, the comparative slaughter method has been used, where the heat production is not directly measured but obtained by the difference between the metabolizable energy consumption and the energy retained in the empty body, which are obtained directly. Estimating the heat production ($PC = CEM - ER$), it becomes possible to perform the calculations referring to the energy requirements for maintenance. However, the method of comparative slaughtering is costly and laborious. The use of respirometric chambers, direct or indirect, is an alternative to obtain the necessary data for the estimation of the energy requirements for maintenance. Differently from the comparative slaughter method, metabolizable energy consumption and the production of heat (directly or indirectly) are determined by gas exchange, obtaining the energy retained by difference.

Thus, the objective of this work was to determine the nutritional requirements of protein and energy for maintenance and weight gain, the efficiencies of use of metabolizable energy for maintenance and weight gain, and also the net energy requirements to maintain using (Nelore) and crossbred (F1 Angus x Nelore) calves were submitted to a feedlot.

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CHAPTER 1

Do dietary protein content and genetic group affect performance, feed efficiency and carcass traits of beef cattle at different stages of feedlot?

Abstract

An experiment was conducted to evaluate whether a reduction in dietary crude protein and genetic group (GG) affects animal performance, feed efficiency and carcass traits of beef cattle at different stages of feedlot. Forty-four animals (22 Nellore and 22 crossbred F1 Angus x Nellore), with 8 months and initial shrunk BW (SBW) = 218.6 ± 2.15 kg (Nellore = 211.5 kg; Angus x Nellore = 225.7 kg) were used in this experiment. Eight animals (4 from each genetic group) slaughtered at the beginning of the experiment were used as baseline reference to estimate initial chemical body composition. The 36 remaining bulls were randomly assigned to one of three dietary treatments. The experiment was conducted in a completely randomized design with six replicates, in a 2x3 factorial scheme. The factors were two genetic groups (Nellore and crossbred F1 Angus x Nellore - A×N) and three crude protein contents (CP) (100, 120 and 140 g/kg DM). The experimental period lasted 224 days, being divided in 2 growing stages (GS; Growing (GR) = 112 days, and Finishing (FS) = 112 days). At the end of the experiment, all animals were slaughtered to evaluate their chemical body composition, and carcass characteristics. Crossbred A×N had a higher DMI (%BW) (19.28) compared to Nellore (18.26), and also higher ADG (A×N = 1.18; Nellore = 0.88 kg/d), regardless of growth stage or CP. Crossbred A×N had a higher intake of all nutrients regardless the CP or growth stage. Crossbred A×N ended up the experiment heavier than Nellore, since they showed a greater ADG and were more efficient when compared to Nellore animals. DMI did not differ ($P > 0.05$) among CP

contents within growth stages, however it increased over time. Animals fed 14% CP had a lower DMI (g/BW) during growing stage, however differences were vanished on finishing stage. During growing stage, animals fed 10% CP had a lower ADG compared with animals fed 12 and 14% CP, which did not differ between them. However, during finishing stage there was no difference among CP on ADG. Differences among CP regarding efficiency were also eliminated during finishing stage. Our study found that crossbred animals have, not only greater performance, but also show better carcass traits compared to Nellore, representing an option to increase productivity. We also found that it is possible to adjust diets according to feedlot stage. During the growing stage, 12% of CP should be used, being reduced to 10% during finishing stage of feedlot, without adversely affects performance or carcass characteristics.

Introduction

Nutrition is a costly item in livestock production and protein is known to be the most expensive nutrient in beef cattle diets. However, excessive use of nitrogen (N) is quite common in feedlot, leading to economic and environmental implications. In general, ruminant animals convert about 20-30% of their dietary nitrogen (N) into animal protein, consequently about 70-80% is excreted in the urine and feces (Doranali et al., 2011). The excessive dietary N excreted may accumulate in the atmosphere, soil, and groundwater (Cole et al., 2012), being detrimental to the ecosystem. Using balanced diets that meet protein requirements during the different growing stages is a way to avoid this problem by reducing N excess excreted into the environment. Thus, we consider the possibility of adopting feed systems that adjust diets according to the animal's growth stage an essential tool to environmentally adequate systems, since it is known that the animal requirements change over time.

Moreover, the use of crossbred animals, F1 Bos taurus x Bos indicus, with greater growth potential, could be an alternative to reduce feedlot period and optimize the effects of

breed complementarity, leading to performance improvements. Trials has been conducted to evaluate the nitrogen usage efficiency in different genetic groups, however, to our knowledge, there are no data comparing these differences during different stages of feedlot.

Therefore, we hypothesize that crossbred F1 Angus x Nellore leads to performance and carcass characteristics improvements. Secondly, it is possible to reduce CP during finishing stage of feedlot, without adversely affecting animal performance and efficiency. This hypothesis will be tested by comparing Nellore vs Angus × Nellore animals, under three dietary CP contents, at two stages of feedlot (growing and finishing), by evaluating intake, performance, feed efficiency, and carcass characteristics.

Materials and methods

The experiment was carried out at the Animal Science Department of the Universidade Federal de Viçosa (Viçosa - MG). All animal care and handling followed the ethics committee guidelines of the Federal University of Viçosa (CEUAP, protocol # 05/2013).

Animals and Experimental Design

Forty-four bulls (22 Nellore and 22 crossbred F1 Angus x Nellore - A×N) were used in this study to determine the influence of dietary CP contents on growth performance, feed efficiency and carcass traits of growing and finishing cattle fed silage-corn based diets.

The bulls were approximately 8 mo of age at the beginning of the experiment and initial shrunk BW (SBW) = 218.6 ± 2.15 kg (Nellore = 211.5 kg; Angus x Nellore = 225.7 kg). All the experimental animals were submitted to a 30-day adaptation prior the beginning of the experiment. Eight animals (4 from each genetic group) were randomly selected to compose the reference group. This group was used as baseline reference of initial chemical body composition, being slaughtered after the adaptation period. The remaining 36 bulls were weighed after 16

hours of fasting and were randomly assigned to one of three dietary treatments. The experiment was conducted in a completely randomized design with six replicates, in a 2×3 factorial arrangement. The factors were two genetic groups and three crude protein contents (100, 120 and 140g CP/kg DM). Experimental diets were formulated according to the Brazilian Tables of Nutrient Requirements of Zebu Beef Cattle - BR-CORTE(Valadares Filho et al., 2010) to achieve an ADG of 1.2 kg (considering an average BW of 350 kg).

Animals were housed in individual pens, with 30m² of total area per animal being 8m² covered. The experimental period lasted 224 days, being divided in 2 growing stages (GS: Growing (GR) = 112 days, and Finishing (FS) = 112 days). Animals were weighed at the beginning and end of each GS, after undergoing 16 hours of fasting, for performance evaluation. The forage:concentrate ratio was 50:50, using corn silage as forage source. The total amount of corn silage was provided at 0700 h with half of the daily amount of concentrate, and the other half was given at 1500 h. Feed intake was adjusted to maintain the orts within 5 to 10% of the amount offered. Chemical composition and proportion of feeds in diets are shown in Table 1.

Sample processing

Feeds and orts were weighed daily, sampled, and frozen. A weekly sample (composite sample of corn silage and composite sample of orts of each animal) was oven-dried (55 °C) for 72 h and grinded at 1 and 2 mm with a Wiley mill (TECNAL Equipamentos para Laboratorio, SP, Brazil). The total DM was evaluated using a drying oven at 105 °C for 16 h. Based on the amount of DM from each animal leftover, composite samples were made for each 28 d period. Ingredients of concentrate were sampled directly from animal feed mill silos.

Digestion trials were conducted at the beginning (during growing stage) and at the end of the experiment (during finishing stage) to determine diet digestible energy (DE). Feces samples

were collected for three days at 0600 h on d 1, at 1200 h on d 2 and at 1800 h on d 3 of each digestion trial. A composite sample from each animal was created per period and they were processed as described for silage and leftovers. Indigestible neutral detergent fiber (iNDF) was used as a marker to estimate fecal DM excretion.

Chemical analysis

The composite sample for each material (silage, concentrate, orts, and feces) was used to determine the content of DM, OM, and N and EE according to the methods described by AOAC (AOAC, 2012; method number 934.01, 930.05, 981.10, and AOAC, 2006; method number 945.16, respectively). The NDF content was estimated according to Mertens et al. (2002), without the addition of sodium sulfite, using thermostable alpha-amylase to the detergent. The NDFap content was estimated according to Mertens (2002) and Licitra et al. (1996), respectively. The analyses of NDF were performed by using a fiber analyser (Ankom®200, Ankom Technology, Macedon, NY, USA). The fecal DM excretion was obtained by dividing the iNDF intake by the fecal iNDF concentration. To quantify iNDF, the fecal samples, concentrate, leftovers, and corn silage were placed in F57 bags (Ankom®) and incubated in the rumen of a cannulated animal for 288 h (Valente et al., 2015). Non-fiber carbohydrates (NFC) was calculated according to Detmann & Valadares Filho (2010), as follows: $NFC = 100 - [(\%CP - \%CP \text{ from urea} + \%urea) + \%NDFap + \%EE + \%ash]$. The total digestible nutrients (TDN) content of the diets was estimated as the sum of digestible nutrients, following the equation: $TDN (\%) = dCP + dNDF + dNFC + 2.25 dEE$, (NRC, 2001), where: dCP = digestible crude protein; dNDF = digestible neutral detergent fiber; dNFC = digestible non-fiber carbohydrates; dEE = digestible ether extract.

Carcass evaluation

Prior to the slaughter, all animals were fasted for 16 hours. The animals were randomly selected for slaughter on each day, being slaughtered six animals per day. Animals were slaughtered via captive bolt followed by bleeding. After slaughtering, the gastrointestinal tract (i.e., rumen, reticulum, omasum, abomasum, and small and large intestines) of each animal was emptied, washed, and weighed. The weight of the empty gastrointestinal tract was added to the weights of the remaining parts of the body (blood, organs, head, hide, tail, hoofs and carcasses) to determine the empty body weight (EBW).

A blood sample was obtained during the course of bleeding. The rumen, reticulum, omasum, abomasum, small and large intestines, visceral fat (renal, pelvic and cardiac fats plus mesenteric fat), liver, heart kidneys, lungs, tongue, spleen, diaphragm, esophagus, trachea, and reproductive tract were ground for 20 minutes by using an industrial cutter to create a homogeneous sample of organs and viscera. The head and limbs had their hide removed, and then were ground by using a grinding machine to reduce the bones size. The hide was sampled in multiple parts, which overall represented the entire hide.

The carcass of each animal was divided into two halves that were weighed and cooled in a cold chamber at 4 °C for 24 h. After 24 h post-mortem, carcasses were re-weighed to quantify cold carcass weight, and the subcutaneous fat thickness (SFT) was measured at the 12th rib. The right half-carcass was dissected to separate the muscle + fat from bones, and each portion was weighed. The muscle + fat of each animal were ground and homogenized to obtain a composite sample of muscle and fat proportional to their natural weight in the EBW. The bones from each animal were sliced to obtain a bone sample. All samples were lyophilized and, subsequently, ground in an industrial blender (Eberbach Model No. E8017.00) using liquid nitrogen to enable sample grinding, since the components had not been partially degraded. Upon completion of

grinding, each sampled content (blood, hide, head and hoofs, organs and viscera, muscle + fat, and bones) were proportionally taken, to build a single sample per animal, representing EBW.

Statistical analysis

All statistical procedures were performed using PROC MIXED of SAS (SAS Inst. Inc., Cary, NC) with animal being the experimental unit.

Data were analyzed in two different models, where data from growth stages were evaluated in a completely randomized design, in a repeated measures scheme, with three CP and two GG. When observed significance, a t test was used to identify differences at $P < 0.05$ and trends at $P < 0.10$. Secondly, data averaged from the entire experiment (without aspects of growth stage) were evaluated in a completely randomized design, with three CP and two GG. When observed significance, a t test was used to identify differences at $P < 0.05$ and trends at $P < 0.10$, and orthogonal contrast were used to identify linear and quadratic effects of CP when necessary.

Results

Intake

Increasing CP did not affect DMI ($P > 0.05$) during growing, nor during finishing stage, however consumption increased over time (Fig.1-A). There was a significant interaction ($P < 0.05$) between genetic group (GG) and growth stage for DMI. Independent of the growth stage, A×N had higher DMI. However, differences between crossbred and Nellore animals regarding DMI were greater during the finishing stage (Fig.2-A). In addition, A×N had also higher DMI (g/BW) (19.57) when compared to Nellore (18.24) regardless the GS or CP (Table 2). Growth stage affected DMI (g/BW) ($P < 0.05$), and a reduction in DMI (g/BW) was observed

during the finishing stage (Table 2). Finally, DMI (g/BW) was also affected by CP ($P < 0.05$). Animals fed 14% CP had a lower DMI (g/BW) when compared with animals fed 10 and 12% CP (Table 2).

In resemblance to DMI (kg/d), organic matter intake (OMI), CP intake (kg/d), NDFap intake (kg/d), and NFC intake (kg/d) were affected ($P > 0.05$) by a GG×GS interaction (Table 2). Similarly to dry matter intake, A×N had a higher intake of all nutrients regardless of CP or GS, however the differences increased during finishing stage (Fig.2-C).

There was a CP×GS interaction on CP intake (Table 2). During growing stage animals fed 10% CP had the lowest CP intake, however there was no difference ($P > 0.05$) between 14 and 12% due to low DMI of animals fed 14% CP. On finishing stage, CP intake was higher for 14%, followed by 12% and lower for 10% CP.

There was also a CP×GS interaction ($P < 0.05$) on non-fiber carbohydrates (NFC) intake. During growing stage animals fed 14% CP had the lowest NFC intake, however differences disappeared over time (Fig.1-D).

Lastly, the TDN intake of A×N animals was greater ($P < 0.001$) than Nellore animals (5.85 and 4.74 kg, respectively), and was also greater on finishing stage when compared to growing stage ($P < 0.001$). No interactions were observed in TDN intake ($P > 0.05$), also CP did not affect this variable ($P > 0.465$).

Digestibility

Dry matter (75.2%) and OM digestibility (77.6%) were not affected ($P > 0.05$) by CP, nor by GG (Table 2). However, there was a trend of higher OM digestibility during growing stage ($P = 0.054$).

The CP digestibility was affected by CP ($P < 0.001$) and GS ($P < 0.001$). The higher the CP, the higher the crude protein digestibility (Table 2), and the digestibility of CP was greater on growing stage (74.6%) when compared to finishing stage (72.0%).

The GG affected ($P < 0.013$) NDFap and NFC digestibilities ($P < 0.035$). Nellore animals had a greater NDFap digestibility compared to A×N, on the other hand, NFC digestibility behaved inversely, being greater for A×N (Table 2).

Performance and carcass traits

There was a trend of CP×GS interaction ($P < 0.055$) on ADG (Table 2). During growing stage, animals fed 10% CP had a lower ADG compared with animals fed 12 and 14% CP, which did not differ between them. However, during finishing stage there was no difference among diets (Fig.1-E).

There was also a GG effect on ADG ($P < 0.001$). Crossbred animals gained more weight compared to Nellore (1.19 and 0.89 kg/d, respectively) regardless CP or GS (Table 2).

There was triple interaction effect ($P < 0.05$) on final body weight (Fig. 3). Crossbred A×N had a higher fBW, regardless of the CP or growth stage, compared to Nellore. Nellore bulls fed 12% CP achieve the same fBW that animals fed 14% during growing and also during finishing stages. However crossbred A×N did not respond equally. The fBW of crossbred A×N fed 10% CP did not differ from animals fed 14% CP, during growing and also during finishing stage.

A CP×GS interaction was observed on EFI ($P < 0.001$). In the growing stage, animals fed 14% CP proved superior than animals receiving other CP. However, there was no differences among CP regarding EFI in finishing stage (Fig 5), thus differences in EFI among the CP disappear over time.

There was also a CP×GS interaction on EFI ($P < 0.023$). The differences between GG on efficiency reduced as CP increased. Differences among Nellore and A×N fed 10% CP regarding EFI were higher than differences on EFI observed on animals fed 12% CP. However, there were no differences between GG regarding EFI when animals were fed 14% CP.

Evaluations across the total trial

There was a GG effect on fBW ($P < 0.05$), where A×N were heavier than Nellore bulls at the end of the trial (Table 3). Crossbred animals also gained more carcass and non-carcass during the experiment compared with Nellore ($P < 0.05$).

There was a quadratic effect of CP ($P < 0.05$) on carcass gains (total and daily). Animals fed 10% CP had lowest carcass gain, compared to animals fed 12 or 14% CP (Table 3). The CP also affected total non-carcass gain ($P < 0.05$), where animals fed 12% CP gained more non-carcass content compared to animals fed 10% CP. However, there was no difference among CP ($P > 0.05$) when assessing daily non-carcass gain.

Crossbred A×N retained more protein and fat ($P < 0.05$) in their bodies compared to Nellore animals (Table 3). There was a quadratic effect of CP on retained CP ($P < 0.05$). Animals fed 10% CP retained less protein in their bodies than animals fed 12 and 14% CP (Table 3).

There was GG effect on subcutaneous fat thickness (SFT). A×N completed the experiment with a greater SFT compared to Nellore bulls.

Finally, ADG was also affected by GG ($P < 0.05$). A×N proved superior than Nellore bulls on ADG (Table 3). Average daily gain was also affected by CP ($P < 0.05$), where animals fed 10% CP had the lowest ADG, however there were no differences when comparing 12 and 14% CP (Table 3).

Discussion

Genetic groups effects and growth stage

In our study A×N had a greater DMI when compared to Nellore bulls, regardless GS, however differences were greater on finishing stage. It is known that changes in DMI are highly correlated with the body weight of the animal. Crossbred A×N started slightly heavier than Nellore (data not shown), despite being contemporary animals and coming from the same farm, which may have contributed to the highest DMI. Furthermore, the NRC (1987) and the AFRC (1993) proposed adjustment factors in DMI prediction for different breeds of beef cattle, and reported that *Bos taurus* breeds had greater potential for DMI, and that is why even when analyzing DMI as a function of BW (g/BW), crossbred A×N also presented greater values compared to Nellore bulls.

The intake (kg/d) of all analyzed nutrients were higher in A×N compared to Nellore, what can be attributed to the behavior observed for DMI (kg/d). This pattern has affected TDN intake, which was also greater to A×N compared to Nellore. The NDF_{ap} digestibility was also affected by GG. The greater DMI by crossbred animals could have affected fiber digestibility. A higher DMI promotes an increase in diet passage rate, leading to a reduction in the fiber digestibility (Scholljegerdes et al., 2004).

Crossbred animals showed a greater ADG compared to Nellore, regardless the GS or CP, justifying the increased differences regarding DMI during the finishing stage of feedlot, what had influenced directly the fBW of the animals. Crossbred A×N showed a higher fBW, regardless the GS or CP, not only because they started the trial slightly heavier, but also because they gained more weight over time.

When evaluating fBW, we see the possibility of working with different CP for different GG. Crossbred AxN fed 10% CP had the same fBW that animals fed 12 and 14% CP, both in

growth and in finishing stage. Crossbred animals had a greater DMI, therefore, regardless of dietary CP, these animals had a higher CP intake compared to Nellore animals.

However, Nellore animals fed 10% CP had lower fBW than animals fed 12 or 14% CP. Thus, for Nellore bulls, 12% CP would be recommended both the growing phase and in the finishing phase.

Crossbred A×N showed a higher efficiency than Nellore when fed diets with 10 or 12% CP. However, when the animals were fed 14% CP diets, there was no difference in GG regarding efficiency. Nellore animals had a lower DMI compared to crossbred, possibly not reaching the optimal substrate availability for microbial growth by consuming diets with lower CP (Bach et al., 2005). It is possible that by consuming a diet with a higher protein content, these animals had a greater substrate for fermentation, which would allow greater ADG, increasing efficiency.

Crossbred animals showed greater carcass and non-carcass gain (total and kg/d), however the proportion of non-carcass in the gain didn't differ between GG. This can be confirmed by carcass dressing data, when it did not differ between GG. Therefore, we suggest that the greatest gain of crossbred animals is linked to a greater carcass gain. This is even clearer when evaluating protein and fat retained on the EBW. Crossbred A×N proved superior in relation to protein and fat deposition (kg/d and %fBW), compared to Nellore, and also had greater SFT deposition. Therefore, A×N gained more weight and had more fat and protein retained in their carcass, leading to higher carcass yield. As known, crossbred animals has precocious puberty compared with Nellore animals, achieving maturity earlier, which would justify the higher fat deposition. However, even depositing more fat, AxN receiving 12 or 14% CP did not reduce efficiency and rate of protein gain, possibly stimulated by the higher amount of protein in the diet.

Crude protein levels and growth stage

Increasing CP did not affect dry matter intake during growing, nor during finishing stage. This behavior confirms the results obtained in previous studies with beef cattle fed CP ranging from 9 to 15% CP (Menezes et al, 2015; Prates et al 2015; Amaral et al, 2014.).

On the contrary, CP affected DMI (g/BW), where animals fed 14% CP had a lower DMI (g/BW) compared with animals fed 10 and 12% CP. Animals fed 14% had a greater ADG, ending up growing stage heavier than animals fed 10 and 12% CP, causing DMI (g/BW) dilution. Furthermore, variations in DMI are frequently associated with changes in nutrient intake, which may explain the absence of effect on the intake (kg/d) of OM, NDFap, and TDN. Finally, dry matter intake was greater during growing stage when compared with finishing stage. This behavior was expected, since animals reduce the consumption as they grow. Leptin is synthesized and secreted by adipose tissue, and leptin concentrations in plasma are positively correlated with the amount of body fat in cattle (Delavaud et al., 2002). Once mammals reach their mature size, most subsequent growth occurs in the form of adipose tissue deposition, increasing circulating concentrations of leptin (Geary et al., 2003). One function of leptin is to signal to the hypothalamus to reduce feed intake (Foot et al., 2015). Thus, as the animal achieves maturity (increase the amount of fat deposited in the body), a reduction in DMI is expected, which most likely occurred during finishing stage.

The lack of CP effect on CP intake during growing stage between 12 and 14% CP was due to the lower DMI (g/BW) of animals fed 14% CP. Even consuming a diet with higher protein concentration, the lowest DMI led to a dilution of the total protein consumed. On finishing stage, there were no differences on DMI (g/BW) and CP intake, being higher for 14%, followed by 12% and lower for 10% CP.

Levels and sources of crude protein in the diet may explain the variations in the responses on digestibility of nutrients reported in the literature (Dung et al., 2014). However, there was no effect of CP on DM and OM digestibilities. Similar results were observed by Menezes et al. (2016) and Silva et al. (2005), where increasing dietary CP on finishing Nelore cattle did not influence intake and nutrient digestibility.

The CP digestibility was affected by CP. The higher the protein level, the higher CP digestibility. Increased dietary protein level leads to a metabolic fecal nitrogen dilution. In other words, there is a reduced participation of endogenous fraction (Broderick, 2003). Furthermore, by increasing CP, there is a consequent increase in consumption of more digestible nutrients.

The interaction between CP and GS ($P < 0.055$) on ADG could be explained by the lower DMI (g/BW) of animals fed 14% CP during the growing stage. The DMI (g/BW) affected ADG, explaining the lack of differences on ADG between animals fed 12 and 14% CP during growing stage. However, during finishing stage, where DMI (g/BW) did not differ among CP, there was no difference on ADG.

Differences in efficiency among the CP disappear over time. In the growing stage, animals fed 14% CP proved superior than animals receiving other CP, since they had a greater ADG (numerically) even having had a lower DMI (g/BW). During the growing stage, protein retention is greater than fat retention, and as protein retains more water in the gain than fat (ARC, 1980), it leads to a higher efficiency compared to finishing stage, when animals are closest to maturity, and start retaining more fat on their carcasses.

On the finishing stage, ADG and DMI did not differ among CP, leading to same efficiencies even receiving different CP.

The carcass traits of animals fed 10% CP were inferior (weight gain, SFT, dressing) to animals fed 12 and 14% CP. Based on these results, the supply of diets with 10% CP does not allow animals to have their best performance and, consequently, better carcass traits. Therefore it is inadvisable to work with 10% CP from the beginning of the feedlot. It is noteworthy that in this experiment the diets with different CP were provided throughout the entire experiment. Thus, the possible responses of a reduction of CP from 12 or 14% to 10% only in the finishing stage are not yet known, and further studies are needed to evaluate the CP reduction only at the final stage to enlighten whether this reduction is detrimental in any stage of life or only during the finishing stage of feedlot..

When evaluating fBW and ADG considering the entire trial, animals fed 12% CP did not differ from animals fed 14% CP, The carcass gain (total and kg/d) of animals fed 12% CP did not differ from animals fed 14% CP, as well as the retained CP (kg/d). Furthermore, the non-carcass gain also did not differ between animals fed 12 and 14%, as well as dressing percentage, subcutaneous fat thickness or retained fat, Suggesting that the supply of 12% crude protein, from the beginning of the feedlot, leads to the same fBW results as feeding 14% CP during the entire feedlot stage.

Conclusion

Crossbred animals (F1Angus x Nellore) have, not only greater performance, but also show better carcass traits compared to Nellore, representing an option to increase productivity.

We also concluded that it is possible to adjust diets according to GSs. During the growing stage, 12% of CP should be used, being reduced to 10% during finishing stage of feedlot without adversely affecting performance or carcass characteristics. This feed management could, not only have a great impact in economic issues, but also reduce the environmental damage caused by livestock system.

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Table 1 – Proportion of feeds and chemical composition of diets

Proportion of feeds in diets (% of diet DM)			
	<u>Diet 10%</u>	<u>Diet 12%</u>	<u>Diet 14%</u>
Corn silage	50.0	50.0	50.0
Corn grain	39.7	39.6	39.6
Wheat meal	5.9	3.0	0.0
Mix ¹	2.2	5.3	8.3
Sodium chloride	0.5	0.5	0.5
Mineralized salt ²	0.5	0.5	0.5
Sodium bicarbonate	0.8	0.8	0.8
Magnesium oxide	0.3	0.3	0.3
Chemical composition (% of diet DM)			
Items	<u>10% CP</u>	<u>12% CP</u>	<u>14% CP</u>
DM	55.5	55.6	55.7
OM	95.4	94.9	94.4
CP	9.9	12.1	14.2
RDP ³	66.8	69.6	71.6
EE ⁴	4.0	4.0	4.0
NDF _{ap} ⁵	31.4	30.7	29.9
NFC ⁶	50.7	49.4	48.2
TDN (%) ⁷	80.2	79.3	79.7
DE ⁸	3.47	3.45	3.50

¹Mix = 83,3% Soybean meal and 16,7% of urea/ammonia sulphate (U/AS); ²Mineralized salt = 223 g/kg of Ca; 174 g/kg of P; 24 g/kg of S; 100 mg/kg of Co; 1.250,0 mg/kg of Cu; 1.795,0 mg/kg of Fe; 90 mg/kg of I; 2.000,0 mg/kg of Mn; 15,00 mg/kg of Se; 5.270,00 mg/kg of Zn and 1.740,00 mg/kg de F. ³Rumen degradable protein; ⁴EE = Ether extract; ⁵NDF_{ap} = Neutral detergent fiber corrected to ash and protein; ⁶NFC = Non-fiber carbohydrates; ⁷TDN(%) = Total digestible nutrients; ⁸DE = Digestible energy .

Table 2 – Intake and performance of Nellore and crossbred Angus x Nellore fed different crude protein levels during growing and finishing stages

Item ¹	GG ²		CP			Growth Stage ³		SEM	p-value ⁴						
	Nel	A×N	10%	12%	14%	GR	FS		CP	GG	GS	CP×GG	CP×GS	GG×GS	CP×GG×GS
Intake															
DM,kg	5.72	6.90	6.20	6.45	6.29	5.42	7.21	0.337	0.714	<.001	<.001	0.459	0.097	<.001	0.117
DM, g/BW	18.24	19.57	19.33a	19.34a	18.05b	19.67	18.14	0.547	0.012	0.001	<.001	0.153	0.124	0.811	0.105
OM, kg	5.45	6.57	5.94	6.14	5.94	5.16	6.86	0.320	0.736	<.001	<.001	0.451	0.105	<.001	0.116
CP, kg	0.71	0.84	0.64	0.80	0.89	0.68	0.87	0.042	<.001	<.001	<.001	0.686	<.001	<.001	0.202
NDF _{ap} , kg	1.73	2.09	1.85	1.93	1.94	1.62	2.20	0.097	0.552	<.001	<.001	0.217	0.989	0.003	0.126
NFC, kg	2.84	3.45	3.25	3.24	2.95	2.72	3.57	0.177	0.162	<.001	<.001	0.731	0.017	<.001	0.107
TDN, kg	4.74	5.85	5.08	5.42	5.38	4.86	5.73	0.331	0.465	<.001	<.001	0.871	0.571	0.107	0.375
Digestibility															
DM, %	75.3	75.2	75.4	75.2	75.1	75.3	75.1	1.01	0.936	0.894	0.666	0.744	0.898	0.691	0.124
OM, %	77.5	77.7	77.8	77.5	77.5	78.0	77.2	0.96	0.913	0.816	0.054	0.637	0.848	0.926	0.291
CP, %	73.4	73.2	70.6c	73.7b	75.6a	74.6	72.0	1.02	<.001	0.691	<.001	0.714	0.785	0.867	0.159
NDF _{ap} , %	67.0	64.2	65.2	64.5	67.1	66.3	64.9	1.72	0.117	0.013	0.165	0.371	0.813	0.758	0.100
NFC, %	84.9	86.5	86.7	85.7	84.8	85.4	86.0	1.10	0.136	0.035	0.233	0.640	0.661	0.783	0.396
Performance															
fBW,kg	362.7	425.5	373.0	396.7	412.5	335.4	452.8	13.39	0.022	<.001	<.001	0.606	0.306	<.001	0.042
ADG,kg	0.89	1.19	0.94	1.10	1.09	1.04	1.05	0.071	0.015	<.001	0.741	0.274	0.055	0.484	0.365
EFI,kg/kg	0.16	0.18	0.15	0.17	0.18	0.19	0.14	0.007	<.001	<.001	<.001	0.023	<.001	0.563	0.880

¹fBW = final body weight; EFI = efficiency;

²GG = genetic group; Nel = Nellore; A×N = crossbred Angus × Nellore;

³GS = growth stage, GR = growing stage; FS = finishing stage;

⁴P = CP level effect; GG = genetic group effect; S = growth stage effect; PxGG = interaction effect between CP level and genetic group; PxS = interaction effect between CP level and growth stage; GG×GS = interaction effect between genetic group and growth stage; PxGG×S = interaction effect among CP level, genetic group and growth stage.

Tabela 3: Carcass characteristics of Nellore and crossbred Angus x Nellore fed different crude protein levels

Item	GeneticGroup		CP			SEM	Main effect ¹			Contrast ²	
	Nel	A×N	10%	12%	14%		CP	GG	CP×GG	L	Q
fBW,kg	412.1	493.4	429.7b	458.3ab	470.4a	14.51	0.032	<.001	0.336	0.011	0.504
Total Carcassgain,kg	129.5	171.7	136.0b	159.5a	156.2a	7.73	0.011	<.001	0.248	0.173	0.048
Total Non-carcass gain,kg	64.2	81.6	66.8b	77.4a	74.6ab	3.84	0.029	<.001	0.436	0.057	0.052
Carcassgain,kg/d	0.58	0.77	0.61b	0.71a	0.70a	0.034	0.011	<.001	0.248	0.173	0.048
Non-carcass gain,kg/d	0.28	0.36	0.30	0.34	0.33	0.017	0.052	<.001	0.558	0.062	0.103
CP onfEBW, %	16.5	16.5	16.1	16.9	16.5	0.41	0.163	0.969	0.335	0.328	0.100
EE onfEBW, %	21.8	23.2	22.0	23.1	22.3	1.09	0.538	0.125	0.592	0.773	0.286
Retained CP, kg/d	0.136	0.178	0.139b	0.172a	0.160a	0.009	0.005	<.001	0.708	0.043	0.008
Retainedfat, kg/d	0.246	0.337	0.266	0.313	0.295	0.028	0.272	<.001	0.682	0.331	0.196
Dressing,%	64.2	64.9	64.1	64.8	64.7	0.45	0.283	0.065	0.461	0.235	0.288
SFT ³ , mm	4.86	7.43	5.84	6.71	5.89	0.979	0.606	0.003	0.517	0.957	0.322
ADG,kg	0.90	1.19	0.94b	1.10a	1.09a	0.053	0.009	<.001	0.204	0.011	0.059
Total Efficiency, kg/kg	0.158	0.177	0.152	0.169	0.180	0.004	<.001	<.001	0.023	<.001	0.338

¹CP = Crude protein content; GG = genetic group;

²L = Linear effect of CP level; Q = quadratic effect of CP level;

³SFT = Subcutaneous fat thickness.

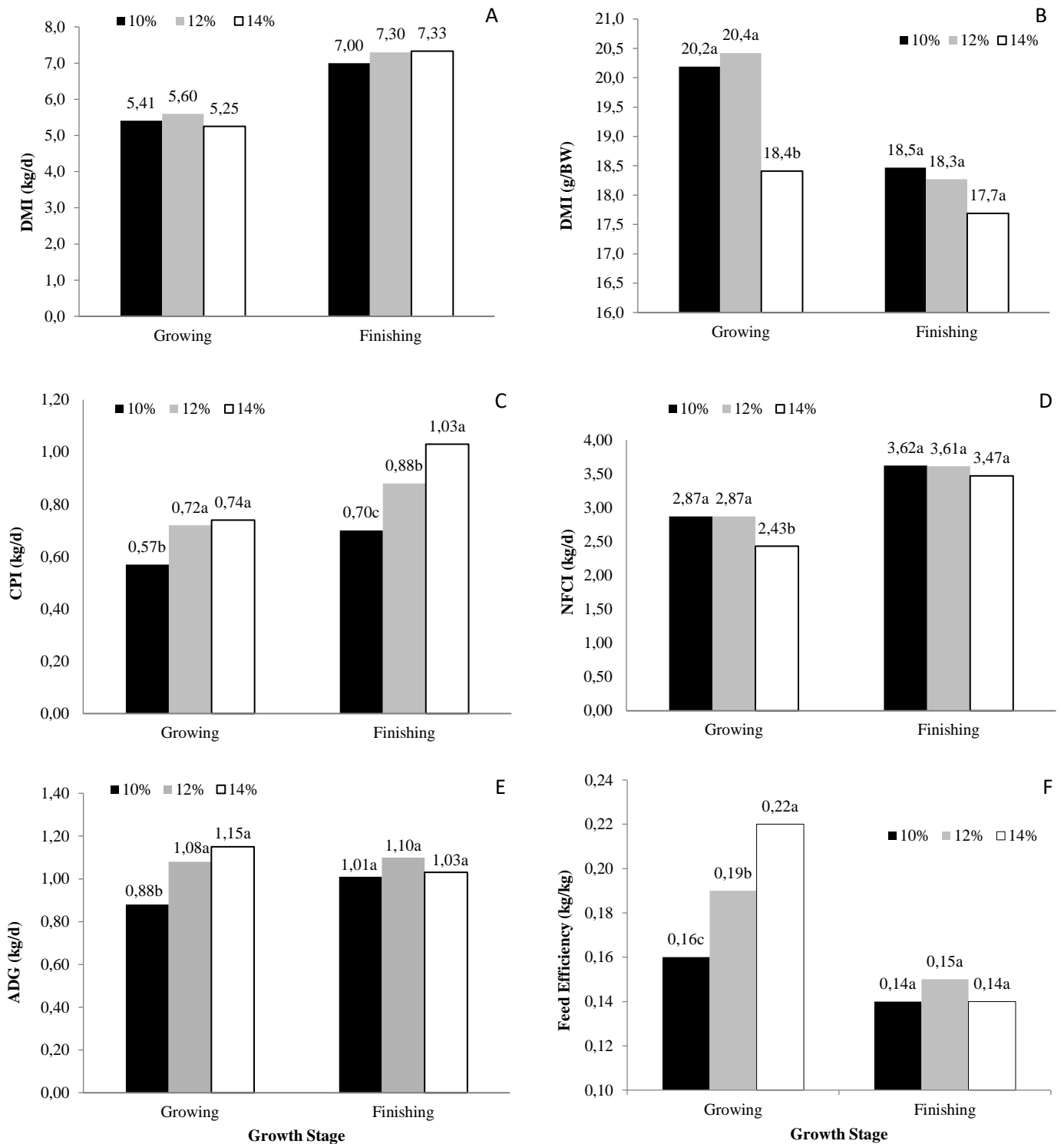


Fig 1: Dry matter intake (kg/d, A), dry matter intake (g/BW, B), crude protein intake (kg/d, C), non-fiber carbohydrates intake (kg/d, D), average daily gain (kg/d, E), and feed efficiency (kg/kg, F) of bulls fed different CP over two growth stages.

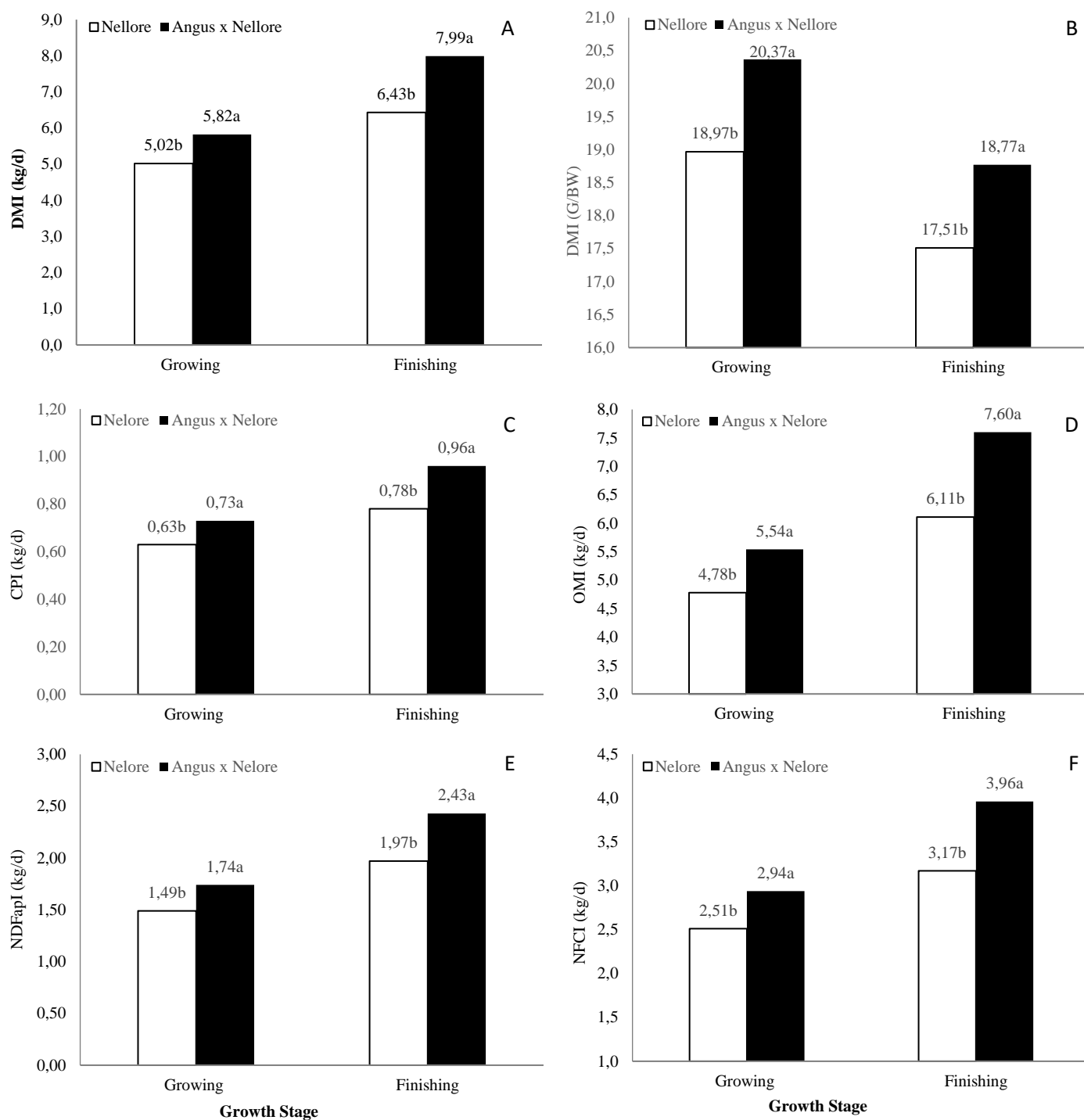


Fig 2: Dry matter intake (kg/d, A); Dry matter intake (g/BW, B); Crude protein, organic matter intake, neutral detergent fiber and non-fiber carbohydrates intakes (kg/d, C) of Nellore and crossbred Angus × Nellore fed different CP over two growth stages.

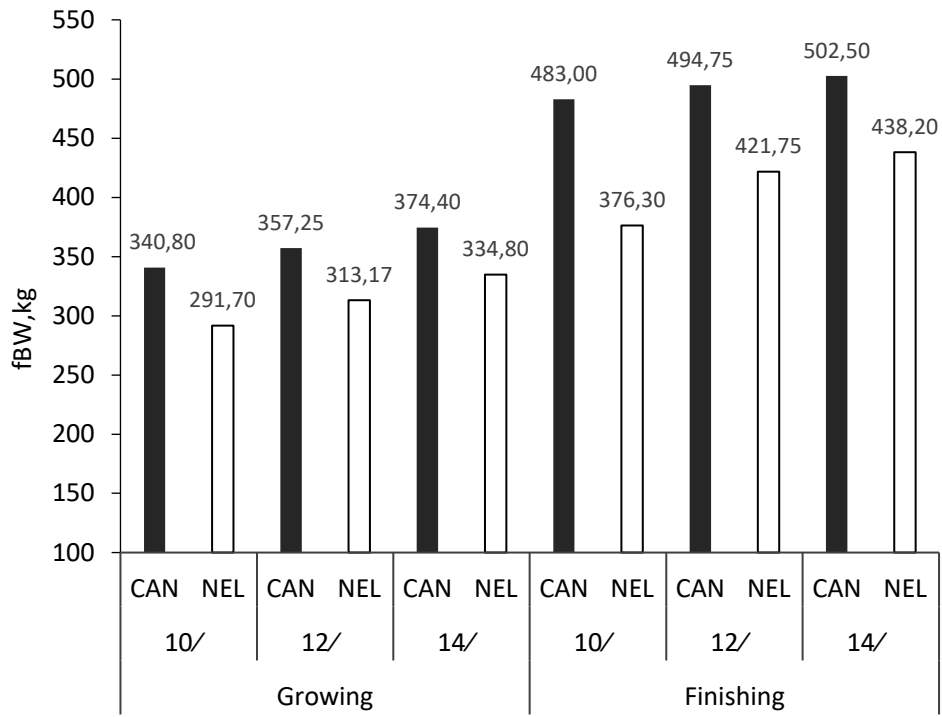


Fig 3: Final BW (kg) of Nellore and crossbred A x N fed different CP over growth stages

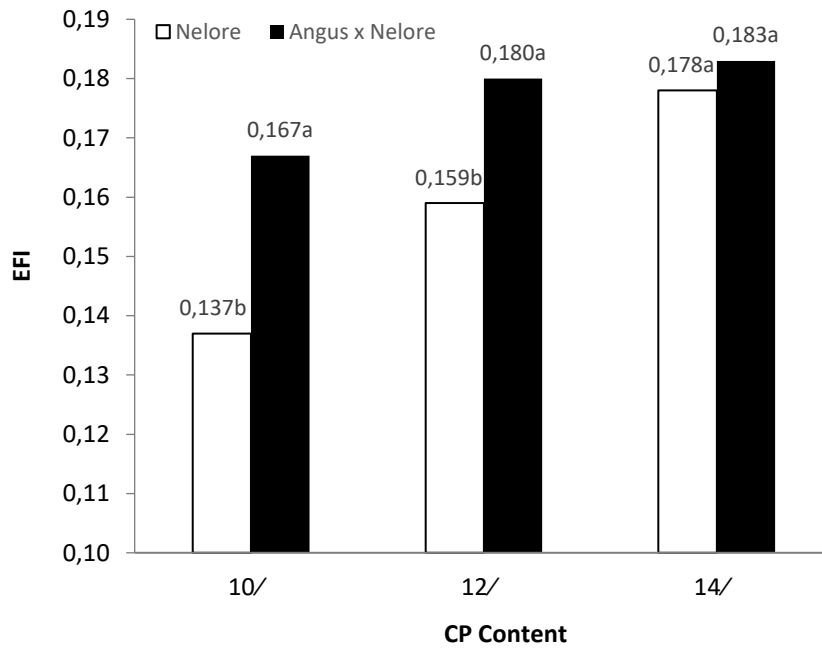


Fig 4: Efficiency of Nellore and crossbred A x N fed different CP

CHAPTER 2

Amino acids requirements of Nellore and crossbred A×N fed different crude protein content

Abstract

It is expected to work with AA instead of CP requirements in order to provide the correct amount of essential AA to meet maintenance and production requirements. Despite the advances in determining microbial protein synthesis and AA digestibility in small intestine (Rutherford et al., 2016), lesser advances were done in intermediate metabolism of AA and especially in net requirements of AAs (Tedeschi et al., 2015). Furthermore, the use of crossbred animals has been increasingly, however, there is no data in the literature comparing AAs requirements between GG, or correlating efficiency with amino acids requirements. Therefore, the objective of this study was to evaluate the proportion and retention of AA in the EBW and also to determine net requirements of AA in purebred (Nellore) and crossbred (A×N) bulls, fed different protein content. Fifty-two bulls, (26 Nellore and 26 crossbred F1 Angus x Nellore) with 8 months and initial shrunk BW (SBW) = 215.0 ± 15.0 kg (Nellore = 208.0 ± 12.78 kg; Angus x Nellore = 221.9 ± 14.16 kg) were used in this experiment. Eight animals (4 from each genetic group) slaughtered at the beginning of the experiment were used as baseline reference to estimate initial chemical body composition. The 36 remaining bulls were randomly assigned to one of three dietary treatments. The experiment was conducted in a completely randomized design with six replicates, in a 2x3 factorial scheme. The factors were two genetic groups (Nellore and crossbred F1 Angus x Nellore - A×N) and three crude protein contents (CP) (100, 120 and 140 g/kg DM). The experimental period lasted 224 days, and at the end of the experiment, all animals were

slaughtered to evaluate their amino acid body composition and the net requirements of amino acids for gain. In the present study it is observed that some AA as lysine and phenylalanine have high correlation with muscle, however, other amino acids (e.g. methionine) have a stronger correlation with body's composition. It is worth mentioning that this behavior is not constant among different weights, showing that more studies like this need to be conducted to determine more precisely the net requirements of amino acids.

Introduction

Nutritional models for feeding protein have evolved from basic CP (NRC, 1978; ARC, 1980) to more complex systems based on rumen-degradable and rumen-undegradable protein (INRA, 1988; NRC, 1985, 1989; AFRC, 1992; NRC, 2001). However, amino acids (AA), and not protein per se, are the required nutrient. Thus, to ensure a more accurate nutrition, it is expected to work with AA instead of CP requirements in order to provide the correct amount of essential AA to meet maintenance and production requirements.

Microbial crude protein synthesis in the rumen provides the majority of protein supplied to the small intestine of ruminants, accounting for 50 to 80% of total absorbable protein (Bach et al., 2005). The more similar is the profile of essential AA available for absorption in the small intestine to the animal's requirement, the higher will be the AA usage efficiency for protein synthesis (NRC, 2001). However, the unique features of the intermediate metabolism, in addition to the complex feed transformation during ruminal fermentation, and the difficulties to determine the AA available for absorption in the duodenum, pose a challenge to formulate diets based on AA requirements (Silva et al., 2002).

Due to these challenges, there is a deficiency of AA requirement data in the literature, especially for beef cattle. However, with the increased use of protected AA, there is a growth in

this research field, and, consequently, a greater understanding of the microbial protein modeling (NRC, 2016) and the amino acids digestibility. Thus, with this advances in research, it is expected to associate the absorption of amino acids in the small intestine with the retention of amino acids on the carcass. However despite the advances in determining microbial protein synthesis, and AA digestibility in small intestine (Rutherford et al., 2016), lesser advances were done in intermediate metabolism of AA and especially in net requirements of AAs (Tedeschi et al., 2015).

The Brazilian herd consists mostly by Zebu animals, however the use of crossbred animals has been increasingly common, aiming to optimize performance and productivity of beef production system. Studies in tropical conditions have shown that there are differences in the use of feed and productive responses between genetic groups (GG), and greater performances have been observed in crossed animals compared to zebu (Marcondes et al., 2011). However, there is no data in the literature comparing AAs requirements between GG, or correlating efficiency with amino acids requirements.

Thus, we hypothesized that net requirements of AAs do not vary between genetic groups, however the final composition of AAs in the body may vary between these groups. Therefore, the objective of this study was to evaluate the proportion and retention of AA in carcass and also to determine net requirements of AA in purebred (Nellore) and crossbred (A×N) bulls, fed different protein content.

Materials and methods

The experiment was conducted at the Animal Science Department of the Federal University of Viçosa (Viçosa - MG). All animal care and handling followed the ethics committee guidelines of the Federal University of Viçosa (CEUAP, protocol # 05/2013)

Animals and Experimental Design

Fifty-two bulls (26 Nellore and 26 crossbred F1 Angus x Nellore) with approximately 8 months of age at the beginning of the experiment and initial shrunk BW (SBW) = 215.0 ± 15.08 kg (Nellore = 208.0 ± 12.78 kg; Angus x Nellore = 221.9 ± 14.16 kg) were used in this experiment. All the experimental animals were submitted to a 30-day adaptation period prior the beginning of the experiment.

The bulls were subdivided into 3 groups (reference, maintenance, and ad libitum). Eight bulls (four Nellore and four F1 A×N) were designated to slaughter on day zero as a reference group to estimate the initial body weight (EBW) and initial body composition. Eight bulls (four Nellore and four F1 A×N) were fed to maintenance level (1.2% of BW) and 36 bulls (eighteen Nellore and eighteen F1 A×N) were fed ad libitum.

The experiment was conducted in a completely randomized design with six replicates, in a 2×3 factorial scheme. The factors were two (GG) and three crude protein contents (100, 120 and 140g CP/kg DM). Experimental diets were formulated according to the Brazilian Tables of Nutrient Requirements of Zebu Beef Cattle - BR-CORTE (Valadares Filho et al., 2010) to achieve an ADG of 1.2 kg (considering an average BW of 350 kg). Animals were housed in individual pens, with 30m² of total area per animal being 8m² covered. The experimental period lasted 224 days.

The forage:concentrate ratio was 50:50, using corn silage as forage source. The total amount of corn silage was provided at 0700 h with half of the daily amount of concentrate, and the other half was given at 1500 h. Feed intake was adjusted to maintain the leftovers within 5 to 10% of the amount offered. Chemical composition and proportion of feeds in diets is shown in Table 1. Proportion of amino acids in the ingredients and in the diets is shown in Table 2.

Carcass evaluation

Prior to the slaughter, all animals were solids fasted for 16 hours. The animals were randomly selected for slaughter on each day, being slaughtered six animals per day. Animals were slaughtered via captive bolt followed by bleeding. After slaughtering, the gastrointestinal tract (i.e., rumen, reticulum, omasum, abomasum, and small and large intestines) of each animal was emptied, washed, and weighed. The weight of the empty gastrointestinal tract was added to the weights of the remaining parts of the body (blood, organs, head, hide, tail, hoofs and carcasses) to determine the empty body weight (EBW).

A blood sample was obtained during the course of bleeding. The rumen, reticulum, omasum, abomasum, small and large intestines, visceral fat (renal, pelvic and cardiac fats plus mesenteric fat), liver, heart kidneys, lungs, tongue, spleen, diaphragm, esophagus, trachea, and reproductive tract were ground for 20 minutes by using an industrial cutter to create a homogeneous sample of organs and viscera. The head and limbs had their hide removed, and then were ground by using a grinding machine to reduce the bones size. The hide was sampled in multiple parts, which overall represented the entire hide.

The carcass of each animal was divided into two halves that were weighed and cooled in a cold chamber at 4 °C for 24 h. After 24 h post-mortem, the right half-carcass was dissected to separate the muscle + fat from bones, and each portion was weighed. The muscle + fat of each

animal were ground and homogenized to obtain a composite sample of muscle and fat proportional to their natural weight in the EBW. The bones from each animal were sliced to obtain a bone sample. All samples were lyophilized and, subsequently, ground in an industrial blender (Eberbach Model No. E8017.00) using liquid nitrogen to enable sample grinding, since the components had not been partially degraded. Upon completion of grinding, each sampled content (blood, hide, head and hoofs, organs and viscera, muscle + fat, and bones) were proportionally taken, to build a single sample per animal, representing EBW.

Chemical analysis

The determination of amino acid concentrations were carried out by high-performance liquid chromatography (HPLC). The methodology involves the reaction of the precolumn derivatization with phenylisothiocyanate (PITC) to form the PTC amino acids which were quantitated by HPLC in reverse phase (30ul loop injection, pH 6.40, in a binary linear gradient with a flow of 1 mL / min and column temperature 58 ° C) using UV detection at 254nm.

Net Requirements of Amino Acids for gain

The AA content (AAC; kg) or CP content (CPB) in the body of all animals (including baseline group) was regressed as a function of the empty body weight (EBW), according to the allometric model (ARC, 1980):

$$AAC \text{ or } CPB = a \times EBW^b, \text{ [Equation 1]}$$

where: AAC = amino acid content (kg); CPB = CP content in the body; EBW = empty body weight (kg); “a” and “b” = regression parameters.

The net requirements of AA (AA_g , kg/d) or CP (CP_g , kg) for gain were estimated from the derivative of the Equation 1, according to the model (ARC, 1980):

$$AA_g \text{ or } CP_g = a \times b \times EBW^{(b-1)} \times EBW, \text{ [Equation 2]}$$

where: AA_g = net requirements of AA for gain (kg/d), CP_g = net requirements of CP for gain (kg/d), EBW = empty body weight (kg); “a” and “b” = parameters of the model, EBG = empty body gain.

Statistical analysis

The final AA in the EBW (g/100 of CP) and AA retention (kg/d) were evaluated according to a completely randomized design, with three CP content (CP) and two GG. When observed significance of CP, orthogonal contrast was used to identify linear and quadratic effects of CP when necessary. Differences were declared significant at $P < 0.05$ and trends at $P < 0.10$. All procedures were done using PROC MIXED of SAS (SAS Inst. Inc., Cary, NC) and Kenward-Roger approximation was used to calculate the residual degrees of freedom.

The parameters of Equation 1 were determined using PROC NLMIXED of SAS (SAS Inst. Inc., Cary, NC), using GG as fixed effect and CP as random effect. The GG effect was tested through command ESTIMATE, and when its effect was declared non-significant at $P < 0.05$, it was removed from the model, and a single Equation was determined for both GG.

Results and discussion

There was no interaction ($P > 0.05$) between CP and GG to none of the variables evaluated. Thus, we will discuss the factors separately (Tables 3 and 4).

Genetic groups effects

There was GG effect ($P < 0.05$) on the amount of protein retained on carcass. Crossbred A×N retained more protein in their carcasses when compared to Nellore bulls (Table 3). NRC (1987) and the AFRC (1993) reported that *Bos taurus* breeds had greater potential for DMI and gain. In general, a greater DMI leads to a greater ADG and, hence, greater BW, leading to a greater protein retention in crossbred animal's carcasses. In this study, A×N presented both

greater DMI and ADG than Nellore animals (Amaral et al., preparing for submission), leading to a greater CP deposition in the body.

The retention of essential amino acids (EAA) followed exactly the same pattern of protein deposition, regarding GG (Table 3). Crossbred A×N retained more EAA in their carcasses than Nellore animals. There was a greater deposition of EAA for A×N as these animals gained more weight, however it is important to assess the concentration of EAA on the gain to verify differences between GG.

When we assessed the final proportion of EAA (g/100g of CP) in the EBW, there was no difference ($P > 0.05$) between GG. Thus, it can be inferred that there is no difference in the EAA composition on the EBW of A×N and Nellore animals, and therefore, the EAA are deposited in the same proportion, regardless of GG. Previous research have demonstrated that CPB of GG can affect fat proportion in the muscle (Nuernberg et al., 2005; Smet et al., 2004; Campo et al., 2000), however, to our knowledge, all evidences indicate that EAA proportion in the muscle is fixed, and linked more to cell metabolism (Wagenmakers et al., 1999) than to breed or diets.

Of the eight non-essential amino acids (NEAA) evaluated, only two did not follow the behavior observed for protein deposition, regarding GG. The glycine and proline deposition (kg) did not differ between GG, however a trend for greater proportion in N×A animals ($P = 0.076$ and $P = 0.077$, respectively) was observed. Nevertheless, when assessing the proportion of the amino acids in the EBW gain, only proline was greater in Nellore animals (Table 4). In addition, there was a trend of greater glycine and alanine ($P = 0.058$) in Nellore animals.

Crude protein contents effect

CP did not affect ($P > 0.05$) the retention of none of the NEAA, except by cystine (Table 3). CP quadratically affected protein retention ($P = 0.008$). There was also a quadratic effect ($P <$

0.05) on the retention of histidine, phenylalanine, leucine, isoleucine, valine and tryptophan (Table 3). Animals fed 12% CP had a greater retention of these EAA on their carcasses compared to animals fed 10 or 14% CP, following the behavior observed to protein retention (Table 3). Presumably, it would be occurring a protein excess when the animals are fed with 14%. The AA could be being deamidated and used as an energy source for animals, explaining the quadratic behavior. The EAA lysine, methionine and threonine were affected linearly by CP ($P < 0.05$). There is an increase in these AA retention as the CP increase (Table 3). These amino acids would be used for muscle deposition. This indicates that the proportion of AA in the small intestine is still not ideal. The advances in determination of AAs in the small intestine, leads to opportunity for a better aa balance, which would increase Protein retention and also the ADG. However, studies have yet to be conducted, with better control of AA availability in the gut and their correlation with AA deposition in the body.

Moreover, when we assessed the proportion of retained AA (g/100 g of CP), the behavior was not the same as observed to the amount (kg) of AA deposited in the body (Table 4). CP did not affect none of the EAA ($P > 0.05$). Regarding the NEAA, only glycine and alanine concentration were affected ($P < 0.05$) by CP. An increase in CP promoted a quadratic effect on these AA (Table 4).

The AA composition of the EBW observed in our study closely agree with those of Silva et al. (2002) and also with EAA profile presented by Ainslie et al. (1993).

Total AA in the empty body weight

An allometric equation was generated for each the analyzed AA in order to contrast with the protein in the EBW. The GG effect was tested on the parameters a and b of each equations, and it did not affect any of the AA evaluated, nor the CP content in the body ($P > 0.05$).

The EAA Arginine, leucine and lysine followed the same CP behavior, increasing with the EBW increase (Figure 1). Others EAA also increased with EBW, however it happened in a lower proportion compared with protein deposition. Glutamic acid and glycine were the NEAA that behaved the closest to protein (Figure 2). Theoretically, the AA which follows protein retention behavior are those with higher correlation with muscular deposition. In contrast, amino acids which do not have the same behavior of the protein would be connected to other tissues deposition, such as gastrointestinal tissue or organs, and also could be more related with protein turnover. It is known that cysteine and methionine are highly demanded on organs and viscera deposition (Fang et al., 2009; Jankowski et al., 2014), and that most of gastrointestinal deposition is done during earlier stages of life (until 200 kg of EBW) (Bauchart-Thevretet al., 2009), therefore it is suggested that those AAs. On the other hand, mainly leucine (Burdet al., 2015; Kimball et al., 2004) and glutamine (Aproet al., 2010), the most abundant muscle amino acid, act on several kinases to stimulate the initiation of protein, synthesis translation.

The behavior of the methionine and tryptophan (EAA – Fig. 1) and cysteine and tyrosine (NEAA – Fig.2) is the least closely resembles to the protein behavior, suggesting that the demand for such AA does not increase with increased BW as the same rate as protein does.

The relation between lysine and methionine slightly increases as EBW increased. A much strong increasing was observed to lysine:tryptophan ratio, suggesting that lysine becomes increasingly important with the EBW increasing, while methionine and tryptophan are less necessary. On the contrary, Lysine:Histidine ratio diminishes as EBW increase, suggesting that Histidine becomes less important to tissue deposition with the increase in EBW.

The equations for determining the requirements of AAs and CP are presented on Table 5. Net requirements were estimated (g / 100g CP) for three EBW (200, 300, and 400 kg) using these equation and then, the results were compared with literature data (Table 6).

Phenylalanine, histidine, isoleucine, leucine, lysine, methionine, threonine, tryptophan, valine, cysteine, glutamic acid and tyrosine have their requirements increased when the EBW increases. On the other hand, arginine, alanine, aspartic acid, proline and serine diminish their requirements when the EBW increase. Comparing the data of this experiment with the average literature data, some amino acids meet exactly the same requirements (e.g. Methionine, arginine and serine). However, other amino acids requirements found in this study, differ from literature, as lysine, isoleucine and glutamine. In the present study it is observed that some AA, as lysine and phenylalanine, have high correlation with muscle, however, other amino acids (e.g. methionine) have a stronger correlation with body's composition.

Conclusion

There has always been an understanding that the net requirements of amino acids are equal to the AA composition in the body or muscle. In the present study it is observed that some AA, as lysine and phenylalanine, have high correlation with muscle, however, other amino acids (e.g. methionine) have a stronger correlation with body's composition. It is worth mentioning that amino acid requirements is not constant among different weights, showing that more studies need to be conducted to determine more precisely the net requirements of amino acids.

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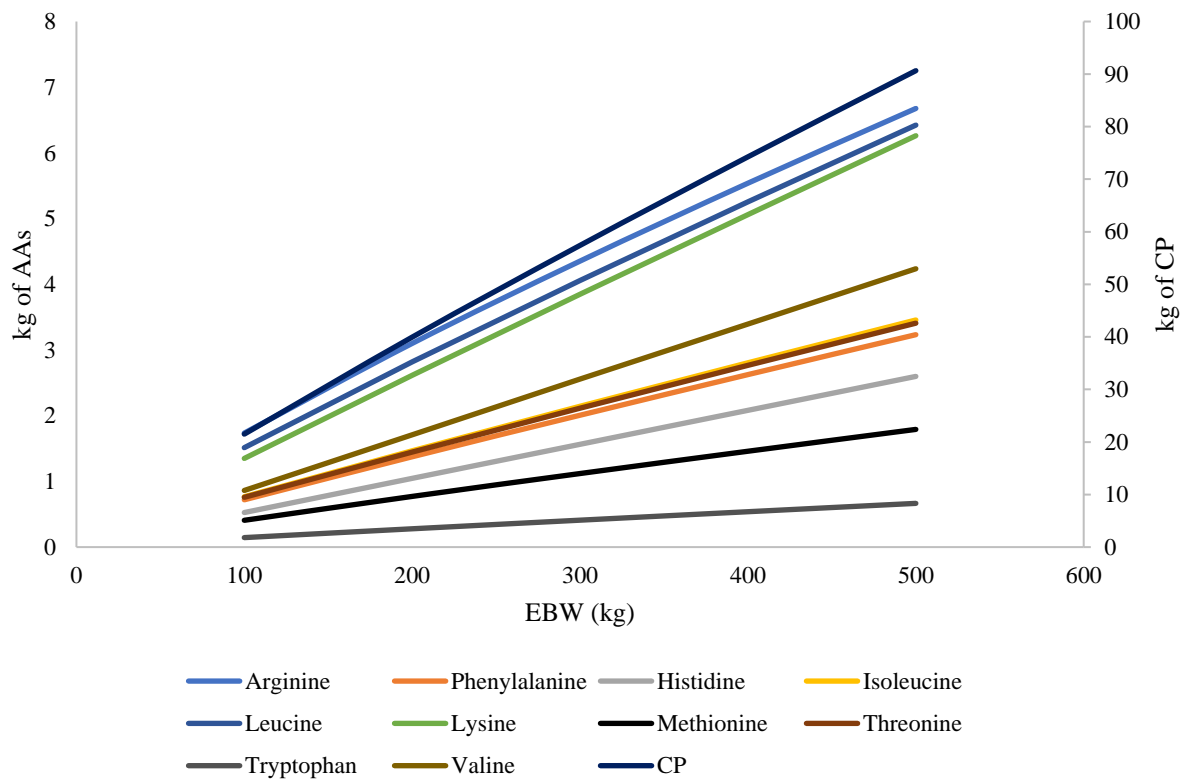


Figure 1. Content of essential amino acids and crude protein in the EBW of growing and finishing beef cattle.

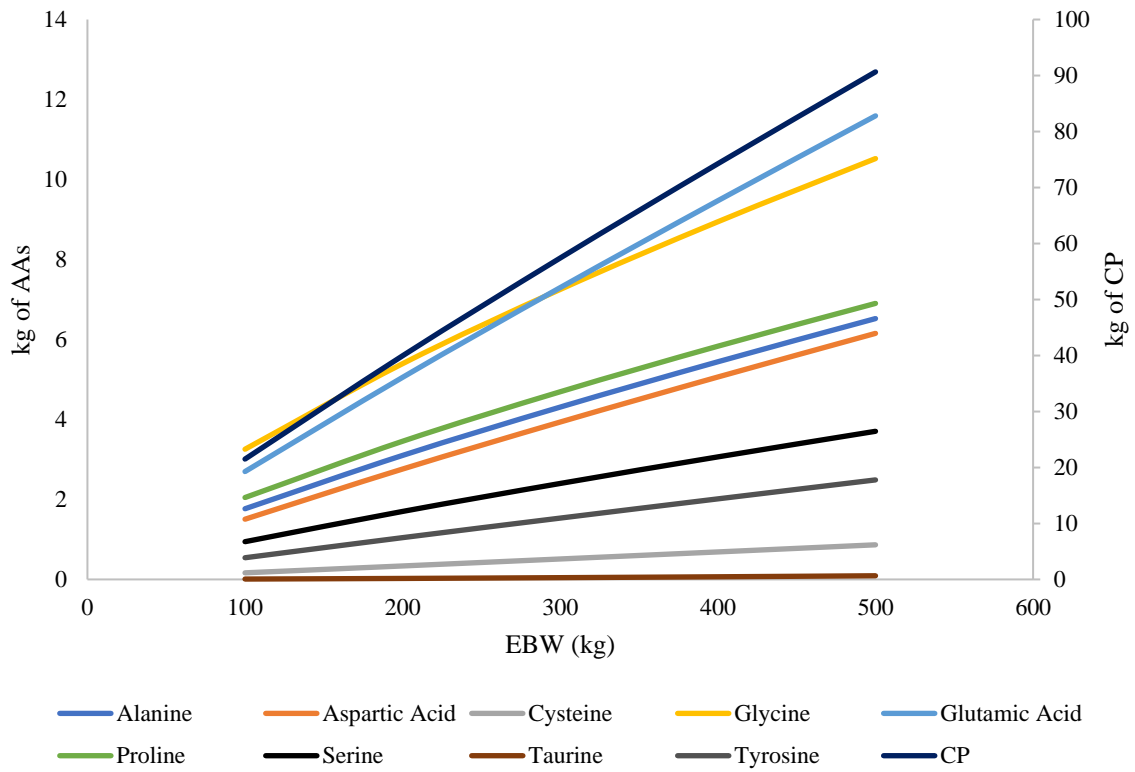


Figure 2. Content of non-essential amino acids and crude protein in the EBW of growing and finishing beef cattle.

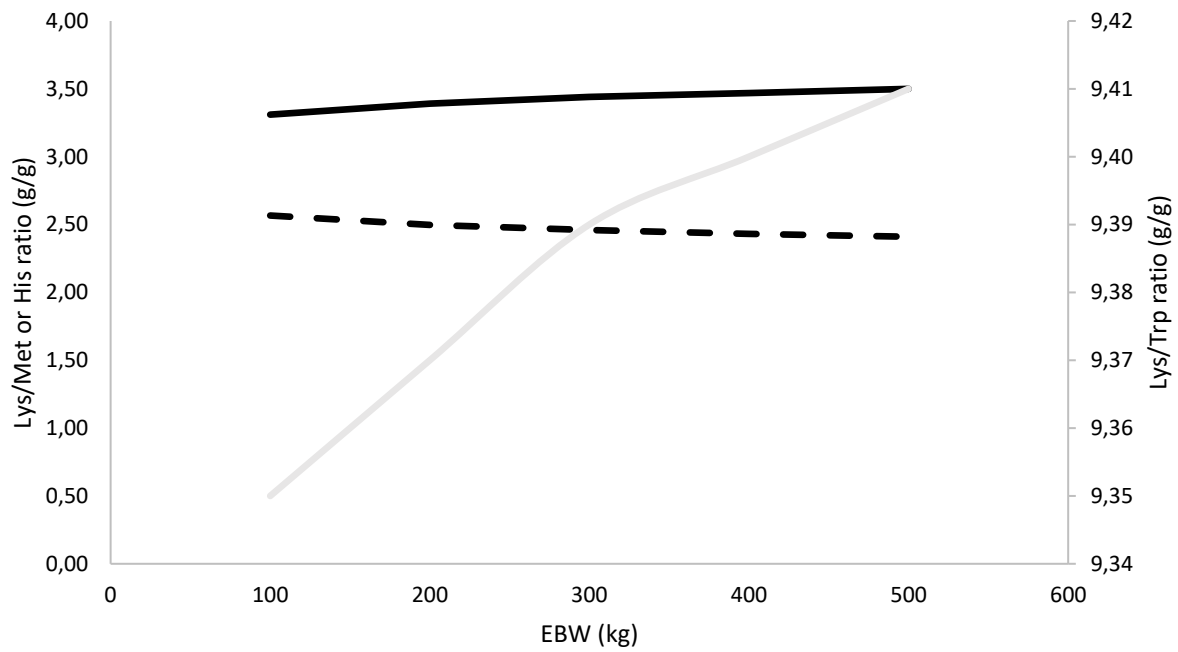


Figure 3. Ratios between Lysine and Methionine (solid black line), Histidine (dashed black line) or Tryptophan (solid grey line) in growing and finishing beef cattle.

Table 1 – Proportion of feeds and chemical composition of diets

Proportion of feeds in diets (% of diet DM)			
	Diet 10%	Diet 12%	Diet 14%
Corn silage	50.0	50.0	50.0
Corn grain	39.7	39.6	39.6
Wheat meal	5.9	3.0	0.0
Mix ¹	2.2	5.3	8.3
Sodium chloride	0.5	0.5	0.5
Mineralized salt ²	0.5	0.5	0.5
Sodium bicarbonate	0.8	0.8	0.8
Magnesium oxide	0.3	0.3	0.3
Chemical composition (% of diet DM)			
Items	10% CP	12% CP	14% CP
DM	55.5	55.6	55.7
OM	95.4	94.9	94.4
CP	9.9	12.1	14.2
RDP ³	66.8	69.6	71.6
EE ⁴	4.0	4.0	4.0
NDF _{ap} ⁵	31.4	30.7	29.9
NFC ⁶	50.7	49.4	48.2
TDN (%) ⁷	80.2	79.3	79.7
DE ⁸	3.47	3.45	3.50

¹Mix = 83,3% Soybean meal and 16,7% of urea/ammonia sulphate (U/AS); ²Mineralized salt = 223 g/kg of Ca; 174 g/kg of P; 24 g/kg of S; 100 mg/kg of Co; 1.250,0 mg/kg of Cu; 1.795,0 mg/kg of Fe; 90 mg/kg of I; 2.000,0 mg/kg of Mn; 15,00 mg/kg of Se; 5.270,00 mg/kg of Zn and 1.740,00 mg/kg de F. ³Rumen degradable protein; ⁴EE = Ether extract; ⁵NDF_{ap} = Neutral detergent fiber corrected to ash and protein; ⁶NFC = Non-fiber carbohydrates; ⁷TDN(%) = Total digestible nutrients; ⁸DE = Digestible energy .

Tabela2. Proportion of amino acids in the ingredients and in the diets

Item	Ingredients				Diet (%CP)		
	Corn silage	Corn Grain	Wheat meal	Soybean meal	10%	12%	14%
	%DM						
	Essentials AA						
Arginine	0.36	0.40	1.77	3.99	0.54	0.59	0.65
Histidine	0.07	0.18	0.61	1.32	0.17	0.19	0.21
Isoleucine	0.22	0.24	0.79	2.40	0.31	0.35	0.39
Leucine	0.49	0.90	1.54	4.09	0.80	0.86	0.93
Lysine	0.27	0.36	1.01	4.13	0.43	0.51	0.60
Methionine	0.05	0.13	0.26	0.53	0.11	0.11	0.12
Phenylalanine	0.21	0.34	0.98	2.68	0.36	0.40	0.45
Threonine	0.18	0.25	0.76	2.05	0.29	0.32	0.35
Tryptophan	0.03	0.13	0.20	0.36	0.21	0.21	0.22
Valine	0.28	0.31	1.04	2.11	0.37	0.40	0.43
	Non-essential AA						
Alanine	0.54	0.56	1.13	2.41	0.62	0.65	0.69
Aspartic	0.29	0.48	1.89	5.96	0.58	0.69	0.79
Cystine	0.04	0.08	0.27	0.49	0.08	0.09	0.09
Glutamine	0.62	1.37	4.35	9.46	1.72	1.84	1.97
Glycine	0.30	0.36	1.37	2.56	0.44	0.47	0.49
Proline	0.38	0.79	1.49	3.02	0.67	0.71	0.75
Serine	0.17	0.40	1.14	2.97	0.49	0.54	0.59
Tyrosine	0.12	0.22	0.69	1.77	0.23	0.58	0.62

Table 3. Retention of protein and amino acids in the fEBW (g/kg)

Item	CP			Genetic Group		SEM	p-value			Contrasts	
	10%	12%	14%	N	A×N		CP	GG	CP×GG	Linear	Quadratic
Retained protein	158.3	189.2	185.2	155.1	201.5	9.60	0.002	<0.01	0.734	0.012	0.008
Essential AA											
Methionine	3.18	3.85	3.99	3.25	4.10	0.38	0.091	<0.01	0.878	0.043	0.397
Lysine	10.81	13.55	13.73	10.82	14.57	1.04	0.013	<0.01	0.630	<0.01	0.141
Histidine	4.71	5.96	5.22	4.55	6.04	0.46	0.027	<0.01	0.062	0.278	0.013
Phenylalanine	5.58	7.23	6.60	5.51	7.44	0.51	<0.01	<0.01	0.440	0.054	0.010
Threonine	6.03	7.37	7.23	5.98	7.78	0.53	0.029	<0.01	0.472	0.031	0.095
Leucine	10.79	13.78	12.77	10.74	14.16	0.91	<0.01	<0.01	0.380	0.037	0.011
Isoleucine	6.14	7.84	7.44	6.23	8.06	0.55	0.010	<0.01	0.842	0.025	0.027
Valine	7.50	9.90	8.56	7.33	9.98	0.83	0.019	<0.01	0.493	0.211	0.010
Arginine	12.13	13.17	13.58	11.52	14.40	0.97	0.320	<0.01	0.275	0.148	0.693
Tryptophan	1.07	1.57	1.37	1.19	1.48	0.16	<0.01	0.026	0.858	0.062	0.011
Non essential AA											
Aspartic	8.64	11.99	11.47	7.92	13.48	1.81	0.145	<0.01	0.684	0.129	0.202
Serine	6.34	7.41	7.29	6.08	7.95	0.55	0.114	<0.01	0.345	0.097	0.196
Glutamic	20.03	23.32	23.44	18.55	25.98	2.17	0.213	<0.01	0.779	0.127	0.377
Glycine	18.67	17.24	19.65	16.89	20.15	2.23	0.527	0.076	0.697	0.662	0.300
Alanine	11.63	12.17	12.82	10.86	13.56	1.01	0.502	<0.01	0.562	0.245	0.951
Tyrosine	4.72	5.70	5.46	4.69	5.90	0.46	0.089	<0.01	0.511	0.115	0.115
Cystine	1.57	2.05	1.98	1.68	2.05	0.20	0.048	0.030	0.887	0.054	0.110
Proline	11.96	12.14	13.12	11.39	13.42	1.39	0.668	0.077	0.505	0.413	0.723

Table 4. Proportion of amino acids (g/100g of CP) in the fEBW

Item	CP level			Genetic Group		SEM	p-value			Contrasts	
	10%	12%	14%	N	A×N		CPL	GG	CPL×GG	Linear	Quadratic
Essentials AA											
Methionine	1.95	1.97	2.02	2.00	1.96	0.09	0.743	0.549	0.559	0.472	0.797
Lysine	6.67	6.86	6.97	6.78	6.88	0.20	0.316	0.495	0.809	0.137	0.804
Histidine	2.61	2.76	2.57	2.62	2.67	0.09	0.115	0.577	0.062	0.650	0.043
Phenylalanine	3.47	3.64	3.50	3.51	3.56	0.09	0.132	0.471	0.779	0.746	0.049
Threonine	3.72	3.77	3.76	3.76	3.74	0.11	0.861	0.866	0.840	0.685	0.719
Leucine	6.99	7.25	7.04	7.10	7.08	0.17	0.271	0.916	0.778	0.802	0.113
Isoleucine	3.73	3.88	3.80	3.81	3.80	0.13	0.505	0.884	0.823	0.632	0.289
Valine	4.47	4.76	4.44	4.50	4.61	0.18	0.137	0.452	0.774	0.881	0.049
Arginine	7.66	7.32	7.49	7.61	7.37	0.16	0.119	0.073	0.235	0.323	0.068
Tryptophan	0.68	0.77	0.72	0.73	0.71	0.05	0.150	0.546	0.490	0.474	0.070
Non-essential AA											
Aspartic	6.44	6.83	6.78	6.33	7.04	0.48	0.683	0.075	0.410	0.499	0.587
Serine	4.15	4.11	4.11	4.15	4.10	0.08	0.849	0.562	0.400	0.649	0.735
Glutamic	12.79	12.60	12.76	12.54	12.89	0.43	0.886	0.321	0.452	0.948	0.629
Glycine	12.74	11.29	12.13	12.45	11.66	0.50	0.018	0.058	0.921	0.232	<0.01
Alanine	7.58	7.10	7.36	7.48	7.21	0.17	0.024	0.058	0.761	0.215	0.013
Tyrosine	2.72	2.73	2.77	2.71	2.77	0.11	0.898	0.561	0.693	0.669	0.836
Cystine	0.92	0.97	0.95	0.97	0.93	0.05	0.560	0.421	0.998	0.509	0.398
Proline	8.14	7.53	7.91	8.13	7.60	0.31	0.130	0.038	0.957	0.456	0.060

Table 5. Amino acids net requirements for gain

Amino Acid	Net Requirements for gain
Arginine	$Y=0.0308 \times EBW^{-0.1629} \times EBG$
Phenylalanine	$Y=0.0091 \times EBW^{-0.0669} \times EBG$
Histidine	$Y=0.0054 \times EBW^{-0.0075} \times EBG$
Isoleucine	$Y=0.0097 \times EBW^{-0.0646} \times EBG$
Leucine	$Y=0.0217 \times EBW^{-0.1017} \times EBG$
Lysine	$Y=0.0159 \times EBW^{-0.0465} \times EBG$
Methionine	$Y=0.0054 \times EBW^{-0.0803} \times EBG$
Threonine	$Y=0.0096 \times EBW^{-0.0658} \times EBG$
Tryptophan	$Y=0.0017 \times EBW^{-0.0504} \times EBG$
Valine	$Y=0.009 \times EBW^{-0.0106} \times EBG$
Alanine	$Y=0.0342 \times EBW^{-0.1886} \times EBG$
Aspartic Acid	$Y=0.0235 \times EBW^{-0.1255} \times EBG$
Cysteine	$Y=0.0015 \times EBW^{0.0298} \times EBG$
Glycine	$Y=0.0827 \times EBW^{-0.2710} \times EBG$
Glutamic Acid	$Y=0.0377 \times EBW^{-0.094} \times EBG$
Proline	$Y=0.0478 \times EBW^{-0.2449} \times EBG$
Serine	$Y=0.016 \times EBW^{-0.1502} \times EBG$
Tyrosine	$Y=0.0065 \times EBW^{-0.0527} \times EBG$
Crude Protein	$Y=0.3129 \times EBW^{-0.1058} \times EBG$

Table 6. Amino acids composition on empty body at different weights

Amino Acid	Muscles AA proportion ¹	EBW AA proportion ²	Empty body Weight ³		
			200	300	400
Essential amino acids					
Arginine	7.14	7.59	7.27	7.11	6.99
Phenylalanine	3.67	3.34	3.57	3.63	3.67
Histidine	3.13	2.39	2.91	3.02	3.11
Isoleucine	3.68	2.88	3.86	3.92	3.97
Leucine	7.49	6.42	7.09	7.10	7.11
Lysine	7.56	5.91	6.96	7.13	7.25
Methionine	Ne ⁴	2.00	1.98	2.00	2.01
Threonine	4.32	3.52	3.79	3.85	3.90
Tryptophan ²		0.57	0.73	0.75	0.76
Valine	4.11	4.18	4.76	4.95	5.09
Non-essential amino acids					
Alanine	5.97	7.78	7.05	6.82	6.66
Aspartic Acid	8.83	7.88	6.77	6.71	6.67
Cysteine	1.86	1.15	0.98	1.04	1.08
Glycine	5.67	13.79	11.01	10.30	9.82
Glutamic Acid	15.36	13.36	12.83	12.89	12.93
Proline			7.31	6.91	6.64
Serine	3.94	3.91	4.04	3.97	3.92
Tyrosine	3.09	2.29	2.75	2.81	2.86

¹Amino acid proportion on muscle - data from Silva et al. (2002); ²Amino acid proportion on empty body weight - data from Silva et al. (2002); ³ Amino acid requirement at different weight; ⁴non-estimable

Capítulo 3

Exigências de energia e proteína de zebuínos puros (Nelore) e cruzados (F1 Angus x Nelore) não castrados em confinamento

Resumo

Dois experimentos foram conduzidos com o objetivo de avaliar as exigências nutricionais de proteína e energia para manutenção e ganho de peso e as eficiências de utilização da energia metabolizável para manutenção e ganho de peso, usando a técnica do abate comparativo. No segundo experimento, as exigências líquidas de energia para manutenção também foram estimadas utilizando-se a produção de calor mensurada em câmara respirométrica. No experimento 1, foram utilizados 52 bovinos machos, não castrados, com idade média de 8 meses e peso médio inicial de $215,0 \pm 15,08$ kg (Nelore = $208,0 \pm 12,78$ kg; F1 Angus x Nelore = $221,9 \pm 14,16$ kg). O experimento foi conduzido em delineamento inteiramente casualizado com seis repetições, em esquema fatorial 2×3 . Os fatores foram dois grupos genéticos (Nelore e cruzado F1 Angus x Nelore - A x N) e três conteúdos de proteína bruta (PB) (100, 120 e 140 g/kg MS). Os animais selecionados para consumo voluntário foram redistribuídos aleatoriamente em três grupos que receberam uma das 3 dietas experimentais. Os animais em manutenção foram alimentados com 12g de MS por kg de peso corporal, com a dieta de 12% de PB na base da MS. Os animais designados ao grupo referência foram abatidos para avaliação do peso PCVZ inicial e da composição química inicial do PCVZ. Ao final de 225 dias os animais foram abatidos. Parte do experimento 2 foi realizada na Universidade Federal de Minas Gerais, onde, a produção de calor (PC) e de metano de oito animais (4 Nelores e 4 cruzados Angus x Nelore) foram mensuradas em câmara respirométrica. Logo após, esses mesmos animais voltaram para a UFV, onde um experimento de digestibilidade foi conduzido utilizando um QL 4×4 . A relação entre PCJ e o

PCVZ encontrada neste estudo foi de 0,925. Houve diferença ($P < 0,05$) entre GG para a relação GMD x GPCVZ, sendo obtida a relação média de 0,966 para animais Nelore, e 0,947 para animais cruzados. O valor encontrado para Elm foi de 74,6 Kcal/PCVZ^{0,75}/dia, não havendo diferença entre os GG. A EMm encontrada para animais Nelore foi de 122 kcal/PCVZ^{0,75}/dia e de 119 kcal/PCVZ^{0,75}/dia para animais cruzados. Não houve diferença entre os GG ($P > 0,05$) para ELg, sendo obtida a equação: $ER = 0,0643 \times PCVZ^{0,75} \times GPCVZ^{0,6191}$. As eficiências de conversão da ELm para EMm de animais Nelore e cruzados neste experimento foram, respectivamente, 61,1 e 62,7%. A equação obtida para estimar a exigência líquida de proteína para ganho foi: $PR = 188,37 \times GPCVZ - 9,39 \times ER$. A eficiência de utilização da proteína metabolizável paraganho (k) foi de 0,3302. No experimento 2, as digestibilidades aparentes dos nutrientes não diferiram ($P > 0,05$) entre os GG. Da mesma forma, não houve diferença para as digestibilidades aparentes dos nutrientes ($P > 0,05$) quando se comparou consumo restrito e voluntário. O CPB aumentou linearmente ($P < 0,05$) com os níveis de PB. Houve efeito linear dos níveis dietéticos de PB ($P < 0,05$) sobre as digestibilidades aparentes da MS e MO. Uma Elm de 85,2 kcal/PCVZ^{0,75}/dia e uma EMm de 114 kcal/PCVZ^{0,75}/dia foram obtidas com a utilização da câmara respirométrica, sendo esses valores superior e inferior, respectivamente, aos obtidos pelo abate comparativo.

Introdução

O cruzamento industrial vem sendo cada vez mais difundido no país como uma forma de otimizar o desempenho e melhorar os índices zootécnicos da bovinocultura de corte. Este tipo de cruzamento disponibiliza animais com alto potencial de desempenho e com uma maior resistência ao clima e parasitas, comparados com animais de raças europeias puras criados em

clima tropical. Entretanto, as informações sobre as exigências de animais cruzados ainda são escassas na literatura nacional.

A importância em se determinar as exigências nutricionais de bovinos de corte no Brasil, está em se obter informações mais próximas à realidade do nosso rebanho e prover informações adequadas às nossas condições, notadamente distintas daquelas presentes em países de clima temperado. Trabalhar com informações adequadas à real situação da atividade pecuária nacional pode gerar um grande impacto econômico e na qualidade do sistema de produção de carne.

Considerando a necessidade de um sistema de exigências nutricionais de bovinos sob tais condições, (Valadares Filho et al., 2010) publicaram a 2ª edição das Tabelas Brasileiras de Exigências Nutricionais de Zebuínos (BR-CORTE), cujo banco de dados utiliza animais com características similares aos animais que compõem o rebanho brasileiro, criados sob condições características do território nacional. Entretanto, poucos trabalhos foram conduzidos para a determinação das exigências nutricionais de energia e proteína para animais provenientes de cruzamento industrial (F1 Angus x Nelore).

De acordo com o BCNRM (2016), os requerimentos de proteína decrescem na fase de terminação dos animais, corroborando com Menezes et al. (2016) e Amaral et al. (2014). Entretanto, os confinamentos comerciais têm utilizado altos níveis de proteína nas dietas de terminação à fim de reduzirem o período de confinamento dos animais. Millen et al. (2014) relataram que a concentração média de proteína bruta (na base da MS) recomendada para dietas de terminação é 13,4% (moda = 13,5%). O BR-CORTE (2010) recomenda para bovinos de 450kg de peso corporal exigências de proteína bruta (PB) média de aproximadamente 1300 gramas para ganho de um kg de peso corporal. Considerando um consumo médio de MS de 10 kg por dia, isto representaria uma dieta com 13% de PB. Entretanto, ao analisar os dados de

consumo de PB de experimentos realizados sob condições tropicais, pode-se observar que em geral há um excesso de PB nas dietas fornecidas aos animais. Isso pode ser verificado ao comparar os consumos de PB observados nos experimentos, com os consumos de PB preditos pelo sistema de exigências nutricionais de bovinos sob tais condições (BR-CORTE, 2010). Dessa forma, pode-se inferir que os valores preditos pelo BR-CORTE (2010) precisam ser ajustados, e considera-se a possibilidade de se adotar sistemas alimentares que reduzam os teores de proteína bruta das dietas sem que haja o comprometimento do desempenho animal, o que resultaria em redução da quantidade de proteína bruta ingerida e de compostos nitrogenados excretados para o meio ambiente, representando uma opção tecnológica para alcançar o desempenho desejado, com menores custos de produção e minimizando os danos ambientais.

Para o cálculo das exigências de energia para bovinos de corte, têm-se utilizando o método do abate comparativo, onde a produção de calor não é diretamente mensurada, mas obtida pela diferença entre o consumo de energia metabolizável e a energia retida no corpo vazio, que são obtidos diretamente. Estimando-se a produção de calor ($PC = CEM - ER$), torna-se possível realizar os cálculos referentes às exigências de energia para manutenção. Entretanto, o método do abate comparativo mostra-se oneroso e laborioso. A utilização de câmaras de respirométricas, diretas ou indiretas, é uma alternativa para se obter os dados necessários para a estimativa das exigências de energia para manutenção. Diferentemente do método do abate comparativo, determina-se consumo de energia metabolizável e a produção de calor (direta ou indiretamente) via troca de gases, obtendo-se a energia retida por diferença.

Sendo assim, o objetivo deste trabalho foi determinar as exigências nutricionais de proteína e energia para manutenção e ganho de peso, as eficiências de utilização da energia metabolizável para manutenção e ganho de peso, e também as exigências líquidas de energia para

manutenção utilizando-se a produção de calor mensurada em câmara calorimétrica, de bovinos puros (Nelore) e cruzados (F1 Ângus x Nelore) não castrados terminados em confinamento.

Material e Métodos

Foram realizados dois experimentos, ambos com animais contemporâneos e provenientes da mesma propriedade. O experimento 1 foi conduzido no Laboratório Animal do Departamento de Zootecnia da Universidade Federal de Viçosa (UFV) em Viçosa – MG. Os procedimentos de cuidado e manejo humanitário dos animais seguiram as orientações do comitê de ética da Universidade Federal de Viçosa (CEUAP, processo nº 05/2013). O experimento 2 foi realizado na Universidade Federal de Minas Gerais (UFMG), seguindo, também, as orientações do comitê de ética da Universidade Federal de Viçosa (processo nº 06/2013).

Manejo animal, delineamento experimental e dietas

No experimento 1, foram utilizados 52 bovinos machos, não castrados, com idade média de 8 meses e peso médio inicial de $215,0 \pm 15,08$ kg (Nelore = $208,0 \pm 12,78$ kg; Cruzados - F1 Angus \times Nelore = $221,9 \pm 14,16$ kg). O experimento foi realizado em delineamento inteiramente casualizado, com seis repetições em esquema fatorial 2×3 , em que os fatores foram dois grupos genéticos (Nelore ou Cruzado - F1 Angus \times Nelore) e três níveis de PB na dieta (10, 12 ou 14% de PB com base na MS). Os animais foram confinados em baias individuais, com piso de concreto, providas de comedouro e bebedouro de concreto, com área total de 30 m^2 , dos quais 8 m^2 eram cobertos.

Foi realizado um período de adaptação às condições experimentais de 30 dias, durante o qual os animais foram identificados e tratados contra ecto e endoparasitas. Após o período de

adaptação às condições experimentais, os animais foram pesados em jejum de sólidos de 16 horas e aleatoriamente distribuídos em três grupos: referência (oito animais: quatro Nelores e quatro F1 Angus × Nelore), manutenção (oito animais: quatro nelores e quatro F1 angus x nelore) e consumo voluntário (36 animais: 18 nelores e 18 F1 angus x nelore).

Os animais selecionados para consumo voluntário foram redistribuídos aleatoriamente em três grupos (12 bovinos em cada grupo) que receberam uma das 3 dietas experimentais. Os animais em manutenção foram alimentados com 12g de MS por kg de peso corporal, com a dieta de 12% de PB na base da MS durante todo o período experimental. Os animais designados ao grupo referência foram abatidos para avaliação do peso de corpo vazio (PCVZ) inicial e da composição química inicial dos animais experimentais. A partir do PCVZ e da composição química do PCVZ dos animais referência foram estimados os PCVZ e a composição química do PCVZ iniciais dos animais que permaneceram no experimento. O período experimental teve duração de 225 dias. Para determinação do peso corporal em jejum inicial (PCJi) e peso corporal em jejum final (PCJf), os animais foram pesados ao início e ao final do experimento após serem submetidos a jejum de sólidos de 16 horas.

Também foi realizado um segundo experimento, no qual oito animais (4 Nelores e 4 cruzados Angus × Nelore) foram enviados à Universidade Federal de Minas Gerais (UFMG) para mensurações da produção de calor em câmara respirométrica. Os animais utilizados nesse experimento eram contemporâneos e provenientes da mesma propriedade dos animais do experimento 1.

As dietas experimentais utilizadas foram as mesmas em ambos os experimentos, tendo sido adaptadas baseando-se nas exigências de PB descritas no BR-CORTE (2010). A proporção dos

ingredientes utilizados nas dietas, bem como a composição nutricional das mesmas com base na matéria seca (MS) é apresentada na Tabela 1.

Fixou-se a relação entre volumoso e concentrado em 50:50, utilizando-se a silagem de milho como volumoso. O volumoso foi fornecido em sua totalidade às 07h00 juntamente com metade da quantidade diária do concentrado, sendo a outra metade fornecida aos animais às 15h00. A silagem foi retirada de silo tipo trincheira, diariamente, uma hora antes do fornecimento aos animais.

As quantidades de volumoso e de concentrado, bem como as sobras dos animais foram pesadas diariamente. A dieta fornecida aos animais foi ajustada para manter as sobras em torno de 5 a 10% do ofertado, com água permanentemente à disposição dos mesmos.

Amostragens dos alimentos e das sobras

Os volumosos e as sobras de cada animal foram diariamente amostrados, e posteriormente acondicionados em freezer a - 20°C. Semanalmente, amostras compostas da silagem de milho, e das sobras de cada animal foram submetidas à secagem em estufa de circulação forçada (55°C) e moídas em moinho de facas (1 e 2 mm). Posteriormente, foi avaliada a matéria seca total dessas amostras por meio da secagem em estufa a 105° por 16 horas. Com base na quantidade de matéria seca de sobras de cada animal, ou da quantidade de matéria seca da silagem de milho oferecida, foram realizadas amostras compostas para cada período de 28 dias.

Os ingredientes que compuseram o concentrado foram amostrados diretamente dos silos da fábrica de ração nos dias das misturas dos mesmos.

Tabela 1 – Proporção dos alimentos e composição química das dietas

Itens	Dietas		
	10% PB	12% PB	14% PB
Proporção dos alimentos nas dietas (% MS da dieta)			
Silagem de Milho	50.0	50.0	50.0
Milho grão	39.7	39.6	39.6
Farelo de Trigo	5.9	3.0	0.0
Mix ¹	2.2	5.3	8.3
Cloreto de Sódio	0.5	0.5	0.5
Sal mineral ²	0.5	0.5	0.5
Bicarbonato de sódio	0.8	0.8	0.8
Óxido de magnésio	0.3	0.3	0.3
Composição química (% MS da dieta)			
MS	55.5	55.6	55.7
MO	95.4	94.9	94.4
PB	9.9	12.1	14.2
PDR ³	66.8	69.6	71.6
EE ⁴	4.0	4.0	4.0
FDNcp ⁵	31.4	30.7	29.9
CNF ⁶	50.7	49.4	48.2
NDT(%) ⁷	80.2	79.3	79.7
ED ⁸	3.47	3.45	3.50

¹Mix = 83,3% Farelo de soja e 16,7% de uréia/sulfato de amônia (U/S.A); ²Sal mineral = 223 g/kg de Ca; 174 g/kg de P; 24 g/kg de S; 100 mg/kg de Co; 1.250,0 mg/kg de Cu; 1.795,0 mg/kg de Fe; 90 mg/kg de I; 2.000,0 mg/kg de Mn; 15,00 mg/kg de Se; 5.270,00 mg/kg de Zn e 1.740,00 mg/kg de F. ³Proteína degradável no rúmen; ⁴EE = Extrato etéreo; ⁵FDNcp = Fibra em detergente neutro corrigida para cinzas e proteína; ⁶CNF = Carbohidratos não fibrosos; ⁷NDT(%) = Nutrientes digestíveis totais; ⁸ED = Energia digestível.

Mensurações na Câmara Respirométrica

O sistema adotado para mensurações em câmara respirométrica foi o de circuito aberto e foi descrito por Rodríguez et al. (2007), no qual o consumo de oxigênio e a produção de metano e dióxido de carbono pelo animal são calculados pela diferença entre a quantidade de um gás no ar externo e a quantidade deste mesmo gás na amostra obtida no interior da câmara respirométrica.

Antes da mensuração da produção e consumo de gases pelo animal, foi realizada uma calibração dos analisadores de gás, na qual se ajustava o fluxo do fluxômetro de massa de acordo com o peso vivo do animal, e conferia-se o bom funcionamento dos sistemas de circulação e refrigeração do ar, de forma a garantir o mínimo de desconforto para o animal alojado no interior da câmara respirométrica. Após a calibração, o animal era conduzido à câmara. Imediatamente antes do início da leitura, o animal era alimentado para que dados de produção de gás (e como consequência, a produção de calor) associados à alimentação não fossem perdidos. A câmara era então fechada e assim iniciava-se a leitura, que prosseguia até o dia seguinte (totalizando, em média, 20h de leitura).

Para a obtenção da exigência de energia, é necessária a mensuração da energia perdida pelo animal como calor. São duas as fontes de produção de calor no metabolismo animal: o metabolismo basal e o calor oriundo do processo digestivo ou incremento calórico da dieta (IC). A produção de calor é obtida pela equação proposta por Brouwer (1965), que considera o volume de gases consumidos e produzidos pelo animal, além do nitrogênio excretado na urina (Nu), como segue: $PC = (3,866 \times O_2) + (1,200 \times CO_2) - (0,518 \times CH_4) - (1,431 \times Nu)$.

Por sua vez, os volumes (em litros) de oxigênio (O₂) consumido e dióxido de carbono (CO₂) e metano (CH₄) produzidos são determinados por respirometria. A determinação do calor oriundo do metabolismo basal corresponde à PC do animal em jejum alimentar por um período de, pelo menos, 72h (PCJ), que por sua vez, também corresponde à exigência de energia líquida para manutenção do animal.

Ensaio de digestibilidade

Para avaliação da digestibilidade das dietas no experimento 1, foram realizadas coletas pontuais de fezes de todos os animais ao início e ao final do experimento. As coletas

foram realizadas durante três dias consecutivos, às 6h00 do dia 1, às 12h00 do dia 2 e às 18h00 do dia 3. As amostras foram secas em estufa com ventilação forçada (55°C) por 72 horas e moídas em moinho de facas a 1 e 2 mm. Foi realizada uma amostra composta por período de coleta para cada animal. Para estimar a produção de matéria seca fecal foi utilizada a fibra em detergente neutro indigestível como indicador.

No experimento 2, realizou-se um ensaio de digestibilidade, com coleta total de fezes durante três dias consecutivos. Ao final de cada dia de coleta (24 horas) as fezes foram pesadas, homogeneizadas e uma amostra diária foi submetida à secagem parcial em estufa de ventilação forçada (55°C) por 72 horas e moída em moinho de facas com peneiras de 2 e 1 mm, respectivamente (Wiley mill; A. H. Thomas, Philadelphia, PA). Posteriormente, foi feita uma amostra composta das fezes de cada animal por período, com base no peso seco de cada dia de coleta.

Também foi realizada a coleta total de urina de cada animal, durante as mesmas 72 horas da coleta total de fezes. Foram utilizados funis coletores acoplados à mangueiras, que conduziram a urina até recipientes plásticos contendo 200 ml de H₂SO₄ a 20%, para a conservação do N. Ao final de cada dia de coleta (24 horas) o volume urinário diário foi quantificado e uma amostra proporcional ao volume diário excretado de cada animal foi armazenada. Posteriormente foi feita uma amostra composta de urina por período, para cada animal, proporcional ao total excretado em cada um dos dias de coleta. A amostra composta foi armazenada a -20°C para posterior análise de N urinário.

Abates e amostragens

Ao final dos 225 dias do experimento 1, os animais foram abatidos, sendo selecionados aleatoriamente e abatidos 7 animais por dia.

Os animais foram submetidos a jejum de sólidos de 16 horas antes ao abate. O abate foi realizado via insensibilização e secção da jugular para sangramento total, seguido de lavagem do aparelho gastrintestinal (rúmen, retículo, omaso, abomaso e intestinos delgado e grosso). Os pesos do coração, pulmões, fígado, baço, rins, gordura interna, carne industrial, mesentério, cauda e aparas (traqueia, esôfago e aparelho reprodutor), juntamente com os do trato gastrintestinal lavado, foram somados aos das demais partes do corpo (carcaça, cabeça, couro, pés e sangue) para determinação do PCVZ.

O rúmen, retículo, omaso, abomaso, intestino delgado, intestino grosso, mesentério, gordura interna, coração, fígado, rins, pulmão, baço, carne industrial, língua e aparas (esôfago, traqueia e aparelho reprodutor) foram moídos em um cutter industrial para obtenção de uma amostra referente aos órgãos e vísceras.

Durante a sangria foi coletada uma amostra do sangue. Após a esola dos animais, amostrou-se o couro dos mesmos, sendo este coletado em diferentes porções. A cabeça e os membros dos animais foram moídos em moedor de ossos industrial após a retirada do couro dos mesmos, e uma amostra foi coletada.

Após o abate, a carcaça de cada animal foi dividida em duas metades que foram pesadas e, em seguida, resfriadas em câmara fria a 4°C durante 24 horas. Posteriormente todas as metades carcaças direitas foram desossadas para separação dos ossos e músculo mais gordura, que também foram coletados.

Todos os itens amostrados (sangue, couro, órgãos e vísceras, cabeça e membros, músculo mais gordura e ossos) foram liofilizados por 72 horas e, posteriormente moídos em liquidificador industrial (EBERBACH, modelo nº E8017.00) utilizando-se nitrogênio líquido para possibilitar a moagem uma vez que os componentes amostrados não foram parcialmente desengordurados.

Elaborou-se, então, uma única amostra por animal, composta por cada ítem amostrado (sangue, couro, órgãos e vísceras, cabeça e membros, músculo mais gordura e ossos), proporcionalmente em relação ao peso de corpo vazio do mesmo.

Análises químicas

As amostras dos volumosos, ingredientes do concentrado, sobras e fezes foram avaliadas quanto aos teores de matéria seca (MS) segundo método INCT - CA G-003/1, matéria mineral (MM) segundo método INCT-CA M-001/1, proteína bruta (PB) segundo método INCT - CA N-001/1, fibra em detergente neutro (FDN) segundo método INCT - CA F-001/1 e correções para proteína e cinzas, respectivamente, segundo métodos INCT - CA N-004/1 e INCT - CA M-002/1, extrato etéreo (EE) segundo método INCT - CA G-004/1, e lignina segundo método INCT-CA F-005/1, conforme descritas por Detmann et al. (2012). A fibra em detergente neutro indigestível (FDNi), foi obtida após a incubação das amostras de fezes, digesta reticular e omasal, volumosos e concentrado moídos a 2mm, em sacos F57 (Ankom®) in situ por 288 horas, de acordo com o método INCT- CA F-008/1, conforme descritos por Detmann et al. (2012).

A quantificação dos carboidratos não fibrosos (CNF) foi realizada de acordo com (Detmann and Valadares Filho 2010): $CNF = 100 - [(\%PB - \%PB \text{ da uréia} + \% \text{ de uréia}) + \%FDN_{cp} + \%EE + \%MM]$ em que: FDN_{cp} = fibra em detergente neutro corrigida para cinzas e proteína.

As determinações das concentrações dos aminoácidos foram realizadas por intermédio de cromatografia líquida de alta eficiência (HPLC). A metodologia envolve a reação de derivatização pré-coluna com fenilisotiocianato (PITC), formando os PTC aminoácidos que foram quantificados por HPLC em fase reversa (loop de injeção de 30uL, pH 6,40, em gradiente

linear binário com fluxo de 1mL/min e temperatura da coluna 58°C), utilizando detecção em UV a 254nm.

Cálculos para as exigências nutricionais e análises estatísticas

O consumo de energia digestível (CED) pelos animais foi obtido pela multiplicação da fração digestível de cada nutriente pelo seu respectivo valor calórico : $CED = 5,6 \times CPBd + 9,4 \times CEEd + 4,2 \times CFDN_{cpd} + 4,2 \times CNFd$, conforme NRC (2001), em que CED = consumo de energia digestível (Mcal/dia); CPBd = consumo de proteína bruta digestível (kg/dia); CEEd = consumo de extrato etéreo digestível (kg/dia); $CFDN_{cpd}$ = consumo de fibra em detergente neutro corrigida para cinzas e proteína digestível (kg/dia) e CNFd = consumo de carboidratos não fibrosos digestíveis (kg/dia). O consumo de energia metabolizável (CEM) foi calculado multiplicando-se o CED por 0,82.

O consumo de proteína metabolizável (CPmet) foi estimado somando-se a proteína microbiana verdadeira digestível e a proteína não degradável no rúmen digestível. Utilizou-se o valor de síntese de proteína microbiana de 120 g/kg de NDT (BR-CORTE, 2010), sendo sua fração verdadeira adotada como 80% e considerando sua digestibilidade de 80% (NRC, 2001). O consumo de proteína não degradada no rúmen foi estimado como o consumo de proteína bruta menos a produção microbiana, e sua digestibilidade considerada como 80%.

A determinação da energia corporal foi obtida a partir dos teores corporais de proteína e gordura e seus respectivos equivalentes calóricos, 5,6405 e 9,3929 respectivamente, conforme equação preconizada pelo ARC (1980): $CE = PB \times 5,6405 + EE \times 9,3929$, em que CE = conteúdo de energia corporal (Mcal), PB = proteína no corpo vivo (kg) e EE = extrato etéreo no corpo vivo (kg).

Ajustou-se uma equação de regressão entre a energia retida (ER) e o ganho diário de PCVZ (GPCVZ), para determinar PCVZ metabólico ($PCVZ^{0,75}$) para os animais em manutenção e desempenho, utilizando-se o seguinte modelo: $ER = a \times PCVZ^{0,75} \times GPCVZ^b$, em que ER = energia retida (Mcal/dia), $PCVZ^{0,75}$ = peso de corpo vazio metabólico ($kg^{0,75}$), GPCVZ = ganho de peso de corpo vazio (kg/dia) e 'a' e 'b' são parâmetros da regressão.

A produção de calor (PC), em Mcal/ $PCVZ^{0,75}$ /dia, foi obtida pela diferença entre o CEM (Mcal/ $PCVZ^{0,75}$ /dia) e a ER (Mcal/ $PCVZ^{0,75}$ /dia). A exigência de energia líquida para manutenção (EL_m , Mcal/ $PCVZ^{0,75}$ /dia) foi calculada a partir do intercepto (β_0) da regressão exponencial entre a PC e o CEM (Ferrell & Jenkins, 1998), segundo o modelo: $PC = \beta_0 \times e^{(\beta_1 \times CEM)}$, em que PC = produção de calor (Mcal/ $PCVZ^{0,75}$ /dia), CEM = consumo de energia metabolizável (Mcal/ $PCVZ^{0,75}$ /dia), β_0 e β_1 são parâmetros da regressão.

A EMm (Mcal/ $PCVZ^{0,75}$ /dia) foi determinada por método iterativo, sendo a EMm estimada como sendo o valor em que a PC é igual ao CEM. A eficiência de utilização da energia metabolizável para manutenção (k_m) foi obtida a partir da relação entre as exigências de energia líquida e metabolizável para manutenção (EL_m/EM_m).

A exigência de proteína metabolizável para manutenção foi baseada na regressão linear entre o consumo de proteína metabolizável e o ganho de peso de corpo vazio, conforme modelo: $CP_{met} = \beta_0 + GPCVZ \times \beta_1$, em que CP_{met} = consumo de proteína metabolizável (g/dia), GPCVZ = ganho de peso de corpo vazio (kg/dia) e β_0 e β_1 são parâmetros da regressão. A divisão do intercepto dessa regressão pelo peso metabólico médio dos animais estima os requerimentos de proteína metabolizável para manutenção: $PM_m = (\beta_0/PC^{0,75})$, em que PM_m = exigência de proteína metabolizável para manutenção (g/ $PC^{0,75}$ /dia), β_0 = intercepto e $PC^{0,75}$ = peso corporal em jejum metabólico médio (kg).

A exigência líquida de proteína para ganho foi estimada através de um modelo envolvendo GPCVZ e energia retida no corpo, como segue: $PLg = \beta_1 \times GPCVZ + \beta_2 \times ER$, em que PR = proteína retida (g/dia), GPCVZ = ganho de peso de corpovazio (kg/dia), ER = energia retida (Mcal/dia) e β_1 e β_2 são os parâmetros da regressão. As exigências de proteína metabolizável paraganho (PMg) foram calculadas dividindo-se as exigências líquidas de proteína paraganho pela eficiência de utilização da proteína metabolizável paraganho.

Os modelos lineares utilizados foram ajustados por intermédio do PROC REG do SAS e para os modelos não-lineares foi utilizado o PROC NL MIXED do SAS, sendo esses últimos ajustados pelo método de Gauss-Newton. Para verificar a significância dos parâmetros dos modelos e identificar efeitos de grupo genético sobre os requerimentos dos animais foi utilizado 5% como nível crítico de probabilidade.

Resultados e discussão

- Experimento 1:

Relação entre PCJ e PCVZ e GMD e GPCVZ

Os dados foram contrastados por meio de uma regressão linear sem intercepto, testando-se o efeito de grupo genético. Não houve diferença entre os grupos genéticos ($P > 0,05$) e a equação conjunta obtida foi a seguinte: $PCVZ = 0,925 \times PCJ$, em que PCVZ = peso de corpovazio em jejum (kg) e PCJ = peso corporal em jejum (kg).

A relação entre PCJ e o PCVZ encontrada neste estudo (0,925) foi similar aos valores de 0,915 descritos por Menezes (2016) e de 0,914 obtidos por Costa e Silva (2012), e inferior ao valor recomendado pelo BR-CORTE de 0,895. Já o NRC (2000) recomenda o valor médio de 0,891, mas relata que essa relação pode variar de 85 a 95%.

Houve diferença ($P < 0,05$) entre GG para a relação GMD x GPCVZ, sendo obtida a relação média de 0,966 para animais Nelore, e 0,947 para animais cruzados. A relação obtida é bastante próxima à descrita pelo NRC (2000) de 0,956. A diferença obtida para grupo genético pode estar associada ao maior efeito de enchimento nos animais cruzados quando comparado à animais Nelore, justificando a menor relação GMD x GPCVZ.

Exigências de Energia para Manutenção

A relação exponencial entre a produção de calor e o consumo de energia metabolizável gerou as seguintes equações: $PC = 0,0746 \times e^{(4,0335 \times CEM)}$, para animais Nelore; e $PC = 0,0746 \pm 0,0020 \times e^{(3,9407 \pm 0,1033 \times CEM)}$, para animais cruzados Angus x Nelore, em que PC = produção de calor (Mcal/PCVZ^{0,75}/dia) e CEM = consumo de energia metabolizável (Mcal/PCVZ^{0,75}/dia).

Não houve efeito de grupo genético sobre o parâmetro β_0 do modelo ($P = 0,0899$), o que indica não haver diferenças na estimativa da ELM. Por outro lado, foi observado efeito de grupo genético sobre o parâmetro β_1 do modelo descrito acima ($P = 0,0392$), o que indica que existem efeitos raciais sobre a eficiência de utilização da energia metabolizável para manutenção.

O valor encontrado para ELM no presente experimento foi de 74,6 Kcal/PCVZ^{0,75}/dia é similar ao valor recomendado pelo BR-CORTE de 74,2.

O valor de EMmfo calculado como o momento em que o consumo de energia metabolizável se torna igual a produção de calor, ou seja, quando todo o CEM for perdido na forma de calor não haverá retenção de energia, sendo este CEM equivalente à exigência de EMm. A EMm encontrada para animais Nelore foi de 122 kcal/PCVZ^{0,75}/dia e de 119 kcal/PCVZ^{0,75}/dia para animais cruzados. Os valores obtidos para EMm nesse experimento são superiores ao valor apresentado no BR-CORTE (2010) de 112,4 kcal/PCVZ^{0,75}/dia.

Exigências de Energia Líquida para Ganho

A energia líquida de ganho é definida como o teor de energia do tecido depositado, que, por sua vez, é uma função da proporção de gordura e proteína no ganho de tecido do corpo vazio (Garrett et al., 1959). A composição do ganho de corpo vazio é o principal determinante das exigências de energia para ganho de peso, que são estimadas a partir da energia retida no corpo (BR-CORTE, 2010).

Para cálculo da energia retida (ER) ou energia líquida para ganho (ELg), foi ajustada uma equação de regressão entre a energia retida em função do GPCVZ para estimar as exigências líquidas de energia para ganho para todos os pesos e ganhos de peso.

Não houve diferença estatística entre os grupos genéticos ($P > 0,05$), e o modelo obtido foi o seguinte: $ER = 0,0643 \times PCVZ^{0,75} \times GPCVZ^{0,6191}$, em que ER = energia retida ou requerimento de energia líquida (ELg) (Mcal/dia), $PCVZ^{0,75}$ = peso de corpo vazio médio metabólico (kg) e GPCVZ = ganho de peso de corpo vazio (kg/dia).

O expoente do GPCVZ obtido (0,6191) foi numericamente menor do que o descrito por Chizzotti et al. (2008) de 1,070 e por Gionbelli (2010) de 1,1216. De acordo com os dados deste experimento, a ER aumenta de forma menos acentuada com o aumento do GPCVZ em comparação ao modelo de Chizzotti et al. (2008) e de Gionbelli (2010), sugerindo uma maior proporção de proteína no GPCVZ.

Eficiência de Utilização da Energia Metabolizável para Manutenção e Ganho

Os principais sistemas de exigências nutricionais (NRC, 2000; ARC, 1980; AFRC, 1993; CSIRO, 2007) utilizam separadamente as informações de eficiência de utilização da energia metabolizável para manutenção (k_m) e paraganho (k_g).

O valor de k_m pode ser obtido dividindo-se o valor de ELM pelo valor de EMm. As eficiências de conversão da ELM para EMm de animais Nelore e cruzados neste experimento foram, respectivamente, 61,1 e 62,7%. Este valor está um pouco abaixo da faixa preconizada por Ferrell & Jenkins (1998) para animais taurinos e seus cruzados, que sugerem que o valor de k_m fique entre 0,65 e 0,69.

Diversas variáveis alteram os valores de k_m obtidos. Determinar os fatores que afetam a k_m é exatamente um dos desafios enfrentados pelos nutricionistas, já que, reconhecidamente, a PC é afetada pela taxa e pela composição do ganho de peso (Willians & Jenkins, 2003). Garrett (1980) apontou dentre eles a proporção de ácidos graxos voláteis absorvidos, o nível de fibra dietética, o nível de consumo de EM, turnover protéico, entre outros. O autor ainda explicou que a síntese e degradação protéica poderiam explicar diferenças entre k_m de raças que são terminadas com maior e menor peso. Marcondes et al. (2010), estudando os efeitos de diversas variáveis sobre a k_m , observaram que os requisitos de manutenção são afetados pelo desempenho dos animais, e propuseram o seguinte modelo: $k_m = 0,513 + 0,173 \times kg + a \times GPCVZ$, onde a é igual a 0,100 para animais Nelore e 0,073 para animais cruzados Bostaurus x Bos indicus.

Assim como para o valor de k_m , na maioria dos trabalhos nacionais tem-se estimado valores estáticos de k_g . O BR-CORTE (2010) cita que, o k_g pode ser obtido através do coeficiente de inclinação da regressão linear entre a ER e o CEM. Neste trabalho, o valor de k_g foi estimado desta mesma forma (Figura 1), sendo a inclinação do modelo de 0,2933.

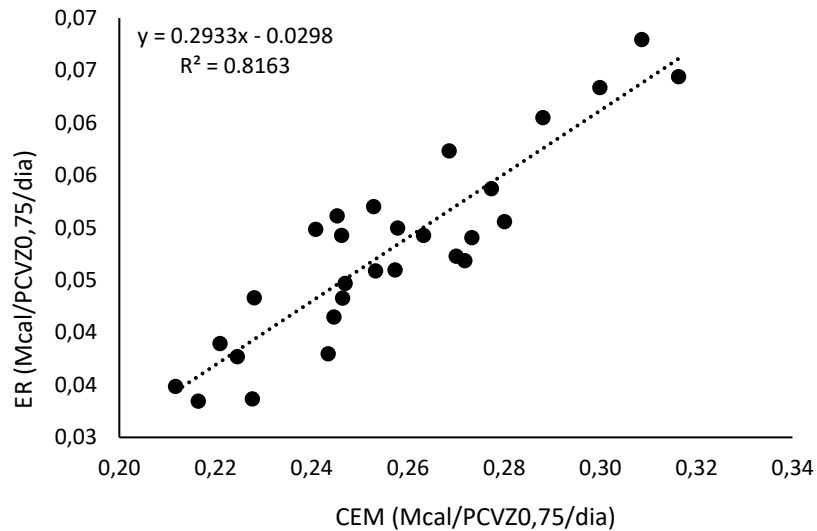


Figura 1 - Energia retida em função do consumo de energia metabolizável.

Exigências de Proteína para Ganho

As exigências de PR foram estimadas conforme preconizado pelo BR CORTE (2010). A equação obtida foi a seguinte: $PR = 188,37 \times GPCVZ - 9,39 \times ER$, em que PR = proteína retida (g/dia), GPCVZ = ganho de peso de corpovazio (kg/dia) e ER = energia retida (Mcal/dia).

De acordo com Geay (1984), a proporção de proteína no ganho cai linearmente com o aumento da deposição de energia, e as exigências líquidas de proteína para ganho são mais baixas em animais tardios em comparação a bovinos precoces.

A equação recomendada para machos não castrados pelo BR- CORTE (2010) foi: $PR = 219,43 \times GPCVZ - 15,01 \times ER$ para animais cruzados e $PR = 238,79 \times GPCVZ - 15,68 \times ER$ para animais Nelore.

Fox & Black (1984) apresentaram modelos de crescimento de tecidos que mostram que alterações nas curvas de crescimento ocorrem de forma mais pronunciada em animais na fase de crescimento. Ressaltam, ainda, que as exigências líquidas de proteína para bovinos em terminação dependem da composição corporal dos animais. Assim, os requerimentos variam

de acordo com o peso, a taxa de ganho de peso, raça, sexo, efeitos dietéticos e manejo nutricional.

Para conversão das exigências líquidas de proteína (PR), em exigências de proteína metabolizável (PM) paraganho, deve-se dividir o valor de PR pela eficiência de utilização da proteína metabolizável paraganho (k), que nesse experimento foi de 0,33, valor obtido quando se avaliou a proteína retida em função do consumo de proteína metabolizável (Figura 2).

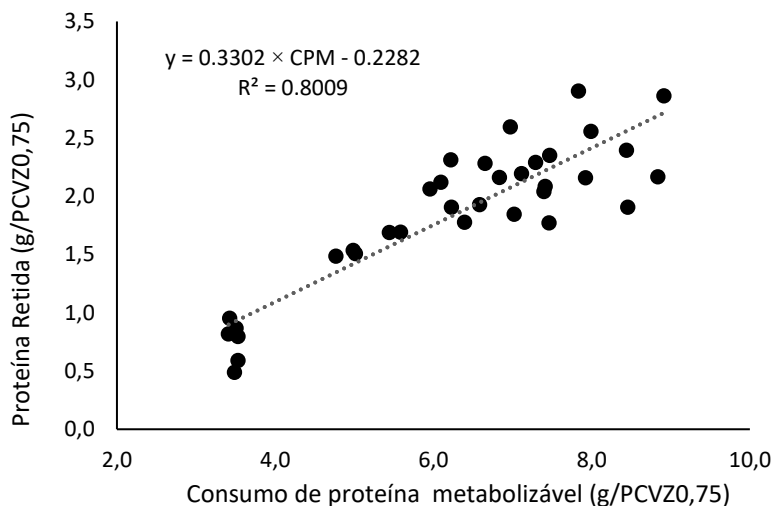


Figura 2: Proteína retida em função do consumo de proteína metabolizável

Marcondes et al. (2010), trabalhando com machos castrados Nelore e cruzados em terminação, encontraram eficiência de conversão de PR em PM de 0,38. O NRC (2000), baseado no trabalho de Wilkerson et al. (1993), preconizou que a eficiência de conversão da PR em PM decresce com o aumento do peso do animal em função da diminuição da deposição de proteína. O NRC (2000) considera valor fixo de 0,492 para animais com peso acima de 300kg, enquanto o BR CORTE (2010) recomenda usar 0,469 para animais com peso acima de 350kg.

- Experimento 2:

Consumo e digestibilidade

O consumo (kg/dia) e a digestibilidade aparente dos nutrientes são apresentados na Tabela 2. Não houve interação significativa ($P > 0,05$) entre grupo genético e níveis dietéticos de PB para nenhuma das variáveis de consumo e digestibilidade aparente avaliadas. Animais cruzados apresentaram maior CMS expresso em kg/dia ($P < 0,05$) em relação aos da raça Nelore.

Não foi verificado efeito significativo ($P > 0,05$) dos níveis de PB sobre o CMS. Este comportamento corrobora com os resultados obtidos em estudos com bovinos de corte alimentados com diferentes níveis de PB (Menezes et al., 2016; Prates et al., 2015; Amaral et al., 2013).

Os consumos dos nutrientes (kg/dia) foram maiores ($P < 0,05$) para os animais cruzados em relação aos zebuínos puros, isto pode ser atribuído ao comportamento verificado para o CMS em kg/dia.

De maneira geral, as digestibilidades aparentes dos nutrientes não diferiram ($P > 0,05$) entre grupos genéticos. Da mesma forma, não houve diferença para as digestibilidades aparentes dos nutrientes ($P > 0,05$), quando comparou-se consumo restrito e voluntário.

O CPB aumentou linearmente ($P < 0,05$) com os níveis de PB, demonstrando que maiores concentrações de PB nas dietas estão relacionadas com maiores consumos de N em bovinos (NoftsgerandSt-Pierre, 2003).

Houve efeito do nível de PB ($P < 0,05$) sobre o consumo de NDT (CNDT). O CNDT cresceu linearmente com o aumento dos níveis de PB na dieta, seguindo o comportamento do CPB.

Houve efeito linear dos níveis dietéticos de PB ($P < 0,05$) sobre as digestibilidades aparentes da MS e MO. O aumento da digestibilidade da MS tem sido relacionado positivamente com as quantidades de PB nas dietas (Allen, 2000; Archibeque et al., 2007).

Houve efeito linear ($P < 0,05$) sobre a digestibilidade aparente da PB em resposta aos níveis crescentes de PB. Supõe-se que essa diferença possa ser atribuída à maior inclusão de uma fonte de nitrogênio não proteico, uma vez que a concentração de N-amoniaco no rúmen é indispensável para o crescimento microbiano. O metabolismo de proteína no rúmen é resultado da atividade metabólica dos microrganismos e envolve dois importantes eventos: a degradação da proteína, que fornece fontes de N para as bactérias ruminais e a síntese de microbiana (Bach et al., 2005).

Exigências de Energia para Manutenção – Câmara de respirometria calorimétrica

A mensuração da produção de calor dos animais do experimento 2, foi obtida pela equação proposta por Brouwer (1965), após a passagem dos mesmos pela câmara respirométrica. A relação exponencial entre a produção de calor mensurada, e o consumo de energia metabolizável gerou a seguinte equação: $PC = 0,0852 \pm 0,0146 \times e^{(2,5315 \pm 0,6522 \times CEM)}$, em que PC = produção de calor (Mcal/PCVZ^{0,75}/dia) e CEM = consumo de energia metabolizável (Mcal/PCVZ^{0,75}/dia), sendo a ELM = 85,2 Kcal/PCVZ^{0,75}/dia. O valor de ELM encontrada pela técnica da câmara respirométrica (85,2) foi 12% superior ao valor de ELM encontrado pelo método do abate comparativo (74,6).

A EMm encontrada, foi de 114 kcal/PCVZ^{0,75}/dia, valor bastante semelhante ao descrito por Menezes et al. (2016) de 114,41 kcal/PCVZ^{0,75}/dia. O método do abate comparativo gerou valores de EMm superiores aos estimados por esta técnica, sendo 122 para animais Nelore e 119 kcal/PCVZ^{0,75}/dia para animais cruzados.

A eficiência de conversão da ELM para EMm (km) foi de 74,5%. O valor obtido em câmara respirométrica foi superior aos valores de km obtidos pelo método do abate comparativo, de 61,1% e 62,7% para Nelore e para animais cruzados, respectivamente.

Tabela 2: Consumo e digestibilidade animais experimento de metano

Item	Grupo Genético		Nível de PB ¹				EPM	Efeito principal ²			Contraste ³		
	Nelore	Cruzado	CR	10	12	14		GG	N	GG×N	CR × CV	L	Q
Consumo de nutrientes													
MS	7,48	9,58	5,49	9,50	9,37	9,77	0,542	0,012	<,001	0,069	<,001	0,352	0,301
MO	7,15	9,14	5,24	9,11	8,95	9,28	0,005	0,012	<,001	0,073	<,001	0,553	0,324
PB	0,90	1,14	0,65	0,94	1,13	1,36	0,068	0,018	<,001	0,121	<,001	<,001	0,604
FDN	2,38	3,11	1,83	3,08	2,98	3,08	0,172	0,007	<,001	0,051	<,001	0,716	0,348
CNF	3,89	4,94	2,79	5,10	4,87	4,91	0,309	0,024	<,001	0,135	<,001	0,254	0,342
NDT	5,50	7,01	4,04	6,68	6,88	7,41	0,442	0,018	<,001	0,111	<,001	0,025	0,516
Digestibilidade dos nutrientes													
MS	69,40	69,19	67,01	68,82	71,65	69,71	0,019	0,915	0,079	0,290	0,709	0,012	0,730
MO	70,31	70,31	70,61	68,11	69,95	72,58	1,937	0,998	0,091	0,388	0,788	0,014	0,782
PB	68,43	67,34	66,53	63,77	68,58	72,66	1,941	0,509	0,001	0,260	0,282	<,001	0,824
FDN	49,15	48,14	50,78	45,72	46,35	51,73	3,713	0,783	0,199	0,310	0,329	0,080	0,408
EE	78,69	79,68	80,72	75,97	80,61	79,43	3,046	0,788	0,145	0,943	0,293	0,123	0,132
CNF	81,27	82,08	81,93	79,83	81,68	83,25	1,376	0,581	0,055	0,177	0,734	0,007	0,885

¹CR = consumo restrito;

²GG = grupo genético; N = nível dietético de PB;

³CR × CV = consumo restrito × consumo voluntário ; L = efeito linear do nível de proteína; Q = efeito quadrático.

Resumo das Equações Geradas e Cálculo das Exigências Nutricionais

A partir do resumo das equações geradas neste trabalho e algumas citadas pelo BR CORTEem 2010 (Valadares Filho et al., 2010) estimaram-se os requerimentos nutricionais de energia e proteína de zebuínos puros (Nelore) e cruzados (F1 Angus×Nelore) não castrados em confinamento (Tabela3).

Tabela 3 – Resumo dos modelos de estimativa das exigências nutricionais de energia e proteína parabolinos Nelore e Cruzados (F1 AxN) não castrados em terminação

Item	Equação	Unidade
PCVZ	$0,925(\pm 0,00185) \times PCJ$	kg
GPCVZ	Nelore: $0,966 \times GMD$	kg/dia
	Cruzado: $0,947 \times GMD$	kg/dia
ELm	74,6	Kcal/PCVZ ^{0,75} /dia
EMm	Nelore: 122	Kcal/PCVZ ^{0,75} /dia
	Cruzado: 119	Kcal/PCVZ ^{0,75} /dia
k _m	Nelore: 0,61	-
	Cruzado: 0,63	-
ELg	$0,0643 \times PCVZ^{0,75} \times GPCVZ^{0,6191}$	Mcal/dia
k _g	0,29	-
EMg	ELg/k _g	Mcal/dia
EM	EMm + EMg	Mcal/dia
ED	EM × 0,82	Kcal/PCVZ ^{0,75} /dia
NDT	ED × 4,409	kg/dia
PLg	$188,37 \times GPCVZ - 9,32 \times ER$	g/dia
k	0,33	-
PMg	PLg/k	g/dia
PM	PMm + PMg	g/dia
PBmic	PB _{mic} = 120 × NDT	g/dia
PDR	(PB _{mic} × 1,11)	g/dia
PNDR	$(PM_{total} - (PB_{mic} \times 0,64))/0,8$	g/dia

Conclusão

Utilizando-se o método do abate comparativo, as exigências de machos não castrados terminados em confinamento são:

Exigência de energia líquida para manutenção é de 74,6 kcal/PCVZ^{0,75}/dia.

A exigência de energia metabolizável para manutenção é de 122 kcal/PCVZ^{0,75}/dia para animais Nelore, e 119 kcal/PCVZ^{0,75}/dia para animais cruzados.

A eficiência de utilização da energia metabolizável para manutenção é de 61,1% para animais Nelore e 62,7% para animais cruzados.

A eficiência de utilização da energia metabolizável para ganho é de 29,33%.

A exigência de proteína metabolizável para ganho de machos não castrados terminados em confinamento pode ser calculada pela seguinte equação: $PR = 188,37 \times GPCVZ - 9,32 \times ER$.

A eficiência de uso da proteína metabolizável para ganho é igual a 33,02%.

Utilizando-se a câmara respirométrica, as exigências de machos não castrados terminados em confinamento são:

Exigência de energia líquida para manutenção é de 85,2 kcal/PCVZ^{0,75}/dia.

A exigência de energia metabolizável para manutenção é de 114 kcal/PCVZ^{0,75}/dia.

A eficiência de utilização da energia metabolizável para manutenção é igual a 74,5%.

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