HERLON MENEGUELLI ALHADAS

IMPACT OF DIFFERENT LEVELS OF LOW-FAT DRIED DISTILLERS GRAINS IN FINISHING DIETS ON PERFORMANCE, NUTRIENT INTAKE AND DIGESTIBILITY, IN SITU DEGRADABILITY AND ON PROTEIN AND ENERGY INTAKE AND RETENTION AND ESTIMATION OF PROTEIN AND ENERGY REQUIREMENTS OF YOUNG NELLORE BULLS

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

Adviser: Sebastião de Campos Valadares Filho

VIÇOSA - MINAS GERAIS
2022

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T
A397i
2022

Alhadas, Herlon Meneguelli, 1993-
Impact of different levels of low-fat dried distillersgrains in finishing diets on performance, nutrient intake and digestibility, insitu degradability and on protein and energy intake and retention andestimation of protein and energy requirements of young Nellore bulls / Herlon Meneguelli Alhadas. – Viçosa, MG, 2022.
1 tese eletrônica (156 f.): il.

Texto em inglês.
Orientador: Sebastião de Campos Valadares Filho.
Tese (doutorado) - Universidade Federal de Viçosa, Departamento de Zootecnia, 2022.
Inclui bibliografia.
DOI: https://doi.org/10.47328/ufvbbt.2022.231
Modo de acesso: World Wide Web.

1. Bovinos - Nutrição. 2. Nelore (Bovino). 3. Grãos. I. Filho, Sebastião de Campos Valadares, 1955-. II. Universidade Federal de Viçosa. Departamento de Zootecnia. Programa de Pós-Graduação em Zootecnia. III. Título.

CDD 22. ed. 636.0852

Bibliotecário(a) responsável: Bruna Silva CRB6/2552

HERLON MENEGUELLI ALHADAS

IMPACT OF DIFFERENT LEVELS OF LOW-FAT DRIED DISTILLERS GRAINS IN FINISHING DIETS ON PERFORMANCE, NUTRIENT INTAKE AND DIGESTIBILITY, IN SITU DEGRADABILITY AND ON PROTEIN AND ENERGY INTAKE AND RETENTION AND ESTIMATION OF PROTEIN AND ENERGY REQUIREMENTS OF YOUNG NELLORE BULLS

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

APPROVED: February 23, 2022

Assent:

Herlon Meneguelli Alhadas
Author

Sebastião de Campos Valadares Filho
Adviser

ACKNOWLEDGEMENTS

Mesmo que descrito nestas poucas linhas, jamais serei capaz de agradecer a cada um que fez parte dessa construção que hoje marca minha personalidade, inteligência e caráter. Sou eternamente grato à Deus por me permitir trilhar estes passos. Fui um privilegiado em uma sociedade marcada pela desigualdade. Embora nunca tenha tido os bens materiais em abundância, não precisei trabalhar para estudar ou me preocupar se teria o que comer. E se hoje me coloco sob avaliação para tentar adquirir o mais alto grau de qualificação intelectual que existe na sociedade, é pela garra de uma alma que hoje ocupa a posição de minha mãe, Elaine. Digo que hoje ocupa esta posição pois tenho a convicção que este não é nosso primeiro encontro. Espero ainda podermos trilhar juntos muitos caminhos. Sem dúvida uma das pessoas com maior coração que conheço. Obrigado minha mãe. Se eu puder oferecer para um filho 10% do que fez pela nossa família estarei muito feliz. E como nada é por acaso, fomos reunidos em um lar de três filhos, Herlon, Marcony e Rayane. Cada um com suas características e afinidades, e que foram desde sempre exemplos para mim. Muito desta conquista se deve a vocês, tanto pelo apoio financeiro quanto pelo exemplo moral. Eu amo vocês. E falando em amor, em um dia você é o "Herlon" e no outro você é o "Tio Herlon". Quanta vida você trouxe para nossos lares Arthur. Quanta energia e alegria em uma só alma! O tio te ama! Mesmo que do outro lado desta vida, agradeço também a você meu pai, José Alípio, que permitiu a reunião de nossas almas e que nos deixou uma herança incalculável, a doutrina espírita. Talvez eu já tivesse sucumbido se não fosse por conhecer as verdades que o espiritismo nos permite. Mas a família também é composta por aqueles que escolhemos ao longo de nossa jornada. E tenho o privilégio de poder ter amigos no mais puro significado desta palavra. Vocês fizeram e fazem meus dias mais alegres. Obrigado por tantas risadas, reflexões, carinho e atenção. Esses são os combustíveis que nos movem! Os caminhos da pós graduação, para aqueles que querem fazer um bom trabalho, são muitas vezes penosos. Mas poderia ter sido muito pior, se não tivesse

tido o privilégio de ser orientado por um dos maiores profissionais que tive a oportunidade de conhecer, Professor Dr. Sebastião de Campos Valadares Filho. Quanta determinação e vontade! Liderar a equipe LabNur é para quem tem muita balha na agulha, e muito conhecimento! Obrigado professor, o senhor realmente faz jus ao termo "Orientador". E falando em excelência de trabalho, fui também um privilegiado acadêmico. A experiência internacional nos deixa muitas marcas, e claro, é sempre um desafio. Mas fomos (digo fomos porque tive a oportunidade de passar por esta etapa com um grande amigo, vulgo Marquim) recebidos de braços abertos pelo Professor Dr. Luis Orlindo Tedeschi, uma das pessoas mais inteligentes que já convivi, e que nos deu uma aula de como acolher pessoas. Muito obrigado professor, pela sua contribuição na estruturação de minha tese e na minha trajetória profissional. E de forma geral, mas sem dúvida nem um pouco menos importante, agradeço a todos os professores da graduação e pós-graduação. Infelizmente a sociedade ainda não está preparada para entender o papel da educação e da ciência em nossas vidas. E estendo o meu agradecimento a todos os profissionais da ciência e da educação, pois sem vocês a humanidade entra em ruínas.

Agradeço ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), pela concessão da bolsa de estudos.

Agradeço ao Instituto nacional de ciência e tecnologia de ciência animal (INCT) pelo suporte ao projeto.

Agradeço Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), pelo suporte ao projeto.

Agradeço à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pelo suporte ao projeto.

ABSTRACT

ALHADAS, Herlon Meneguelli, D.Sc., Universidade Federal de Viçosa, February, 2022**. Impact of different levels of low-fat dried distillers grains in finishing diets on performance, nutrient intake and digestibility, in situ degradability and on protein and energy intake and retention and estimation of protein and energy requirements of young Nellore bulls.** Adviser: Sebastião de Campos Valadares Filho.

This study was developed in two experiments, one with performance animals and the other with cannulated animals. Each of the two experiments originated two chapters of this thesis, totaling four chapters. In the first chapter, the effect of including low-fat dried distillers grains (**DDG**) on young Nellore bulls performance, nutritional parameters and nitrogen metabolism was evaluated. Thirty-five Nellore cattle were randomly divided into four diets: without DDG (**D0**) or with the inclusion of DDG at 150 g/kg (**D150**), 300 g/kg (**D300**), or 450 g/kg (**D450**). The evaluation period lasted 126 days and three periods of collection of feces and urine were carried out. Final body weight ($P = 0.099$) and average daily gain ($P = 0.097$) tended to decrease linearly; the digestibility of dry matter (**DM**, $P < 0.001$), organic matter (**OM**, $P < 0.001$), ether extract (**EE**, $P < 0.001$) and non-fiber carbohydrates (**NFC**, $P < 0.001$), and intakes of total digestible nutrients (**TDN**, $P < 0.001$) decreased linearly. The increase in crude protein intake (**CP**, $P < 0.001$) did not result in an increase in the amount of nitrogen retained (**N**, $P = 0.540$). In the second chapter, the effect of including low-fat DDG in finishing diets on protein and energy intake and retention and to estimate the protein and energy requirement of young Nellore bulls was evaluated. Thirty-five animals were used: baseline (n = 4), maintenance (n = 4), and ad libitum intake (n = 27). Ad libitum animals were divided into four groups: diets with the inclusion of DDG at the levels of 0, 150, 300 and 450 g/kg (DM basis). At the end of the experiment all animals were slaughtered. There was a linear reduction with increasing DDG levels in the TDN ($P = 0.008$), metabolizable energy (**ME**) intake ($P < 0.010$), in total retained energy ($P = 0.065$), and in heat production ($P < 0.065$). Metabolizable protein (**MP**) intake increased linearly ($P < 0.010$), but retained protein did not differ ($P = 0.499$). Daily net energy and ME requirement for maintenance was 75.9 and 122 kcal/$kg^{0.75}$ EBW, respectively. Daily MP for maintenance was 3.6 g/$kg^{0.75}$ shrunk body weight. In the third chapter it was evaluated the effect of including levels of low-fat DDG in high-concentrate diets on intake and digestibility. Four rumen-cannulated Nellore cattle received four dietary treatments in a 4×4 Latin square experimental design. Treatments included diets without DDG (**D0**) and varying inclusion levels of DDG at 150 g/kg (**D150**), 300 g/kg (**D300**), and 450 g/kg (**D450**). Four

periods of total feces and urine collection were performed to estimate nutrient intake and digestibility and estimate nitrogen balance. Collections of omasum digesta were used to estimate ruminal degradability. The ruminal emptying technique was performed to estimate the ruminal rates of ingestion, passage, and digestion (**kd**). The inclusion of DDG reduced the intake of TDN ($P < 0.010$), DM ($P = 0.020$), OM ($P = 0.020$), NFC ($P < 0.010$) and EE ($P = 0.060$). The intake of neutral detergent fiber (**NDF**) increased linearly ($P < 0.01$), whereas CP had a quadratic pattern ($P = 0.020$). The total digestibility in kg/d of DM ($P < 0.010$), OM ($P < 0.010$), NFC ($P < 0.010$) and EE ($P = 0.020$) decreased linearly. The total digestibility in kg/d of the NDF increased linearly ($P < 0.010$) and the CP had a quadratic pattern ($P = 0.010$). The kd of DM ($P = 0.010$), OM ($P < 0.010$) decreased linearly and NDF increased linearly ($P < 0.010$). The amount of N retained did not change ($P = 0.720$), but the intake of MP increased ($P = 0.03$). And in the fourth chapter, it was evaluated ruminal *in situ* degradation of diets containing different levels of **DDG** replacing soybean meal, urea and corn and the effect of these diets on *in situ* ruminal degradation of DDG, and to propose alternative methods to estimate *in vivo* digestibility of diets. Four rumen-cannulated Nellore bulls were used in a 4×4 Latin square design, for both *in situ* incubation and *in vivo* digestibility. We evaluated the following treatments: diet without DDG (**D0**) or containing 150 (**D150**), 300 (**D300**), or 450 g/kg (**D450**) of inclusion. For *in situ* evaluation, DDG was incubated in all animals, and diets only in those fed with the same diet that would be incubated. To estimate *in vivo* digestibility, three days of total feces collection were performed in each period. Data was analysed in PROC MIXED, PROC NLIN and PROC REG of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). There was no effect of diet on the *in situ* degradation parameters of the DDG ($P > 0.050$). However, for the diets, the readily soluble fraction (parameter "**a**") ($P < 0.010$), and the rate constant for degradation (parameter **kd**) of the potentially degradable fraction in the rumen (parameter "**b**") ($P = 0.050$) of DM, decreased linearly. On the other hand, the parameter "b" ($P < 0.01$) and kd rate ($P = 0.03$) of NDF of the diets increased linearly. The *in situ* incubation time to estimate the *in vivo* DM digestibility ranged from 27.2-h to 37.7-h. For the NDF, the time was 60-h for all diets. In addition, to estimate the *in vivo* digestibility of DM (**DMD**), and the residual NDF (**RNDF**) of diets, two equations were suggested for each one: DMD = 804.8 - 0.092 × level, and DMD = 658 + 0.468 × a; RNDF = 393.9 - 0.138 × level, and RNDF = 874.3 - (0.602 × b) - (0.805 × I) + (1,662 × kd), where I = indigestible NDF. Therefore, the inclusion of low-fat DDG in finishing diets reduces performance, nutrient digestibility and energy intake.

Keywords: Dried distillers grains. Finishing diet. Nellore.

RESUMO

ALHADAS, Herlon Meneguelli, D.Sc., Universidade Federal de Viçosa, fevereiro de 2022**. Impacto de diferentes níveis de inclusão de grãos secos de destilaria de baixa gordura em dietas de terminação sobre o desempenho, consumo e digestibilidade, degradabilidade in situ e sobre o consumo, retenção e exigências de proteína e energia de bovinos Nelore machos não-castrados superprecoces.** Orientador: Sebastião de Campos Valadares Filho.

Este estudo foi desenvolvido em dois experimentos, um com animais em desempenho e outro com animais canulados. Cada um dos dois experimentos originou dois capítulos desta tese, totalizando quatro capítulos. No primeiro capítulo, avaliou-se o efeito da inclusão de grãos secos de destilaria com baixo teor de gordura (DDG) sobre o desempenho, parâmetros nutricionais e metabolismo de nitrogênio de bovinos Nelore machos não-castrados superprecoces. Trinta e cinco bovinos Nelore foram divididos aleatoriamente em quatro dietas: sem DDG (D0) ou com inclusão de DDG a 150 g/kg (D150), 300 g/kg (D300) ou 450 g/kg (D450) . O período de avaliação durou 126 dias e foram realizados três períodos de coleta de fezes e urina. O peso corporal final (P=0,099) e o ganho médio diário (P = 0,097) tenderam a diminuir linearmente; a digestibilidade da matéria seca (MS, P < 0,001), matéria orgânica (MO, P < 0,001), extrato etéreo (EE, P<0,001) e carboidratos não fibrosos (CNF, P < 0,001), e os consumos de nutrientes digestíveis totais (NDT, P < 0,001) diminuíram linearmente. O aumento da ingestão de proteína bruta (PB, P < 0,001) não resultou em aumento da quantidade de nitrogênio retido (N, P = 0,540). No segundo capítulo, avaliou-se o efeito da inclusão de DDG em dietas de terminação sobre a ingestão e retenção de proteína e energia e estimou-se a exigência de proteína e energia de bovinos Nelore machos não-castrados superprecoces. .Trinta e cinco animais foram utilizados: referência (n = 4), manutenção (n = 4) e ingestão ad libitum (n = 27). Os animais ad libitum foram divididos em quatro grupos: dietas com inclusão de DDG nos níveis de 0, 150, 300 e 450 g/kg (base MS). Ao final do experimento todos os animais foram abatidos. Com o aumento dos níveis de DDG na dieta, houve redução linear no consumo de NDT (P = 0,008), consumo de energia metabolizável (EM) (P < 0,010), na energia total retida (P = 0,065) e na produção de calor (P < 0,065). A ingestão de proteína metabolizável (PM) aumentou linearmente (P < 0,010), mas a proteína retida não diferiu (P = 0,499). A exigência de energia líquida e a exigência de EM para mantença foram de 75,9 e 122 kcal/$kg^{0,75}$ PCV, respectivamente. A exigência de PM diária para manutenção foi de 3,6 g/$kg^{0,75}$ do peso corporal em jejum. No terceiro capítulo avaliou-se o efeito da inclusão de DDG em dietas de alto concentrado sobre o consumo e a digestibilidade dos nutrientes. Quatro bovinos Nelore

canulados no rúmen receberam quatro tratamentos dietéticos em um delineamento experimental tipo quadrado latino 4×4. Os tratamentos incluíram dietas sem DDG (D0) e níveis variados de inclusão de DDG em 150 g/kg (D150), 300 g/kg (D300) e 450 g/kg (D450). Foram realizados quatro períodos de coleta total de fezes e urina para estimar a ingestão e digestibilidade dos nutrientes e estimar o balanço de nitrogênio. Coletas de digesta omasal foram usadas para estimar a degradabilidade ruminal. A técnica de esvaziamento ruminal foi realizada para estimar as taxas ruminais de ingestão, passagem e digestão (kd). A inclusão de DDG reduziu o consumo de NDT ($P < 0,01$), MS ($P = 0,02$), MO ($P = 0,02$), CNF ($P < 0,01$) e EE ($P = 0,06$). A ingestão de fibra em detergente neutro (FDN) aumentou linearmente ($P < 0,01$), enquanto a PB apresentou padrão quadrático ($P = 0,02$). A digestibilidade total em kg/d de MS ($P < 0,01$), MO ($P < 0,01$), NFC ($P < 0,01$) e EE ($P = 0,02$) decresceram linearmente. A digestibilidade total em kg/d da FDN aumentou linearmente ($P < 0,01$) e a PB apresentou padrão quadrático ($P = 0,01$). O kd de MS ($P = 0,01$), MO ($P < 0,01$) diminuiu linearmente e o da FDN aumentou linearmente ($P < 0,01$). A quantidade de N retida não se alterou ($P = 0,72$), mas a ingestão de proteína metabolizável aumentou ($P = 0,03$). E no quarto capítulo, avaliou-se a degradação ruminal in situ de dietas contendo diferentes níveis de DDG em substituição ao farelo de soja, uréia e milho e o efeito dessas dietas na degradação ruminal in situ do DDG, e foram propostos métodos alternativos para estimar a digestibilidade in vivo de dietas. Quatro touros Nelore canulados no rúmen foram utilizados em delineamento quadrado latino 4×4, tanto para incubação in situ quanto para digestibilidade in vivo. Foram avaliados os seguintes tratamentos: dieta sem DDG (D0) ou contendo 150 (D150), 300 (D300) ou 450 g/kg (D450) de inclusão. Para avaliação in situ, o DDG foi incubado em todos os animais, e as dietas apenas naqueles alimentados com a mesma dieta que seria incubada. Para estimar a digestibilidade in vivo, foram realizados três dias de coleta total de fezes em cada período. Os dados foram analisados em PROC MIXED, PROC NLIN e PROC REG do SAS (versão 9.4, SAS Institute Inc., Cary, NC, EUA). Não houve efeito da dieta sobre os parâmetros de degradação in situ do DDG ($P > 0,05$). No entanto, para as dietas, a fração prontamente solúvel (parâmetro "a") ($P < 0,01$), e a constante de velocidade para degradação (parâmetro kd) da fração potencialmente degradável no rúmen (parâmetro "b") ($P = 0,05$) de MS, diminuiu linearmente. Por outro lado, o parâmetro "b" ($P < 0,01$) e a taxa kd ($P = 0,03$) da FDN das dietas aumentaram linearmente. O tempo de incubação in situ para estimar a digestibilidade da MS in vivo variou de 27,2 horas a 37,7 horas. Para a FDN, o tempo foi de 60 horas para todas as dietas. Além disso, para estimar a digestibilidade in vivo da MS (DMS) e o resíduo de FDN (RFDN) das dietas, foram sugeridas duas equações para cada uma: $DMS = 804,8 - 0,092 \times \text{nível (DDG)}$, e $DMS = 658 + 0,468 \times a$;

RFDN = 393,9 - 0,138 × nível (DDG), e RFDN = 874,3 - (0,602 × b) - (0,805 × I) + (1.662 × kd), onde I = FDN indigestível. Portanto, a inclusão de DDG com baixo teor de gordura nas dietas de terminação reduz o desempenho, a digestibilidade dos nutrientes e o consumo de energia.

Palavras-chave: Grãos secos de destilaria. Dietas de terminação. Nelore.

SUMMARY

INTRODUCTION

Distillers grains are co-products of the alcohol production industry, being used as a source of protein [inclusion levels of up to 150 g/kg in the dry matter (**DM**) of the diet] or energy (levels above 150 g/kg DM base) in beef cattle diets (Klopfenstein, 2001). It has been demonstrated for quite some time that after fermentation, the composition of the residue differs from the original grain, having a lower concentration of starch, and higher concentrations of crude protein (**CP**), neutral detergent fiber (**NDF**), and ether extract (**EE**) (Klopfenstein et al., 2008). The heating process can also cause changes in the CP profile, which can present around 730 g/kg of rumen undegradable protein (**RUP**) (NASEM, 2016). Many studies evaluating levels of inclusion of distillers grains in finishing cattle diets have been performed (Al-Suwaiegh et al., 2002; Buckner et al., 2007; Corrigan et al., 2007), but most studies evaluated the inclusion of wet distillers grains plus solubles (**WDGS**) or DDG plus solubles (**DDGS**) with little data for DDG without solubles. Also, there is a large variation in the distillers grains composition among ethanol plants and within an ethanol plant on different days (Buckner et al., 2011), and the change in this product's chemical composition can generate changes in the digestibility and use of nutrients.

Our study aimed to evaluate: 1 - the performance and digestive parameters of young Nellore bulls when receiving finishing diets with different levels of inclusion of low-fat DDG without solubles; 2 - the effect of including low-fat DDG in finishing diets on intake and digestibility of nutrients, protein and energy intake and retention, and to evaluate the whole-body chemical composition and estimate the protein and energy requirements for the maintenance and growth of young Nellore bulls; 3 - the effect of low-fat DDG levels in finishing diets on nutrient intake and digestibility; 4 - evaluate the ruminal *in situ* degradation of diets containing different levels of DDG inclusion, and the

effect of these diets on ruminal *in situ* degradation of DDG, and to find the number of hours of *in situ* incubation of diets to estimate their *in vivo* digestibility and to develop models based on the level of inclusion of DDG and based on *in situ* degradation parameters of diets to evaluate their *in vivo* digestibility.

CHAPTER 1

Impact of different levels of low-fat dried distillers grains on performance of young Nellore bulls during the finishing phase[1]

H. M. Alhadas,[2,†,‡] S. C. Valadares Filho,[†] L. O. Tedeschi,[‡] R. S. R. Vilela,[†] G. A. P. Souza,[†] B. C. Lage,[†] B. C. Silva ,[†] L. N. Rennó,[†] M. F. Paulino,[†]

[1]This study and scholarship of the first author were supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ, Brazil).

[†]Department of Animal Science, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil, 36570-900.

[‡]Department of Animal Science, Texas A&M University, College Station, Texas, USA, 77843-2471.

[2]Corresponding author: H. M. Alhadas, E-mail: herlonmalhadas@gmail.com

https://orcid.org/0000-0003-2406-9726

ABSTRACT

This study evaluated the effect of including low-fat dried distillers grains (**DDG**) on young Nellore bulls performance, nutritional parameters and nitrogen metabolism. Thirty-five Nellore cattle were randomly divided into four diets: without dried distillers grains (**D0**) or with the inclusion of DDG at 150 g/kg (**D150**), 300 g/kg (**D300**), or 450 g/kg (**D450**). The evaluation period lasted 126 days and three periods of collection of feces and urine were carried out. Final body weight (P = 0.099) and average daily gain (P = 0.097) tended to decrease linearly; the digestibility of dry matter (P <0.001), organic matter (P <0.001), ether extract (P <0.001) and non fiber carbohydrates (P <0.001), and intakes of total digestible nutrients (**TDN**, P <0.001) decreased linearly. The increase in crude protein intake (P <0.001) did not result in an increase in the amount of nitrogen retained (P = 0.540). We concluded that the inclusion of low-fat DDG in finishing diets up to the level of 450 g/kg tends to reduce animal performance and the intake of TDN.

Key words: Distillery grains, finishing phase, growth, Nellore.

INTRODUCTION

The use of corn for ethanol production is growing worldwide. In the United States, from 2002 to 2019, the area of corn destined for ethanol production increased 5.38 times (USDA, 2019). In Brazil, the estimate is that ethanol production from corn will increase 61.1% in the 2020 harvest, reaching 2.7 million liters (CONAB, 2020). As a result, the production of distillers grains also increased, and most of the byproducts were used to feed ruminants. One of the byproducts generated by the fermentation process is the dried distillers grain (**DDG**) with or without solubles. Because it is easily transported and stored due to its reduced humidity obtained by the drying process, it can be fed to ruminant production systems far from the ethanol plants (Morris et al., 2005). According to Klopfenstein (2001), distillers grains are used in finishing diets of beef cattle as a source of protein with inclusions of up to 150 g/kg, but also as an energy source when levels of inclusion are higher. Consequently, it is important to know the animal response concerning the levels of inclusion of distillers grains in the finishing diet of cattle, and whether different types of cattle have similar performances.

Many studies evaluating levels of inclusion of distillers grains in finishing cattle diets have been performed (Al-Suwaiegh et al., 2002; Buckner et al., 2007; Corrigan et al., 2007), but most studies evaluated the inclusion of wet distillers grains plus solubles (**WDGS**) or DDG plus solubles (**DDGS**) with little data for DDG without solubles. Also, there is a large variation in the distillers grains composition among ethanol plants and within an ethanol plant on different days. Buckner et al. (2011) evaluated the composition of WDGS and modified distillers grains plus solubles (**MDGS**) from six ethanol plants. They collected ten samples per day for five days in four different months. They reported that the smallest variation occurred for crude protein (**CP**) and phosphorus, while the fat content varied between plants. The lowest and the highest values reported for fat for Buckner et al. (2011) were 102 g/kg and 133 g/kg. Fat is one of the main constituents of distillers grains, and it is responsible for increasing the

energy equivalent. Its nutritive values described in different publications are within the values described previously (MacDonald et al., 2007). However, in some ethanol plants, distillers grains are defatted, with fat values close to that of corn. Consequently, animal performance may be different than anticipated when feeding this type of distillers grains, mainly because the composition of the gain might be altered.

Therefore, the hypothesis is that the high inclusion of low-fat DDG in finishing diets can reduce the performance and prejudice of the digestive parameters of young Nellore bulls. Our study aimed to evaluate the performance and digestive parameters of young Nellore bulls when receiving finishing diets with different levels of inclusion of low-fat DDG without solubles.

MATERIAL AND METHODS

The trial was conducted in the experimental feedlot of the Department of Animal Science of the Federal University of Viçosa, Viçosa, Brazil, MG, observing the evaluation of the Ethics Committee for Animal Use (Protocol CEUAP/DZO/UFV 01/2019). A total of 35 young Nellore bulls from the beef cattle farm of the Federal University of Viçosa were used. The minimum sample size per treatment was calculated to be 8, which was estimated based on the methodology described by Ryan (2013), considering the average daily gain (**ADG**) coefficient of variation of 5% and an expectation of reducing treatment differences by 8.97% from the results of a meta-analysis (Klopfenstein et al., 2008), and assuming 5% for the Type-I error and 95% for the power (1 - β).

Animals, design, and experimental diets. Thirty five animals in the performance growth (average initial weight of 267 kg) were allocated in collective pens (48 m^2, with eight or nine animals per pen) containing feeders (Model AF-1000 Master; Intergado, Contagem, MG, Brazil) and electronic waterers (Model WD-1000 Master; Intergado, Contagem, MG, Brazil). A tag (FDX-ISO11784/11785; Allflex, Joinville, SC, Brazil) with an exclusive number fixed

to each animal's ear to facilitate the identification of animals in the electronic system and to have access to individual intakes. Before starting the experimental diets, animals underwent an initial adaptation period of 30 days. The evaluation period lasted 126 days, consisting of 6 periods of 21 days each. The bulls were randomly divided into four groups to receive the treatments (diets). On the first and last experimental day, they were weighed at 07.00 h after fasting for 16 h of solids. These weights were considered the initial and final weights and used to estimate the ADG. Four treatments consisted of levels of inclusion of DDG in the diets, which were formulated according to BR-CORTE recommendations (Valadares Filho et al., 2016). All diets contained 280 g/kg of corn silage, and levels of 0 g/kg (**D0**), 150 g/kg (**D150**), 300 g/kg (**D300**), and 450 g/kg (**D450**) DDG in the dry matter (**DM**) of the total diet. The animals were fed the complete mixed diets twice daily, at 07.00 h and 16.00 h. The inclusion of DDG in the diets provided a partial replacement of corn meal and total replacement of soybean meal. The D0 and D150 diets had levels close to CP, around 123 g/kg. However, the D300 and D450 diets had CP levels around 152 g/kg and 187 g/kg, respectively. The chemical composition of the diet ingredients is listed in Table 1. The chemical composition of the diets, as well as the level of inclusion of each ingredient, are shown in Table 2.

Nutrients digestibility and nitrogen (N) balance. Three periods of collection of feces and urine (Amaral et al., 2014; Prados et al., 2017) were carried out at 25 days, 71 days, and 110 days of feeding. These collection periods overlapped the second, fourth, and sixth experimental periods, respectively. Each collection period lasted for three days, with collections taking place at different times on each day (06.00 on the first day, 12.00 on the second day, 18.00 h on the third day). The feces were collected directly from the rectum of the animals. The samples were immediately placed in a forced ventilation oven at 55°C for partial drying for 72 h. Subsequently, they were ground using a knife mill with 1-mm and 2-mm sieves (Fortinox, Piracicaba, São Paulo, Brazil). Composites of the three collections were obtained for further

chemical analyses. The internal indigestible neutral detergent fiber (**iNDF**) marker was used to estimate the amount of feces excreted per animal per day. The calculation was performed by dividing the iNDF intake by the concentration of iNDF in the feces. The digestibility coefficient of each nutrient was estimated based on the total intake of each nutrient. With the nutrient digestibility coefficients, the intake of total digestible nutrients (**TDN**) was estimated by the following equation: TDN = dCP + dNDF + (dEE x 2.25) + dNFC, where dCP = digestible crude protein, dNDF = digestible neutral detergent fibre, dEE = digestible ether extract, dNFC = digestible non-fibre carbohydrates.

At each fecal collection, spot urine collections were obtained to estimate the purine derivatives and the urinary excretion of N. To preserve nitrogenous compounds, 5 ml of urine was diluted in 20 ml of 0.018 mol/L sulfuric acid and stored in a freezer at -20°C for each collection day. It was necessary to estimate the urinary volume. Thus, daily urine creatine excretion was estimated according to Valadares Filho et al. (2016) (Equation 1). The urinary creatinine concentration was also obteined, and the urinary volume was obtained by the ratio between daily urine creatine excretion and urinary creatine concentration. For the N balance, data of N intake, fecal N, and urinary N were used.

Rumen degradable protein, rumen undegradable protein, and synthesis of microbial protein. Urinary purine derivatives methodology was used to estimate microbial protein synthesis. This method assumes that the nucleic acids that leave the rumen and reach the intestine are of microbial origin (McAlian and Smith, 1973). In cattle, microbial nucleic acids are mainly converted to purine derivatives allantoin and uric acid, which are mainly excreted in the urine (Tas and Susenbeth, 2007). A urinary recovery rate for purines derivatives of 80% was adopted as recommended by Valadares Filho et al. (2016). A microbial RNA digestion rate of 93% and the endogenous fraction of urinary for purine derivatives of 0.3 mmol/kg$^{0.75}$ body weight (**BW**) was adopted, as found by Barbosa et al. (2011). In addition, the ratio of 70 mg of

N per mmol of purine and 0.1369 of N in purines and total N in bacteria were adopted (Barbosa et al., 2011). The first step was to calculate the absorbed purines (**AP**, mmol/d) (Equation 2). The second step was to calculate the microbial N synthesis (**MNS**, g/d) (Equation 3). Microbial protein synthesis (**MPS**) was found by multiplying the value found for MNS by 6.25. The rumen degradable protein (**RDP**) was adopted as equivalent to the microbial protein synthesis (Valadares Filho et al., 2016), and the rumen undegradable protein (**RUP**) was estimated by the difference between CP intake and RDP. With the values of RUP and RDP, the metabolizable protein (**MP**) was estimated (Equation 4).

$$\text{Daily excretion of creatinine (mg/d)} = 37.88 \times BW^{0.9316} \quad (1)$$

$$PD = (PA - 0.30 \times kg^{0.75} BW)/0.80 \quad (2)$$

$$MNS = (70 \times PA)/(0.93 \times 0.1369 \times 1000) \quad (3)$$

$$MP = (RDP \times 0.64) + (RUP \times 0.8) \quad (4)$$

where BW = body weight; MNS = microbial N synthesis; MP = metabolizable protein; PA = purine absorbed; PD = purine derivatives; RUP = rumen undegradable protein; RDP = rumen degradable protein.

Laboratory analyses. Feces samples and dietary ingredients were analyzed for DM (method #934.01), N (method #981.10), ash (method #930.05), according to AOAC (2012), and ether extract (**EE**) (method #2003.05) according to AOAC (2005). The CP content was estimated by multiplying the N content by 6.25, and the organic matter (**OM**) content was calculated by subtracting the ash content from the DM content. The neutral detergent fiber (**NDF**) content was estimated, according to Mertens et al. (2002), with the addition of thermostable α-amylase to neutral detergent (Ankom Tech. Corp., Fairport, NY, USA), without the addition of sodium sulphite, and corrected for contamination with ash and protein (**apNDF**).

The iNDF content was determined by ruminal incubation of the sample for 288 h, as described by Valente et al. (2011). The non-fiber carbohydrates (**NFC**) content was estimated as suggested by Detmann and Valadares Filho (2010): NFC = OM - CP - apNDF – EE. Urine samples were analyzed for allantoin, creatinine, and uric acid using HPLC (Agilent 1100 series, Agilent Technologies, Waldbronn, Germany) as described by George et al. (2006). Urine samples were also analyzed for N.

Statistical analysis. The data were analyzed as a completely randomized design, and individual animals were used as the experimental units for all parameters. When the effect of treatment (i.e., levels of DDG) was significant, the linear, quadratic, and cubic effects were evaluated using orthogonal contrasts that were obtained with PROC MIXED of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). The least-square means were used to determine statistical significance among treatments. Initial body weight was used as a covariate. The $P = 0.05$ was used as the critical level of probability for the Type-I error, and tendencies were considered at $0.05 < P \leq 0.10$.

RESULTS

Performance, intake, and digestibility. As shown in Table 3, the final BW ($P = 0.099$) and ADG ($P = 0.097$) tended to reduce linearly, but feed efficiency ($P = 0.137$) did not differ between treatments. The tendency for quadratic increase in the intake of DM ($P = 0.071$) and OM ($P = 0.071$), together with the increasing levels of CP and NDF in the diet, resulted in a linear increase in the consumption of these nutrients ($P < 0.001$ and $P < 0.001$, respectively) (Table 4), with a lowest value for CP intake for D0 (0.79 kg/d) and with the highest value for D450 (1.13 kg/d). The inclusion of DDG also resulted in a quadratic increase for EE intake ($P = 0.039$). The NFC ($P < 0.001$) and TDN ($P < 0.001$) intake was reduced linearly. Increasing levels of DDG in the diet changed the digestibility of nutrients, in such a way that the digestibilities of DM (g/kg, $P < 0.001$; and kg/d, $P = 0.001$), OM (g/kg, $P < 0.001$; and kg/d, P

= 0.001), NFC (g/kg, P <0.001; and kg/d, P <0.001) and EE (g/kg, P <0.001) decreased linearly (Table 4). The digestibility of the EE in kg/d tended to have a quadratic effect (P = 0.063), with a higher value for D150. However, the digestibility of NDF (g/kg, P <0.001; and kg/d, P = 0.001) increased linearly, and the digestibility of CP (g/kg, P <0.001; and kg/d, P <0.001) showed a quadratic effect with lower value for D300 for g/kg digestibility and lower value for D150 for digestibility in kg/d.

N balance and rumen protein metabolism. In addition to the increase in protein, the inclusion of DDG in the diet changed the profile of this protein, causing a linear increase in RUP intake (P <0.001), the relationship between RUP intake and CP intake (P <0.001), MP intake (P <0.001), and MPS efficiency (P <0.001) (Table 5). However, as the MPS was not affected by the treatments (P = 0.220), and the CP intake increased linearly, the relationship between MPS and CP intake decreased linearly (P <0.001). The linear increase in N intake (P <0.001), did not increase the N retained (P = 0.540) (Table 5). This was because there was a linear increase in the N excreted in the feces (P <0.001) and a quadratic increase with a minimum point in D150 in the N excreted in the urine (P = 0.001). Thus, as there was an increase in N intake and retention was not different, the partial efficiency of the use of this compound reduced linearly (P = 0.012).

DISCUSSION

Performance, intake, and digestibility. The inclusion of DDG in the diet tended to reduce performance data. However, there are other studies that have evaluated levels of distillers grains in cattle diets and found different patterns. In this sense, Depenbusch et al. (2009) evaluated diets with or without the inclusion of 150 g/kg DDGS and reported no difference in performance. On the other hand, there are studies describing a quadratic increase in the ADG of animals fed with increasing levels of DDGS (Klopfenstein et al., 2008). Besides that, some studies that evaluated the inclusion of dry grains in diets for cattle in the finishing phase found

greater feed value for DDG or DDGS compared to corn, explaining the improvement in the performance of the animals up to a certain level of inclusion (Buckner et al., 2007; Ham et al., 1994). Two are the main factors described by the authors for this improvement: the increase in the concentration of CP and an increase in the concentration of EE. However, the increased intake of CP did not affect the amount of protein retained and the fat concentration of the diets used in this study was very similar, varying only 3.8 g/kg units from the diet with the highest concentration (**D450**) to the diet with the lowest concentration (**D0**). The DDG used did not present high values of EE as reported in the literature, 120 g/kg (MacDonald et al., 2007), having a value close to that of corn (48.6 g/kg).

Corroborating the performance data, there was a reduction in the digestibility of almost all nutrients. This is associated with the DDG production process. As DDG is a co-product obtained from starch fermentation, which is the main carbohydrate in the grain, its inclusion in the diet promoted a linear reduction in the concentration of NFC, which have greater and faster fermentation compared to fiber (Miron et al., 2001). This modification may be associated with the digestibility values found for DM and OM. In addition, the heating process by which this co-product is subjected can increase the damage caused to the protein and increase the concentration of acid detergent insoluble N (**ADIN**), which has a negative correlation with the digestibility of DM (Tedeschi et al., 2009). These data may also be associated with a reduction in the g/kg digestibility of CP, since ADIN correlates linearly and negatively with the digestibility of non-forage CP (r = 0.81; Nakamura et al., 1994) and forage CP (r = 0.93; Yu and Thomas, 1976), although ADIN in distillers grains can have a digestibility of 600 g/kg (Van Soest, 1994).

Although DDGS has different characteristics from DDG, Gilroyed et al. (2015) evaluated the inclusion of DDGS in cattle diets and indicated a reduction in the digestibility of nutrients, as in our study. These authors described an increase in the total gross energy of feces

from animals fed with higher levels of DDGS inclusion in the diet, which ranged from 0 to 400 g/kg. Additionally, the authors described higher concentrations of recalcitrant compounds in the feces of animals fed with the highest levels, which was a result of the process of fermentation of the starch of the grain and concentration of the other constituents. The authors also cited a reduction in OM digestibility, being 820, 780, and 750 g/kg respectively for diets with 0, 200, and 400 g/kg of DDGS inclusion, values close to those found in this study.

The only component that showed an increase in digestibility was NDF. The DDG used showed a high fiber content (546 g/kg). However, the fiber digestibility of this co-product is high and can reach 863 g/kg in *in vitro* tests during 48 hours (Tedeschi et al., 2009). The reduction in dietary starch associated with an increase in fiber content may contribute to a reduction in subacute acidosis (Klopfenstein et al., 2008), which may explain the increase in NDF digestibility.

Although the intake of DM and OM showed a quadratic pattern, the reduction in the digestibility of nutrients linearly reduced the intake of TDN, indicating less use of energy in the diet, also explaining the reduction in performance. Regarding the intake pattern, it is also possible to find different responses in the literature, since factors such as the type of grain used to obtain the distillers grains and the level of inclusion can interfere in the response (Merayo et al., 2020). In this sense, Walter et al. (2010) evaluated diets with the inclusion of 200 or 400 g/kg of DDGS obtained from corn or wheat and found a quadratic increase in DMI for wheat DDGS and a linear reduction for corn DDGS. On the other hand, some authors found no difference in DMI (Salim et al., 2014; Vander Pol et al., 2009).

N balance and rumen protein metabolism. The increase in the concentration of RUP may have occurred due to some factors. The main protein in corn is zein, which has an increased concentration in DDG. However, the digestibility of this protein is low, around 400 g/kg

(McDonald, 1954). In addition, a contribution of the total protein of the distillers grains is the remaining yeasts, which, when going through the heating process, become more resistant to microbial degradation (Bruning and Yokoyama, 1988). Consequently, the escape of rumen protein (RUP) tends to increase with the inclusion of distillers grains in diets (Aines et al., 1987). The result of the increase in RUP is the increase in MP likely because it presents greater efficiency of use compared to RDP (80% according to the adopted calculations).

However, the increased intake of N and of MP did not increase N retention. Confirming this data, Menezes et al. (2016) evaluated levels of CP of 100, 120, and 140 g/kg in finishing diets and also found no difference in N retention, with increased N excreted in the urine. In this sense, Cole et al. (2005) and Todd et al. (2006) found a reduction in urinary N excretion in diets with 115 g/kg of CP compared to diets with 130 g/kg of CP. Consequently, the data found show the importance of assessing how much of the N absorbed is actually retained in the animal's body.

The protein concentration of the diets was increasing, with D0 and D150 showing similar concentrations. However, the g/kg digestibility of the protein reduced around 100 units of the control diet for the other diets. These two factors associated with the quadratic pattern of DM intake caused the amount of digested protein to present a quadratic trend with a minimum value for the D150 diet, as previously described. This data may be associated with the lower urinary N value found for D150.

CONCLUSION

The inclusion levels of low-fat DDG of up to the level of 450 g/kg (DM basis) in diets of Nellore cattle during the finishing phase reduced performance, and TDN intake, as well as reduced the digestibility of DM, OM, CP, EE and NFC (g/kg). Although the protein profile of the diet might

be altered by increasing the RUP/CP ratio and, consequently, the dietary MP content, the higher protein intake does not seem to modify the protein retained.

Declarations of interest: none

Funding: This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ, Brazil; 465377-2014/9).

LITERATURE CITED

Aines, G., Klopfenstein, T., Stock, R., 1987. Distillers Grains. MP51, Nebraska Agric. Res. Div., Lincoln.

Al-Suwaiegh, S., Fanning, K.C., Grant, R.J., Milton, C.T., Klopfenstein, T.J., 2002. Utilization of distillers grains from the fermentation of sorghum or corn in diets for finishing beef and lactating dairy cattle. J. Anim. Sci. 80. https://doi.org/10.2527/2002.8041105x

Amaral, P.M., Valadares Filho, S.C., Detmann, E., Santos, S.A., Prados, L.F., Mariz, L.D.S., Alves, L.C., Menezes, A.C.B., Villadiego, F.A.C., Novaes, M.A.S., Silva, F.A.S., 2014. Effect of phase-feeding crude protein on performance and carcass characteristics of crossbred beef bulls: An application to reduce nitrogen compounds in beef cattle diets. Trop. Anim. Health Prod. 46, 419–425. https://doi.org/10.1007/s11250-013-0507-z

AOAC, 2012. Official Methods of Analysis of AOAC International, 19th ed. Association of Official Analysis Chemists International. Gaithersburg, Maryland.

AOAC, 2005. Official Methods of Analysis of AOAC International, 18th ed, Association of Official Analysis Chemists International. Gaithersburg, Maryland.

Barbosa, A.M., Valadares, R.F.D., Valadares Filho, S.C., Pina, D.S., Detmann, E., Leão, M.I., 2011. Endogenous fraction and urinary recovery of purine derivatives obtained by different methods in Nellore cattle1. J. Anim. Sci. 89, 510–519. https://doi.org/10.2527/jas.2009-2366

Bruning, C.L., Yokoyama, M.T., 1988. Characteristics of Live and Killed Brewer's Yeast Slurries and Intoxication by Intraruminal Administration to Cattle. J. Anim. Sci. 66, 585. https://doi.org/10.2527/jas1988.662585x

Buckner, C.D., Erickson, G., Mader, T., Colgan, S.L., Karges, K.K., Gibson, M.L., 2007. Optimum Levels of Dry Distillers Grains with Solubles for Finishing Beef Steers. Nebraska Beef Cattle Reports.

Buckner, C.D., Wilken, M.F., Benton, J.R., Vanness, S.J., Bremer, V.R., Klopfenstein, T.J., Kononoff, P.J., Erickson, G.E., 2011. Nutrient variability for distillers grains plus solubles and dry matter determination of ethanol by-products. Prof. Anim. Sci. 27, 57–64. https://doi.org/10.15232/S1080-7446(15)30445-9

Cole, N.A., Clark, R.N., Todd, R.W., Richardson, C.R., Gueye, A., Greene, L.W., McBride, K., 2005. Influence of dietary crude protein concentration and source on potential ammonia emissions from beef cattle manure1,2,3. J. Anim. Sci. 83, 722–731. https://doi.org/10.2527/2005.833722x

CONAB, 2020. Companhia Nacional de Abastecimento - Safra da cana segue voltada ao açúcar e mostra novo aumento do etanol de milho [WWW Document]. URL https://www.conab.gov.br/ultimas-noticias/3357-safra-da-cana-segue-voltada-a-producao-de-acucar-e-mostra-novo-aumento-do-etanol-de-milho (accessed 9.8.20).

Corrigan, M.E., Erickson, G., Klopfenstein, T., Pol, K.J. Vander, Greenquist, M.A., Luebbe, M., 2007. Effect of Corn Processing and Wet Distillers Grains Inclusion Level in Finishing Diets. Nebraska Beef Cattle Reports.

Depenbusch, B.E., Loe, E.R., Sindt, J.J., Cole, N.A., Higgins, J.J., Drouillard, J.S., 2009. Optimizing use of distillers grains in finishing diets containing steam-flaked corn. J. Anim. Sci. 87. https://doi.org/10.2527/jas.2008-1358

Detmann, E., Valadares Filho, S.C., 2010. Sobre a estimação de carboidratos não fibrosos em alimentos e dietas. Arq. Bras. Med. Vet. e Zootec. 62, 980–984. https://doi.org/10.1590/S0102-09352010000400030

George, S.K., Dipu, M.T., Mehra, U.R., Singh, P., Verma, A.K., Ramgaokar, J.S., 2006. Improved HPLC method for the simultaneous determination of allantoin, uric acid and creatinine in cattle urine. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 832, 134–137. https://doi.org/10.1016/j.jchromb.2005.10.051

Gilroyed, B.H., Li, C., Reuter, T., Beauchemin, K.A., Hao, X., McAllister, T.A., 2015. Influence of distiller's grains and condensed tannins in the diet of feedlot cattle on biohydrogen production from cattle manure. Int. J. Hydrogen Energy 40, 6050–6058. https://doi.org/10.1016/j.ijhydene.2013.07.014

Ham, G.A., Stock, R.A., Klopfenstein, T.J., Larson, E.M., Shain, D.H., Huffman, R.P., 1994. Wet corn distillers byproducts compared with dried corn distillers grains with solubles as a source of protein and energy for ruminants. J. Anim. Sci. 72, 3246–3257. https://doi.org/10.2527/1994.72123246x

Klopfenstein, T., 2001. Distillers Grains for Beef Cattle. In National Corn Growers Association, Ethanol Co-Products Workshop, Lincoln, Neb.

Klopfenstein, T.J., Erickson, G.E., Bremer, V.R., 2008. BOARD-INVITED REVIEW: Use of distillers byproducts in the beef cattle feeding industry. J. Anim. Sci. 86, pp. 1223-1231.

MacDonald, J.C., Klopfenstein, T.J., Erickson, G.E., Griffin, W.A., 2007. Effects of dried distillers grains and equivalent undegradable intake protein or ether extract on performance and forage intake of heifers grazing smooth bromegrass pastures1. J. Anim. Sci. 85, 2614–2624. https://doi.org/10.2527/jas.2006-560

McAlian, A.B., Smith, R.H., 1973. Degradation of nucleic acids in the rumen. Br. J. Nutr. 29, 331–345. https://doi.org/10.1079/bjn19730107

McDonald, I.W., 1954. The extent of conversion of feed protein to microbial protein in the rumen of sheep. Biochem. J. 56, pp.120–125.

Menezes, A.C.B., Valadares Filho, S.C., Costa e Silva, L.F., Pacheco, M.V.C., Pereira, J.M.V., Rotta, P.P., Zanetti, D., Detmann, E., Silva, F.A.S., Godoi, L.A., Rennó, L.N., 2016. Does a reduction in dietary crude protein content affect performance, nutrient requirements, nitrogen losses, and methane emissions in finishing Nellore bulls? Agric. Ecosyst. Environ. 223, 239–249. https://doi.org/10.1016/j.agee.2016.03.015

Merayo, M., Pighin, D., Grigioni, G., 2020. Meat quality traits and feeding distillers grains to cattle: a review. Anim. Prod. Sci. 60, 1123. https://doi.org/10.1071/AN18382

Mertens, D.R., Allen, M., Carmany, J., Clegg, J., Davidowicz, A., Drouches, M., Frank, K., Gambin, D., Garkie, M., Gildemeister, B., Jeffress, D., Jeon, C.S., Jones, D., Kaplan, D., Kim, G.N., Kobata, S., Main, D., Moua, X., Paul, B., Robertson, J., Taysom, D., Thiex, N., Williams, J., Wolf, M., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. J. AOAC Int.

Miron, J., Yosef, E., Ben-Ghedalia, D., 2001. Composition and in vitro digestibility of monosaccharide constituents of selected byproduct feeds. J. Agric. Food Chem. 49, 2322–2326. https://doi.org/10.1021/jf0008700

Morris, S., Klopfenstein, T.J., Adams, D.C., Erickson, G.E., Vander Pol, K.J., Pol, V., Morris Terry J Klopfenstein Don C Adams Galen E Erickson Kyle J Vander Pol, S.E., 2005. The Effects of Dried Distillers Grains on Heifers Consuming LowHigh Quality Forage or High Quality Forage. Nebraska Beef Cattle Reports. 172.

Nakamura, T., Klopfenstein, T.J., Britton, R.A., 1994. Evaluation of acid detergent insoluble nitrogen as an indicator of protein quality in nonforage proteins. J. Anim. Sci. 72, 1043–1048. https://doi.org/10.2527/1994.7241043x

Prados, L.F., Sathler, D.F.T., Silva, B.C., Zanetti, D., Valadares Filho, S.C., Alhadas, H.M., Detmann, E., Santos, S.A., Mariz, L.D.S., Chizzotti, M.L., 2017. Reducing mineral usage in feedlot diets for Nellore cattle: II. Impacts of calcium, phosphorus, copper, manganese, and zinc contents on intake, performance, and liver and bone status1. J. Anim. Sci. 95, 1766–1776. https://doi.org/10.2527/jas.2016.1085

Ryan, T.P., 2013. Sample size determination and power. John Wiley & Sons, Ltd, p. 404.

Salim, H., Wood, K.M., McEwen, P.L., Vandervoort, G., Miller, S.P., Mandell, I.B., Cant, J.P.,

Swanson, K.C., 2014. Influence of feeding increasing level of dry or modified wet corn distillers grains plus solubles in whole corn grain-based finishing diets on growth performance, carcass traits, and feeding behavior in finishing cattle. Livest. Sci. 161, 53–59. https://doi.org/10.1016/j.livsci.2013.12.020

Tas, B.M., Susenbeth, A., 2007. Urinary purine derivates excretion as an indicator of in vivo microbial N flow in cattle: A review. Livest. Sci. https://doi.org/10.1016/j.livsci.2007.05.010

Tedeschi, L.O., Kononoff, P.J., Karges, K., Gibson, M.L., 2009. Effects of chemical composition variation on the dynamics of ruminal fermentation and biological value of corn milling (co)products. J. Dairy Sci. 92, 401–413. https://doi.org/10.3168/jds.2008-1141

Todd, R.W., Cole, N.A., Clark, R.N., 2006. Reducing Crude Protein in Beef Cattle Diet Reduces Ammonia Emissions from Artificial Feedyard Surfaces. J. Environ. Qual. 35, 404–411. https://doi.org/10.2134/jeq2005.0045

USDA ERS - Feed Grains: Yearbook Tables [WWW Document], 2019. URL https://www.ers.usda.gov/data-products/feed-grains-database/feed-grains-yearbook-tables/ (accessed 7.16.20).

Valadares Filho, S.C., Costa e Silva, L.F., Gionbelli, M.P., Rotta, P.P., Marcondes, M.I., Chizzotti, M.L., Prados, L.F., 2016. Nutrient Requirements of Zebu and Crossbred Cattle - BR-CORTE, in: Exigências Nutricionais de Zebuínos Puros e Cruzados - BR-CORTE. https://doi.org/10.5935/978-85-8179-111-1.2016b002

Valente, T.N.P., Detmann, E., Queiroz, A.C., Valadares Filho, S.C., Gomes, D.I., Figueiras, J.F., 2011. Evaluation of ruminal degradation profiles of forages using bags made from different textiles. Rev. Bras. Zootec. https://doi.org/10.1590/S1516-35982011001100039

Van Soest, P.J., 1994. Nutritional ecology of the ruminant, 2nd ed. Ithaca: Cornell University

Press, pp. 476.

Vander Pol, K.J., Luebbe, M.K., Crawford, G.I., Erickson, G.E., Klopfenstein, T.J., 2009. Performance and digestibility characteristics of finishing diets containing distillers grains, composites of corn processing coproducts, or supplemental corn oil1. J. Anim. Sci. 87, 639–652. https://doi.org/10.2527/jas.2008-1036

Walter, L.J., Aalhus, J.L., Robertson, W.M., McAllister, T.A., Gibb, D.J., Dugan, M.E.R., Aldai, N., McKinnon, J.J., 2010. Evaluation of wheat or corn dried distillers' grains with solubles on performance and carcass characteristics of feedlot steers. Can. J. Anim. Sci. 90, 259–269. https://doi.org/10.4141/CJAS09089

Yu, Y., Thomas, J.W., 1976. Estimation of the Extent of Heat Damage in Alfalfa Haylage by Laboratory Measurement. J. Anim. Sci. 42, 766–774. https://doi.org/10.2527/jas1976.423766x

Table 1 - Chemical composition of diet ingredients

Ingredients	DM[1]	OM[2]	NDF[3]	CP[4]	EE[5]
		g/kg			
Corn silage	307	936	498	62.2	24.9
DDG[6]	897	989	540	327	48.6
Corn meal	894	990	90.2	91.7	42.4
Soybean meal	885	939	95.4	505	23.6
Urea/A.S[7]	997	1000	-	2610	-
Premix[8]	1000	-	-	-	-
Virginiamycin	1000	-	-	-	-

[1]Dry matter, [2]Organic matter, [3]Neutral detergent fiber, [4]Crude protein, [5]Ether extract, [6]Dry distillers' grains, [7]urea and ammonium sulfate (9:1), [8]Premix guarantees (per kg of DM): 200 - 220 g of Ca, 10 mg of Co (Min), 500 mg of Cu (Min), 22 g of S (Min), 333 mg of Fe (Min), 178.41 mg of F (Max), 10 g of P (Min), 25 mg of I (Min), 17 g of Mg (Min), 1500 mg of Mn (Min), 1100 mg of monensin, 6.6 mg of Se (Min), 50 g of Na (Min), and 2000 mg of Zn (Min). The unit g/kg for dry matter represents grams of dry matter per kilogram of raw material, and for the other constituents represents grams of the constituent per kilogram of dry matter.

Table 2 - Proportion of ingredients and composition of experimental diets

Items	Diets			
	D0	D150	D300	D450
	g/kg			
Ingredients				
Corn silage	280	280	280	280
DDG	-	150	300	450
Corn meal	649	537	399	249
Soybean meal	40.0	12.0	-	-
Urea/A.S[2]	10.0	-	-	-
Premix	20.0	20.0	20.0	20.0
Virginiamycin	1.00	1.00	1.00	1.00
Analyzed composition				
DM	587	589	589	589
OM[3]	952	953	954	954
NDF[4]	202	270	337	405
CP[5]	123	122	152	187
EE[6]	35.6	37.3	38.4	39.4
NFC[7]	608	525	426	322

D0 = diet without DDG, D150 = diet with 150 g/kg of DDG, D300 = diet with 300 g/kg of DDG, D450 = diet with 450 g/kg of DDG, [1]Dry matter, [2]urea and ammonium sulfate (9:1), [3]Organic matter, [4]Neutral detergent fiber, [5]Crude protein, [6]Ether extract, [7]Non-fiber carbohydrates. In the "Ingredients" section, the unit g/kg represents grams of the ingredient per kilogram of dry matter in the diet. In the "Analyzed composition" section, the unit g/kg for dry matter represents grams of dry matter per kilogram of raw material, and for the other constituents represents grams of the constituent per kilogram of dry matter.

Table 3 – Effect of DDG inclusion levels in finishing diets on the performance and efficiency of young Nellore

Items	Diets				SEM[1]	P - value	
	D0	D150	D300	D450		L	Q
Initial BW[2], kg	268	265	271	262	10.57	0.845	0.758
Final BW, kg	425	421	415	411	6.047	0.099	0.934
ADG[3], kg/d	1.26	1.23	1.19	1.15	0.048	0.097	0.733
G:F[4], kg/kg	0.20	0.19	0.18	0.19	0.007	0.372	0.137

D0 = diet without DDG, D150 = diet with 150 g/kg of DDG, D300 = diet with 300 g/kg of DDG, D450 = diet with 450 g/kg of DDG, [1]Standard error of mean, [2]Body weight, [3]Average daily gain, [4]Relationship between gain and feed. Values on the same line, marked by different letters, are statistically different ($P = 0.05$) by the least-squares means test.

Table 4 - Effect of DDG inclusion levels in finishing diets on nutrient intake and apparent digestibility

Items	Diets				SEM[1]	*P*-value	
	D0	D150	D300	D450		L	Q
Dry matter							
Intake, kg/d	6.43	6.63	6.61	6.06	0.198	0.225	0.071
Digestibility, g/kg	799^{A}	745^{B}	725^{BC}	719^{C}	0.542	<0.001	<0.001
Digestibility, kg/d	5.13^{A}	4.94^{A}	4.79^{AB}	4.35^{B}	0.151	0.001	0.421
Organic matter							
Intake, kg/d	6.12	6.32	6.30	5.78	0.189	0.238	0.068
Digestibility, g/kg	814^{A}	757^{B}	735^{BC}	733^{C}	0.734	<0.001	0.001
Digestibility, kg/d	4.98^{A}	4.79^{A}	4.62^{AB}	4.23^{B}	0.137	0.001	0.483
Neutal detergent fiber							
Intake, kg/d	1.29^{D}	1.79^{C}	2.24^{B}	2.47^{A}	0.066	<0.001	0.054
Digestibility, g/kg	614^{BC}	612^{C}	643^{B}	687^{A}	0.753	<0.001	0.005
Digestibility, kg/d	0.79^{D}	1.10^{C}	1.44^{B}	1.69^{A}	0.045	<0.001	0.590
Crude protein							
Intake, kg/d	0.79^{C}	0.81^{C}	1.00^{B}	1.13^{A}	0.031	<0.001	0.071
Digestibility, g/kg	800^{A}	709^{C}	703^{C}	736^{B}	0.693	<0.001	<0.001
Digestibility, kg/d	0.63^{BC}	0.57^{C}	0.70^{B}	0.84^{A}	0.023	<0.001	<0.001
Ether extract							
Intake, kg/d	0.23^{A}	0.24^{A}	0.24^{A}	0.22^{A}	0.007	0.984	0.039
Digestibility, g/kg	945^{A}	929^{A}	898^{B}	887^{B}	0.690	<0.001	0.768
Digestibility, kg/d	0.21^{AB}	0.22^{A}	0.22^{AB}	0.20^{B}	0.007	0.137	0.063
Non fiber carbohidrates							
Intake, kg/d	3.89^{A}	3.54^{B}	2.82^{C}	1.96^{D}	0.094	<0.001	0.010
Digestibility, g/kg	880^{A}	830^{B}	811^{C}	771^{D}	0.595	<0.001	0.472
Digestibility, kg/d	3.42^{A}	2.94^{B}	2.29^{C}	1.51^{D}	0.082	<0.001	0.069
Total digestible nutrients							
Intake, kg/d	5.32^{A}	5.11^{A}	4.92^{AB}	4.48^{B}	0.156	<0.001	0.477

D0 = diet without DDG, D150 = diet with 150 g/kg of DDG, D300 = diet with 300 g/kg of DDG, D450 = diet with 450 g/kg of DDG, [1]Standard error of mean. Values on the same line, marked by different letters, are statistically

different ($P = 0.05$) by the least-squares means test. The values shown in the table are the means obtained from the three collection periods (25, 71 and 110 days).

Table 5 - Effect of DDG inclusion levels in finishing diets on N metabolism

Items	Diets				SEM[1]	P - value	
	D0	D150	D300	D450		L	Q
N intake, g/d	127^{C}	129^{C}	161^{B}	181^{A}	0.005	<0.001	0.071
Fecal N, g/d	26.4^{C}	35.4^{B}	46.8^{A}	48.2^{A}	1.744	<0.001	0.041
Urine N, g/d	62.6^{BC}	50.1^{C}	70.1^{B}	92.6^{A}	4.646	<0.001	0.001
Retained N, g/d	39.0	41.4	41.5	43.4	3.497	0.552	0.540
Retained N, g/kg N intake	370^{A}	368^{A}	306^{AB}	248^{B}	2.961	0.012	0.382
RUP[2], g/d	318^{C}	311^{C}	518^{B}	619^{A}	31.07	<0.001	0.089
MPS[3], g/d	476	494	488	514	19.14	0.220	0.853
RUP, g/kg CP[4] intake	399^{B}	384^{B}	511^{A}	541^{A}	0.021	<0.001	0.298
MPS, g/kg CP intake	601^{A}	616^{A}	489^{B}	459^{B}	0.207	<0.001	0.299
MPS/DOM[5], g/kg	95.8^{B}	103.6^{B}	105.8^{B}	122.8^{A}	4.829	<0.001	0.329
MP[6] intake, g/d	559^{C}	565^{C}	724^{B}	824^{A}	24.03	<0.001	0.066

D0 = diet without DDG, D150 = diet with 150 g/kg of DDG, D300 = diet with 300 g/kg of DDG, D450 = diet with 450 g/kg of DDG, [1]Standard error of mean, [2]Rumen undegradable protein, [3]Microbial protein synthesis = Rumen degradable protein, [4]Crude protein, [5]Digestible organic matter, [6]Metabolizable protein. Values on the same line, marked by different letters, are statistically different ($P = 0.05$) by the least-squares means test. The values shown in the table are the means obtained from the three collection periods (25, 71 and 110 days).

CHAPTER 2

Effect of inclusion levels of low-fat dried distillers grains in finishing diets on protein and energy intake and retention and estimation of protein and energy requirements of young Nellore bulls fed with high concentrate diets[1]

H. M. Alhadas,[2,†,‡] S. C. Valadares Filho,[†] L. O. Tedeschi,[‡] R. S. R. Vilela,[†] G. A. P. Souza,[†] B. C. Lage,[†] B. C. Silva ,[†] L. N. Rennó,[†] M. F. Paulino,[†]

[1]This study and scholarship of the first author were supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ, Brazil).

[†]Department of Animal Science, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil, 36570-900.

[‡]Department of Animal Science, Texas A&M University, College Station, Texas, USA, 77843-2471.

[2]Corresponding author: H. M. Alhadas, E-mail: herlonmalhadas@gmail.com

https://orcid.org/0000-0003-2406-9726

ABSTRACT

The objective was to evaluate the effect of including low-fat dried distillers grains (**DDG**) in finishing diets on protein and energy intake and retention and to estimate the protein and energy requirement of young Nellore bulls. Thirty-five animals were used: baseline (n = 4), maintenance (n = 4), and ad libitum intake (n = 27). Ad libitum animals were divided into four groups: diets with the inclusion of DDG at the levels of 0, 150, 300 and 450 g/kg (dry matter basis). At the end of the experiment all animals were slaughtered. There was a linear reduction with increasing DDG levels in the total digestible nutrients intake ($P = 0.008$), metabolizable energy (**ME**) intake ($P < 0.010$), in total retained energy ($P = 0.065$), and in heat production ($P < 0.001$). Metabolizable protein (**MP**) intake increased linearly ($P < 0.010$), but retained protein did not differ ($P = 0.499$). Daily net energy and ME requirement for maintenance was 75.9 and 122 kcal/kg$^{0.75}$ EBW, respectively. Daily MP for maintenance was 3.6 g/kg$^{0.75}$ shrunk body weight. DDG inclusion in finishing diets reduces energy intake and deposition and we recommend the equations of this study to estimate the requirements of young Nellore bulls.

Key words: Co-product, energy requirement, Nellore, protein requirement

INTRODUCTION

Distillers grains are co-products of the alcohol production industry, being used as a source of protein [inclusion levels of up to 150 g/kg in the dry matter (**DM**) of the diet] or energy (levels above 150 g/kg DM base) in beef cattle diets (Klopfenstein, 2001). However, there are several types of distillers grains, such as dried grains (**DDG**) or wet grains (**WDG**) with the addition (**DDGS** and **WDGW**) or not of solubles. Consequently, the composition of each type of this co-product will be different, and the animal response may also be different. It is possible to find in the literature studies evaluating the use of distillers grains (Al-Suwaiegh et al., 2002; Buckner et al., 2007; Corrigan et al., 2007). However, most of these studies have evaluated WDGS or DDGS, and data on DDG are scarce.

In addition, for the precision nutrition, knowledge of nutritional requirements is necessary. In this sense, an important factor is the breed of the animal that will be evaluated, since there are physiological and metabolic differences when different breeds are compared (NASEM, 2016; Valadares Filho et al., 2016). In this context, the estimation of the Bos indicus animals' requirements is necessary, due to its importance in world meat production. According to Cooke et al. (2020), Bos indicus represent a large portion of the world herd, with more than half of the animals in tropical environments. In addition, the same authors point out that Bos indicus animals play an important role in the heterosis of herds in the United States, especially in hot and humid areas. Besides that, the Brazilian herd is predominantly Zebu (Bos indicus) (Ferreira et al., 2020), with the Nellore breed and its hybrids showing up to 80% of animals (Bonin et al., 2021).

Accurate determination of requirements is directly linked to the sustainability of production systems, so that feed and nutrients can be used at their optimum point. Diets formulated based on incorrectly estimated requirements provide nutrient losses through feces and urine, which is unwanted both economically and environmentally. In this context, in

addition to the energy requirement, the correct use of protein requirements is necessary, since this nutrient is considered the most expensive component in cattle diets, and has the potential for pollution (Appuhamy et al., 2014). Although it is possible to find in the literature studies evaluating the requirements of Nellore cattle (Chizzotti et al., 2008; Tedeschi et al., 2002), data on young Nellore bulls are scarce, and some points may be different. Therefore, understanding the importance and relevance of this topic, the aim of this study was to evaluate the effect of including low-fat DDG in finishing diets on intake and digestibility of nutrients, protein and energy intake and retention, and to evaluate the whole-body chemical composition and estimate the protein and energy requirements for the maintenance and growth of young Nellore bulls.

MATERIAL AND METHODS

The experiment was conducted in the experimental feedlot of the Department of Animal Science of the Federal University of Viçosa, Viçosa, Brazil, MG, observing the evaluation of the Ethics Committee for Animal Use (Protocol CEUAP/DZO/UFV 01/2019).

Animals, design, and experimental diets. Thirty-five young Nellore bulls (average weight of 261kg and an initial age of 8 months) were used in this study. The animals were allocated in collective pens (48m^2) containing feeders (Model AF-1000 Master; Intergado, Contagem, MG, Brazil) and electronic waterers (Model WD-1000 Master; Intergado, Contagem, MG, Brazil). A tag (FDX-ISO11784/11785; Allflex, Joinville, SC, Brazil) with an exclusive number fixed to each animal's ear to facilitate the identification of animals in the electronic system and to have access to individual intakes. Before starting the experimental diets, animals underwent an initial adaptation period of 30 days. The experiment lasted 126 days, consisting of 6 periods of 21 days each. These animals were initially divided into 3 groups: baseline (n = 4), maintenance level of intake (n = 4), and ad libitum intake (i.e., growth performance) (n = 27). Animals in the baseline group were slaughtered at the beginning of the experiment to estimate the initial body composition. The animals in the maintenance group

were fed at 12 g/kg body weight (**BW**) of DM during the whole period. Animals in the ad libitum group were randomly allocated to the treatments, which were levels of inclusion of DDG in the diet (DM basis) as follow: 0 g/kg (n = 7), 150 g/kg (n = 7), 300 g/kg (n = 7) and 450 g/kg (n = 6). Diets were formulated according to BR-CORTE recommendations (Valadares Filho et al., 2016). Each animal in the maintenance group received one of the diets previously described. Animals were fed the complete mixed diets twice daily, at 07.00 h and 16.00 h. Animals from group 3 were slaughtered at the end of the 126 days, together with the animals of the maintenance group. The three groups were used to estimate nutritional requirements, however only the ad libitum group of animals were used to evaluate the effect of treatments (DDG levels) on protein and energy intake and retention. The chemical composition of the diet ingredients is listed in Table 1. The chemical composition of the diets, as well as the level of inclusion of each ingredient, are shown in Table 2.

Nutrients Digestibility. To determine the digestibility coefficient of each nutrient, three digestibility periods (Amaral et al., 2014; Prados et al., 2017) were carried out at 25 days, 71 days, and 110 days of feeding. Each collection period lasted for three days, with collections taking place at different times on each day (06.00 h on the first day, 12.00 h on the second day, 18.00 h on the third day). The feces were collected directly from the rectum of the animals. The samples were immediately placed in a forced ventilation oven at 55°C for partial drying for 72 h. Subsequently, they were ground using a knife mill with 1-mm and 2-mm sieves (Fortinox, Piracicaba, São Paulo, Brazil). Composites of the three collections were obtained for further chemical analyses. The internal indigestible neutral detergent fiber (**iNDF**) marker was used to estimate the amount of feces excreted per animal per day (Alhadas et al., 2021; Prados et al., 2017) . The calculation was performed by dividing the iNDF intake by the concentration of iNDF in the feces. The digestibility coefficient of each nutrient was estimated based on the total intake of each nutrient (DM basis).

Energy and protein intakes. Digestible energy (**DE**) intake was obtained by multiplying digestible nutrients by their respective energy values (NRC, 2001): DE = 5.6 x dCPI + 9.4 x dEEI + 4.2 x dNDFI + 4.2 x dNFCI, where dCPI is digestible crude protein intake, dEEI is digestible ether extract intake, dNDFI is digestible neutral detergent fiber intake and dNFCI is digestible non-fiber carbohydrates intake. With the DE values, the metabolizable energy (**ME**) was estimated using the equation suggested by Valadares Filho et al. (2016): ME = 0.9455 x DE - 0.3032. To estimate metabolizable protein intake, rumen undegradable protein (RUP) and rumen degradable protein (RDP) fractions were first calculated using urine purine methodology. For this, at each feces collection time, urine samples were collected, and to preserve nitrogenous compounds, 5 ml of urine was diluted in 20 ml of 0.018 mol/L sulfuric acid and stored in a freezer at -20°C for each collection day.

Urine purine methodology assumes that nucleic acids arriving in the intestine are of microbial origin (McAlian and Smith, 1973), and in cattle they are mainly converted to allantoin and uric acid and excreted via urine (Tas and Susenbeth, 2007). A purine derivative recovery rate of 80% was used, as recommended by Valadares Filho et al. (2016). Furthermore, a microbial RNA digestion rate of 93% and an endogenous fraction of 0.3 mmol/kg$^{0.75}$ BW was adopted, as described by Barbosa et al. (2011). The ratio of 70 mg of N per mmol of purine and 0.1369 of N in purines and total N in bacteria were adopted (Barbosa et al., 2011). Therefore, the absorbed purine (**AP**, mmol/d) was first calculated by Equation 1. Subsequently, the microbial N synthesis (**MNS**, g/d) was estimated by Equation 2, and the Microbial protein synthesis (**MPS**) was calculated by multiplication of MNS by 6.25. Then, the RDP was assumed to be equivalent to MPS (Valadares Filho et al., 2016), and the RUP was calculated by the difference between crude protein (**CP**) intake and RDP. This methodology was also based on the recommendations of the NASEM (2016), which assumes one hundred percent efficiency of

nitrogen use for microbial synthesis. The metabolizable protein was estimated by Equation 3 (Valadares Filho et al., 2016).

$$PD = (AP - 0.30 \times kg^{0.75} BW)/0.80 \quad (1)$$

$$MNS = (70 \times PA)/(0.93 \times 0.1369 \times 1000) \quad (2)$$

$$MP = (RDP \times 0.64) + (RUP \times 0.8) \quad (3)$$

where AP = absorbed purine; BW = body weight; MNS = microbial N synthesis; MP = metabolizable protein; PD = purine derivatives; RUP = rumen undegradable protein; RDP = rumen degradable protein.

Slaughter and samplings. Before the slaughter, animals were fasted from solids for 16 h to estimate the shrunk body weight (**SBW**). The animal slaughter was performed using a captive bolt followed by bleeding. All organs and viscera were removed, washed, and weighed to obtain empty body weight (**EBW**). Subsequently, cleaned organs and viscera were ground in an industrial cutter to obtain a homogeneous sample, which, together with the samples of the limbs, head, leather, and blood, was used to estimate the chemical composition of the non-carcass components. All samples were collected in aluminum trays, weighed, and lyophilized. After removing the non-carcass components, the carcass was divided into two halves, which were weighed and cooled in a cold chamber at 4°C for 18 hours. After this period, the half carcasses were weighed again. The section between the ninth and eleventh ribs (Hankins and Howe, 1946) of the left half carcass was removed to estimate the chemical composition of the carcass components. For this, it was performed in this section the separation of the muscle plus the fat from the bone was carried out, which were ground and sampled in aluminum trays for lyophilization.

Body composition and carcass and non-carcass components. All compositions were estimated based on EBW using the equations described by Marcondes et al. (2012) (Equations 4, 5, and 6) as follows below. The body energy content was obtained by sum the ether extract (**EE**) and CP content multiplied by their respective caloric values (ARC, 1980) (Equation 7). The amount of protein, fat and water as a function of EBW was estimated using the non-linear model: $Ci = a \times EBW^b$, where Ci is the "i" body component of the bull, which is the CP, EE or water content (kg), and "a" and "b" are the regression parameters. In the same way, non-linear models were developed as described above for the carcass weight (**CW**, kg), the non-carcass weight (**NCW**, kg) and the EBW (kg) based on the SBW (kg), and for the empty weight gain (**EWG**, kg) based on average daily gain (**ADG**, kg/d).

$$CP_{EBW}\,(\%) = 10.78 + 0.47 \times \%CP_{Cor} - 0.21 \times \%VF \quad (4)$$

$$EE_{EBW}\,(\%) = 2.75 + 0.33 \times \%EE_{Cor} + 1.80 \times \%VF \quad (5)$$

$$W_{EBW}\,(\%) = 38.31 + 0.33 \times \%W_{Cor} - 1.09 \times \%VF + 0.50 \times \%OV \quad (6)$$

$$EC = 5.6405 \times BP + 9.3929 \times BF \quad (7)$$

where CP_{EBW} = %CP in empty body weight; BF = body fat (kg); BP = body protein (kg); CP_{Cor} = %CP in the 9^{th} to 11^{th} rib section; EC = energy content (Mcal); EE_{Cor} = %EE in the 9^{th} to 11^{th} rib section; EE_{EBW} = %EE in empty body weight; OV = % organs and viscera per unit of EBW; VF = % visceral fat (renal, pelvic, cardiac, and mesenteric fat) per unit of EBW; W_{Cor} = % water in the 9^{th} to 11^{th} rib section; W_{EBW} = %water in empty body weight.

Protein and Energy requirements. Heat production (**HP,** Mcal/$kg^{0.75}$ EBW) was estimated by the difference between ME intake (**MEI,** Mcal/$kg^{0.75}$ EBW) and retained energy (**RE,** Mcal/$kg^{0.75}$ EBW), as described by Tedeschi et al. (2002). Total RE (Mcal/$kg^{0.75}$ EBW) was estimated as the sum of RE as fat (Mcal/$kg^{0.75}$ EBW) and RE as protein (Mcal/$kg^{0.75}$ EBW)

considering their respective caloric values, as described above. The net energy requirement for maintenance (**NE_m**, Mcal/$kg^{0.75}$ EBW) was obtained by regressing HP on MEI, considering the intercept of Equation 8. The metabolizable maintenance energy (**ME_m**, Mcal/$kg^{0.75}$ EBW) was obtained by an iterative method, where HP equals MEI, and the efficiency of use of metabolizable energy (**k_{ma}**) was obtained by dividing NE_m/ME_m. The net energy requirement for gain (**NE_g**, Mcal/d) was calculated by Equation 9. The partial efficiency of the use of the ME for gain (**k_{gain}**) was obtained by the linear regression between the RE and the MEI to gain, which was considered the intercept of the model. The partitioning of MEI to RE as fat and protein was computed a multiple regression as shown in Equation 10, and the deposition efficiencies in the form of fat (**k_f**) and protein (**k_p**) were calculated as the inverse of the coefficients β1 and β2, which represent the amounts of ME required to deposit 1 Mcal of ME as fat or protein, respectively. To estimate the metabolizable protein requirement for maintenance (**MP_m**, g/$kg^{0.75}$ SBW), Equation 11 was first calculated, and the MP_m value was obtained by dividing the model's intercept by the animals' average metabolic weight, as described by Valadares Filho et al. (2016). The net protein for gain (**NP_g**, g/d) was estimated by the Equation 12.

$$HP = \beta0 \times e^{\beta1 \times MEI} \quad (8)$$

$$NE_g = \beta0 \times EBW^{0.75} \times EWG^{\beta1} \quad (9)$$

$$MEI = \beta0 + \beta1 \times RE_f + \beta2 \times RE_p \quad (10)$$

$$MPI = \beta0 + \beta1 \times EWG \quad (11)$$

$$NP_g = \beta0 \times EWG - \beta1 \times RE \quad (12)$$

Where β0, β1 and β2 are the regression parameters; $EBW^{0.75}$ = metabolic empty body weight; EWG = empty weight gain (kg/d); HP = heat production (Mcal/$kg^{0.75}$ EBW); MEI =

metabolizable energy intake (Mcal/kg$^{0.75}$ EBW); MPI = metabolizable protein intake (g/d); NE_g = net energy for growth represented as the energy retained in the body (Mcal/d); NP_g = retained protein or the net protein requirement for growth (g/d); RE = retained energy (Mcal/d), RE_f = retained energy as fat (Mcal/kg$^{0.75}$ EBW), RE_p = retained energy as protein (Mcal/kg$^{0.75}$ EBW).

Laboratory analyses. Feces samples and dietary ingredients were analysed for DM (method #934.01), nitrogen (method #981.10), ash (method #930.05), according to AOAC (2012), and EE (method #2003.05) according to AOAC (2005). The CP content was estimated by multiplying the nitrogen content by 6.25, and the organic matter (**OM**) content was calculated by subtracting the ash content from the DM content. The neutral detergent fiber (**NDF**) content was estimated, according to Mertens et al. (2002), with the addition of thermostable α-amylase to neutral detergent (Ankom Tech. Corp., Fairport, NY, USA), without the addition of sodium sulphite, and corrected for contamination with ash and protein (**apNDF**). The iNDF content was determined by ruminal incubation of the sample for 288 h, as described by Valente et al. (2011). For this, the samples were weighed in F57 filter bags (25 μm; ANKOM 256 Technology, Macedon, NY) and incubated in five cannulated Nellore bulls. The animals received a diet with the same ingredients as those used in the experimental diet, with the average inclusion of DDG. Subsequently, the bags were removed from the rumen and washed under running water until the wash water ran clear. Subsequently, the samples went through the same procedure as described for NDF analyses, and after oven-drying at 55°C for 72 h and later at 105°C for 16 h, the residue was considered the iNDF. The non-fiber carbohydrates (**NFC**) content was estimated as suggested by Detmann and Valadares Filho (2010): NFC = OM - CP - apNDF – EE. Body tissue samples were analysed for ash, nitrogen and EE using the same methods previously described. Urine samples were analysed for purine derivatives using HPLC (Agilent 1100 series, Agilent Technologies, Waldbronn, Germany) as described by George et al. (2006).

Statistical analyses. To assess the effect of including DDG levels on protein and energy intake and retention, and on nutrient intake and digestibility, animals from the ad libitum group were used, and data were analysed as a completely randomized design, with each animal used as an experimental unit. When the treatment effect (i.e., levels of DDG) was significant, linear, quadratic and cubic effects were evaluated using orthogonal contrasts obtained with the PROC MIXED of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). To determine statistical difference between treatments, the least-square means were used. The $P = 0.05$ was used as a critical level of probability for the Type-I error, and trends were considered at $0.05 < P \leq 0.10$.

The coefficients of the models used to estimate energy and protein requirements were analysed by treatment using confidence intervals (a = 0.05), and no significant differences were observed. Therefore, the requirements were estimated for the entire database and the treatments were not considered in the statistical analyses. Furthermore, to estimate nutritional requirements, different levels of intake and deposition are necessary, since the main tool used is linear and non-linear regression models. Coefficients, standard error and confidence intervals were presented in the supplementary data (Tables S1-11) together with the performance data (Table S12) from the ad libitum group. Linear models were performed using the PROC REG of SAS (SAS Institute Inc., Cary, NC). Non-linear models were performed using PROC NLIN of SAS, and $P = 0.05$ was adopted for the Type-I error probability.

RESULTS

Effect of DDG inclusion on the nutrient intake and digestibility, and on MEI, MPI, Total RE, RE as fat and protein, HP and EBW. There was no cubic effect for any of the evaluated parameters and it was removed from the tables. As the requirements data for this study came from the performance work published in the Animal Science Journal (Alhadas et al., 2021b), nutrient intake and digestibility data were presented as supplementary data (Table S13). There was no effect of DDG including on the intake of DM ($P = 0.205$), OM (P = 0.200)

and EE ($P = 0.137$). However, intake of NDF ($P < 0.001$) and CP ($P < 0.001$) increased linearly and intake of NFC ($P < 0.01$) and TDN ($P = 0.008$) decreased linearly (Table S13). On the other hand, the DDG inclusion in the diets reduced the digestibility of DM ($P < 0.001$), OM ($P < 0.001$), CP ($P < 0.001$), EE ($P < 0.010$) and NFC ($P < 0.001$). Only NDF digestibility increased linearly ($P < 0.001$).

The inclusion of DDG reduced MEI ($P < 0.010$), HP ($P < 0.010$), and tended to reduce total RE ($P = 0.065$) linearly (Table 3). The RE as protein ($P = 0.550$) did not differ between treatments. However, RE as fat ($P = 0.010$) reduced linearly. Although MPI increased linearly ($P < 0.010$), the retained protein did not differ ($P = 0.499$) with the inclusion of DDG in the diet. EBW also did not differ between treatments ($P = 0.428$).

Body composition and relationship between EBW and SBW, and EWG ADG. Body composition equations [CP, EE and water (kg)] estimated from EBW (kg) (Figure 1), the equations generated to estimate the contribution of carcass components and non-carcass components from SBW (Figure 2), and the relationships between body weights and body weight gains were described in Table 4.

Energy and protein requirements. The equations that better described the HP, NE_g, MEI, and NP_g is described in Table 5. HP is also depicted in Figure 3. For the evaluated data, the daily NE_m requirement found was 75.9 kcal/kg$^{0.75}$ EBW, and the daily ME_m requirement was 122 kcal/kg$^{0.75}$ EBW. Consequently, the k_{ma} was 62.2%. The efficiency of using the ME for gain was 36% (Figure 4). We find a value of 0.81 for k_f and 0.28 for k_p, and the daily MP_m requirement found was 3.6g/kg$^{0.75}$ SBW (Figure 5).

DISCUSSION

Effect of DDG inclusion on the nutrient intake and digestibility, and on MEI, MPI, total RE, RE as fat and protein, HP and EBW. DDG is a co-product obtained from the

fermentation of corn starch to produce alcohol. As starch is one of the main constituents of NFC, after fermentation DDG presents a reduction in the concentration of this fraction and an increase in the concentration of NDF, which presents a lower and slower fermentation rate (Miron et al., 2001). Consequently, by including DDG in the diet, the NDF concentration increased from 202 g/kg (D0) to 405 g/kg (D450), while the NFC concentration decreased from 608 g/kg (D0) to 322 g/kg (D450). This change in composition possibly modified the animals' use of dietary energy, which resulted in a reduction in the digestibility of most nutrients. As a result, TDN intake reduced 18.58% from D0 (5.36 kg/d) to D450 (4.52 kg/d). In addition, there was a reduction of 13.92% in the MEI between D0 (MEI = 0.2909 Mcal/kg$^{0.75}$ EBW) and D450 (MEI = 0.2504 Mcal/kg$^{0.75}$ EBW). However, it is possible to find in the literature studies that describe a higher feed value for distillers grains compared to corn (Buckner et al., 2007; Ham et al., 1994). Nevertheless, most of the studies evaluated the DDGS, which presents a different composition from that presented for the DDG in this study. One of the main differences is regarding to the EE concentration. According to MacDonald et al. (2007), it is possible to find distillers grains with the addition of solubles with EE concentrations close to 120 g/kg. Consequently, one of the explanations for the increase in the feed value of the distillers grain would be its higher concentration of fat, which has an energy value higher than carbohydrates and proteins (NASEM, 2016). However, the DDG evaluated did not have the addition of solubles and had its EE concentration close to that of corn (49 g/kg). Thus, these factors together corroborate to the observed pattern of reduction in the MEI.

There are several factors influencing energy retention in the animal's body, with BW, rate of ADG and MEI being some of the main ones (Tedeschi and Fox, 2020). As the EBW increases, the rate of fat addition is greater than that of protein, which makes the animal deposit more fat than protein per kilogram of gain (NASEM, 2016). Furthermore, according to Garrett et al. (1959), fat has 9,367 kcal/g and nonfat organic matter contains an average of 5,686 kcal/g,

which indicates that greater fat deposition provides greater RE. In addition, Owens et al. (1995) and Krehbiel et al. (2006) indicated that there is an increase in RE when the MEI is increased, and this increase is a function of the increase in fat deposition. Our data are in agreement with the studies presented above, since the inclusion of DDG in the diet reduced the MEI and tended to reduce the total RE and reduced the RE as fat. However, a reduction in the final EBW would be expected, which did not occur, although numerically the animals that received the D450 diet had a weight 4.07% lower than the animals that received the D0 diet. Even though MEI and RE have reduced, HP was also lower for diets with greater inclusion of the co-product. This was due to the fact that RE has proportionally reduced more (15.81%) than MEI (13.92%), and the HP is calculated as the difference between these two variables. On the other hand, the higher MPI did not affect protein deposition, indicating that higher nitrogen losses may have occurred for treatments with higher protein concentration.

Body composition and relationship between EBW and SBW, and EWG and ADG. Body composition is a fundamental factor for estimating requirements and for the commercialization of animals. Therefore, in addition to knowing the weight, it is important to know its composition. A point of reference is weight at maturity. As the animal approaches maturity, there is an increase in the amount of fat deposited in relation to the amount of protein in the EBW (Valadares Filho et al., 2016). Some references to maturity can be found in the literature. Tedeschi et al. (2002) estimated the weight at maturity of Nellore animals when they have 22% fat in EBW. The NRC (2001) and BR-CORTE (Valadares Filho et al., 2016) systems estimate this point at 25% fat in EBW. For the present study, the value of 25% was adopted. By associating the body composition equations with the models proposed for the correlation between weights, it is possible to infer at which point of maturity the animal is. Valadares Filho et al. (2016) suggested the following model to estimate SBW (kg) from BW (kg): $SBW = 0.8800 \times BW^{1.0175}$. Considering a 400kg animal, the SBW is 391kg. Using the model proposed

by this study (Equation 18), the EBW of this animal is 363kg. Using the Equations 13, 14 and 15, the content (kg) of CP, EE and water of the EBW is: 64, 75 and 206kg respectively. Therefore, this animal has 20% fat in EBW, and has not reached maturity. This information can be used to decide what will be the ideal slaughter point. Likewise, using Equation 16, it is possible to estimate the CW of this animal, which is the component of commercial interest. For the above example, the CW is 233kg.

It is important to say that some authors describe exponential models for the deposition of fat in EBW. Menezes et al. (2019) proposed the following model for Nellore cattle: EE (kg) = $16.2886 \times e^{(0.0041 \times EBW)}$. If we consider the animal in the hypothetical example of the previous paragraph, we find a value of 72kg of EE in EBW, a value close to that described by the equation proposed by this study (75kg). However, greater differences will be found for higher weights, above maturity. It is important to mention that the average SBW of the animals described by Menezes et al. (2019) (445kg) is higher than the average SBW of the animals evaluated in this study (402kg), which explains the difference in the proposed models. Menezes et al. (2019) also proposed the following equation for estimating EWG (kg/d): EWG = 1.0119 x $ADG^{0.8315}$, with the model coefficients close to those suggested by Equation 19.

Energy and protein requirements. The NE_m can be defined as the heat production of the fasting animal (Valadares Filho et al., 2016) and is related to essential functions such as respiration, circulation, tissue synthesis, and functioning of organs and viscera (Garrett et al., 1959). The value found for this variable in this study is close to the values described in the literature. Menezes et al. (2016) found a NE_m value of 77 kcal/$kg^{0.75}$ EBW for Nellore cattle. Valadares Filho et al. (2016) recommend a NE_m value of 74.9 kcal/$kg^{0.75}$ EBW and NASEM (2016) of 77 kcal/$kg^{0.75}$ SBW. Likewise, Tedeschi et al. (2002) evaluated the energy requirements of Nellore bulls and steers and found a NEm value of 77.2 kcal/$kg^{0.75}$ EBW for both.

The values found for ME_m and k_{ma} in this study were close to the value reported by Menezes et al. (2016), of 122.75kcal/$kg^{0.75}$ EBW and 62.7%, respectively. A similar value for k_{ma} was described by Fox et al. (2004), of 64%. However, in a meta-analyses carried out by Chizzotti et al. (2008) for Nellore cattle, a higher value for k_{ma} than that described in this study was found, 67%. Some variation in these data can be expected, since the efficiency of the use of this energy can be affected by sex, age, genetic group, or even the energy concentration of the diet (AFRC, 1993; CSIRO, 2007; NASEM, 2016). In addition, some authors describe the association of k_{ma} with factors such as animal performance, gain, and weight composition (Marcondes et al., 2010; Tedeschi and Fox, 2020; Williams and Jenkins, 2003).

The parameters β0 (0.055) and β1 (0.6923) used to estimate NE_g for the present study were lower than those suggested by Valadares Filho et al. (2016) (β0 = 0.061 and β1 = 1.035) and NASEM (2016) (β0 = 0.0635 and β1 = 1.097), indicating a lower value of RE. As the RE is a function of the gain composition, the closer the weight to maturity, the more fat the animal deposits, and consequently, the greater the energy requirement for weight gain (Marcondes et al., 2016). The final SBW of the animals evaluated, for example, by Valadares Filho et al. (2016) is higher (451kg) than in the present study (402kg), indicating that the animals were closer to weight at maturity, which explains the higher requirement recommended by the authors in comparison with the present study.

The estimated value of k_{gain} was close to that described by Menezes et al. (2016) and Costa e Silva et al. (2012), 34.4% and 33.0%, respectively. However, Chizzotti et al. (2008) described in a meta-analyses a value of 44% for k_{gain}, a value eight percentage units higher than that found in this study. This efficiency is also affected by several factors, as described for the energy requirement for gain. However, most studies involve information from Nellore in the growth phase (Chizzotti et al., 2008; Costa e Silva et al., 2015; Tedeschi et al., 2002), with more data on animals in the finishing phase being important to estimate this variable more accurately.

According to Tedeschi et al. (2004) and Tedeschi and Fox (2020), the deposition of ME as fat is more efficient due to the energy cost of protein turnover, which raises the required amount of ME for deposition of 1 Mcal of protein. In addition, fat can be deposited from the energy left over from the intake of other compounds, such as carbohydrates, while protein is deposited only from amino acids. Consequently, some variation in this data can be found, depending on, for example, the breed. According to Sainz et al. (2005), protein turnover in Nellore animals is less than in B. taurus animals. Consequently, it is possible to find higher k_p values for Nellore animals. In this sense, Geay (1984) described a value of 0.20 for energy deposition efficiency as a protein, which is 40% lower than that found in this study. However, for the efficiency of deposition as fat, it is possible to find values close to that described in this work, as is the case of the authors Owens et al. (1995), who found a value of 0.79 for k_p. Inconsistencies in the efficiency of fat and protein used for growth might be related to the methods used to determine retained fat and protein (Tedeschi, 2019; Tedeschi et al., 2017; Tedeschi and Fox, 2020).

It is important to say that the animals received a diet containing the additive monensin (22 mg/kg DM in the diet) and virginiamycin (1 g/kg, DM basis). According to NASEM (2016), the use of the monensin additive can increase dietary ME by 2.3%. This value can be considered when the energy models proposed by this study are used.

The value found for MP_m was close to that suggested by Valadares Filho et al. (2016) and NASEM (2016), 3.6 g/kg$^{0.75}$ SBW and 3.8 g/kg$^{0.75}$ SBW, respectively. Similarly, Smuts (1935) determined a value of 3.52 g/kg$^{0.75}$ SBW. However, the equation suggested for NP_g presented coefficients higher than those described by Costa e Silva et al. (2020) (Equation 24), but close to those described by Valadares Filho et al. (2016) (Equation 25). According to Fox and Black, (1984), NP_g requirements are affected by BW, rate of weight gain, diet, and others,

with the percentage of RE deposited as protein decreasing exponentially when RE in the gain increases (Chizzotti et al., 2008), that is, when the animal approaches weight to maturity.

CONCLUSION

Therefore, the inclusion of low-fat dried distillers grains in finishing diets reduced TDN intake, MEI and total RE and RE as fat, although EBW did not differ. The highest MPI did not provide greater protein deposition, indicating that greater losses occurred through the urine, which is undesirable both from an energy and environmental point of view. Regarding nutritional requirements, the NE_m was 75.9 kcal/$kg^{0.75}$ EBW and the ME_m was 122 kcal/$kg^{0.75}$ EBW. Consequently, the k_{ma} was 62.2%. Daily MP_m was 3.6 g/$kg^{0.75}$ SBW. The efficiency of using the ME for gain was 36%. We find a value of 0.81 for k_f and 0.28 for k_p. So, it is possible to use the proposed models to estimate body composition and to estimate energy and protein requirements for maintenance and growth of young Nellore bulls.

Declarations of interest: none

Funding: This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ, Brazil; 465377-2014/9).

LITERATURE CITED

AFRC, 1993. Agricultural and Food Research Council. Energy and Protein Requirements of Ruminants. CAB International, Wallingford, p. 159.

Al-Suwaiegh, S., Fanning, K.C., Grant, R.J., Milton, C.T., Klopfenstein, T.J., 2002. Utilization of distillers grains from the fermentation of sorghum or corn in diets for finishing beef and lactating dairy cattle. Journal of Animal Science 80. https://doi.org/10.2527/2002.8041105x

Alhadas, H.M., Valadares Filho, S.C., Silva, F.F., Silva, F.A.S., Pucetti, P., Pacheco, M.V.C., Silva, B.C., Tedeschi, L.O., 2021a. Effects of including physically effective fiber from sugarcane in whole corn grain diets on the ingestive, digestive, and ruminal parameters of growing beef bulls. Livestock Science 248, 104508. https://doi.org/10.1016/j.livsci.2021.104508

Alhadas, H.M., Valadares Filho, S.C., Tedeschi, L.O., Vilela, R.S.R., Souza, G.A.P., Lage, B.C., Silva, B.C., Rennó, L.N., Paulino, M.F., 2021b. Impact of different levels of low-fat dried distillers grains on performance of young Nellore bulls during the finishing phase. Animal Science Journal 92, e13623. https://doi.org/10.1111/ASJ.13623

Amaral, P.M., Valadares Filho, S.C., Detmann, E., Santos, S.A., Prados, L.F., Mariz, L.D.S., Alves, L.C., Menezes, A.C.B., Villadiego, F.A.C., Novaes, M.A.S., Silva, F.A.S., 2014. Effect of phase-feeding crude protein on performance and carcass characteristics of crossbred beef bulls: An application to reduce nitrogen compounds in beef cattle diets. Tropical Animal Health and Production 46, 419–425. https://doi.org/10.1007/s11250-013-0507-z

AOAC, 2012. Official Methods of Analysis of AOAC International, 19th ed. Association of Official Analysis Chemists International. Gaithersburg, Maryland.

AOAC, 2005. Official Methods of Analysis of AOAC International, 18th ed, Association of Official Analysis Chemists International. Gaithersburg, Maryland.

Appuhamy, J.A.D.R.N., Nayananjalie, W.A., England, E.M., Gerrard, D.E., Akers, R.M., Hanigan, M.D., 2014. Effects of AMP-activated protein kinase (AMPK) signaling and essential amino acids on mammalian target of rapamycin (mTOR) signaling and protein synthesis rates in mammary cells. Journal of Dairy Science 97, 419–429. https://doi.org/10.3168/jds.2013-7189

ARC, 1980. Agricultural Research Council. The nutrient requirements of ruminant livestock. London: The Gresham Press. p. 351.

Barbosa, A.M., Valadares, R.F.D., Valadares Filho, S.C., Pina, D.S., Detmann, E., Leão, M.I., 2011. Endogenous fraction and urinary recovery of purine derivatives obtained by different methods in Nellore cattle1. Journal of Animal Science 89, 510–519. https://doi.org/10.2527/jas.2009-2366

Bonin, M. de N., Pedrosa, V.B., da Luz e Silva, S., Bünger, L., Ross, D., da Costa Gomes, R., de Almeida Santana, M.H., de Córdova Cucco, D., de Rezende, F.M., Ítavo, L.C.V., de Novais, F.J., Pereira, M.W.F., de Mattos Oliveira, E.C., Ferraz, J.B.S., 2021. Genetic parameters associated with meat quality of Nellore cattle at different anatomical points of longissimus: Brazilian standards. Meat Science 171, 108281. https://doi.org/10.1016/j.meatsci.2020.108281

Buckner, C.D., Erickson, G., Mader, T., Colgan, S.L., Karges, K.K., Gibson, M.L., 2007. Optimum Levels of Dry Distillers Grains with Solubles for Finishing Beef Steers. Nebraska Beef Cattle Reports.

Chizzotti, M.L., Tedeschi, L.O., Valadares Filho, S.C., 2008. A meta-analysis of energy and

protein requirements for maintenance and growth of Nellore cattle1. Journal of Animal Science 86, 1588–1597. https://doi.org/10.2527/jas.2007-0309

Cooke, R.F., Daigle, C.L., Moriel, P., Smith, S.B., Tedeschi, L.O., Vendramini, J.M.B., 2020. Cattle adapted to tropical and subtropical environments: social, nutritional, and carcass quality considerations. Journal of Animal Science 98. https://doi.org/10.1093/JAS/SKAA014

Corrigan, M.E., Erickson, G., Klopfenstein, T., Pol, K.J. Vander, Greenquist, M.A., Luebbe, M., 2007. Effect of Corn Processing and Wet Distillers Grains Inclusion Level in Finishing Diets. Nebraska Beef Cattle Reports.

Costa e Silva, L.F., Engle, T.E., Valadares Filho, S.C., Rotta, P.P., Valadares, R.F.D., Silva, B.C., Pacheco, M.V.C., 2015. Intake, apparent digestibility, and nutrient requirements for growing Nellore heifers and steers fed two levels of calcium and phosphorus. Livestock Science 181, 17–24. https://doi.org/10.1016/j.livsci.2015.09.024

Costa e Silva, L.F., Valadares Filho, S.C., Benedeti, P.D.B., Detmann, E., Menezes, A.C.B., Silva, T.E., Silva, F.A.S., 2020. Development of an equation to predict net protein requirements for the growth of Zebu beef cattle. Animal 14, 963–972. https://doi.org/10.1017/S1751731119002684

Costa e Silva, L.F., Valadares Filho, S.C., Zanetti, D., Rotta, P.P., Marcondes, M.I., Prados, L.F., Paulino, M.F., Azevedo, H. de O., 2012. Energy and protein nutritional requirements for Nellore bulls. Revista Brasileira de Zootecnia 41, 1516–1524. https://doi.org/10.1590/S1516-35982012000600028

CSIRO, 2007. Commonwealth Scientific and Industrial Research Organization: Nutrient requirements of domesticated ruminants. Collingwood, AU. p. 270.

Detmann, E., Valadares Filho, S.C., 2010. Sobre a estimação de carboidratos não fibrosos em alimentos e dietas. Arquivo Brasileiro de Medicina Veterinaria e Zootecnia 62, 980–984. https://doi.org/10.1590/S0102-09352010000400030

Ferreira, J.E., Mello, M.R.B. de, Silva, O.R. e, Silenciato, L.N., Couto, S.R.B. do, Mello, R.R.C., 2020. Weaning weight and optimization of reproductive efficiency in Nellore females subjected to timed artificial insemination. Revista Brasileira de Zootecnia 49, 2020. https://doi.org/10.37496/rbz4920190057

Fox, D.G., Black, J.R., 1984. A system for Predicting Body Composition and Performance of Growing Cattle. Journal of Animal Science 58, 725–739. https://doi.org/10.2527/jas1984.583725x

Fox, D.G., Tedeschi, L.O., Tylutki, T.P., Russell, J.B., Van Amburgh, M.E., Chase, L.E., Pell, A.N., Overton, T.R., 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. Animal Feed Science and Technology 112, 29–78. https://doi.org/10.1016/j.anifeedsci.2003.10.006

Garrett, W.N., Meyer, J.H., Lofgreen, G.P., 1959. The Comparative Energy Requirements of Sheep and Cattle for Maintenance and Gain1. Journal of Animal Science 18, 528–547. https://doi.org/10.2527/jas1959.182528x

Geay, Y., 1984. Energy and Protein Utilization in Growing Cattle. Journal of Animal Science 58, 766–778. https://doi.org/10.2527/jas1984.583766x

George, S.K., Dipu, M.T., Mehra, U.R., Singh, P., Verma, A.K., Ramgaokar, J.S., 2006. Improved HPLC method for the simultaneous determination of allantoin, uric acid and creatinine in cattle urine. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences 832, 134–137.

https://doi.org/10.1016/j.jchromb.2005.10.051

Ham, G.A., Stock, R.A., Klopfenstein, T.J., Larson, E.M., Shain, D.H., Huffman, R.P., 1994. Wet corn distillers byproducts compared with dried corn distillers grains with solubles as a source of protein and energy for ruminants. Journal of Animal Science 72, 3246–3257. https://doi.org/10.2527/1994.72123246x

Hankins, O.G., Howe, P.E., 1946. Estimation of the composition of beef carcasses and cuts. US Department of Agriculture 926.

Klopfenstein, T., 2001. Distillers Grains for Beef Cattle. In National Corn Growers Association, Ethanol Co-Products Workshop, Lincoln, Neb.

Krehbiel, C.R., Cranston, J.J., McCurdy, M.P., 2006. An upper limit for caloric density of finishing diets. Journal of Animal Science 84, E34–E49. https://doi.org/10.2527/2006.8413_supplE34x

MacDonald, J.C., Klopfenstein, T.J., Erickson, G.E., Griffin, W.A., 2007. Effects of dried distillers grains and equivalent undegradable intake protein or ether extract on performance and forage intake of heifers grazing smooth bromegrass pastures. Journal of Animal Science 85, 2614–2624. https://doi.org/10.2527/jas.2006-560

Marcondes, M.I., Tedeschi, L.O., Valadares Filho, S.C., 2010. Prediction of partial efficiency of use of metabolizable energy to net energy for gain and maintenance. In: Energy and protein metabolism and nutrition 28, 543–544.

Marcondes, M.I., Tedeschi, L.O., Valadares Filho, S.C., Chizzotti, M.L., 2012. Prediction of physical and chemical body compositions of purebred and crossbred Nellore cattle using the composition of a rib section1. Journal of Animal Science 90, 1280–1290. https://doi.org/10.2527/jas.2011-3839

Marcondes, M.I., Tedeschi, L.O., Valadares Filho, S.C., Costa e Silva, L.F., Silva, A.L., 2016. Using growth and body composition to determine weight at maturity in Nellore cattle. Animal Production Science 56, 1121. https://doi.org/10.1071/AN14750

McAlian, A.B., Smith, R.H., 1973. Degradation of nucleic acids in the rumen. British Journal of Nutrition 29, 331–345. https://doi.org/10.1079/bjn19730107

Menezes, A.C.B., V. Filho, S.C., Pucetti, P., C. Pacheco, M. V, Godoi, L.A., Zanetti, D., Alhadas, H.M., Paulino, M.F., Caton, J.S., 2019. Oscillating and static dietary crude protein supply: II. Energy and protein requirements of young Nellore bulls. Translational Animal Science 3, 1216–1226. https://doi.org/10.1093/tas/txz139

Menezes, A.C.B., Valadares Filho, S.C., Costa e Silva, L.F., Pacheco, M.V.C., Pereira, J.M.V., Rotta, P.P., Zanetti, D., Detmann, E., Silva, F.A.S., Godoi, L.A., Rennó, L.N., 2016. Does a reduction in dietary crude protein content affect performance, nutrient requirements, nitrogen losses, and methane emissions in finishing Nellore bulls? Agriculture, Ecosystems and Environment 223, 239–249. https://doi.org/10.1016/j.agee.2016.03.015

Mertens, D.R., Allen, M., Carmany, J., Clegg, J., Davidowicz, A., Drouches, M., Frank, K., Gambin, D., Garkie, M., Gildemeister, B., Jeffress, D., Jeon, C.S., Jones, D., Kaplan, D., Kim, G.N., Kobata, S., Main, D., Moua, X., Paul, B., Robertson, J., Taysom, D., Thiex, N., Williams, J., Wolf, M., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. Journal of AOAC International.

Miron, J., Yosef, E., Ben-Ghedalia, D., 2001. Composition and in vitro digestibility of monosaccharide constituents of selected byproduct feeds. Journal of Agricultural and Food Chemistry 49, 2322–2326. https://doi.org/10.1021/jf0008700

NASEM, 2016. National Academies of Sciences, Engineering, and Medicine. National Academies Press: Washington, DC, p. 171. https://doi.org/10.17226/19014

NRC, 2001. Nutrient requirements of Dairy Cattle. Natl. Acad. Press, Washington, DC, USA., p. 381.

Owens, F.N., Gill, D.R., Secrist, D.S., Coleman, S.W., 1995. Review of some aspects of growth and development of feedlot cattle. Journal of Animal Science 73, 3152. https://doi.org/10.2527/1995.73103152x

Prados, L.F., Sathler, D.F.T., Silva, B.C., Zanetti, D., Valadares Filho, S.C., Alhadas, H.M., Detmann, E., Santos, S.A., Mariz, L.D.S., Chizzotti, M.L., 2017. Reducing mineral usage in feedlot diets for Nellore cattle: II. Impacts of calcium, phosphorus, copper, manganese, and zinc contents on intake, performance, and liver and bone status1. Journal of Animal Science 95, 1766–1776. https://doi.org/10.2527/jas.2016.1085

Sainz, R.D., Barioni, L.G., Paulino, P. V., Valadares Filho, S.C., Oltjen, J.W., 2005. Growth patterns of Nellore vs. British beef cattle breeds assessed using a dynamic, mechanistic model of cattle growth and composition. In: Nutrient digestion and utilization in farm animals: modelling approaches. CABI Publishin, Wallingford, UK. pp, 160-170.

Smuts, D.B., 1935. The relation between the basal metabolism and the endogenous nitrogen metabolism, with particular reference to the estimation of the maintenance requirement of protein. Journal of Nutrition, 9:403-433.

Tas, B.M., Susenbeth, A., 2007. Urinary purine derivates excretion as an indicator of in vivo microbial N flow in cattle: A review. Livestock Science. https://doi.org/10.1016/j.livsci.2007.05.010

Tedeschi, L.O., 2019. Relationships of retained energy and retained protein that influence the

determination of cattle requirements of energy and protein using the California Net Energy System. Translational Animal Science 3, 1029–1039. https://doi.org/10.1093/tas/txy120

Tedeschi, L.O., Boin, C., Fox, D.G., Leme, P.R., Alleoni, G.F., Lanna, D.P.D., 2002. Energy requirement for maintenance and growth of Nellore bulls and steers fed high-forage diets. Journal of Animal Science 80, 1671–1682. https://doi.org/10.2527/2002.8061671x

Tedeschi, L.O., Fox, D.G., 2020. The Ruminant Nutrition System: Volume I - An Applied Model for Predicting Nutrient Requirements and Feed Utilization in Ruminants. XanEdu, Ann Arbor, MI, p. 291.

Tedeschi, L.O., Fox, D.G., Guiroy, P.J., 2004. A decision support system to improve individual cattle management. 1. A mechanistic, dynamic model for animal growth. Agricultural Systems 79, 171–204. https://doi.org/10.1016/S0308-521X(03)00070-2

Tedeschi, L.O., Galyean, M.L., Hales, K.E., 2017. Recent advances in estimating protein and energy requirements of ruminants. Animal Production Science 57, 2237. https://doi.org/10.1071/AN17341

Valadares Filho, S.C., Costa e Silva, L.F., Gionbelli, M.P., Rotta, P.P., Marcondes, M.I., Chizzotti, M.L., Prados, L.F., 2016. Nutrient Requirements of Zebu and Crossbred Cattle - BR-CORTE, in: Exigências Nutricionais de Zebuínos Puros e Cruzados - BR-CORTE. Editora Federal de Viçosa, p. 163. https://doi.org/10.5935/978-85-8179-111-1.2016b002

Valente, T.N.P., Detmann, E., Queiroz, A.C., Valadares Filho, S.C., Gomes, D.I., Figueiras, J.F., 2011. Evaluation of ruminal degradation profiles of forages using bags made from different textiles. Revista Brasileira de Zootecnia. https://doi.org/10.1590/S1516-35982011001100039

Williams, C.B., Jenkins, T.G., 2003. A dynamic model of metabolizable energy utilization in

growing and mature cattle. I. Metabolizable energy utilization for maintenance and support metabolism. Journal of Animal Science 81, 1371–1381. https://doi.org/10.2527/2003.8161371x

Table 1 - Chemical composition of diet ingredients

Ingredients	DM[1]	OM[2]	NDF[3]	CP[4]	EE[5]
		g/kg			
Corn silage	307	936	498	62	25
DDG[6]	897	989	540	327	49
Corn meal	894	990	90	92	42
Soybean meal	885	939	95	505	24
Urea/A.S[7]	997	1000	-	2610	-
Premix[8]	1000	-	-	-	-
Virginiamycin	1000	-	-	-	-

[1]Dry matter, [2]Organic matter, [3]Neutral detergent fiber, [4]Crude protein, [5]Ether extract, [6]Dried distillers grains, [7]urea and ammonium sulfate (9:1), [8]Premix guarantees (per kg of DM): 200 - 220 g of Ca, 10 mg of Co (Min), 500 mg of Cu (Min), 22 g of S (Min), 333 mg of Fe (Min), 178.41 mg of F (Max), 10 g of P (Min), 25 mg of I (Min), 17 g of Mg (Min), 1500 mg of Mn (Min), 1100 mg of monensin, 6.6 mg of Se (Min), 50 g of Na (Min), and 2000 mg of Zn (Min). The unit g/kg for dry matter represents grams of dry matter per kilogram of raw material, and for the other constituents represents grams of the constituent per kilogram of dry matter.

Table 2 - Proportion of ingredients and composition of experimental diets

Items	Diets			
	D0	D150	D300	D450
	g/kg			
Ingredients				
Corn silage	280	280	280	280
DDG	-	150	300	450
Corn meal	649	537	399	249
Soybean meal	40.0	12.0	-	-
Urea/A.S[2]	10.0	-	-	-
Premix	20.0	20.0	20.0	20.0
Virginiamycin	1.00	1.00	1.00	1.00
Analysed composition				
DM	587	589	589	589
OM[3]	952	953	954	954
NDF[4]	202	270	337	405
CP[5]	123	122	152	187
EE[6]	35.6	37.3	38.4	39.4
NFC[7]	608	525	426	322

D0 = diet without DDG, D150 = diet with 150 g/kg of DDG, D300 = diet with 300 g/kg of DDG, D450 = diet with 450 g/kg of DDG, [1]Dry matter, [2]urea and ammonium sulfate (9:1), [3]Organic matter, [4]Neutral detergent fiber, [5]Crude protein, [6]Ether extract, [7]Non-fiber carbohydrates. In the "Ingredients" section, the unit g/kg represents grams of the ingredient per kilogram of dry matter in the diet. In the "Analysed composition" section, the unit g/kg for dry matter represents grams of dry matter per kilogram of raw material, and for the other constituents represents grams of the constituent per kilogram of dry matter.

Table 3 – Effect of low-fat DDG inclusion levels in finishing diets on the metabolizable energy and protein intake, energy and protein deposition, heat production and on the empty body weight

Items	Diets				SEM[1]	P - value	
	D0	D150	D300	D450		L	Q
Metabolizable energy intake (Mcal/kg$^{0.75}$ EBW)	0.2909^{A}	0.2775^{AB}	0.2666^{BC}	0.2504^{C}	0.006	< 0.010	0.820
Retained energy (Mcal/kg$^{0.75}$ EBW)	0.0683	0.0668	0.0607	0.0575	0.003	0.065	0.689
Retained energy as protein (Mcal/kg$^{0.75}$ EBW)	0.0172	0.0179	0.0177	0.0171	0.001	0.917	0.550
Retained energy as fat (Mcal/kg$^{0.75}$ EBW)	0.0511^{A}	0.0489^{AB}	0.0430^{AB}	0.0404^{B}	0.003	0.010	0.952
Heat production (Mcal/kg$^{0.75}$ EBW)	0.2226^{A}	0.2107^{AB}	0.2059^{BC}	0.1929^{C}	0.005	< 0.001	0.887
Metabolizable protein intake (g/d)	559.8^{B}	563.1^{B}	724.8^{A}	734.8^{A}	25.50	< 0.010	0.898
Retained crude protein (g/d)	231.4	236.0	238.2	222.6	14.41	0.722	0.499
Empty body weight (kg)	402.5	397.7	391.4	386.1	14.79	0.428	0.988

D0 = diet without DDG, D150 = diet with 150 g/kg of DDG, D300 = diet with 300 g/kg of DDG, D450 = diet with 450 g/kg of DDG, [1]Standard error of mean. Values on the same line, marked by different letters, are statistically different (P = 0.05) by the least-squares means test.

Table 4 - Equations to prediction EBW composition, carcass and non-carcass components in the SBW, and the relationships between EBW and SBW, and EWG and ADG.

Item	Equation	R^2	Number
Crude Protein, kg	$0.2112 \times EBW^{0.9689}$	0.98	13
Ether extract, kg	$0.0169 \times EBW^{1.4245}$	0.85	14
Water, kg	$1.0863 \times EBW^{0.8900}$	0.99	15
Carcass weight, kg	$0.4619 \times SBW^{1.0428}$	0.98	16
Non-Carcass weight, kg	$0.4502 \times SBW^{0.9489}$	0.97	17
Empty body weight, kg	$0.8644 \times SBW^{1.0119}$	0.99	18
Empty weight gain, kg	$1.0295 \times ADG^{0.9083}$	0.99	19

ADG = Average daily gain, EBW = empty body weight, SBW = shrunk body weight. Standard errors of the models: 10 (a = 0.0434; b = 0.0343); 11 (a = 0.0146; b = 0.1443); 12 (a = 0.1433; b = 0.0221); 13 (a = 0.0940; b = 0.0336); 14 (a = 0.1078; b = 0.0396); 15 (a = 0.0886; b = 0.0169); 16 (a = 0.0107; b = 0.0367).

Table 5 - Equations to prediction energy and protein requirements for growth and maintenance

Item	Equation	R^2	Number
Heat production, Mcal/kg$^{0.75}$ EBW	$0.0759 \times e^{(3.6987 \times MEI)}$	0.95	20
Net energy requirement to gain, Mcal/d	$0.055 \times EBW^{0.75} \times EWG^{0.6923}$	0.87	21
Metabolizable energy intake, Mcal/kg$^{0.75}$ EBW	$15.31 + 1.23 \times RE_f + 3.55 \times RE_p$	0.34	22
Net protein requirement for growth[1], g/d	$216.31 \times EWG - 7.38 \times RE$	0.99	23
Net protein requirement for growth[2], g/d	$176 \times EWG - 6.24 \times RE$	-	24
Net protein requirement for growth[3], g/d	$210 \times EWG - 10.01 \times RE$	-	25

EBW = empty body weight (kg); $EBW^{0.75}$ = metabolic empty body weight (kg); EWG = empty weight gain (kg/d); MEI = metabolizable energy intake (Mcal/kg$^{0.75}$ EBW); RE = retained energy (Mcal/d); RE_p = retained energy as protein (Mcal/kg$^{0.75}$ EBW); RE_f = retained energy as fat (Mcal/kg$^{0.75}$ EBW). [1]Equation suggested by this study; [2]Equation suggested by Costa e Silva et al. (2020); [3]Equation suggested by Valadares Filho et al., (2016). Standard errors of the models: 17 (a = 0.004; b = 0.1861); 18 (a = 0.002; b = 0.1334); 19 (a = 0.036; b = 0.4012; c = 1.4145); 20 (a = 15.78; b = 4.032).

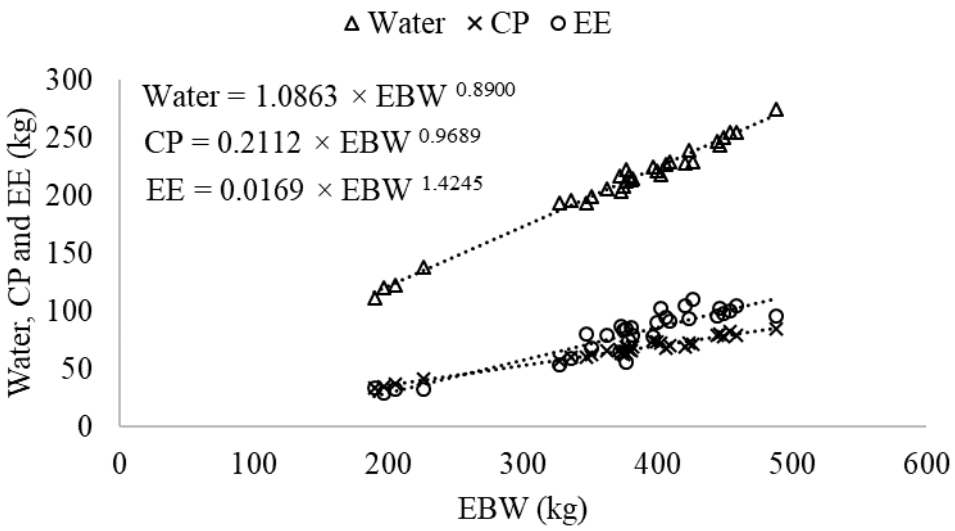

Figure 1 - Relationship between the amount of water, crude protein (CP), ether extract (EE), and empty body weight (EBW).

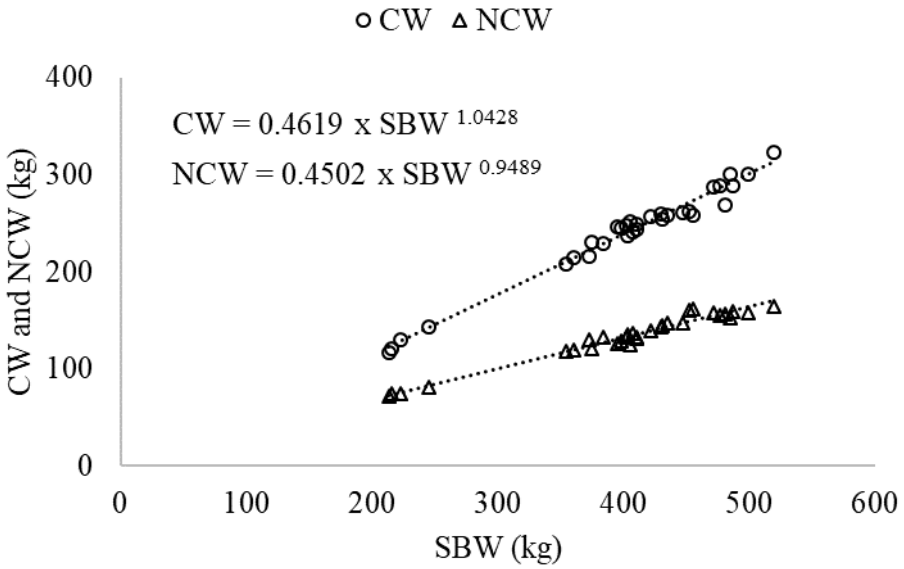

Figure 2 - Relationship between the carcass weight (CW) and non-carcass weight (NCW) and shrunk body weight (SBW).

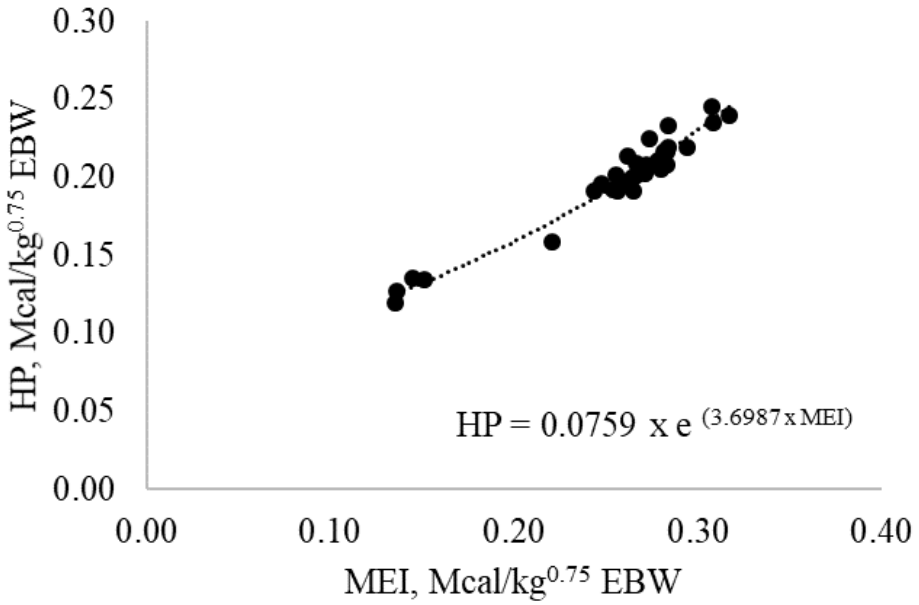

Figure 3 - Relationship between heat production (HP) and metabolizable energy intake (MEI).

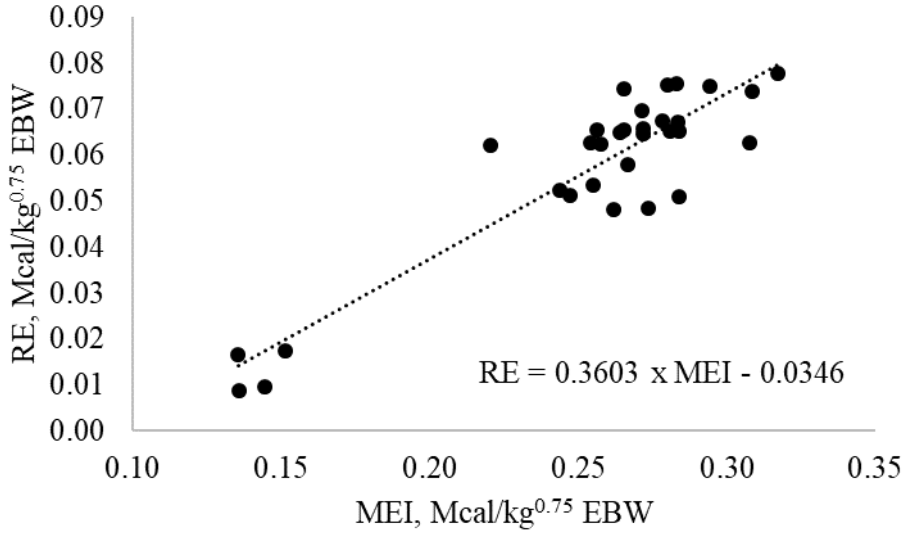

Figure 4 - Relationship between retained energy (RE) and metabolizable energy intake to gain (MEI).

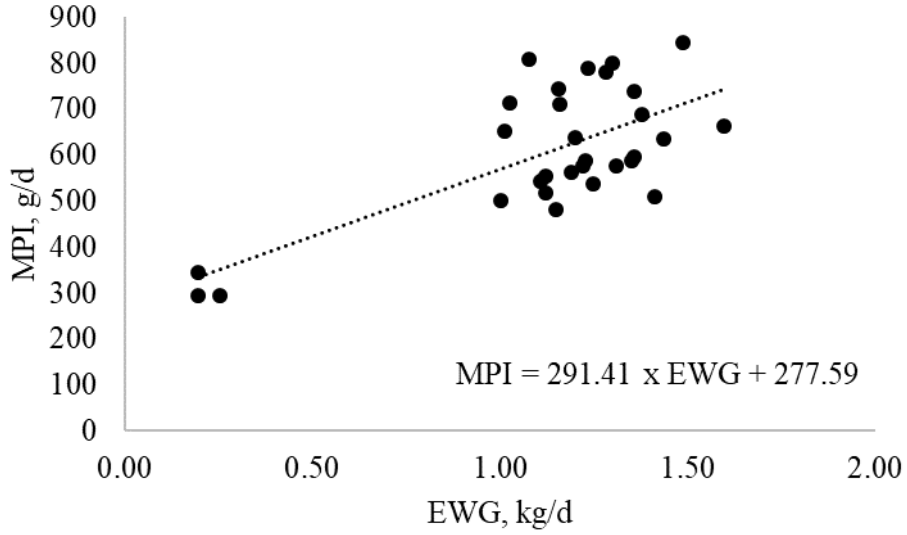

Figure 5 - Relationship between metabolizable protein intake (MPI) and empty weight gain (EWG).

Table S1 - Treatment effect on NE_m estimation model coefficients using confidence interval

Parameter	Estimate	SEM	Confidence Interval Limits	
			Lower	Upper
β0				
D0	0.0707	0.0080	0.0541	0.0873
D150	0.0780	0.0090	0.0594	0.0965
D300	0.0799	0.0105	0.0626	0.1062
D450	0.0750	0.0094	0.0555	0.0945
β1				
D0	3.9663	0.3940	3.1513	4.7813
D150	3.5772	0.4187	2.7111	4.4434
D300	3.5110	0.4770	2.3151	4.2885
D450	3.7403	0.5093	2.6868	4.7938

D0 = diet without DDG, D150 = diet with 150 g/kg of DDG, D300 = diet with 300 g/kg of DDG, D450 = diet with 450 g/kg of DDG, [1]Standard error of mean. The equation used was: $HP = \beta0 \times e^{\beta1 \times MEI}$, where β0 and β1 are the regression parameters; HP = heat production ($Mcal/kg^{0.75}$ EBW); MEI = metabolizable energy intake ($Mcal/kg^{0.75}$ EBW).

Table S2 - Treatment effect on NE_g estimation model coefficients using confidence interval

Parameter	Estimate	SEM[1]	Confidence Interval Limits	
			Lower	Upper
β0				
D0	0.0488	0.0039	0.0408	0.0568
D150	0.0494	0.0042	0.0407	0.0582
D300	0.0453	0.0039	0.0372	0.0533
D450	0.0434	0.0038	0.0355	0.0513
β1				
D0	0.6530	0.2533	0.1278	1.1782
D150	0.5707	0.2922	-0.0353	1.1767
D300	0.5598	0.2998	-0.0619	1.1814
D450	0.6107	0.3901	-0.1984	1.4198

D0 = diet without DDG, D150 = diet with 150 g/kg of DDG, D300 = diet with 300 g/kg of DDG, D450 = diet with 450 g/kg of DDG, [1]Standard error of mean. The equation used was: $NEg = \beta0 \times EBW^{0.75} \times EWG^{\beta1}$, where β0 and β1 are the regression parameters; $EBW^{0.75}$ = metabolic empty body weight; EWG = empty weight gain (kg/d); NEg = net energy for growth (Mcal/d).

Table S3 - Treatment effect on MPI estimation model coefficients using confidence interval

Parameter	Estimate	SEM[1]	Confidence Interval Limits	
			Lower	Upper
β0				
D0	198.20	71.381	50.210	346.30
D150	235.00	68.463	92.977	376.90
D300	163.50	72.537	13.093	314.00
D450	293.50	70.959	146.40	440.70
β1				
D0	285.10	59.873	160.90	409.20
D150	261.20	57.651	141.60	380.80
D300	436.30	62.992	305.70	566.90
D450	375.50	65.410	239.80	511.10

D0 = diet without DDG, D150 = diet with 150 g/kg of DDG, D300 = diet with 300 g/kg of DDG, D450 = diet with 450 g/kg of DDG, [1]Standard error of mean. The equation used was: MPI = β0 + β1 × EWG, where β0 and β1 are the regression parameters; EWG = empty weight gain (kg/d); MPI = metabolizable protein intake (g/d).

Table S4 - Treatment effect on NP_g estimation model coefficients using confidence interval

Parameter	Estimate	SEM[1]	Confidence Interval Limits	
			Lower	Upper
β0				
D0	321.70	38.869	241.10	402.30
D150	153.00	28.919	93.051	261.00
D300	213.60	27.579	156.40	270.80
D450	192.00	32.677	124.30	259.80
β1				
D0	9.4201	9.5337	13.780	53.323
D150	6.7156	7.1733	-23.551	16.202
D300	8.5299	7.4680	-8.7340	22.241
D450	4.8549	9.0303	-18.560	18.896

D0 = diet without DDG, D150 = diet with 150 g/kg of DDG, D300 = diet with 300 g/kg of DDG, D450 = diet with 450 g/kg of DDG, [1]Standard error of mean. The equation used was: NPg = β0 × EWG - β1 × RE, where β0 and β1 are the regression parameters; EWG = empty weight gain (kg/d); RE = retained energy (Mcal/d).

Table S5 - Treatment effect on EBW form SBW estimation model coefficients using confidence interval

Parameter	Estimate	SEM[1]	Confidence Interval Limits	
			Lower	Upper
a				
D0	1.1030	0.2373	0.6108	1.5952
D150	0.7209	0.1478	0.4145	1.0273
D300	0.8817	0.2139	0.4380	1.3254
D450	0.9343	0.1996	0.5205	1.3482
b				
D0	0.9704	0.0355	0.8969	1.0440
D150	1.0428	0.0339	0.9725	1.1131
D300	1.0090	0.0403	0.9254	1.0925
D450	0.9987	0.0355	0.9251	1.0722

D0 = diet without DDG, D150 = diet with 150 g/kg of DDG, D300 = diet with 300 g/kg of DDG, D450 = diet with 450 g/kg of DDG, [1]Standard error of mean. The equation used was: $EBW = a \times SBW^b$, where "a" and "b" are the regression parameters; EBW = empty body weight (kg); SBW = shrunk body weight (kg).

Table S6 - Treatment effect on EWG form ADG estimation model coefficients using confidence interval

Parameter	Estimate	SEM[1]	Confidence Interval Limits	
			Lower	Upper
a				
D0	1.0072	0.0200	0.9657	1.0487
D150	1.0422	0.0221	0.9964	1.0880
D300	1.0367	0.0204	0.9943	1.0790
D450	1.0280	0.0193	0.9879	1.0681
b				
D0	0.9335	0.0615	0.8059	1.0611
D150	0.8620	0.0755	0.7054	1.0185
D300	0.8790	0.0707	0.7323	1.0256
D450	0.8868	0.0854	0.7096	1.0639

D0 = diet without DDG, D150 = diet with 150 g/kg of DDG, D300 = diet with 300 g/kg of DDG, D450 = diet with 450 g/kg of DDG, [1]Standard error of mean. The equation used was: $EWG = a \times ADG^{b}$, where "a" and "b" are the regression parameters; EWG = empty weight gain (kg/d); ADG = average daily gain (kg/d).

Table S7 - Treatment effect on NCW form SBW estimation model coefficients using confidence interval

Parameter	Estimate	SEM[1]	Confidence Interval Limits	
			Lower	Upper
a				
D0	0.4170	0.2199	-0.0391	0.8732
D150	0.3477	0.1693	-0.0035	0.6988
D300	0.2969	0.1769	-0.0698	0.6637
D450	0.6351	0.3205	-0.0297	1.2999
b				
D0	0.9598	0.0870	0.7794	1.1402
D150	0.9946	0.0805	0.8275	1.1616
D300	1.0185	0.0989	0.8135	1.2236
D450	0.8908	0.0839	0.7168	1.0649

D0 = diet without DDG, D150 = diet with 150 g/kg of DDG, D300 = diet with 300 g/kg of DDG, D450 = diet with 450 g/kg of DDG, [1]Standard error of mean. The equation used was: $NCW = a \times SBW^b$, where "a" and "b" are the regression parameters; NCW = non-carcass weight (kg); SBW = shrunk body weight (kg).

Table S8 - Treatment effect on CW form SBW estimation model coefficients using confidence interval

Parameter	Estimate	SEM[1]	Confidence Interval Limits	
			Lower	Upper
a				
D0	0.6874	0.3085	0.0477	1.3271
D150	0.3916	0.1703	0.0384	0.7449
D300	0.5850	0.2958	-0.0285	1.1986
D450	0.4165	0.1893	0.0239	0.8091
b				
D0	0.9762	0.0740	0.8228	1.1297
D150	1.0698	0.0719	0.9207	1.2189
D300	1.0038	0.0839	0.8297	1.1779
D450	1.0600	0.0754	0.9035	1.2164

D0 = diet without DDG, D150 = diet with 150 g/kg of DDG, D300 = diet with 300 g/kg of DDG, D450 = diet with 450 g/kg of DDG, [1]Standard error of mean. The equation used was: $CW = a \times SBW^b$, where "a" and "b" are the regression parameters; CW = carcass weight (kg); SBW = shrunk body weight (kg).

Table S9 - Treatment effect on water in the EBW estimation model coefficients using confidence interval

Parameter	Estimate	SEM[1]	Confidence Interval Limits	
			Lower	Upper
a				
D0	1.0269	0.3174	0.3687	1.6850
D150	1.0383	0.2871	0.4429	1.6336
D300	1.3790	0.4588	0.4275	2.3306
D450	1.1753	0.3491	0.4512	1.8994
b				
D0	0.8988	0.0517	0.7916	1.0060
D150	0.8968	0.0463	0.8008	0.9928
D300	0.8496	0.0560	0.7336	0.9657
D450	0.8772	0.0500	0.7734	0.9809

D0 = diet without DDG, D150 = diet with 150 g/kg of DDG, D300 = diet with 300 g/kg of DDG, D450 = diet with 450 g/kg of DDG, [1]Standard error of mean. The equation used was: Water = a × EBW^b, where “a” and “b” are the regression parameters; EBW = empty body weight (kg).

Table S10 - Treatment effect on ether extract in the EBW estimation model coefficients using confidence interval

Parameter	Estimate	SEM[1]	Confidence Interval Limits	
			Lower	Upper
a				
D0	0.0081	0.0161	-0.0254	0.0416
D150	0.0195	0.0328	-0.0484	0.0875
D300	0.0059	0.0129	-0.0208	0.0327
D450	0.0057	0.0115	-0.0181	0.0296
b				
D0	1.5526	0.3334	0.8612	2.2440
D150	1.4050	0.2795	0.8254	1.9846
D300	1.5988	0.3635	0.8450	2.3526
D450	1.6023	0.3357	0.9062	2.2984

D0 = diet without DDG, D150 = diet with 150 g/kg of DDG, D300 = diet with 300 g/kg of DDG, D450 = diet with 450 g/kg of DDG, [1]Standard error of mean. The equation used was: Ether extract = a × EBW^b, where “a” and “b” are the regression parameters; EBW = empty body weight (kg).

Table S11 - Treatment effect on crude protein in the EBW estimation model coefficients using confidence interval

Parameter	Estimate	SEM[1]	Confidence Interval Limits	
			Lower	Upper
a				
D0	0.1925	0.1031	-0.0214	0.4064
D150	0.1786	0.0854	0.0014	0.3558
D300	0.2710	0.1534	-0.0472	0.5891
D450	0.1971	0.1015	-0.0133	0.4075
b				
D0	0.9834	0.0895	0.7977	1.1690
D150	0.9972	0.0800	0.8313	1.1631
D300	0.9277	0.0952	0.7303	1.1251
D450	0.9814	0.0866	0.8017	1.1610

D0 = diet without DDG, D150 = diet with 150 g/kg of DDG, D300 = diet with 300 g/kg of DDG, D450 = diet with 450 g/kg of DDG, [1]Standard error of mean. The equation used was: Crude protein = $a \times EBW^b$, where “a” and “b” are the regression parameters; EBW = empty body weight (kg).

Table S12 – Effect of low-fat DDG inclusion levels in finishing diets on the performance

Items	Diets				SEM	P - value	
	D0	D150	D300	D450		L	Q
Initial body weight, kg	272	265	272	267	13.45	0.917	0.967
Final body weight, kg	428	427	425	414	7.567	0.219	0.566
Final carcass weight, kg	258	257	260	251	5.655	0.445	0.478
Average daily gain, kg/d	1.27	1.25	1.24	1.15	0.060	0.574	0.314
Carcass weight gain, kg/d	0.85	0.84	0.86	0.79	0.043	0.449	0.513
Gain to feed ratio	0.20	0.19	0.19	0.19	0.007	0.470	0.500

D0 = diet without DDG, D150 = diet with 150 g/kg of DDG, D300 = diet with 300 g/kg of DDG, D450 = diet with 450 g/kg of DDG, [1]Standard error of mean. Values on the same line, marked by different letters, are statistically different ($P = 0.05$) by the least-squares means test.

Table S13 – Effect of low-fat DDG inclusion levels in finishing diets on the nutrient intake and digestibility

Items	Diets				SEM[1]	P - value	
	D0	D150	D300	D450		L	Q
Dry matter							
Intake, kg/d	6.45	6.6	6.62	6.11	0.251	0.403	0.205
Digestibility, g/kg	802^{A}	745^{B}	725^{C}	718^{C}	6.514	< 0.001	0.512
Organic matter							
Intake, kg/d	6.14	6.29	6.32	5.82	0.239	0.418	0.200
Digestibility, g/kg	818^{A}	757^{B}	733^{B}	732^{B}	9.245	< 0.001	0.215
Neutal detergent fiber							
Intake, kg/d	1.29^{C}	1.78^{B}	2.24^{A}	2.48^{A}	0.083	< 0.001	0.165
Digestibility, g/kg	614^{C}	610^{C}	643^{B}	684^{A}	9.395	< 0.001	0.025
Crude protein							
Intake, kg/d	0.80^{C}	0.80^{C}	1.01^{B}	1.14^{A}	0.039	< 0.001	0.111
Digestibility, g/kg	804^{A}	707^{C}	700^{C}	738^{B}	7.910	< 0.001	< 0.001
Ether extract							
Intake, kg/d	0.226	0.239	0.242	0.226	0.009	0.903	0.137
Digestibility, g/kg	949^{A}	925^{B}	901^{C}	895^{C}	6.84	< 0.010	0.184
Non fiber carbohydrates							
Intake, kg/d	3.90^{A}	3.47^{B}	2.83^{C}	1.97^{D}	0.115	< 0.001	0.251
Digestibility, g/kg	884^{A}	833^{B}	812^{C}	770^{D}	7.071	< 0.001	0.503
Total digestible nutrients							
Intake, kg/d	5.36^{A}	5.04^{AB}	4.93^{AB}	4.52^{B}	0.195	0.008	0.814

D0 = diet without DDG, D150 = diet with 150 g/kg of DDG, D300 = diet with 300 g/kg of DDG, D450 = diet with 450 g/kg of DDG, [1]Standard error of mean. Values on the same line, marked by different letters, are statistically different (P = 0.05) by the least-squares means test.

CHAPTER 3

Effects of low-fat dried distillers grains on nutrient intake and digestibility in high-concentrate diets

H. M. Alhadas [a, 1], S. C.Valadares Filho[a], L. O. Tedeschi[b], R. S. R. Vilela[a], G. A. P. Souza[a], B. C. Lage[a], K. R. Oliveira[a], M. V. C. Pacheco[a], P. Pucetti[a], J. T. Silva[a], L. A. Godoi[a], F. A. S. Silva[a], L. N. Rennó[a], M. F. Paulino[a]

[a]*Department of Animal Science, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil, 36570-900.*

[b]*Department of Animal Science, Texas A&M University, College Station, Texas, USA, 77843-2471.*

[1]Corresponding author: H. M. Alhadas, E-mail: herlonmalhadas@gmail.com

https://orcid.org/0000-0003-2406-9726

ABSTRACT

This study aimed to evaluate the effect of including levels of low-fat dried distillers grains without soluble (**DDG**) in high-concentrate diets on intake and digestibility. Four rumen-cannulated Nellore cattle received four dietary treatments in a 4×4 Latin square experimental design. Treatments included diets without DDG (**D0**) and varying inclusion levels of DDG at 150 g/kg (**D150**), 300 g/kg (**D300**), and 450 g/kg (**D450**). Four periods of total feces and urine collection were performed to estimate nutrient intake and digestibility and estimate nitrogen balance. Collections of omasum digesta were used to estimate ruminal degradability. The ruminal emptying technique was performed to estimate the ruminal rates of ingestion, passage, and digestion (**kd**). The inclusion of DDG reduced the intake of total digestible nutrients (**TDN**) ($P < 0.01$), dry matter (**DM**) ($P = 0.02$), organic matter (**OM**) ($P = 0.02$), non-fiber carbohydrates (**NFC**) ($P < 0.01$) and ether extract (**EE**) ($P = 0.06$). The intake of neutral detergent fiber (**NDF**) increased linearly ($P < 0.01$), whereas CP had a quadratic pattern ($P = 0.02$). The total digestibility in kg/d of DM ($P < 0.01$), OM ($P < 0.01$), NFC ($P < 0.01$) and EE ($P = 0.02$) decreased linearly. The total digestibility in kg/d of the NDF increased linearly ($P < 0.01$) and the CP had a quadratic pattern ($P = 0.01$). The kd of DM ($P = 0.01$), OM ($P < 0.01$) decreased linearly and NDF increased linearly ($P < 0.01$). The amount of N retained did not change ($P = 0.72$), but the intake of metabolizable protein increased ($P = 0.03$). Therefore, the inclusion of low-fat DDG in finishing diets reduces TDN intake and reduces the digestibility of almost all nutrients.

Keywords: Beef cattle, finishing phase, coproduct, Nellore.

INTRODUCTION

Dried distillers grain (**DDG**) are coproducts generated after the fermentation of carbohydrates such as starch for alcohol production. The use of corn for this purpose has been growing in many countries, including Brazil and the United States (CONAB, 2020; USDA, 2019), and consequently, the amount of coproduct produced is also increasing. The coproduct is largely used to feed ruminants. It has been demonstrated for quite some time that after fermentation, the composition of the residue differs from the original grain, having a lower concentration of starch, and higher concentrations of crude protein (**CP**), neutral detergent fiber (**NDF**), and ether extract (**EE**) (Klopfenstein et al., 2008). The heating process can also cause changes in the CP profile, which can present around 730 g/kg of rumen undegradable protein (**RUP**) (NASEM, 2016). However, there are processing differences, which may include the addition of solubles, resulting in dried distillers grains with solubles (**DDGS**). Differences in chemical composition in nutrient concentration can also occur (Buckner et al., 2011), and the change in this product's chemical composition can generate changes in the digestibility and use of nutrients.

We hypothesized that the inclusion of low-fat DDG in finishing diets reduces the intake and digestibility of nutrients. The objective was to evaluate the effect of low-fat DDG levels in finishing diets on nutrient intake and digestibility.

MATERIAL AND METHODS

The experiment was conducted in the experimental feedlot facility of the Animal Science Department of the Federal University of Viçosa, Viçosa, Brazil, MG.

Animals, experimental design, and diets

Four rumen-cannulated Nellore bulls (395kg ± 20) at the age of 14 months were used in a 4 × 4 Latin square design. The animals were housed in individual 8-m^2 pens containing individual

water troughs and feeders. Before the start of the experiment, the animals were vaccinated against endo and ectoparasites (Ivermectin 3.5%; 1mL/kg body weight; Ranger LA 3.5%, MSD Saúde Animal, Brazil) and received an injection of vitamin ADE (5mL; ADE Vallée, MSD Saúde Animal, Brazil).

The experiment was conducted using four 23-d periods. Each period consisted of 14-d adaptation to the experimental diets and 9-d for data collection. Four dietary treatments were evaluated, as follows: diet without DDG (**D0**), including 150 g/kg DDG (**D150**), 300 g/kg DDG (**D300**), and 450 g/kg DDG (**D450**), on a dry matter (**DM**) basis. All diets had 280 g/kg corn silage (DM basis) as a source of roughage and were formulated according to Valadares Filho et al. (2016). The animals were fed twice a day at 07h00 and 16h00, and the intake was adjusted to maintain orts at 50 g/kg of the amount on an as-fed basis. The chemical composition of the ingredients and the level of inclusion of each ingredient as well as the the chemical composition of the diets can be seen in Tables 1 and 2 respectively.

Nutrient digestibility

Four digestibility periods were performed with a total collection of feces and urine on d-15, d-16, d-17 of each experimental period. The orts were weighed and sampled between d-15 and d-23 of all experimental periods to estimate the dry matter intake (**DMI**) and the intake of other dietary nutrients. Corn silage was also sampled on these days. Feedstuff were dried in a forced circulation oven (55°C) for 72-h and ground (Fortinox, Piracicaba, São Paulo, Brazil) through a 2-mm screen [indigestible NDF (**iNDF**) analyses] and subsequently with a 1-mm screen for further analyses. The other ingredients of the diet were sampled and processed with each mixture of the feed.

During the three days of collection, the feces were sampled and weighed (300 g, fresh matter) daily in aluminum trays to be dried in a forced circulation oven and ground, as described

above. At the end of each experimental period, a composite sample was mixed to be proportional to the amount excreted each day (DM basis). Urine was collected in gallons containing 200 ml of 20% (v/v) sulfuric acid, and daily its volume was measured and sampled. 5 ml of urine was diluted in 20 ml of 0.036 N sulfuric acid to preserve nitrogenous compounds and then stored in a freezer at -20°C for each collection day. Subsequently, a sample composed based on the volume of each day was obtained for chemical analyses. With the nutrient digestibility coefficients, the intake of total digestible nutrients (**TDN**) was estimated by the following equation (NRC, 2001):

$$TDN = dCP + dNDF + (dEE \times 2.25) + dNFC \quad (1)$$

where dCP = digestible crude protein (kg); dNDF = digestible neutral detergent fiber (kg); dEE = digestible ether extract (kg); and dNFC = digestible non-fiber carbohydrates (kg).

Markers infusion and omasal digesta sampling

Collections of omasal digesta were performed in each experimental period to estimate ruminal degradability and ruminal flow. The digesta flow was estimated using a double marker (Rotta et al., 2014), with Co-EDTA associated with the liquid and the small particles, and the iNDF associated with large particles, as described by Valadares Filho et al. (2011). The Co-EDTA marker was continuously infused (5000 mg/d Co-EDTA - 572 mg/d Co - diluted in 5 L of water) through the animals' cannula using a peristaltic pump (BP600.4; Milan Scientific Equipment, Inc., Colombo, Paraná, Brazil). The infusion was performed on d-15 to d-20, starting three days before the first omasal digesta collection (d-18), and continued until the last day of omasal digesta collection (d-20).

Eight collections were carried out at 9-h intervals during the three days (Huhtanen et al., 1997; Leão, 2002). On d-18, collections were made at 08h00 and 17h00, on d-19 at 02h00, 11h00, and 20h00, and on d-20 at 05h00, 14h00, and 23h00. Approximately 750 ml were

collected each time, with 250 ml used to isolate liquid-associated bacteria (**LAB**) and particle-associated bacteria (**PAB**), 250 ml used to compose the non-representative digesta sample, and 250 ml were filtered in a 100 μm nylon mesh filter with 44% surface pore area (Sefar Nitex 100/44, Sefar, Thal, Switzerland) to obtain a liquid fraction with smaller particles and another fraction with large particles. The non-representative digest was not filtered and contains the digest with all the phases: liquid and small-particles phase and large-particles phase. It is the sample as it was collected in the omasum.

After each collection time, the samples of non-representative digest, liquid and small particles phase and large particles phase were lyophilized and ground in a Wiley mill (Fortinox, Piracicaba, São Paulo, Brazil) at 2- and 1-mm, and subsequently, a composite sample (DM basis) of the eight collections, per period was made for further analyses. The LAB and PAB samples were processed every four collection times, resulting in two centrifugations per period, performed according to Reynal et al. (2005), with modifications suggested by Krizsan et al. (2010). After isolation, the LAB and PAB samples were lyophilized, macerated, and a composite sample was taken per animal and period (DM basis).

At each omasal collection time, rumen pH was measured, and 40 mL of ruminal fluid was sampled to quantify ammonia nitrogen concentration. To preserve nitrogenous compounds, 1 mL of H_2SO_4 (50% v/v) was added to each sample, which was subsequently frozen at -20°C for future analyses.

Ruminal emptying

On d-21 and d-23, the rumen was emptied to determine the pool of each nutrient, and with the data of omasal flows, ruminal rates of ingestion (**ki**), digestion (**kd**), and passage (**kp**) were estimated as described by Allen and Linton (2007). On d-21, sampling was carried out 3-h after

feeding, and on d-23 immediately before. The rumen content was separated into two fractions with the aid of four layers of cheesecloth, with one fraction consisting of liquid and small particles, and the other fraction composed of large particles. The samples were lyophilized and ground, and at the end of each period, a compound was made (DM basis) with two days of sampling for each type of sample per animal.

*Nitrogen (**N**) balance, rumen degradable protein, and rumen undegradable protein*

The N balance was estimated with the N intake, N excreted in the feces, and N excreted in the urine, in such a way that the retained N was obtained by Equation 2. The values of rumen degradable protein (**RDP**) were adopted as equivalent to the microbial protein synthesis (**MPS**) (Valadares Filho et al., 2016), which was estimated with the samples obtained from the omasal digesta collections. The RUP was estimated by the difference between CP intake and RDP. With the values of RDP and RUP, the metabolizable protein (**MP**) was estimated by Equation 3.

$$\text{N retained} = \text{N intake} - \text{N feces} - \text{N urine} \quad (2)$$

$$\text{MP} = (\text{RDP} \times 0.64) + (\text{RUP} \times 0.8) \quad (3)$$

where MP = metabolizable protein (g); N = nitrogen (g); RDP = rumen degradable protein (g); RUP = rumen undegradable protein (g).

Laboratory analyses

Samples of feeds, refusals, feces, and ruminal and omasal digesta were analysed for DM (method 934.01), N (method 981.10), and ash (method 930.05) according to AOAC (2012) and EE (method 2003.05) according to AOAC (2005). The OM concentration was estimated by the difference between the DM content and the ash content, and the CP concentration was estimated by multiplying the N content by 6.25. The NDF was estimated with the addition of thermostable

α-amylase to neutral detergent (Ankom Tech. Corp., Fairport, NY, USA), without the addition of sodium sulphite. The residue was corrected for ash (Mertens et al., 2002) and residual N compounds (Licitra et al., 1996). The iNDF concentration was estimated, according to Valente et al. (2011), through ruminal incubation of samples for 288-h, using F57 filter bags (25 μm; ANKOM Technology, Macedon, NY). The non-fiber carbohydrates (**NFC**) content was estimated as suggested by Detmann and Valadares Filho (2010):

$$NFC = OM - CP - apNDF - EE \quad (4)$$

where apNDF = ash and protein-free NDF (g/kg).

The PAB and LAB samples were also evaluated for DM and N concentration by the same methods. Additionally, the samples of omasum were analysed for the concentration of Co by an atomic absorption spectrometer (Spctr AA-800, Varian spectrometer, Harbor City, CA) after digestion with nitroperchloric acid to estimate the ruminal flow of nutrients. Omasum and LAB and PAB samples were also analysed for N-RNA concentration (N concentration in microbial RNA) to estimate microbial protein flow, according to Zinn and Owens (1980), as modified by Ushida et al. (1985). Analyses of NH_3-N were performed according to Chaney and Marbach (1962).

Statistical analyses

The data were analysed, assuming a 4 × 4 Latin square design, with PROC MIXED of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA), using the following statistical model:

$$Y_{ijk} = \mu + D_i + a_j + p_k + e_{ijk}$$

where: Y_{ijk} = dependent variable, μ = general mean; D_i = fixed effect of dietary DDG content i; a_j = random effect of animal j; p_k = random effect of experimental period k (random); and e_{ijk} = random error taken as normal and independently distributed (NID) (0, σ_e^2).

When the effect of treatment (i.e., levels of DDG) was significant, the linear, quadratic, and cubic effects were evaluated using orthogonal contrasts that were obtained with PROC IML of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA), assuming their equidistance (0, 150, 300, and 450 g/kg). The least square means were used to determine statistical significance among treatments.

Data on NH_3-N concentrations and pH were analysed as repeated measures by PROC MIXED procedures in SAS (version 9.4, SAS Institute Inc., Cary, NC, USA) using the REPEATED statement. The model tested was similar to that described above, including time as a fixed effect and all its interactions. The unstructured covariance structure was assumed for the random factors.

RESULTS

There was no cubic effect for any of the evaluated parameters. Only the linear and quadratic contrasts were presented.

Intake and digestibility

Intake and digestibility of all constituents are shown in Tables 3 and 4. The inclusion of low-fat DDG in the diet tended or resulted in a linear reduction in DMI ($P = 0.02$), organic matter intake (**OMI**, $P = 0.02$), NFC intake ($P < 0.01$) and EE intake ($P = 0.06$). On the other hand, the intake of CP was quadratic with a minimum point in D150 ($P = 0.02$) and the intake of NDF ($P < 0.01$) increased linearly. Changes in the chemical composition of diets, in addition to affecting intake, also affected the digestion of nutrients. The total digestibilities of DM (g/kg, $P < 0.01$; kg/d, $P < 0.01$), OM (g/kg, $P < 0.01$; kg/d, $P < 0.01$), NFC (kg/d, $P < 0.01$) and EE (g/kg, $P = 0.01$; kg/d, $P = 0.02$) reduced linearly. However, the total digestibility of NFC in g/kg did not differ between treatments ($P = 0.38$). Ruminal degradabilities followed the same patterns, with a linear reduction for DM (g/kg, $P = 0.01$; kg/d, $P < 0.01$), OM (g/kg, $P = < 0.01$;

kg/d, $P < 0.01$), and NFC (kg/d, $P < 0.01$). Ruminal degradability of NFC in g/kg did not differ ($P = 0.14$). Total CP digestibility (g/kg) also tended to linearly reduce ($P = 0.08$), and the digestibility in kg/d of this nutrient presented a quadratic response with a minimum point in D150 ($P = 0.01$). Ruminal degradability also decreased linearly (g/kg, $P < 0.01$; kg/d, $P = 0.01$). The increase in low-fat DDG levels in the diet also increased the total digestibility (g/kg, $P < 0.01$; kg/d, $P < 0.01$) and ruminal degradability (g/kg, $P = 0.01$; kg/d, $P < 0.01$) of NDF. With reduced intake and digestibility of almost all dietary constituents, TDN intake decreased linearly ($P < 0.01$).

Ruminal rates

The ruminal rates are described in Table 5. With the addition of low-fat DDG in the diet the ruminal rates ki ($P = 0.02$), kp ($P = 0.10$) and kd ($P = 0.01$) of DM, and ki ($P = 0.01$), kp ($P = 0.04$) and kd ($P < 0.01$) of OM, tended or reduced linearly. However, ki ($P = 0.40$) kp ($P = 0.52$) and kd ($P = 0.36$) rate of NFC did not differ between treatments. On the other hand, ki ($P < 0.01$), kp ($P = 0.04$) and kd ($P < 0.01$) of NDF increased linearly.

pH and ruminal ammonia

The pH increased linearly with the inclusion of low-fat DDG in the diet ($P = 0.01$), and varied quadratically depending on the time of collection ($P < 0.01$) (Figure 1). The concentration of ruminal ammonia decreased linearly according to the level of inclusion ($P < 0.01$) and varied cubically throughout the day ($P < 0.01$) (Figure 2).

Nitrogen balance and microbial protein synthesis

Similar to CP, as expected, N intake showed a quadratic pattern with a minimum point in D150 ($P = 0.02$) (Table 6). The N excreted through the urine tended to show the same response ($P = 0.06$). However, the N excreted in the feces increased linearly ($P = 0.02$). Consequently, the

retained N did not differ between treatments ($P = 0.72$), although the ratio between retained N and N intake did not differ ($P = 0.27$). The addition of low-fat DDG in the diet provided a quadratic pattern or linear increase in RUP ($P < 0.01$), in the ratio RUP and N intake ($P < 0.01$), in microbial efficiency concerning energy use ($P = 0.06$), and the intake of MP ($P = 0.03$). On the other hand, RDP or MPS tended to reduce linearly ($P = 0.09$), and microbial relationship about the use of the protein decreased linearly ($P < 0.01$). The N-RNA/Nmic ratio was not changed ($P = 0.34$).

DISCUSSION

Intake and digestibility

There was a difference of 1.47 kg/d between the diet with the highest DMI (D0) and the lowest DMI (D450). There are sufficient studies in the literature showing different responses regarding DMI when using distillers grains, depending, among other factors, on the type of grain used to make this coproduct and its level of inclusion (Merayo et al., 2020). In this sense, Walter et al. (2010) evaluated the inclusion of wheat or corn dried distillers grains with soluble (DDGS) at levels of 20 or 40% in finishing diets and found a quadratic increase in DMI when wheat DDGS were used and a reduction in DMI when corn DDGS were used, as found in this study. On the other hand, some authors who also evaluated the inclusion of distillers grains in finishing diets found no difference in DMI (Salim et al., 2014; Vander Pol et al., 2009) or found a quadratic pattern (Klopfenstein et al., 2008).

In addition to being influenced by DMI, nutrient intake was also affected by the composition of the diet. DDG is a coproduct obtained from a fermentation process of cereals such as corn, sorghum, or wheat. For this, the raw material is ground and mixed with water to form a slurry, which will be cooked, and its pH adjusted to add an amylase enzyme. Later, yeasts are added to ferment the starch (Merayo et al., 2020). As a result, the concentration of

starch, and consequently NFC, is reduced, and the concentration of cell wall components and CP is increased, resulting in the intake pattern observed for these components. However, an important point in the use of distillers grains is the variation in the composition that exists between the producing plants and within the same plant, as described by Buckner et al. (2011). One point described by these authors is the variation in fat content. Many studies use distillers grains with fat levels between 100 and 130 g/kg (MacDonald et al., 2007), values above the fat concentration of corn. Consequently, the inclusion of this kind of coproduct in the diet provides an increase in the concentration of fat. However, some ethanol plants remove fat from the residue, making the concentration similar to that of corn, as was the case in this study. Consequently, the fat concentration of the diets was close, and their intake followed the DMI.

Also, the increase in the concentration of structural carbohydrates, which show a lower and slower digestion (Miron et al., 2001), and the reduction in the concentration of non-structural carbohydrates, may also be associated with the patterns found for digestibility. The average digestibility of NFC was around 42% higher than the average digestibility of NDF, which may be associated with a reduction in DM and OM digestibility. Likewise, Gilroyed et al. (2015) evaluated levels of inclusion of DDGS in cattle diets that ranged from 0 to 400 g/kg (DM basis) and reported an increase in gross energy lost through feces. The authors described higher concentrations of recalcitrant compounds in animals fed at higher levels, reinforcing the process described above. For the inclusion of 0, 200, and 400 g/kg, the digestibility of OM was 820, 780, and 750 g/kg, values close to that found in this study.

Part of the protein present in the DDG comes from the yeasts that were added for fermentation, and that went through the heating process (Klopfenstein et al., 2008). Consequently, this fraction presents a reduction in its ruminal degradability because the yeasts, when heated, become resistant to lysis and rumen microbial degradation (Bruning and Yokoyama, 1988), reaching only 20% in distillers grains (Herold, 1999). In addition, the

heating process is also related to an increase in the damage caused to the protein with a consequent increase in the concentration of acid detergent insoluble nitrogen (ADIN) (Tedeschi et al., 2009), which negatively correlates with the digestibility of the CP both in non-forages (r = 0.81; Nakamura et al., 1994) as in forages (r = 0.93; Yu and Thomas, 1976). The ADIN fraction also correlates negatively with the digestibility of DM (Tedeschi et al., 2009), in agreement with the data found.

The only constituent that increased digestibility in g/kg was NDF. Both ruminal degradability and total digestibility increased around 10% with the inclusion of low-fat DDG. There are many factors that affect fiber degradation, with the EE concentration of the diet being one of them. Fat, when in contact with fiber, forms protection and hinders colonization by ruminal microorganisms. As a result, the lag time is increased, and the extent and the rate of digestion are reduced (Valadares Filho and Pina, 2006). In addition, fat has a toxic effect on ruminal microorganisms, which limits the number of digestion sites in the feed particle (McAllister et al., 1994). In this sense, Tedeschi et al. (2009) analysed an *in vitro* study and found less lag time for samples of corn milling (co) products defatted than samples with higher concentrations of fat. However, as the DDG used in this study did not have high EE concentrations, this was not a limiting factor for fiber digestion. Another factor associated with fiber digestion is ruminal pH (Alhadas et al., 2021). The fermentation process to form DDG reduces the concentration of starch in this coproduct, which can contribute to a reduction in the occurrence of sub-acute acidosis (Klopfenstein et al., 2008). Fibrolytic bacteria are more sensitive to low pH values than amylolytic bacteria (Russell and Dombrowski, 1980), and they can reduce their growth linearly or exponentially (Tedeschi and Fox, 2020) when the ruminal pH decreases (Owens et al., 1998).

As a final result of the combination of intake and digestibility, the inclusion of low-fat DDG in the diet reduced the intake of TDN. Some authors describe a higher feed value of DDG

or DDGS than corn, which could explain the increased performance of animals up to a certain level of inclusion (Buckner et al., 2007; Ham et al., 1994). Although this study did not evaluate performance data, the parameters evaluated indicate a reduction in the use of nutrients when low-fat DDG replaced corn.

Ruminal rates

Digestion processes in cattle are affected by numerous variables, such as diet, microbial population, removal of fermentation products, pH, and other factors directly linked to the rumen environment (Tedeschi and Fox, 2020). As feed reaches the rumen, three fates co-occur: degradation with reduced size in the rumen, absorption for metabolism, or escape the rumen (Tedeschi and Fox, 2020). An important factor that influences the magnitude of these processes is the intake, in such a way that the kp tends to increase when the intake increases (Balch and Campling, 1965). This relationship was observed for DM, OM, and NDF, where lower intakes resulted in lower rates. This pattern is also described by other authors (Clauss et al., 2009; Coleman et al., 2003; Dias et al., 2011).

The same factors that influenced digestibility also influenced the rate of digestion, in such a way that the concentration of compounds that are more difficult to digest may be associated with a reduction in the kd of DM (D0 = 60.7 g/kg per h; D450 = 45.4 g/kg per h) and OM (D0 = 64.2 g/kg/h; D450 = 45.8 g/kg/h). The reduction was approximately 34% and 40% for DM and OM, respectively. According to Mertens (1993), non-cell wall components may have a kd that varies between 3 and 10 times the kp value, while the cell wall components have values close for kd and kp rates. For the evaluated data, the average kd/kp ratio for the NDF was 1.6, and for the NFC, it was 2.8. However, the NFC kd was around 3.4 times higher than the NDF kd, although the NDF kd increased linearly [from 30.5 g/kg per h (D0) to 50.8 g/kg per h (D450)]. In addition to influencing the degraded amount of fiber, rumen pH can also affect

the rate at which fiber is degraded. According to Tedeschi and Fox (2020), in general, the reduction in ruminal pH reduces the kd rate of the fiber.

pH and ruminal ammonia

The increase in the amount of starch digested in the rumen is usually associated with the rapid accumulation of volatile fatty acids (**VFA**) and a reduction in ruminal pH (NASEM, 2016). Consequently, the addition of carbohydrates with a lower rate of digestion in the diet, such as NDF, also reduces the rate of VFA production, reducing the potential for the occurrence of acidosis. The pH averages per treatment ranged from 6.29 (D0) to 6.52 (D450), with only D0 remaining for a few hours with the pH below 6.00. The oscillation throughout the day was similar for all treatments. The highest observed value was approximately 2 hours before feeding the animals in the morning. After feeding, the pH values decreased until about midday, remaining constant at particular times until the next feeding. After feeding, the carbohydrates that reached the rumen will be fermented and generate VFA's, which quickly dissociate and release H^+ ion in the rumen environment (Aschenbach et al., 2011), resulting in a reduction in pH values. However, animals have mechanisms to control the concentration of ions H^+, including neutralization via bicarbonate from saliva or rumen epithelium (Penner et al., 2009) or the passage of rumen fluid to the omasum. Consequently, there is a tendency to maintain rumen homeostasis. These processes will be affected by some factors, such as pH and fiber concentration in the diet. Under conditions of ruminal pH equal to 7.0, around 99% of VFA are dissociated, reducing to 83% at pH equal to 5.5, which will directly interfere with epithelial absorption and, consequently, epithelial secretion of bicarbonate ions. In the same sense, the increase in dietary fiber is correlated to the increase in liquid flow, increasing the removal of fermentation products, and controlling pH (NASEM, 2016; Tedeschi and Fox, 2020).

The fermentation process that occurs in the rumen allows the transformation of protein and non-protein nitrogen from the diet into microbial protein and NH_3. As previously discussed, the amount of protein degraded in rumen decreased linearly, which may be associated with a linear reduction in rumen ammonia concentration. Average concentrations ranged from 11.1 mmol/dL (D0) to 5.05 mmol/dL (D450). High ammonia concentrations were observed at 08h00 and 17h00, both one hour post feeding. Similar to the results found in this study, other authors describe this pattern when distillers grains is added to the diet (Anderson et al., 2006; Benchaar et al., 2013; Kleinschmit et al., 2006).

Nitrogen balance and microbial protein synthesis

The increase in nitrogen intake did not increase nitrogen retention, as the excretion in feces and urine increased. The lower nitrogen value observed in feces and urine for D150 may be associated with a lower amount of digested CP (0.78 kg) compared to other treatments. This was due to the fact that diets D0 and D150 had similar CP levels [123 and 122 g/kg (DM basis), respectively], but lower than D300 and D450, which, together with the reduction in DMI, resulted in the observed pattern. In this sense, Menezes et al. (2016) evaluated CP levels in finishing diets that varied from 100 to 140 g/kg (DM basis) and found no difference in retained nitrogen, with an increase in nitrogen in the urine. Cole et al. (2005) and Todd et al. (2006) also found a increase in the excretion of nitrogen in the urine when the CP level varied from 115 to 130 g/kg (DM basis). In addition, ruminant diets with high protein levels can increase nitrogen recycling, which, together with increased excretion, increases the energy cost for metabolism (Reynolds and Kristensen, 2008).

Although the concentration of CP in the distillers grains increases compared to corn, the main protein present in this grain is zein, which has a low digestibility around 400 g/kg (McDonald, 1954). Besides, part of the distillers grains protein is made up of yeasts, whichis

more resistant to ruminal degradation with heating. Heating also reduces ruminal protein degradation, as it causes the Maillard reaction between a lysine group and a carbonyl group (NASEM, 2016). As a result, the concentration of RUP increased, and consequently, the MP also increased. However, RDP or MPS was reduced. Several factors affect MPS, and OM's intake and the amount of OM degraded in the rumen are two of them (Clark et al., 1992). The increased intake of OM usually provides a higher rate of passage, which allows more microorganisms to reach the intestine for digestion. In addition, fast-digesting carbohydrates, such as starch, are more efficient at promoting microbial growth than fibrous carbohydrates (Stern and Hoover, 1979). Microbial efficiency is also affected by several factors, but according to Galyean and Tedeschi (2014), MPS decreases with a decreasing in TDN, as verified for the present study.

CONCLUSION

The inclusion of low-fat DDG in finishing diets reduced the TDN intake and reduced the total digestibility in kg/d of DM, OM, NFC, and EE. In addition, the kd rate of DM and OM decreased linearly. However, as the ruminal pH increased linearly, NDF digestion and kd rate increased. Although there was a higher intake of MP for the highest levels of inclusion, the retained nitrogen was the same for all treatments.

ACKNOWLEDGMENT

We thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Fundação de Amparo a Pesquisa do Estado de Minas Gerais (Fapemig) for partial funding to this study.

DECLARATIONS OF INTEREST

None of the authors of this paper have any conflict of interest.

FUNDING

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ, Brazil; 465377-2014/9).

ETHICS STATEMENT

The experimental protocol was approved by the Ethics Committee for Animal Use of University Federal of Viçosa (Protocol CEUAP/DZO/UFV 01/2019).

LITERATURE CITED

Alhadas, H.M., Valadares Filho, S.C., Silva, F.F., Silva, F.A.S., Pucetti, P., Pacheco, M.V.C., Silva, B.C., Tedeschi, L.O., 2021. Effects of including physically effective fiber from sugarcane in whole corn grain diets on the ingestive, digestive, and ruminal parameters of growing beef bulls. Livest. Sci. 248, 104508. https://doi.org/10.1016/j.livsci.2021.104508

Allen, M.S., Linton, J.A., 2007. In vivo methods to measure digestibility and digestion Kinetics of feed fractions in the rumen. In: Proc of 1st Simpósio Internacional Avanços em Técnicas de Pesquisa em Nutrição de Ruminantes. Pirassununga, Brazil: Universidade de São Paulo, pp. 72-89 .

Anderson, J.L., Schingoethe, D.J., Kalscheur, K.F., Hippen, A.R., 2006. Evaluation of dried and wet distillers grains included at two concentrations in the diets of lactating dairy cows. J. Dairy Sci. 89, 3133–3142. https://doi.org/10.3168/jds.S0022-0302(06)72587-5

AOAC, 2012. Official Methods of Analysis of AOAC International, 19th ed. Association of Official Analysis Chemists International. Gaithersburg, Maryland.

AOAC, 2005. Official Methods of Analysis of AOAC International, 18th ed, Association of Official Analysis Chemists International. Gaithersburg, Maryland.

Aschenbach, J.R., Penner, G.B., Stumpff, F., Gäbel, G., 2011. RUMINANT NUTRITION SYMPOSIUM: Role of fermentation acid absorption in the regulation of ruminal pH12. J. Anim. Sci. 89, 1092–1107. https://doi.org/10.2527/jas.2010-3301

Balch, C.C., Campling, R.C., 1965. Rate of passage of digesta through the ruminant digestive tract, in: Physiology of Digestion in the Ruminant. pp. 108–123.

Benchaar, C., Hassanat, F., Gervais, R., Chouinard, P.Y., Julien, C., Petit, H. V., Massé, D.I., 2013. Effects of increasing amounts of corn dried distillers grains with solubles in dairy

cow diets on methane production, ruminal fermentation, digestion, N balance, and milk production. J. Dairy Sci. 96, 2413–2427. https://doi.org/10.3168/jds.2012-6037

Bruning, C.L., Yokoyama, M.T., 1988. Characteristics of Live and Killed Brewer's Yeast Slurries and Intoxication by Intraruminal Administration to Cattle. J. Anim. Sci. 66, 585. https://doi.org/10.2527/jas1988.662585x

Buckner, C.D., Erickson, G., Mader, T., Colgan, S.L., Karges, K.K., Gibson, M.L., 2007. Optimum Levels of Dry Distillers Grains with Solubles for Finishing Beef Steers. Nebraska Beef Cattle Reports.

Buckner, C.D., Wilken, M.F., Benton, J.R., Vanness, S.J., Bremer, V.R., Klopfenstein, T.J., Kononoff, P.J., Erickson, G.E., 2011. Nutrient variability for distillers grains plus solubles and dry matter determination of ethanol by-products. Prof. Anim. Sci. 27, 57–64. https://doi.org/10.15232/S1080-7446(15)30445-9

Chaney, A.L., Marbach, E.P., 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. https://doi.org/10.1093/clinchem/8.2.130

Clark, J.H., Klusmeyer, T.H., Cameron, M.R., 1992. Microbial Protein Synthesis and Flows of Nitrogen Fractions to the Duodenum of Dairy Cows. J. Dairy Sci. https://doi.org/10.3168/jds.S0022-0302(92)77992-2

Clauss, M., Nunn, C., Fritz, J., Hummel, J., 2009. Evidence for a tradeoff between retention time and chewing efficiency in large mammalian herbivores. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. https://doi.org/10.1016/j.cbpa.2009.07.016

Cole, N.A., Clark, R.N., Todd, R.W., Richardson, C.R., Gueye, A., Greene, L.W., McBride, K., 2005. Influence of dietary crude protein concentration and source on potential ammonia emissions from beef cattle manure1,2,3. J. Anim. Sci. 83, 722–731.

https://doi.org/10.2527/2005.833722x

Coleman, S.W., Hart, S.P., Sahlu, T., 2003. Relationships among forage chemistry, rumination and retention time with intake and digestibility of hay by goats. Small Rumin. Res. https://doi.org/10.1016/S0921-4488(03)00116-0

CONAB, 2020. Companhia Nacional de Abastecimento - Safra da cana segue voltada ao açúcar e mostra novo aumento do etanol de milho [WWW Document]. URL https://www.conab.gov.br/ultimas-noticias/3357-safra-da-cana-segue-voltada-a-producao-de-acucar-e-mostra-novo-aumento-do-etanol-de-milho (accessed 9.8.20).

Detmann, E., Valadares Filho, S.C., 2010. Sobre a estimação de carboidratos não fibrosos em alimentos e dietas. Arq. Bras. Med. Vet. e Zootec. 62, 980–984. https://doi.org/10.1590/S0102-09352010000400030

Dias, R.S., Patino, H.O., López, S., Prates, E., Swanson, K.C., France, J., 2011. Relationships between chewing behavior, digestibility, and digesta passage kinetics in steers fed oat hay at restricted and ad libitum intakes. J. Anim. Sci. https://doi.org/10.2527/jas.2010-3156

Galyean, M.L., Tedeschi, L.O., 2014. Predicting microbial protein synthesis in beef cattle: Relationship to intakes of total digestible nutrients and crude protein1. J. Anim. Sci. 92, 5099–5111. https://doi.org/10.2527/jas.2014-8098

Gilroyed, B.H., Li, C., Reuter, T., Beauchemin, K.A., Hao, X., McAllister, T.A., 2015. Influence of distiller's grains and condensed tannins in the diet of feedlot cattle on biohydrogen production from cattle manure. Int. J. Hydrogen Energy 40, 6050–6058. https://doi.org/10.1016/j.ijhydene.2013.07.014

Ham, G.A., Stock, R.A., Klopfenstein, T.J., Larson, E.M., Shain, D.H., Huffman, R.P., 1994. Wet corn distillers byproducts compared with dried corn distillers grains with solubles as

a source of protein and energy for ruminants. J. Anim. Sci. 72, 3246–3257. https://doi.org/10.2527/1994.72123246x

Herold, D.W., 1999. Solvent extracted germ meal for ruminants. PhD Diss. University of Nebraska, Lincoln.

Huhtanen, P., Brotz, P.G., Satter, L.D., 1997. Omasal Sampling Technique for Assessing Fermentative Digestion in the Forestomach of Dairy Cows. J. Anim. Sci. https://doi.org/10.2527/1997.7551380x

Kleinschmit, D.H., Schingoethe, D.J., Kalscheur, K.F., Hippen, A.R., 2006. Evaluation of various sources of corn dried distillers grains plus solubles for lactating dairy cattle. J. Dairy Sci. 89, 4784–4794. https://doi.org/10.3168/jds.S0022-0302(06)72528-0

Klopfenstein, T.J., Erickson, G.E., Bremer, V.R., 2008. BOARD-INVITED REVIEW: Use of distillers byproducts in the beef cattle feeding industry. J. Anim. Sci. 86, pp. 1223-1231.

Krizsan, S.J., Ahvenjärvi, S., Volden, H., Broderick, G.A., 2010. Estimation of rumen outflow in dairy cows fed grass silage-based diets by use of reticular sampling as an alternative to sampling from the omasal canal. J. Dairy Sci. https://doi.org/10.3168/jds.2009-2661

Leão, M.., 2002. Metodologias de coletas de digestas omasal e abomasal em novilhos submetidos a três níveis de ingestão: consumo, digestibilidade e produção microbiana. Tese de doutorado. Universidade Federal de Viçosa, p. 57.

Licitra, G., Hernandez, T.M., Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. https://doi.org/10.1016/0377-8401(95)00837-3

MacDonald, J.C., Klopfenstein, T.J., Erickson, G.E., Griffin, W.A., 2007. Effects of dried distillers grains and equivalent undegradable intake protein or ether extract on

performance and forage intake of heifers grazing smooth bromegrass pastures1. J. Anim. Sci. 85, 2614–2624. https://doi.org/10.2527/jas.2006-560

McAllister, T.A., Bae, H.D., Jones, G.A., Cheng, K.-J., 1994. Microbial attachment and feed digestion in the rumen. J. Anim. Sci. 72, 3004–3018. https://doi.org/10.2527/1994.72113004x

McDonald, I.W., 1954. The extent of conversion of feed protein to microbial protein in the rumen of sheep. Biochem. J. 56, pp.120–125.

Menezes, A.C.B., Valadares Filho, S.C., Costa e Silva, L.F., Pacheco, M.V.C., Pereira, J.M.V., Rotta, P.P., Zanetti, D., Detmann, E., Silva, F.A.S., Godoi, L.A., Rennó, L.N., 2016. Does a reduction in dietary crude protein content affect performance, nutrient requirements, nitrogen losses, and methane emissions in finishing Nellore bulls? Agric. Ecosyst. Environ. 223, 239–249. https://doi.org/10.1016/j.agee.2016.03.015

Merayo, M., Pighin, D., Grigioni, G., 2020. Meat quality traits and feeding distillers grains to cattle: a review. Anim. Prod. Sci. 60, 1123. https://doi.org/10.1071/AN18382

Mertens, D.R., 1993. Rate and extent of digestion, in: Quantitative Aspects of Ruminant Digestion and Metabolism. Englewood Cliffs, pp. 13–51.

Mertens, D.R., Allen, M., Carmany, J., Clegg, J., Davidowicz, A., Drouches, M., Frank, K., Gambin, D., Garkie, M., Gildemeister, B., Jeffress, D., Jeon, C.S., Jones, D., Kaplan, D., Kim, G.N., Kobata, S., Main, D., Moua, X., Paul, B., Robertson, J., Taysom, D., Thiex, N., Williams, J., Wolf, M., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. J. AOAC Int.

Miron, J., Yosef, E., Ben-Ghedalia, D., 2001. Composition and in vitro digestibility of

monosaccharide constituents of selected byproduct feeds. J. Agric. Food Chem. 49, 2322–2326. https://doi.org/10.1021/jf0008700

Nakamura, T., Klopfenstein, T.J., Britton, R.A., 1994. Evaluation of acid detergent insoluble nitrogen as an indicator of protein quality in nonforage proteins. J. Anim. Sci. 72, 1043–1048. https://doi.org/10.2527/1994.7241043x

NASEM, 2016. National Academies of Sciences, Engineering, and Medicine, 8th ed. National Academies Press: Washington, DC. https://doi.org/10.17226/19014

NRC, 2001. Nutrient requirements of beef cattle, 7th ed. Natl. Acad. Press, Washington, DC, USA.

Owens, F.N., Secrist, D.S., Hill, W.J., Gill, D.R., 1998. Acidosis in Cattle: A Review. J. Anim. Sci. https://doi.org/10.2527/1998.761275x

Penner, G.B., Aschenbach, J.R., Gäbel, G., Rackwitz, R., Oba, M., 2009. Epithelial Capacity for Apical Uptake of Short Chain Fatty Acids Is a Key Determinant for Intraruminal pH and the Susceptibility to Subacute Ruminal Acidosis in Sheep. J. Nutr. https://doi.org/10.3945/jn.109.108506

Reynal, S.M., Broderick, G.A., Bearzi, C., 2005. Comparison of four markers for quantifying microbial protein flow from the rumen of lactating dairy cows. J. Dairy Sci. https://doi.org/10.3168/jds.S0022-0302(05)73091-5

Reynolds, C.K., Kristensen, N.B., 2008. Nitrogen recycling through the gut and the nitrogen economy of ruminants: An asynchronous symbiosis1. J. Anim. Sci. 86, E293–E305. https://doi.org/10.2527/jas.2007-0475

Rotta, P.P., Valadares Filho, S.C., Detmann, E., Costa e Silva, L.F., Paulino, M.F., Marcondes, M.I., Lobo, A.A.G., Villadiego, F.A.C., 2014. Digesta sampling sites and marker methods

for estimation of ruminal outflow in bulls fed different proportions of corn silage or sugarcane. J. Anim. Sci. 92, 2996–3006. https://doi.org/10.2527/jas.2013-7364

Russell, J.B., Dombrowski, D.B., 1980. Effect of pH on the efficiency of growth by pure cultures of rumen bacteria in continuous culture. Appl. Environ. Microbiol. https://doi.org/10.1128/aem.39.3.604-610.1980

Salim, H., Wood, K.M., McEwen, P.L., Vandervoort, G., Miller, S.P., Mandell, I.B., Cant, J.P., Swanson, K.C., 2014. Influence of feeding increasing level of dry or modified wet corn distillers grains plus solubles in whole corn grain-based finishing diets on growth performance, carcass traits, and feeding behavior in finishing cattle. Livest. Sci. 161, 53–59. https://doi.org/10.1016/j.livsci.2013.12.020

Stern, M.D., Hoover, W.H., 1979. Methods for Determining and Factors Affecting Rumen Microbial Protein Synthesis: a Review. J. Anim. Sci. 49, 1590–1603. https://doi.org/10.2527/jas1979.4961590x

Tedeschi, L.O., Fox, D.G., 2020. The Ruminant Nutrition System: Volume I - An Applied Model for Predicting Nutrient Requirements and Feed Utilization in Ruminants, 3nd ed. XanEdu, Ann Arbor, MI.

Tedeschi, L.O., Kononoff, P.J., Karges, K., Gibson, M.L., 2009. Effects of chemical composition variation on the dynamics of ruminal fermentation and biological value of corn milling (co)products. J. Dairy Sci. 92, 401–413. https://doi.org/10.3168/jds.2008-1141

Todd, R.W., Cole, N.A., Clark, R.N., 2006. Reducing Crude Protein in Beef Cattle Diet Reduces Ammonia Emissions from Artificial Feedyard Surfaces. J. Environ. Qual. 35, 404–411. https://doi.org/10.2134/jeq2005.0045

USDA ERS - Feed Grains: Yearbook Tables [WWW Document], 2019. URL https://www.ers.usda.gov/data-products/feed-grains-database/feed-grains-yearbook-tables/ (accessed 7.16.20).

Ushida, K., Lassalas, B., Jouany, J.P., 1985. Determination of assay parameters for RNA analysis in bacterial and duodenal samples by spectrophotometry. Influence of sample treatment and preservation. Reprod. Nutr. Dev. https://doi.org/10.1051/rnd:19850804

Valadares Filho, S.C., Costa e Silva, L.F., Gionbelli, M.P., Rotta, P.P., Marcondes, M.I., Chizzotti, M.L., Prados, L.F., 2016. Nutrient Requirements of Zebu and Crossbred Cattle - BR-CORTE, in: Exigências Nutricionais de Zebuínos Puros e Cruzados - BR-CORTE. Editora Federal de Viçosa. https://doi.org/10.5935/978-85-8179-111-1.2016b002

Valadares Filho, S.C., Pina, D.S., 2006. Fermentação ruminal. in: Berchielli, T.T, Pires, A.V, Oliveira, S.G. Nutrição de Ruminantes. Jaboticabal: Funep, pp. 167-182.

Valadares Filho, S.C., Rotta, P.P., Costa E Silva, L.F., 2011. Técnicas de coleta duodenal, abomasal, omasal e reticular na avaliação do fluxo ruminal. In: Rennó, F.P.; Prada E Silva, L.F. III Simpósio Internacional Avanços em Técnicas de Pesquisa em Nutrição de Ruminantes, Pirassununga, SP, pp. 14-45.

Valente, T.N.P., Detmann, E., Queiroz, A.C., Valadares Filho, S.C., Gomes, D.I., Figueiras, J.F., 2011. Evaluation of ruminal degradation profiles of forages using bags made from different textiles. Rev. Bras. Zootec. https://doi.org/10.1590/S1516-35982011001100039

Vander Pol, K.J., Luebbe, M.K., Crawford, G.I., Erickson, G.E., Klopfenstein, T.J., 2009. Performance and digestibility characteristics of finishing diets containing distillers grains, composites of corn processing coproducts, or supplemental corn oil1. J. Anim. Sci. 87, 639–652. https://doi.org/10.2527/jas.2008-1036

Walter, L.J., Aalhus, J.L., Robertson, W.M., McAllister, T.A., Gibb, D.J., Dugan, M.E.R., Aldai, N., McKinnon, J.J., 2010. Evaluation of wheat or corn dried distillers' grains with solubles on performance and carcass characteristics of feedlot steers. Can. J. Anim. Sci. 90, 259–269. https://doi.org/10.4141/CJAS09089

Yu, Y., Thomas, J.W., 1976. Estimation of the Extent of Heat Damage in Alfalfa Haylage by Laboratory Measurement. J. Anim. Sci. 42, 766–774. https://doi.org/10.2527/jas1976.423766x

Zinn, R.A., Owens, F.N., 1980. Rapid procedure for quantifying nucleic acid content of digesta. In: Protein requirements for cattle. Protein Sympos. Oklahoma State Universit, pp. 26-30.

Table 1 - Chemical composition of diet ingredients

Ingredients	DM[1]	OM[2]	NDF[3]	CP[4]	EE[5]
		g/kg			
Corn silage	307	936	498	62.2	24.9
DDG[6]	897	989	540	327	48.6
Corn meal	894	990	90.2	91.7	42.4
Soybean meal	885	939	95.4	505	23.6
Urea/A.S[7]	997	1000	-	2610	-
Premix[8]	1000	-	-	-	-
Virginiamycin	1000	-	-	-	-

[1]Dry matter, [2]Organic matter, [3]Neutral detergent fiber, [4]Crude protein, [5]Ether extract, [6]Dried distillers grains, [7]urea and ammonium sulfate (9:1), [8]Premix guarantees (per kg of DM): 200 - 220 g of Ca, 10 mg of Co (Min), 500 mg of Cu (Min), 22 g of S (Min), 333 mg of Fe (Min), 178.41 mg of F (Max), 10 g of P (Min), 25 mg of I (Min), 17 g of Mg (Min), 1500 mg of Mn (Min), 1100 mg of monensin, 6.6 mg of Se (Min), 50 g of Na (Min), and 2000 mg of Zn (Min). The unit g/kg for dry matter represents grams of dry matter per kilogram of raw material, and for the other constituents represents grams of the constituent per kilogram of dry matter.

Table 2 - Proportion of ingredients and composition of experimental diets

Items	Diets			
	D0	D150	D300	D450
	g/kg			
Ingredients				
Corn silage	280	280	280	280
DDG[1]	-	150	300	450
Corn meal	649	537	399	249
Soybean meal	40.0	12.0	-	-
Urea/A.S[2]	10.0	-	-	-
Premix	20.0	20.0	20.0	20.0
Virginiamycin	1.00	1.00	1.00	1.00
Analysed composition				
DM[3]	587	589	589	589
OM[4]	952	953	954	954
NDF[5]	202	270	337	405
CP[6]	123	122	152	187
EE[7]	35.6	37.3	38.4	39.4
NFC[8]	608	525	426	322

D0 = diet without dried distillers grains, D150 = diet with 150 g/kg of dried distillers grains, D300 = diet with 300 g/kg of dried distillers grains, D450 = diet with 450 g/kg of dried distillers grains, [1]Dried distillers grains, [2]Urea and ammonium sulfate (9:1), [3]Dry matter, [4]Organic matter, [5]Neutral detergent fiber, [6]Crude protein, [7]Ether extract, [8]Non-fiber carbohydrates. In the "Ingredients" section, the unit g/kg represents grams of the ingredient per kilogram of dry matter in the diet. In the "Analysed composition" section, the unit g/kg for dry matter represents grams of dry matter per kilogram of raw material, and for the other constituents represents grams of the constituent per kilogram of dry matter.

Table 3 - Effect of dried distillers grains levels on total digestible nutrients intake and dry matter, organic matter and crude protein intake and digestibilities

Items	Diets				SEM[1]	*P*-value	
	D0	D150	D300	D450		L	Q
TDN[2] intake							
kg/d	7.56^{A}	6.72^{AB}	6.24^{B}	5.91^{B}	0.493	< 0.01	0.38
DM[3] intake							
kg/d	9.00^{A}	8.34^{AB}	7.87^{AB}	7.53^{B}	0.673	0.02	0.64
Ruminal digestibility							
g/kg	597^{A}	534^{B}	509^{B}	453^{C}	14.24	< 0.01	0.80
kg/d	5.31^{A}	4.45^{AB}	3.97^{BC}	3.40^{C}	0.322	< 0.01	0.58
Total digestibility							
g/kg	808^{A}	780^{B}	780^{B}	765^{B}	12.37	< 0.01	0.35
kg/d	7.22^{A}	6.49^{AB}	6.11^{B}	5.76^{B}	0.471	< 0.01	0.46
OM[4] intake							
kg/d	8.56^{A}	7.95^{AB}	7.50^{AB}	7.16^{B}	0.640	0.02	0.66
Ruminal digestibility							
g/kg	607^{A}	543^{B}	529^{B}	473^{C}	15.40	< 0.01	0.81
kg/d	5.15^{A}	4.31^{AB}	3.93^{BC}	3.39^{C}	0.333	< 0.01	0.58
Total digestibility							
g/kg	820^{A}	797^{B}	795^{BC}	777^{C}	11.83	< 0.01	0.68
kg/d	6.99^{A}	6.32^{AB}	5.93^{B}	5.56^{B}	0.449	< 0.01	0.54
CP[5] intake							
kg/d	1.13^{BC}	1.01^{C}	1.19^{B}	1.39^{A}	0.098	< 0.01	0.02
Ruminal digestibility							
g/kg	133^{A}	131^{A}	106^{A}	58.2^{B}	13.01	< 0.01	0.40
kg/d	0.14^{A}	0.13^{A}	0.12^{AB}	0.08^{B}	0.017	0.01	0.30
Total digestibility							
g/kg	793^{A}	767^{A}	758^{A}	758^{A}	12.98	0.08	0.32
kg/d	0.88^{BC}	0.78^{C}	0.90^{B}	1.05^{A}	0.066	0.01	0.01

D0 = diet without dried distillers grains, D150 = diet with 150 g/kg of dried distillers grains, D300 = diet with 300 g/kg of dried distillers grains, D450 = diet with 450 g/kg of dried distillers grains, [1]Standard error of mean, [2]Total digestible nutrients, [3]Dry matter, [4]Organic matter, [5]Crude protein. L and Q represent the effects of the linear and quadratic contrasts, respectively. The superscript letters indicate the differences between treatments by the least-square means.

Table 4 - Effect of dried distillers grains levels on neutral detergent fiber, non-fiber carbohydrates, and ether extract intake and digestibilities

Items	Diets				SEM[1]	*P*-value	
	D0	D150	D300	D450		L	Q
NDF[2] intake							
Kg/d	1.86^{C}	2.30^{C}	2.71^{B}	3.13^{A}	0.212	< 0.01	0.94
Ruminal digestibility							
g/kg	582^{B}	583^{B}	604^{B}	645^{A}	14.72	0.01	0.11
kg/d	1.07^{C}	1.34^{BC}	1.63^{B}	2.01^{A}	0.122	< 0.01	0.61
Total digestibility							
g/kg	607^{B}	617^{B}	653^{AB}	668^{A}	14.00	0.01	0.83
kg/d	1.12^{C}	1.41^{C}	1.76^{B}	2.09^{A}	0.122	< 0.01	0.79
NFC[3] intake							
kg/d	5.43^{A}	4.39^{B}	3.36^{C}	2.43^{D}	0.322	< 0.01	0.76
Ruminal digestibility							
g/kg	744^{A}	746^{A}	742^{A}	736^{A}	33.67	0.14	0.28
kg/d	4.03^{A}	3.27^{B}	2.49^{C}	1.78^{D}	0.233	< 0.01	0.84
Total digestibility							
g/kg	904^{A}	897^{A}	902^{A}	911^{A}	9.910	0.51	0.38
kg/d	4.89^{A}	3.93^{B}	3.03^{C}	2.21^{D}	0.277	< 0.01	0.60
EE[4] intake							
kg/d	0.33^{A}	0.31^{A}	0.30^{A}	0.28^{A}	0.022	0.06	0.88
Total digestibility							
g/kg	909^{A}	866^{AB}	859^{AB}	837^{B}	11.88	0.01	0.47
kg/d	0.30^{A}	0.28^{AB}	0.25^{AB}	0.24^{B}	0.017	0.02	0.53

D0 = diet without dried distillers grains, D150 = diet with 150 g/kg of dried distillers grains, D300 = diet with 300 g/kg of dried distillers grains, D450 = diet with 450 g/kg of dried distillers grains, [1]Standard error of mean, [2]Neutral detergent fiber, [3]Non-fiber carbohydrates, [4]Ether extract. L and Q represent the effects of the linear and quadratic contrasts, respectively. The superscript letters indicate the differences between treatments by the least-square means.

Table 5 – Effect of dried distillers grains levels on the intake, passage, and digestion ruminal rates

Items	Diets				SEM[1]	P-value	
	D0	D150	D300	D450		L	Q
DM[2] rates, g/kg per h							
ki[3]	106^{A}	103^{A}	97.4^{AB}	84.9^{B}	5.594	0.02	0.30
kp[4]	45.2^{A}	45.1^{A}	44.2^{A}	39.5^{A}	2.419	0.10	0.28
kd[5]	60.7^{A}	57.8^{A}	53.2^{AB}	45.4^{B}	3.598	0.01	0.45
OM[6] rates, g/kg per h							
ki	109^{A}	104^{A}	93.1^{AB}	84.2^{B}	5.728	0.01	0.70
kp	44.4^{AB}	44.7^{A}	42.0^{AB}	38.4^{B}	2.637	0.04	0.27
kd	64.2^{A}	59.6^{AB}	51.1^{BC}	45.8^{C}	3.486	< 0.01	0.92
NDF[7] rates, g/kg per h							
ki	51.6^{C}	64.2^{BC}	69.6^{AB}	78.4^{A}	6.226	< 0.01	0.66
kp	21.2^{B}	26.8^{AB}	27.6^{A}	27.6^{A}	2.700	0.04	0.15
kd	30.5^{C}	37.6^{BC}	42.0^{AB}	50.8^{A}	3.961	< 0.01	0.36
NFC[8] rates, g/kg per h							
ki	193^{A}	190^{A}	181^{A}	175^{A}	18.61	0.40	0.92
kp	50.5^{A}	48.1^{A}	51.1^{A}	46.1^{A}	4.331	0.52	0.70
kd	143^{A}	142^{A}	130^{A}	129^{A}	14.89	0.36	0.99

D0 = diet without dried distillers grains, D150 = diet with 150 g/kg of dried distillers grains, D300 = diet with 300 g/kg of dried distillers grains, D450 = diet with 450 g/kg of dried distillers grains, [1]Standard error of mean, [2]Dry matter, [3]Ingestion rate, [4]Passage rate, [5]Digestion rate, [6]Organic matter, [7]Neutral detergent fiber, [8]Non-fiber carbohydrates. L and Q represent the effects of the linear and quadratic contrasts, respectively. The superscript letters indicate the differences between treatments by the least-square means.

Table 6 - Effect of dried distillers grains levels on nitrogen balance, protein profile, and microbial protein synthesis

Items	Diets				SEM[1]	P-value	
	D0	D150	D300	D450		L	Q
N intake, g/d	180^{BC}	162^{C}	190^{B}	222^{A}	15.66	< 0.01	0.02
Fecal N, g/d	38.8^{B}	37.7^{B}	46.8^{AB}	54.0^{A}	5.565	0.02	0.26
Urine N, g/d	90.2^{AB}	75.5^{B}	93.5^{AB}	116^{A}	11.98	0.04	0.06
Retained N, g/d	52.0^{A}	48.5^{A}	50.0^{A}	52.5^{A}	8.404	0.94	0.72
Retained N, g/kg N intake	310^{A}	301^{A}	274^{A}	238^{A}	41.89	0.27	0.77
RUP[2], g/d	394^{BC}	367^{C}	545^{B}	753^{A}	58.49	< 0.01	0.04
MPS[3], g/d	717^{A}	644^{A}	644^{A}	637^{A}	49.07	0.09	0.23
N-RNA/Nmic[4]	0.19^{A}	0.20^{A}	0.19^{A}	0.20^{A}	0.009	0.34	0.61
RUP, g/kg CP[5]intake	338^{C}	362^{C}	453^{B}	541^{A}	23.99	< 0.01	0.26
MPS, g/kg CP intake	662^{A}	638^{A}	547^{B}	459^{C}	23.99	< 0.01	0.26
MPS/TDN[6], g/kg	96.3^{A}	95.8^{A}	103^{A}	108^{A}	3.774	0.06	0.59
MP[7] intake, g/d	784^{BC}	706^{C}	848^{B}	1010^{A}	71.50	< 0.01	0.03

D0 = diet without dried distillers grains, D150 = diet with 150 g/kg of dried distillers grains, D300 = diet with 300 g/kg of dried distillers grains, D450 = diet with 450 g/kg of dried distillers grains, [1]Standard error of mean, [2]Rumen undegradable protein, [3]Microbial protein synthesis = Rumen degradable protein, [4]Relationship between nitrogen from microbial RNA and microbial nitrogen, [5]Crude protein, [6]Total digestible nutrients, [7]Metabolizable protein. L and Q represent the effects of the linear and quadratic contrasts, respectively. The superscript letters indicate the differences between treatments by the least-square means.

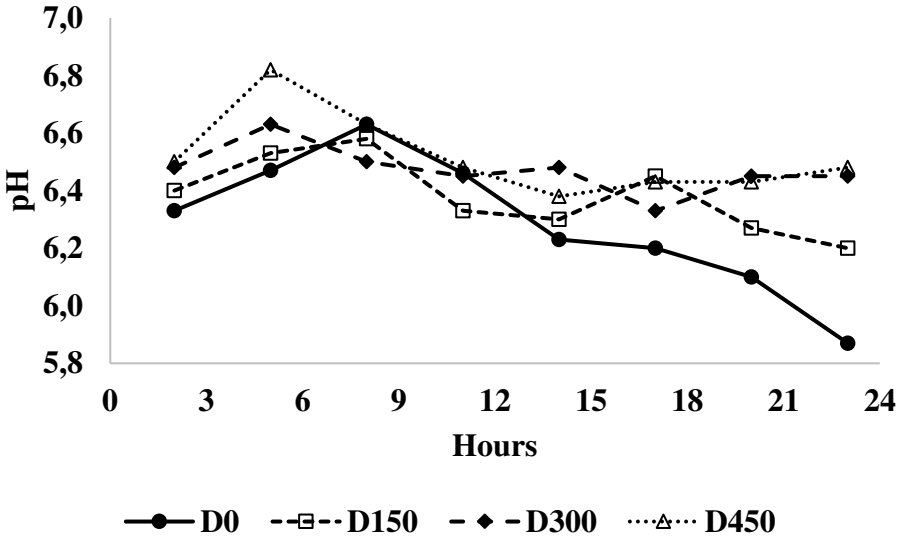

Figure 1 – Effect of dried distillers grains levels on ruminal pH. D0 = diet without dried distillers grains, D150 = diet with 150 g/kg of dried distillers grains, D300 = diet with 300 g/kg of dried distillers grains, D450 = diet with 450 g/kg of dried distillers grains. Averages per treatment (linear effect, $P < 0.01$, SEM = 0.103): D0 = 6.29^{B}, D150 = 6.38^{AB}, D300 = 6.47^{AB}, D450 = 6.52^{A}. Hourly averages (quadratic effect, $P < 0.01$, SEM = 0.107): 2h = 6.43^{BC}, 5h = 6.61^{A}, 8h = 6.58^{AB}, 11h = 6.43^{BC}, 14h = 6.35^{BC}, 17h = 6.35^{C}, 20h = 6.31^{C}, 23h = 6.25^{C}. There was no interaction Level * Hour ($P = 0.83$). The superscript letters indicate the differences between treatments by the least-square means.

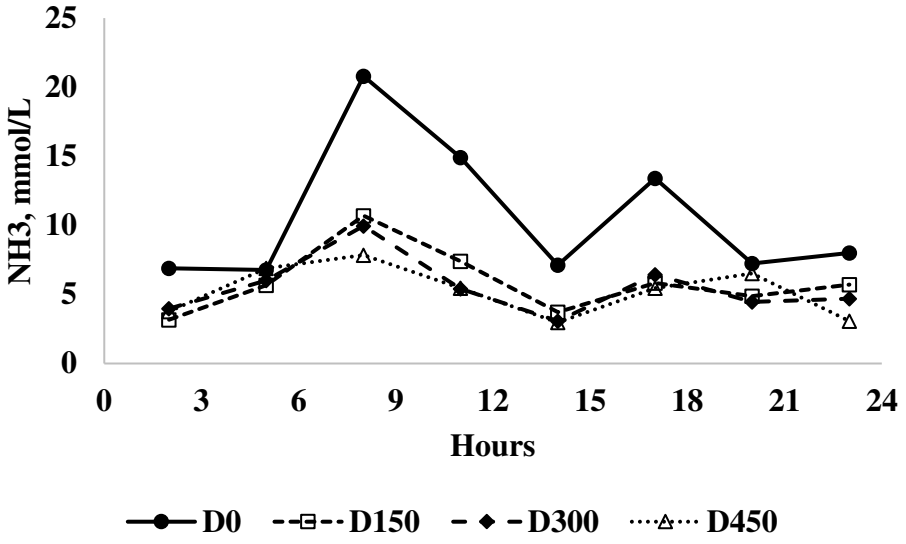

Figure 2 - Effect of dried distillers grains levels on ruminal ammonia concentration. D0 = diet without dried distillers grains, D150 = diet with 150 g/kg of dried distillers grains, D300 = diet with 300 g/kg of dried distillers grains, D450 = diet with 450 g/kg of dried distillers grains. Averages per treatment (linear effect, $P < 0.01$, SEM = 1.112): D0 = 11.1^{A}, D150 = 5.90^{B}, D300 = 5.50^{B}, D450 = 5.05^{B}. Hourly averages (cubic effect, $P < 0.01$, SEM = 1.258): 2h = 4.44^{C}, 5h = 6.34^{BC}, 8h = 12.3^{A}, 11h = 8.28^{B}, 14h = 4.22^{C}, 17h = 7.79^{B}, 20h = 5.78^{BC}, 23h = 6.29^{BC}. There was no interaction Level * Hour ($P = 0.18$). The superscript letters indicate the differences between treatments by the least-square means.

CHAPTER 4

In situ evaluation of dried distillers grains (DDG) and of diets containing different levels of DDG inclusion replacing soybean meal, urea and corn, and development of alternative methods to estimate *in vivo* digestibility of diets

H. M. Alhadas [a, 1], S. C.Valadares Filho[a], L. O. Tedeschi[b], R. S. R. Vilela[a], G. A. P. Souza[a]

[a]*Department of Animal Science, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil, 36570-900.*

[b]*Department of Animal Science, Texas A&M University, College Station, Texas, USA, 77843-2471.*

[1]Corresponding author: H. M. Alhadas, E-mail: herlonmalhadas@gmail.com

https://orcid.org/0000-0003-2406-9726

ABSTRACT

This study aimed to evaluate ruminal *in situ* degradation of diets containing different levels of dried distillers grains (**DDG**) replacing soybean meal, urea and corn and the effect of these diets on *in situ* ruminal degradation of DDG, and to propose alternative methods to estimate *in vivo* digestibility of diets. Four rumen-cannulated Nellore bulls were used in a 4 × 4 Latin square design, for both *in situ* incubation and *in vivo* digestibility. We evaluated the following treatments: diet without DDG (**D0**) or containing 150 (**D150**), 300 (**D300**), or 450 g/kg (**D450**) of inclusion. For *in situ* evaluation, DDG was incubated in all animals, and diets only in those fed with the same diet that would be incubated. To estimate *in vivo* digestibility, three days of total feces collection were performed in each period. Data was analysed in PROC MIXED, PROC NLIN and PROC REG of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). There was no effect of diet on the *in situ* degradation parameters of the DDG ($P > 0.05$). However, for the diets, the readily soluble fraction (parameter "**a**") ($P < 0.01$), and the rate constant for degradation (parameter **kd**) of the potentially degradable fraction in the rumen (parameter "**b"**) ($P = 0.05$) of dry matter (**DM**), decreased linearly. On the other hand, the parameter "b" ($P < 0.01$) and kd rate ($P = 0.03$) of the neutral detergent fiber (**NDF**) of the diets increased linearly. The *in situ* incubation time to estimate the *in vivo* DM digestibility ranged from 27.2-h to 37.7-h. For the NDF, the time was 60-h for all diets. In addition, to estimate the *in vivo* digestibility of DM (**DMD**), and the residual NDF (**RNDF**) of diets, two equations were suggested for each one: DMD = 804.8 - 0.092 × level, and DMD = 658 + 0.468 × a; RNDF = 393.9 - 0.138 × level, and RNDF = 874.3 - (0.602 × b) - (0.805 × I) + (1,662 × kd), where I = indigestible NDF. We conclude that the DDG can be incubated in animals fed diets without DDG or inclusion level up to 450 g/kg (DM basis), and that the inclusion of DDG in finishing diets reduced DM degradability, and it is possible to estimate *in vivo* digestibility using alternative methods.

Key words: beef catlle, coproduct, finishing diet, *in situ* degradability.

INTRODUCTION

The digestibility coefficient and rumen degradation parameters are important factors in beef cattle production because they dictate the absorption of nutrients, impacting performance (Patterson et al., 2006). However, *in vivo* experiments to estimate these parameters are not always feasible, as it is laborious, requires more time, and is more expensive (Stern et al., 1997). Thus, alternative methods were then developed, such as *in situ* and *in vitro* methods (Nocek, 1988; Tilley and Terry, 1963). It is possible to find studies in the literature that correlated alternative methods with the *in vivo* method (Benedeti et al., 2020; Silva et al., 2020). However, diets containing the inclusion of different ingredients may have other chemical characteristics and consequently changes in the degradation parameters (López, 2005). This is the case for diets containing dried distillers grains without soluble (**DDG**), which is a coproduct generated from the fermentation of corn to obtain alcohol. In addition to the reduction in the concentration of starch and non-fibrous carbohydrates (**NFC**), DDG has an increase in the concentration of neutral detergent fiber (**NDF**) and crude protein (**CP**) (Klopfenstein et al., 2008). Therefore, it is necessary that alternative methods be developed with diets that allow assessing these differences. In this context, *in situ* methods have great potential, as they allow the evaluation of a larger number of feedstuffs in a faster and cheaper way.

We hypothesized that the inclusion of DDG in finishing diets reduce its degradability, and that there is no effect of diet on DDG ruminal degradation, and the recommendations in the literature to estimate *in vivo* digestibility of diets using *in situ* technique are not suitable for diets containing DDG. The objectives of this study were to evaluate the ruminal *in situ* degradation of diets containing different levels of DDG inclusion, and the effect of these diets on ruminal *in situ* degradation of DDG, and to find the number of hours of *in situ* incubation of diets to estimate their *in vivo* digestibility and to develop models based on the level of inclusion

of DDG and based on *in situ* degradation parameters of diets to evaluate their *in vivo* digestibility.

MATERIAL AND METHODS

The experiment was conducted in the feedlot of the Animal Science Department of the Federal University of Viçosa, Viçosa, Brazil, MG. The protocol number approved by the Ethics Committee for Animal Use of the Federal University of Viçosa was CEUAP/DZO/UFV 01/2019.

Animals, experimental design, and diets

Four rumen-cannulated Nellore bulls (body weight of 395kg ± 20 and 14 months) were used in a 4 x 4 Latin square design, for both *in vivo* digestibility estimation and *in situ* incubations. The animals were trated for endo and ectoparasites (Ivermectin 3.5%; 1mL/kg body weight; Ranger LA 3.5%, MSD Saúde Animal, Brazil) and were housed in individual 8-m2 pens containing individual drinkers and feeders. Four treatments were evaluated, both *in vivo* and *in situ*, which consisted of diets with increasing levels of DDG as follows: diet without DDG (**D0**), and diets with levels of 150 g/kg (**D150**), 300 g/kg (**D300**) and 450 g/kg (**D450**) of DDG [dry matter (**DM**) basis]. All diets had 280 g/kg of corn silage and were formulated according to Valadares Filho et al. (2016) (Table 2). The inclusion of DDG in the diet resulted in total replacement of soybean meal and partial replacement of corn, resulting in protein levels of 123, 122, 152 and 187 g/kg (DM basis) for diets D0, D150, D300 and D450 respectively. In addition, to balance the protein, D0 diet had the addition of urea, which was incorporated into the diet together with the concentrate ingredients.

The animals were fed twice a day, at 0700h and 1700h. Orts were maintained at around 50 g/kg (fresh matter basis). The chemical composition of each ingredient can be seen in Table

1, and the level of inclusion of each ingredient and the chemical composition of each diet can be seen in Table 2.

In vivo digestibility

Four digestibility periods were carried out, each lasting 17 days, and 14 days were used for animal adaptation and three days for total feces collection. The intake was monitored daily, and the orts were sampled on d-15, d-16, and d-17 of each experimental period to estimate the dry matter intake (**DMI**). The orts were dried in an oven at 55°C for 72-h, and at the end of each period, a composite sample proportional to the quantity supplied was made for each animal (DM basis). The feces were collected on the d-15, d-16, and d-17 directly from the floor and stored in barrels, and at the end of each day, they were homogenized and sampled (300 g fresh matter). Subsequently, they were dried in an oven at 55°C for 72-h, and a composite sample proportional to the amount excreted per day was made for each animal (DM basis). The digestibility of DM, organic matter (**OM**) and NDF was estimated based on total intake (DM basis). It is important to say that the floor of the pens was made of concrete coated with rubber to facilitate the collection of feces and facilitate cleaning of the place, avoiding contamination of the sample. In addition, the collection was monitored 24-hours a day, thus ensuring the quality of the samples.

In situ degradation procedures

On d-10, d-11, d-12, d-13, and d-14 of each experimental period, *in situ* incubations of DDG and experimental diets were performed. For this, the same ingredients sampled to estimate the composition of the diets were used. For the incubation of diets, the corn silage was sampled and dried in a forced-air oven (55°C) for 72-h, and ground in a knife mill (Tecnal, Piracicaba, São Paulo, Brazil) with a 2-mm sieve. After being sampled, the concentrate ingredients were also ground in the same mill described above in a 2-mm sieve. Subsequently, each ingredient of the

concentrate was mixed proportionally (DM basis). The corn silage and concentrate were weighed proportionally (DM basis) and separately so that 5 g of sample was obtained in each nylon bag (Sefar Nitex, Switzerland; 50-μm porosity, 400 cm^2 surface area), in a ratio of 12.5 mg/cm^2. The DDG was weighed following the same proportion.

Eight incubation times were used for diets and DDG: 0, 3, 6, 12, 24, 48, 72, and 96-h to estimate the degradation curves. To obtain a sufficient amount of residue for analyses, more bags were used for the most extended incubation times. Inside the rumen, the bags were attached to a metal chain with a weight at the end to ensure that all were immersed. The incubation was carried out in decreasing order of time so that all the bags were removed simultaneously from the rumen in the end. After removal, all were washed manually with running water until they became translucent (Zanetti et al., 2017). The zero-time bags were not incubated; they were only washed with the other bags. Subsequently, all bags with the residues were dried in an oven at 55°C for 72-h, and the samples were ground in a knife mill (Tecnal, Piracicaba, São Paulo, Brazil) with a 1-mm sieve for analyses. Each diet was incubated only in the animal that was being fed that respective diet. Then, the D0 diet was incubated in the rumen of the animal that was receiving the D0 diet. Diet D150 was incubated in the rumen of the animal that was being fed diet D150, and so on. Oppositely, DDG, in all periods, was incubated in the rumen of all animals (Figure 1).

Laboratory Analyses

Samples of feeds were analysed for DM (method 934.01) nitrogen (**N**, method 981.10) and ash (method 930.05) according to AOAC (2012), and for ether extract (**EE**, method, 2003.05) according to AOAC (2005). The difference between DM and ash estimated OM content. NDF concentration was estimated with the addition of thermostable α-amylase to neutral detergent (Ankom Tech. Corp., Fairport, NY, USA) and without the addition of sodium sulfite. The NDF

residue was corrected for ash (Mertens et al., 2002) and N compounds (Licitra et al., 1996). The CP concentration was estimated by multiplying the N concentration by 6.25. The NFC was estimated as suggested by Detmann and Valadares Filho (2010): NFC = OM − CP − apNDF − EE, where apNDF is the NDF corrected for ash and residual N compounds, as described above. The samples of orts, feces, and residues from *in situ* incubations were analysed for DM, OM and NDF by the methods described above.

Calculations and statistical analyses

The data were analysed as a 4 x 4 Latin square in the PROC MIXED of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA), using the following statistical model:

$$Y_{ijk} = \mu + D_i + a_j + p_k + e_{ijk}$$

where: Y_{ijk} = dependent variable, μ = general mean; D_i = fixed effect of dietary DDG content i; a_j = random effect of animal j; p_k = random effect of experimental period k (random); and e_{ijk} = random error taken as normal and independently distributed (NID) (0, σ_e^2). It is important to emphasize that the statistical design described above was used to assess *in vivo* digestibility and *in situ* incubations. Regarding *in situ* incubations, unlike diets, DDG was incubated in all animals at all periods. This enabled the evaluation of the effect of diet on the DDG degradation parameters. As mentioned above, the diets were incubated in each period only in the animal that was receiving the same diet that would be evaluated. Therefore, the results presented for the degradability of diets should be understood as the variation in degradation parameters in diets with different levels of DDG. However, the results presented for DDG degradability should be understood as the effect of diets on DDG degradation parameters (Figure 1).

Linear, quadratic and cubic orthogonal contrasts were tested when the treatment effect (DDG level) was significant. The contrasts were considered equidistant (0, 150, 300, and 450 g/kg of DDG), and the analyses were performed at PROC IML of SAS (version 9.4, SAS

Institute Inc., Cary, NC, USA). The least-square means were used to determine statistical significance among treatments. For all comparisons and tests, significance was declared at $P \leq 0.05$, and tendencies were considered at *P* between 0.05 and 0.10.

In vivo digestibility and in situ degradability

In vivo digestibility was calculated based on the intake of each constituent (DM basis) as follows: Digestibility (g/kg) = [Intake (kg) – excreted (kg)] / Intake (kg) × 1000. *In vivo* digestibility was used only for comparisons with alternative methods and was not evaluated statistically in this study.

In situ degradation profile of DM and OM was estimated by Equation 1, proposed by Orskov and Mcdonald (1979). The NDF degradation parameters were estimated by Equation 2, proposed by Van Milgen et al. (1991). It is important to say that the equation used for DM and OM provides the degraded fraction, and the equation used for NDF provides the non-degraded residue. Therefore, NDF degradation was calculated by difference: Degradation (g/kg) = 1000 – residue.

$$Y(t) = a + b \times (1 - e^{(-kd \times t)}) \quad (1)$$

$$RNDF(t) = b \times [1 + (\lambda \times t)] \times e^{(-\lambda \times t)} + I \quad (2)$$

where a = readily soluble fraction (g/kg); b = potentially degradable fraction in the rumen (g/kg); I = undegradable fraction (g/kg); kd = rate constant for degradation of b (g/kg per h); t = time independent variable (h); RNDFt = non-degraded NDF residue at time "t" (g/kg); Yt = degraded fraction of DM and OM (g/kg); λ = joint fractional rate of latency and degradation (h^{-1}).

The NDF degradation rate (Equation 3) was calculated based on λ, using the properties of the $\Gamma(2)$ distribution (Ellis et al., 1994).

$$kd = (0.59635 \times \lambda) \times 1000 \quad (3)$$

where kd = rate constant for degradation of b (g/kg per h); λ = joint fractional rate of latency and degradation (h^{-1}).

The parameters "a", "b", kd, λ and "I" of the *in situ* incubation models were estimated using the PROC NLIN procedure (version 9.4, SAS Institute Inc., Cary, NC, USA), and assuming the Marquardt algorithm for convergence.

Estimation of in vivo digestibility by the number of hours of in situ incubation

For each *in vivo* digestibility of DM, OM, and NDF of diets, a confidence interval (1 − α = 0.95) was calculated based on the standard deviation. With the mean digestibility of diets, a higher value for digestibility (**upper limit =** mean + confidence interval) and a lower value for digestibility (**lower limit** = mean − confidence interval) were estimated. Subsequently, the *in situ* degradation curves of DM, OM, and NDF of diets were estimated by the models, with the times (t) varying from 0-h to 96-h. Then, *in vivo* digestibility (mean, upper limit, and lower limit) and *in situ* degeneration curves were plotted on the same graph (time x digestibility/degradability), as shown in Figure 2. The points that the *in situ* degradation curves intersect with the *in vivo* digestibility curves are the points in which the methods are equal. Therefore, the number of hours of *in situ* incubation to estimate *in vivo* digestibility of diets was considered the interval where the *in situ* degradation curve intersects the lower limit of *in vivo* digestibility to the point where it intercepts the upper limit. For DM and OM, this time was calculated using Equation (4). For the NDF, the time was estimated by the iterative method.

$$T = -(\ln(1 - (\text{in vivo digestibility} - a) / b)) / kd \quad (4)$$

where, a = readily soluble fraction (g/kg); b = potentially degradable fraction in the rumen (g/kg); kd = rate constant for degradation of b (g/kg per h); T = estimated time (h).

Estimation of in vivo digestibility by regression models

In order to estimate *in vivo* digestibility of diets by alternative methods, linear regression analyses were also performed according to the level of inclusion of DDG in the diet and according to the parameters of *in situ* degradation. It was used the PROC REG procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA), and $\alpha = 0.05$ was adopted to choose the parameters that would be used in the models.

Comparison between observed value (in vivo) and value predicted by the regression models

After the models were generated by regression analyses, the database itself was used to generate the predicted digestibility values for DM and NDF of diets. The predicted values were compared with the observed values of *in vivo* digestibility using the Model Evaluation System (MES version 3.2.2). The analysis was performed using the following regression model: $y = \beta0 + \beta1 \times x$, where x = predicted value, y = observed value, $\beta0$ = intercept and $\beta1$ = slope. The tested hypotheses were: H0: $\beta0 = 0$ and $\beta1 = 1$; Ha = not H0. When H0 was not rejected, the proposed model can estimate *in vivo* digestibility. The correlation coefficient (R) and the correlation and concordance coefficient (CCC) were also estimated as described by Tedeschi (2006). When the CCC value is close to 1.0, the model has good precision and accuracy. The CCC index was used to indicate the best model. The mean square error of the prediction and its components (Tedeschi, 2006) were also measured by the equations below:

$$MSEP = SB + MaF + MoF = \Sigma_i (Y_i - f(X_1,\ldots, X_p)_i)^2 \quad (5)$$

$$SB = (f(X_1,\ldots,X_p) - Y)^2; \quad (6)$$

$$MaF = (s_{f(X_1,\ldots,X_p)} - r \times s_y)^2; \quad (7)$$

$$MoF = 2 \times (1 - r) \times s_{f(X_1,\ldots,X_p)} \times s_y \quad (8)$$

where MaF = magnitude of random fluctuation; MoF = model random fluctuation; MSEP = mean square error of prediction; r = correlation between observed and predicted value; SB = square bias; s_x = standard deviation of predicted value; s_y = standard deviation of observed value; X = predicted value; Y = observed value.

RESULTS AND DISCUSSION

DDG in situ degradation

There was no effect of diet on the DDG *in situ* degradation for any evaluated parameter (Table 3). This means that the ingredient can be incubated for characterization in animals receiving diets without the inclusion of DDG or levels up to 450 g/kg. The fractions "a", "b" and kd of DM were on average 110 g/kg, 707 g/kg and 24.6 g/kg h^{-1}, respectively. The average *in situ* ruminal degradation was: 24-h = 420 g/kg, 48-h = 591 g/kg and 72-h = 687 g/kg.

DDG is a coproduct from corn fermentation, which does not have the addition of soluble substances at the end of processing, as is the case with DDGS. Consequently, its composition is different from the initial raw material (corn) and from the other coproducts, and these differences in the composition may affect the degradability of this feed. Lykos and Varga (1995) studied the *in situ* degradation parameters of corn processed using different methods and carried out incubations up to 48-h. The authors described for the DM of finely ground corn the fractions "a", "b" and kd of 215 g/kg, 781 g/kg and 69 g/kg h^{-1}, respectively. The fraction "a" of DM was 105 units higher when compared to the DDG evaluated in this study, and the kd fraction of DM was 44.4 units higher, indicating the changes that occur during the fermentation process. On the other hand, Nedelkov et al. (2017) evaluated *in situ* ruminal degradations of DDGS sources and reported the DM fractions "a", "b" and kd of 316 g/kg, 578 g/kg and 62 g/kg h^{-1}, respectively. Although the fraction "b" of DM was smaller, the fractions "a" and kd of DM were, respectively, 206 and 37.4 units higher than the DDG evaluated in this study. In addition,

the ruminal DM degradability of DDGS in 48-h was 829 g/kg, 238 units higher than the value found for the DDG.

The "a" fraction, understood as the water-soluble fraction is formed by the soluble fraction of each component. When considering carbohydrates, this fraction comprises compounds such as organic acids, sugar, and fructans (Tedeschi and Fox, 2020). For proteins, the fraction "a" contains water-soluble non-protein nitrogen (NPN) compounds, such as nitrate, amines, ammonia, and single amino acids (Pichard and Van Soest, 1977). As opposed to OM, ash can also contribute to the soluble fraction of DM. As mentioned earlier, DDG does not get back its fraction of soluble, which may have resulted in the lowest value for the fraction "a" found for DM.

For the NDF, the average of the fractions "b", λ, "I" and kd were 763 g/kg, 0.05 h^{-1}, 222 g/kg and 27.7 g/kg h^{-1}, respectively. The degradabilities were: 24-h = 270 g/kg, 48-h = 531 g/kg and 72-h = 669 g/kg. Although NDF has a lower degradation rate compared to non-fiber carbohydrates (Mertens, 1993), the fibrous fraction of DDG has a high degradation coefficient (Tedeschi et al., 2009). However, many factors can influence NDF degradation, such as the concentration of ether extract of the feed or diet (Tedeschi et al., 2009) and ruminal pH (Alhadas et al., 2021; Russell and Dombrowski, 1980).

Diets in situ degradation

The DM and OM degradation parameters are described in Table 4. The inclusion of DDG did not change the fraction “b” of DM ($P = 0.35$) and OM ($P = 0.53$). However, the fraction “a” decreased linearly for both (DM, $P < 0.01$; OM, $P < 0.01$). Likewise, the DM kd rate ($P = 0.05$) and the OM kd rate ($P = 0.04$) decreased linearly. Consequently, the ruminal degradation estimated at 24-h ($P < 0.01$), 48-h ($P < 0.01$) and 72-h ($P < 0.01$) for DM and the ruminal

degradation estimated at 24-h ($P < 0.01$), 48-h ($P < 0.01$) and 72-h ($P < 0.01$) for OM reduced linearly.

The inclusion of DDG in the diets caused changes in their chemical compositions. The NDF concentration increased from 202 g/kg (D0, DM basis) to 405 g/kg (D450, DM basis). On the other hand, the NFC concentration decreased from 608 g/kg (D0, DM basis) to 322 g/kg (D450, DM basis). For grains, one of the main carbohydrates of the NFC fraction is starch, which has a kd rate that can reach 10 times its passage rate, while for NDF the kd rate is close to the passage rate (Mertens, 1993). Consequently, as the fermentation process reduces the starch concentration of DDG and increases the concentration of NDF, the inclusion of DDG in the diet reduced the kd rate of DM and OM, also reducing degradabilities in 24, 48 and 72-h.

On the other hand, for NDF the fraction "b" ($P < 0.01$), fraction λ ($P = 0.03$) and kd rate ($P = 0.03$) increased linearly (Table 4). However, the parameters "I" reduced linearly ($P = 0.02$). Although the estimated degradability of NDF in 24-h did not differ between treatments ($P = 0.11$), the degradability in 48-h ($P < 0.01$) and in 72-h ($P < 0.01$) increased linearly. According to Klopfenstein et al. (2008), the use of DDG can help to reduce sub-acute acidosis, and consequently favor the activity of fibrolytic microorganisms. Therefore, the improvement in ruminal pH can contribute to the increase in the kd rate of the fiber and consequently increase in its degradation (Alhadas et al., 2021; Tedeschi and Fox, 2020). In addition, the DDG used had an EE concentration close to that of corn (48.6 g/kg, DM basis), and its inclusion in the diet increased the EE concentration in a few units (3.8 units). Therefore, possibly, fat did not increase the lag time for particle colonization (Tedeschi et al., 2009) and did not reduce NDF degradation.

Estimation of in vivo digestibility through the number of hours of in situ incubation

The mean, upper limit and lower limit of *in vivo* digestibility of DM, OM and NDF, and *in situ* incubation time required to estimate *in vivo* digestibility (equivalence time) are described in Table 5. It was not possible to find a single time incubation for all diets. For DM, the times ranged from 23.4-h (lower limit D0) to 45.0-h (upper limit D450). For OM, the times ranged from 24.1-h (lower limit D0) to 47.6-h (upper limit D450). However, to estimate the *in vivo* digestibility of DM, it is possible to group the D0 and D150 diets, and the D300 and D450 diets. The incubation time of 27.2-h was common for the D0 (upper limit) and D150 (lower limit) diets, and the interval from 36.6-h to 37.7-h was common for the D300 and D450 diets.

Silva et al. (2020) evaluated finishing diets containing corn or sorghum processed with different methods and found no effect of type of grain and type of processing. A *in situ* incubation time of 24-h was recommended by the authors to estimate the *in vivo* digestibility of DM and OM for all diets. This incubation time would be sufficient only for the D0 diet, which does not contain DDG, and this time is not present in the confidence interval of the other diets. As previously mentioned, DDG has different characteristics, and it is necessary to develop methods that take these differences into account.

For the NDF, the *in situ* incubation times ranged from 48.0-h (upper limit D450) to 82.0-h (upper limit D450). However, the incubation time of 60.0-h was common to all diets, and can be used to estimate the *in vivo* digestibility of NDF from diets containing DDG inclusion levels of up to 450 g/kg (DM basis).

Although the *in vivo* digestibility of DDG has not been estimated, Valadares Filho et al. (2016) suggest a value of 434 g/kg for the digestible fiber of this feed. Using the fractions found in this study for the DDG NDF, the necessary *in situ* incubation time to obtain 434 g/kg of digestibility is 37.0-h.

Estimation of in vivo digestibility of DM and NDF using regression models

Based on the significant parameters, three equations were described to estimate the *in vivo* DMD and four equations to estimate the RNDF (Table 6). No models have been suggested for OM because its pattern of degradation is similar to that of DM. For the NDF, the residue was estimated, and not the digestibility, because the model used to estimate the *in situ* degradability was based on the residue (Equation 2). For both DM (Equation 9) and NDF (Equation 12), an equation based on the level of inclusion of DDG in the diet has been described. In addition, for DM, an equation based on parameter "a" (Equation 10) and on parameter kd (Equation 11) of *in situ* incubation has been proposed. For NDF, equations based on parameters "b" (Equation 13), "b" and "I" (Equation 14), "b", "I" and kd (Equation 15) have been proposed.

The comparison between the predicted and observed values for DMD and RNDF are described in Table 7 and Table 8, respectively. All the equations evaluated were able to predict the studied variable ($P \geq 0.05$). For DM, the equation with the highest CCC value was Equation 9 (CCC = 0.52), based on the level of inclusion. Of the equations based on the *in situ* degradation parameters, the one with the highest CCC value was Equation 10 (CCC = 0.49), based on the "a" parameter. On the other hand, the best equation to predict RNDF was Equation 15, based on the variables "b", "I" and kd (CCC = 0.90). Equation 12, based on the level of inclusion of DDG, presented a CCC of 0.65.

The suggested model for estimating DMD and RNDF based on the level of DDG inclusion is important because it is not always possible to perform an *in situ* degradation analyses. According to Klopfenstein et al. (2008), distillery grains can be used as a source of protein in finishing diets, and for this purpose, they are included in levels ranging from 150 to 200 g/kg. Considering an inclusion level of 200 g/kg, and using Equation 9 and Equation 12, the digestibility of DM and RNDF would be 786 and 366 g/kg, respectively. The digestibility of NDF would be: Digestibility = 1000 - 366 = 634 g / kg.

Benedeti et al. (2020) evaluated 23 diets from six experiments and proposed the following model based on the kd (*in situ*) parameter to estimate the *in vivo* digestibility of OM:

$$OMD = 569.5204 + 2859.7612\ kd, \quad (16)$$

where OMD = *in vivo* digestibility of organic matter; kd = degradation rate of fraction "b" of organic matter.

With the data obtained in this study, a comparison was made between the observed OMD value and the value predicted by the model proposed by Benedeti et al. (2020) (Table 9). However, the values observed and predicted by the proposed model for the OMD were statistically different ($P < 0.001$). This reinforces that the *in situ* incubation times to estimate the *in vivo* digestibility of diets, and the models proposed in the literature are not appropriate when using diets containing DDG.

CONCLUSION

The *in situ* characterization of DDG can be performed in animals receiving diets without DDG or with the inclusion of up to 450 g/kg. The inclusion of DDG in finishing diets linearly reduced the ruminal degradability and the "a" and kd fractions of DM and OM. However, NDF ruminal degradability and kd rate have increased. It is possible to estimate the *in vivo* digestibility of DM and NDF of diets containing DDG with less expensive and faster alternative methods, as is the case with *in situ* methods. For diets containing 0 and 150 g/kg of DDG, DMD can be estimated with ruminal incubation for 27.2-h. Diets containing 300 and 450 g/kg DDG, the incubation time can vary from 36.6-h to 37.7-h. For NDF, the recommended time for all diets is 60.0-h. In addition, among the models suggested to estimate DMD and RNDF of diets based on the *in situ* parameters, the authors suggest Equation 10 ($DMD = 658 + 0.468 \times a$) for DMD, and Equation 15 [$RNDF = 874.3 - (0.602 \times b) - (0.805 \times I) + (1{,}662 \times kd)$] for RDNF, which were those with the highest CCC value. However, if it is not possible to carry out *in situ*

incubations, it is possible to estimate DMD and RNDF using Equation 9 (DMD = 804.8 - 0.092 × level) and Equation 12 (RNDF = 393.9 - 0.138 × level) respectively.

ACKNOWLEDGMENT

We thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the Fundação de Amparo a Pesquisa do Estado de Minas Gerais (Fapemig) for partial funding to this study.

DECLARATIONS OF INTEREST

None of the authors of this paper have any conflict of interest.

FUNDING

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPQ, Brazil; 465377-2014/9).

LITERATURE CITED

Alhadas, H.M., Valadares Filho, S.C., Silva, F.F., Silva, F.A.S., Pucetti, P., Pacheco, M.V.C., Silva, B.C., Tedeschi, L.O., 2021. Effects of including physically effective fiber from sugarcane in whole corn grain diets on the ingestive, digestive, and ruminal parameters of growing beef bulls. Livest. Sci. 248, 104508. https://doi.org/10.1016/j.livsci.2021.104508

AOAC, 2012. Official Methods of Analysis of AOAC International, 19th ed. Association of Official Analysis Chemists International. Gaithersburg, Maryland.

AOAC, 2005. Official Methods of Analysis of AOAC International, 18th ed, Association of Official Analysis Chemists International. Gaithersburg, Maryland.

Benedeti, P.D.B., Valadares Filho, S.C., Zanetti, D., Fonseca e Silva, F., Silva, B.C., Alhadas, H.M., Pereira, J.M. V, Pachec, M.V.C., Pucetti, P., Menezes, A.C.B., Silva, F.A.S., Godoi, L.A., Santos, S.A., 2020. Prediction of in vivo organic matter digestibility of beef cattle diets from degradation parameters estimated from in situ and in vitro incubations. Artic. J. Agric. Sci. https://doi.org/10.1017/S0021859620000180

Detmann, E., Valadares Filho, S.C., 2010. Sobre a estimação de carboidratos não fibrosos em alimentos e dietas. Arq. Bras. Med. Vet. e Zootec. 62, 980–984. https://doi.org/10.1590/S0102-09352010000400030

Ellis, W.C., Matis, J.H., Hill, T.M., Murphy, M.R., 1994. Methodology for Estimating Digestion and Passage Kinetics of Forages. John Wiley & Sons, Ltd, pp. 682–756. https://doi.org/10.2134/1994.foragequality.c17

Klopfenstein, T.J., Erickson, G.E., Bremer, V.R., 2008. BOARD-INVITED REVIEW: Use of distillers byproducts in the beef cattle feeding industry. J. Anim. Sci. 86, pp. 1223-1231.

Licitra, G., Hernandez, T.M., Van Soest, P.J., 1996. Standardization of procedures for nitrogen

fractionation of ruminant feeds. Anim. Feed Sci. Technol. https://doi.org/10.1016/0377-8401(95)00837-3

López, S., 2005. In vitro and in situ techniques for estimating digestibility, in: Dijkstra J, Forbes JM and France J (Eds.), Quantitative Aspects of Ruminant Digestion and Metabolism, 2nd Edn. Wallingford, UK: CAB International. pp. 87–121.

Lykos, T., Varga, G.A., 1995. Effects of Processing Method on Degradation Characteristics of Protein and Carbohydrate Sources In Situ. J. Dairy Sci. 78, 1789–1801. https://doi.org/10.3168/jds.S0022-0302(95)76804-7

Mertens, D.R., 1993. Rate and extent of digestion, in: Quantitative Aspects of Ruminant Digestion and Metabolism. Englewood Cliffs, pp. 13–51.

Mertens, D.R., Allen, M., Carmany, J., Clegg, J., Davidowicz, A., Drouches, M., Frank, K., Gambin, D., Garkie, M., Gildemeister, B., Jeffress, D., Jeon, C.S., Jones, D., Kaplan, D., Kim, G.N., Kobata, S., Main, D., Moua, X., Paul, B., Robertson, J., Taysom, D., Thiex, N., Williams, J., Wolf, M., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. J. AOAC Int.

Nedelkov, K., Todorov, N., Girginov, D., Simeonov, M., and Ribarski, S., 2017. Comparison of the rumen degradability and intestinal digestibility of dm and cp of dried distillers's by-products from bulgarian distillery companies, Bulgarian Journal of Agricultural Science.

Nocek, J.E., 1988. In situ and Other Methods to Estimate Ruminal Protein and Energy Digestibility: A Review. J. Dairy Sci. https://doi.org/10.3168/jds.S0022-0302(88)79781-7

Orskov, E.R., Mcdonald, I., 1979. The estimation of protein degradability in the rumen from

incubation measurements weighted according to rate of passage. J. Agric. Sci. 92, 499–503. https://doi.org/10.1017/S0021859600063048

Patterson, H.H., Adams, D.C., Klopfenstein, T.J., Lardy, G.P., 2006. Application of the 1996 NRC to Protein and Energy Nutrition of Range Cattle. Prof. Anim. Sci. 22, 307–317. https://doi.org/10.15232/S1080-7446(15)31113-X

Pichard, G., Van Soest, P.J., 1977. Protein solubility of ruminant feeds, in: In: Proc. Cornell Nutr. Conf. Cornell Univ Ithaca, NY.

Russell, J.B., Dombrowski, D.B., 1980. Effect of pH on the efficiency of growth by pure cultures of rumen bacteria in continuous culture. Appl. Environ. Microbiol. https://doi.org/10.1128/aem.39.3.604-610.1980

Silva, B.C., Pacheco, M.V.C., Godoi, L.A., Silva, F.A.S., Zanetti, D., Menezes, A.C.B., Pucetti, P., Santos, S.A., Paulino, M.F., Valadares Filho, S.C., 2020. In situ and in vitro techniques for estimating degradation parameters and digestibility of diets based on maize or sorghum. J. Agric. Sci. 158, 150–158. https://doi.org/10.1017/S0021859620000271

Stern, M.D., Bach, A., Calsamiglia, S., 1997. Alternative techniques for measuring nutrient digestion in ruminants. J. Anim. Sci. 75, 2256. https://doi.org/10.2527/1997.7582256x

Tedeschi, L.O., 2006. Assessment of the adequacy of mathematical models. Agric. Syst. https://doi.org/10.1016/j.agsy.2005.11.004

Tedeschi, L.O., Fox, D.G., 2020. The Ruminant Nutrition System: Volume I - An Applied Model for Predicting Nutrient Requirements and Feed Utilization in Ruminants, 3nd ed. XanEdu, Ann Arbor, MI.

Tedeschi, L.O., Kononoff, P.J., Karges, K., Gibson, M.L., 2009. Effects of chemical composition variation on the dynamics of ruminal fermentation and biological value of

corn milling (co)products. J. Dairy Sci. 92, 401–413. https://doi.org/10.3168/jds.2008-1141

Tilley, J.M.A., Terry, R.A., 1963. A two-stage technique for the in vitro digestion of forage crops. Grass Forage Sci. 18, 104–111. https://doi.org/10.1111/j.1365-2494.1963.tb00335.x

Valadares Filho, S.C., Costa e Silva, L.F., Gionbelli, M.P., Rotta, P.P., Marcondes, M.I., Chizzotti, M.L., Prados, L.F., 2016. Nutrient Requirements of Zebu and Crossbred Cattle - BR-CORTE, in: Exigências Nutricionais de Zebuínos Puros e Cruzados - BR-CORTE. Editora Federal de Viçosa. https://doi.org/10.5935/978-85-8179-111-1.2016b002

Van Milgen, J., Murphy, M.R., Berger, L.L., 1991. A Compartmental Model to Analyze Ruminal Digestion. J. Dairy Sci. 74, 2515–2529. https://doi.org/10.3168/jds.S0022-0302(91)78429-4

Zanetti, D., Menezes, A.C.B., Silva, F.A.S., Costa, S.E., Rotta, P.P., Detmann, E., Engle, T.E., Valadares Filho, S.C., 2017. In situ and in vitro estimation of mineral release from common feedstuffs fed to cattle. J. Agric. Sci. 155, 1160–1173. https://doi.org/10.1017/S002185961700034X

Table 1 - Chemical composition of diet ingredients

Ingredients	DM[1]	OM[2]	NDF[3]	CP[4]	EE[5]
		g/kg			
Corn silage	307	936	498	62.2	24.9
DDG[6]	897	989	540	327	48.6
Corn meal	894	990	90.2	91.7	42.4
Soybean meal	885	939	95.4	505	23.6
Urea/A.S[7]	997	1000	-	2610	-
Premix[8]	1000	-	-	-	-
Virginiamycin	1000	-	-	-	-

[1]Dry matter; [2]Organic matter; [3]Neutral detergent fiber; [4]Crude protein; [5]Ether extract; [6]Dried distillers grains; [7]urea and ammonium sulfate (9:1); [8]Premix guarantees (per kg of DM): 200 - 220 g of Ca; 10 mg of Co (Min); 500 mg of Cu (Min); 22 g of S (Min); 333 mg of Fe (Min); 178.41 mg of F (Max); 10 g of P (Min); 25 mg of I (Min); 17 g of Mg (Min); 1500 mg of Mn (Min); 1100 mg of monensin; 6.6 mg of Se (Min); 50 g of Na (Min); and 2000 mg of Zn (Min). The unit g/kg for dry matter represents grams of dry matter per kilogram of raw material; and for the other constituents represents grams of the constituent per kilogram of dry matter.

Table 2 - Proportion of ingredients and composition of experimental diets

Items	Diets			
	D0	D150	D300	D450
	g/kg			
Ingredients				
Corn silage	280	280	280	280
DDG[1]	-	150	300	450
Corn meal	649	537	399	249
Soybean meal	40.0	12.0	-	-
Urea/A.S[2]	10.0	-	-	-
Premix	20.0	20.0	20.0	20.0
Virginiamycin	1.00	1.00	1.00	1.00
Analysed composition				
DM[3]	587	589	589	589
OM[4]	952	953	954	954
NDF[5]	202	270	337	405
CP[6]	123	122	152	187
EE[7]	35.6	37.3	38.4	39.4
NFC[8]	608	525	426	322

D0 = diet without dried distillers grains; D150 = diet with 150 g/kg of dried distillers grains; D300 = diet with 300 g/kg of dried distillers grains; D450 = diet with 450 g/kg of dried distillers grains; [1]Dried distillers grains; [2]Urea and ammonium sulfate (9:1); [3]Dry matter; [4]Organic matter; [5]Neutral detergent fiber; [6]Crude protein; [7]Ether extract; [8]Non-fiber carbohydrates. In the "Ingredients" section; the unit g/kg represents grams of the ingredient per kilogram of dry matter in the diet. In the "Analysed composition" section; the unit g/kg for dry matter represents grams of dry matter per kilogram of raw material; and for the other constituents represents grams of the constituent per kilogram of dry matter.

Table 3 - Effect of diets on *in situ* degradation parameters of dry matter, organic matter and neutral detergent fiber of the dried distillers grains

Items	DDG				SEM[1]	*P*-value	
	D0	D150	D300	D450		L	Q
DM[2] parameters,							
a[3], g/kg	108[A]	108 [A]	110 [A]	114 [A]	6.621	0.53	0.77
b[4], g/kg	694 [A]	732 [A]	703 [A]	697 [A]	38.47	0.88	0.46
kd[5], g/kg h^{-1}	25.3 [A]	24.2 [A]	24.5 [A]	24.4 [A]	2.612	0.84	0.83
DM ruminal degradation, g/kg							
at 24h	423 [A]	429 [A]	413 [A]	415 [A]	19.24	0.57	0.89
at 48h	594 [A]	609 [A]	580 [A]	582 [A]	23.21	0.35	0.68
at 72h	688 [A]	709 [A]	675 [A]	676 [A]	24.36	0.16	0.34
OM[6] parameters							
a[3], g/kg	107 [A]	107 [A]	114 [A]	116 [A]	5.690	0.23	0.89
b[4], g/kg	695 [A]	732 [A]	700 [A]	695 [A]	39.01	0.82	0.48
kd[5], g/kg h^{-1}	25.3 [A]	24.2 [A]	24.5 [A]	24.4 [A]	2.612	0.84	0.83
OM ruminal degradation, g/kg							
at 24h	422 [A]	429 [A]	415 [A]	416 [A]	19.06	0.66	0.86
at 48h	594 [A]	608 [A]	582 [A]	583 [A]	22.96	0.40	0.66
at 72h	687 [A]	709 [A]	676 [A]	677 [A]	24.10	0.18	0.32
NDF[7] parameters							
b, g/kg	759 [A]	728 [A]	781 [A]	782 [A]	34.93	0.40	0.61
λ[8], h^{-1}	0.05 [A]	0.05 [A]	0.05 [A]	0.05 [A]	0.005	0.95	0.97
I[9], g/kg	225 [A]	254 [A]	205 [A]	204 [A]	35.41	0.46	0.64
kd, g/kg h^{-1}	29.6 [A]	29.8 [A]	29.6 [A]	29.9 [A]	2.952	0.95	0.97
NDF ruminal degradation, g/kg							
at 24h	269 [A]	265 [A]	269 [A]	275 [A]	28.24	0.84	0.83
at 48h	524 [A]	522 [A]	535 [A]	544 [A]	36.63	0.54	0.83
at 72h	660 [A]	655 [A]	676 [A]	685 [A]	31.17	0.21	0.69

D0 = diet without dried distillers grains; D150 = diet with 150 g/kg of dried distillers grains; D300 = diet with 300 g/kg of dried distillers grains; D450 = diet with 450 g/kg of dried distillers grains; [1]Standard error of mean; [2]Dry matter; [3]Readily soluble fraction; [4]Potentially degradable fraction in the rumen; [5]Rate constant for degradation of "b"; [6]Organic matter; [7]Neutral detergent fiber; [8]Joint fractional rate of latency and degradation; [9]Undegradable fraction. L and Q represent the effects of the linear and quadratic contrasts; respectively. The superscript letters indicate the differences between treatments by the least-square means.

Table 4 - Effect of dried distillers grains levels on *in situ* degradation parameters of dry matter, organic matter, and neutral detergent fiber of the diets

Items	Diets				SEM[1]	*P*-value	
	D0	D150	D300	D450		L	Q
DM[2] parameters							
a[3], g/kg	308^{A}	271^{B}	258^{BC}	237^{C}	10.88	< 0.01	0.37
b[4], g/kg	652^{A}	644^{A}	643^{A}	641^{A}	15.70	0.35	0.70
kd[5], g/kg per h	59.0^{A}	51.5^{AB}	50.3^{AB}	43.0^{B}	5.776	0.05	0.98
DM ruminal degradation, g/kg							
at 24h	798^{A}	727^{B}	699^{BC}	645^{C}	22.16	< 0.01	0.71
at 48h	918^{A}	860^{B}	831^{B}	791^{C}	14.22	< 0.01	0.52
at 72h	949^{A}	899^{B}	875^{B}	844^{C}	9.547	< 0.01	0.24
OM[6] parameters							
a, g/kg	299^{A}	261^{B}	245^{BC}	221^{C}	11.50	< 0.01	0.41
b, g/kg	674^{A}	672^{A}	669^{A}	666^{A}	16.88	0.53	0.95
kd, g/kg per h	59.3^{A}	51.5^{AB}	49.3^{AB}	42.3^{B}	5.699	0.04	0.94
OM ruminal degradation, g/kg							
at 24h	805^{A}	737^{B}	698^{BC}	640^{C}	22.34	< 0.01	0.81
at 48h	929^{A}	876^{B}	837^{C}	792^{D}	14.67	< 0.01	0.70
at 72h	961^{A}	916^{B}	884^{C}	850^{D}	10.91	< 0.01	0.44
NDF[7] parameters							
b, g/kg	441^{B}	487^{B}	598^{A}	631^{A}	16.25	< 0.01	0.72
λ[8], h^{-1}	0.06^{B}	0.07^{AB}	0.08^{AB}	0.09^{A}	0.007	0.03	0.61
I[9], g/kg	350^{A}	341^{AB}	312^{AB}	304^{B}	13.31	0.02	0.98
kd, g/kg per h	35.4^{B}	42.6^{AB}	48.6^{AB}	52.3^{A}	4.318	0.03	0.70
NDF ruminal degradation, g/kg							
at 24h	388^{A}	416^{A}	438^{A}	448^{A}	23.94	0.11	0.73
at 48h	535^{B}	580^{AB}	627^{A}	642^{A}	20.61	< 0.01	0.45
at 72h	602^{C}	636^{BC}	675^{AB}	685^{A}	15.24	< 0.01	0.36

D0 = diet without dried distillers grains; D150 = diet with 150 g/kg of dried distillers grains; D300 = diet with 300 g/kg of dried distillers grains; D450 = diet with 450 g/kg of dried distillers grains; [1]Standard error of mean; [2]Dry matter; [3]Readily soluble fraction; [4]Potentially degradable fraction in the rumen; [5]Rate constant for degradation of "b"; [6]Organic matter; [7]Neutral detergent fiber; [8]Joint fractional rate of latency and degradation; [9]Undegradable fraction. L and Q represent the effects of the linear and quadratic contrasts, respectively. The superscript letters indicate the differences between treatments by the least-square means.

Table 5 - Effect of dried distillers grains levels on the time length needed for *in situ* incubation to estimate the *in vivo* digestibility of dry matter, organic matter, and neutral detergent fiber of diets

Items	Diets			
	D0	D150	D300	D450
In vivo digestibility, %		DM[1]		
lower limit	797	757	755	745
mean	808	780	780	765
upper limit	830	803	804	785
Equivalence time, h				
lower limit	23.4	27.2	29.6	36.6
mean	25.2	30.3	33.3	40.5
upper limit	27.2	33.9	37.7	45.0
In vivo digestibility, %		OM[2]		
lower limit	811	780	776	757
mean	820	797	795	777
upper limit	843	814	814	797
Equivalence time, h				
lower limit	24.1	28.7	32.3	39.0
mean	25.9	31.0	35.4	42.9
upper limit	27.9	33.7	38.9	47.6
In vivo digestibility, %		NDF[3]		
lower limit	592	595	631	644
mean	606	617	653	668
upper limit	620	639	676	692
Equivalence time, h				
lower limit	59.0	49.0	48.0	48.0
mean	65.0	57.0	56.0	56.0
upper limit	73.0	69.0	71.0	82.0

D0 = diet without dried distillers grains; D150 = diet with 150 g/kg of dried distillers grains; D300 = diet with 300 g/kg of dried distillers grains; D450 = diet with 450 g/kg of dried distillers grains; [1]Dry matter; [2]Organic matter; [3]Neutral detergent fiber. Mean = mean of *in vivo* digestibility; Upper limit = upper limit of *in vivo* digestibility found with the confidence interval ($1 - \alpha = 0.95$) (upper limit = mean + confidence interval); Lower limit = lower limit of *in vivo* digestibility found with the confidence interval ($1 - \alpha = 0.95$) (lower limit = mean − confidence interval). Each diet has an interval composed of three equivalence times, which represent the points where the *in situ* degradation curve intersects the lower limit, the mean and the upper limit of *in vivo* digestibility.

Table 6 - Regression models based on the dried distillers grains inclusion level in the diet and based on the parameters of *in situ* degradation to estimate *in vivo* dry matter digestibility and residual neutral detergent fiber of diets

Equations	Number
$DMD = 804.8 - 0.092 \times level$	9
$DMD = 658 + 0.468 \times a$	10
$DMD = 727.5 + 1.077 \times kd$	11
$RNDF = 393.9 - 0.138 \times level$	12
$RNDF = 519.1 - 0.291 \times b$	13
$RNDF = 813.1 - (0.452 \times b) - (0.633 \times I)$	14
$RNDF = 874.3 - (0.602 \times b) - (0.805 \times I) + (1.662 \times kd)$	15

a = Readily soluble fraction; b = Potentially degradable fraction in the rumen; DMD = dry matter digestibility; I = Undegradable fraction; kd = Rate constant for degradation of "b"; level = dried distillers grains inclusion level in the diet; RNDF = residual neutral detergent fiber.

Table 7 - Descriptive statistics of the relationship between the observed value (*in vivo*) and the predicted value (*in situ* and dried distillers grains inclusion level) for dry matter digestibility of diets

Parameters	DMD			
	Observed	level	a	kd
Mean, g/kg	784.5	784.0	784.3	782.3
Standard deviation, g/kg	27.93	16.00	15.61	12.80
Maximum, g/kg	835.26	804.78	809.03	810.40
Minimum, g/kg	745.7	763.2	758.4	761.9
R	-	0.60	0.57	0.43
CCC	-	0.52	0.49	0.32
Regression				
Intercept	-	-34.36 ± 293.1	-20.86 ± 307.2	49.36 ± 412.0
Slope	-	1.040 ± 0.374	1.030 ± 0.392	0.940 ± 0.527
P-value (H0: $\beta0 = 0$ and $\beta1 = 1$)	-	0.99	0.99	0.94
MSEP	-	470.1	490.7	601.1
SB	-	0.264	0.062	4.760
MaF	-	0.475	0.166	0.558
MoF	-	469.4	490.4	595.8

a = Readily soluble fraction; CCC = correlation and concordance coefficient; DMD = dry matter digestibility; kd = rate constant for degradation of potential degradable fraction; level = DDG inclusion level in the diet MaF = magnitude of random fluctuation; MoF = model random fluctuation; MSEP = mean square error of prediction; R = correlation between observed and predicted value; SB = square bias. The unit g/kg that follows the Mean, Standard deviation, Maximum, Minimum values refer to the observed digestibility and the digestibility predicted by the models for the dry matter of the diets.

Table 8 - Descriptive statistics of the relationship between the observed value (*in vivo*) and the predicted value (*in situ* and dried distillers grains inlcusion level) for residual neutral detergent fiber of diets

Parameters	RNDF				
	Observed	level	b, I, kd	b, I	kd
Mean, g/kg	362.3	362.8	361.5	362.5	362.5
Standard deviation, g/kg	34.02	23.95	30.33	28.43	24.8
Maximum, g/kg	416.7	393.9	420.8	398.8	395.2
Minimum, g/kg	296.9	331.7	302.2	303.6	325.1
R	-	0.67	0.90	0.83	0.72
CCC	-	0.65	0.90	0.82	0.70
Regression					
Intercept	-	8.590 ± 100.3	-3.450 ± 46.93	2.106 ± 64.75	2.486 ± 91.90
Slope	-	0.980 ± 0.276	1.010 ± 0.129	0.990 ± 0.178	0.993 ± 0.253
P-value (H0: $\beta0 = 0$ and $\beta1 = 1$)	-	0.99	0.98	0.99	0.99
MSEP	-	574.2	202.9	336.7	516.7
SB	-	0.1848	0.6600	0.0300	0.0200
MaF	-	0.333	0.120	0.030	0.030
MoF	-	573.7	202.1	336.6	516.7

CCC = correlation and concordance coefficient; I = Undegradable fraction; kd = rate constant for degradation of potential degradable fraction; level = DDG inclusion level in the diet; MaF = magnitude of random fluctuation; MoF = model random fluctuation; MSEP = mean square error of prediction; R = correlation between observed and predicted value; RNDF = residual neutral detergent fiber; SB = square bias. The unit g/kg that follows the Mean, Standard deviation, Maximum, Minimum values refer to the observed digestibility and the digestibility predicted by the models for the residual neutral detergent fiber of diets.

Table 9 – Descriptive statistics of the relationship between the observed value (*in vivo*) and the predicted value (Benedeti et al., 2020) for organic matter digestibility of diets

Parameters	OMD	
	Observed	Benedeti et al. (2020)
Mean, g/kg	798.6	715.2
Standard deviation, g/kg	26.79	33.97
Maximum, g/kg	848.1	789.7
Minimum, g/kg	758.1	661.0
R	-	0.53
CCC	-	0.11
Regression		
Intercept	-	502.2 ± 128.4
Slope	-	0.415 ± 0.179
P-value (H0: $\beta0 = 0$ and $\beta1 = 1$)	-	<0.001
MSEP	-	7821
SB	-	6963
MaF	-	370.9
MoF	-	487.0

CCC = correlation and concordance coefficient; MaF = magnitude of random fluctuation; MoF = model random fluctuation; MSEP = mean square error of prediction; OMD = organic matter digestibility; R = correlation between observed and predicted value; SB = square bias. Equation suggested by Benedeti et al. (2020): OMD = 569.5204 + 2859.7612 × kd. The unit g/kg that follows the Mean, Standard deviation, Maximum, Minimum values refer to the observed digestibility and the digestibility predicted by the models for the organic matter of diets.

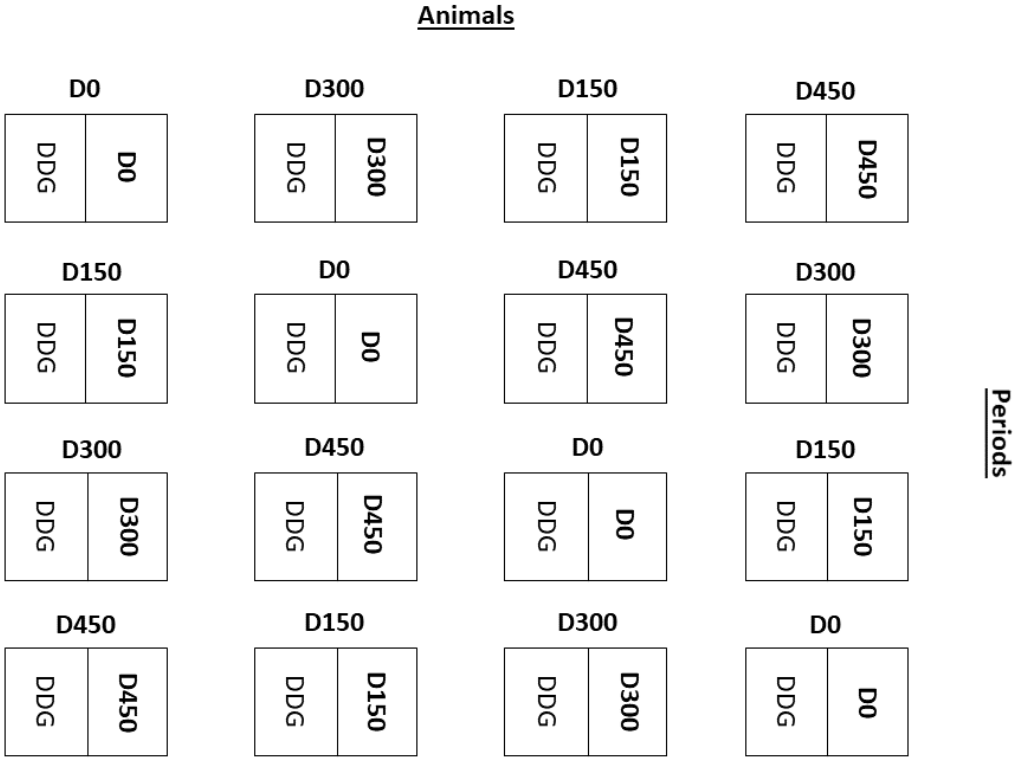

Figure 1 - Experimental design to evaluate the *in vivo* digestibility and *in situ* degradability of dried distillers grains and diets. Each column represents an animal and each row represents a period, with the intersection between row and column being the experimental unit. Therefore, animal 1, represented by column 1, received in period 1 the diet D0 (abbreviation written on the rectangles), in period 2 the diet D150, in period 3 the diet D300, and so on. The abbreviations "DDG" and "D0, D150, D300 and D450" inside each rectangle (animal x period), represent which samples were incubated in each experimental unit, being: DDG = died distillers grains; D0 = diet without dried distillers grains; D150 = diet with 150 g/kg of dried distillers grains; D300 = diet with 300 g/kg of dried distillers grains; D450 = diet with 450 g/kg of dried distillers grains. Therefore, DDG was incubated in all animals all periods, and the diets were only incubated in the animal that was receiving the same diet that would be incubated.

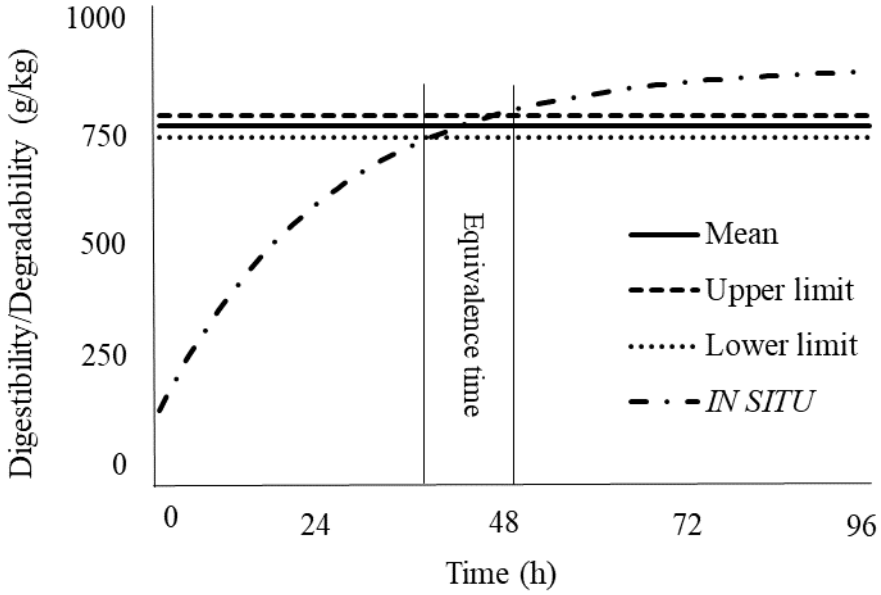

Figure 2 - Graphical representation of the methodology used to find the number of hours of *in situ* incubation to estimate *in vivo* digestibility. The points where the *in situ* degradation curve touches the lower limit and the upper limit of *in vivo* digestibility delimits the equivalence time. Mean = mean of *in vivo* digestibility; Upper limit = upper limit of *in vivo* digestibility found with the confidence interval ($1 - \alpha = 0.95$) (upper limit = mean + confidence interval); Lower limit = lower limit of *in vivo* digestibility found with the confidence interval ($1 - \alpha = 0.95$) (lower limit = mean − confidence interval).