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Use of high-protein distillers dried grains (DDG) in the backgrounding and finishing of early-maturing beef bulls

Dhones Rodrigues de Andrade
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DHONES RODRIGUES DE ANDRADE

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Adviser: Sebastiao de C. V. Filho

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"It does not do to dwell on dreams and forget to live."
Alvo Dumbledore, Harry Potter and the Sorcerer's Stone

ABSTRACT

ANDRADE, Dhones Rodrigues de, D.Sc., Universidade Federal de Viçosa, July, 2025. **Use of high-protein distillers dried grains (DDG) in the backgrounding and finishing of early-maturing beef bulls**. Adviser: Sebastiao de Campos Valadares Filho.

This thesis is composed of two chapters. Chapter 1 – The objective was to evaluate the effects of dried distillers grains (DDG) containing different amino acid concentrations: AA(+), with higher density of essential and non-essential amino acids, and AA(-), with a lower density; on the productive performance of Nellore steers during the backgrounding phase under intensive feedlot conditions. A total of 48 Nellore steers (8 ± 1.0 months of age; 219.74 ± 24.70 kg of body weight) were used, distributed into two dietary treatments: Group 1 received 1.00 kg/animal/day of AA(+) DDG, while Group 2 received 1.00 kg/animal/day of AA(-) DDG plus 0.110 kg/animal/day of urea to ensure isonitrogenous diets. The experimental period lasted 68 days, with 12 days for adaptation and 56 days for data collection. Nutrient intake was similar between treatments (P > 0.05), except for amino acid intake, which was higher (P < 0.05) in animals supplemented with AA(+) DDG. The digestibility of dry matter (DM), ash- and protein-free neutral detergent fiber (apNDF), and organic matter (OM) was also greater (P < 0.05) in animals supplemented with AA(+) DDG. The digestibility of non-fibrous carbohydrates (NFC), crude protein (CP), and ether extract (EE) did not differ between treatments (P > 0.05). Urinary and fecal nitrogen excretion, nitrogen retention, and the ratio between retained and consumed nitrogen (Nr:Nc) were affected (P < 0.05) by AA(+) DDG supplementation, with lower urinary and fecal excretion and higher nitrogen retention and Nr:Nc ratio in animals supplemented with AA(+) DDG, while nitrogen intake and absorbed nitrogen were not significantly different (P > 0.05) between DDG sources. These results suggest that the higher amino acid concentration in AA(+) DDG may favor greater nitrogen utilization efficiency, stimulating muscle protein synthesis and reducing losses to the environment. Microbial efficiency was also unaffected by the treatments (P > 0.05). Animals supplemented with AA(+) DDG showed greater (P < 0.05) final shrunk body weight (fSBW), total shrunk body weight gain (TSBWG), and average daily gain (ADG) compared to animals in the AA(-) DDG group, indicating improved productive performance and reinforcing the potential of AA(+) DDG as a viable alternative to conventional protein sources in intensive backgrounding systems. Chapter 2 – This chapter aimed to evaluate the effects of high-protein dried distillers grains (HP-DDG) inclusion on animal performance, nutrient digestibility, microbial efficiency, nitrogen balance, carcass traits, greenhouse gas emissions, and the expression of genes related to protein metabolism in Nellore cattle during the finishing phase. Forty-four Nellore steers (301.33 ± 34.31 kg; 10 ± 1 months) were used, randomly assigned to four dietary treatments (0, 4, 8, and 12% HP-DDG inclusion, based on total dietary DM) in a completely randomized design. The diets had a forage-to-concentrate ratio of 20:80 and were fed for 100 days, following a 14-day adaptation period. Increasing HP-DDG inclusion levels led to a linear trend of reduced dry matter intake (DMI; P = 0.072), organic matter intake (OMI; P = 0.083), and crude protein intake (CPI; P = 0.063), and significantly reduced (compared to the control diet) metabolizable energy intake (MEI; P < 0.05), non-fibrous carbohydrates (NFC; P < 0.05), and rumen degradable protein (RDP; P < 0.05). A linear increase was observed in ether extract (EE; P < 0.05) and rumen undegradable protein (RUP; P < 0.05) intake. There was a significant treatment effect (P = 0.007), with a linear reduction in TDN intake (P = 0.001) as HP-DDG inclusion increased, ranging from 5.59 kg/d (0% HP-DDG) to 4.79 kg/d (12% HP-DDG), indicating reduced energy intake with higher HP-DDG inclusion. No significant effects were observed on apNDF intake, metabolizable protein intake, or the DMI-to-shrunk body weight ratio (DMI/SBW; P > 0.10). HP-DDG inclusion significantly influenced (P < 0.05) the digestibility of dry matter (DM), organic matter (OM), crude protein (CP), and non-fibrous carbohydrates (NFC), with lower values observed in HP-DDG treatments compared to the control. Conversely, EE digestibility increased (P < 0.05) with HP-DDG inclusion, especially up to the 8% level. Nitrogen (N) intake, urinary N excretion, and N absorption were reduced (P < 0.05), and nitrogen utilization efficiency (N retained/N absorbed) increased (P = 0.026), particularly up to the 8% level, while fecal N and N retention did not differ between treatments (P > 0.05). Microbial protein production (MP) and its efficiency (g MP/kg TDN and g MP/kg digestible OM) were not affected by treatments (P > 0.10). HP-DDG inclusion did not result in significant effects (P > 0.10) on absolute or relative methane (CH_4) and carbon dioxide (CO_2) production. Hot carcass gain was reduced (P < 0.05), and empty body weight gain showed a trend toward reduction (P = 0.052) in HP-DDG treatments compared to the control. Regarding gene expression in liver and muscle tissues, a quadratic response was observed for glutamate-oxaloacetate transaminase 1 (GOT1; P = 0.022) and Forkhead box O1 (FOXO1; P = 0.016), while insulin-like growth factor 1 (IGF1) and AMP-activated protein kinase catalytic subunit alpha 1 (PRKAA1) were not influenced by treatments (P > 0.10). The replacement of soybean meal and urea by HP-DDG promoted nutritional and metabolic adjustments without compromising animal performance, supporting its use as a bypass protein source.

Keywords: amino acid; bypass protein; nutrient digestibility; nitrogen balance; zebu

RESUMO

ANDRADE, Dhones Rodrigues de, D.Sc., Universidade Federal de Viçosa, julho de 2025. **Uso de grãos secos de destilaria (DDG) com alto teor de proteína na recria e terminação de bovinos de corte superprecoces**. Orientador: Sebastiao de Campos Valadares Filho.

Esta tese foi composta por dois capítulos. Capítulo 1 - Objetivou-se avaliar os efeitos de grãos secos de destilaria (DDG) contendo diferentes concentrações de aminoácidos: AA(+), com maior densidade de aminoácidos essenciais e não essenciais, e AA(-), com menor densidade; sobre o desempenho produtivo de novilhos Nelore durante a fase de recria em confinamento intensivo. Foram utilizados 48 novilhos Nelore (8 ± 1,0 meses de idade; 219,74 ± 24,70 kg de peso corporal), distribuídos em dois tratamentos dietéticos: o grupo 1 recebeu 1,00 kg/animal/dia de DDG AA(+), enquanto o grupo 2 recebeu 1,00 kg/animal/dia de DDG AA(-) mais 0,110 kg/animal/dia de ureia, para que as dietas fossem isoproteicas. O período experimental teve duração de 68 dias, sendo 12 dias para adaptação e 56 dias para coleta de dados. O consumo de nutrientes foi semelhante entre os tratamentos (P > 0,05), exceto para o consumo de aminoácidos, com maior (P < 0,05) consumo para os animais suplementados com DDG AA(+). A digestibilidade da matéria seca (MS), da fibra em detergente neutro corrigida para cinzas e proteína (FDNcp) e da matéria orgânica (MO) foi maior (P < 0,05) também nos animais suplementados com DDG AA(+). As digestibilidades dos carboidratos não fibrosos (CNF), da proteína bruta (PB) e do extrato etéreo (EE) não diferiram entre os tratamentos (P > 0,05). A excreção urinária e fecal de nitrogênio, o nitrogênio retido e a relação entre nitrogênio retido e consumido (Nr:Nc) foram influenciados (P < 0,05) pela suplementação com DDG AA(+), com menores valores de excreção urinária e fecal e maiores valores de N retido e para a relação Nr:Nc para os animais suplementados com o DDG AA(+), enquanto o consumo de N e o nitrogênio absorvido não apresentaram diferenças significativas (P > 0,05) pela suplementação com diferentes DDG AA(+) e AA(-). Esses resultados sugerem que a maior concentração de aminoácidos no DDG AA(+) pode favorecer maior eficiência na utilização do nitrogênio, estimulando a síntese proteica muscular e reduzindo perdas para o ambiente. A eficiência microbiana também não foi afetada pelos tratamentos (P >0,05). Os animais suplementados com DDG AA(+) apresentaram maiores (P < 0,05) peso corporal final (PCF), ganho de peso total (GPT) e ganho médio diário (GMD) em comparação aos animais do grupo DDG AA(-), evidenciando melhor desempenho produtivo e reforçando o potencial do

DDG AA(+) como alternativa viável a fontes proteicas convencionais em sistemas intensivos de recria. Capítulo 2 – Nesse capítulo objetivou-se avaliar os efeitos da inclusão de grãos secos de destilaria com alto teor de proteína (HP-DDG) sobre o desempenho animal, digestibilidade dos nutrientes, eficiência microbiana, balanço de nitrogênio, características de carcaça, emissão de gases de efeito estufa e a expressão de genes relacionados ao metabolismo proteico em bovinos Nelore durante a fase de terminação. Foram utilizados 44 novilhos Nelore (301,33 ± 34,31 kg; 10 ± 1 meses), distribuídos aleatoriamente em quatro tratamentos dietéticos (0, 4, 8 e 12% de inclusão de HP-DDG, com base na MS total da dieta) em delineamento inteiramente casualizado. As dietas apresentavam relação volumoso:concentrado de 20:80 e foram fornecidas por 100 dias após 14 dias de adaptação. Com o aumento dos níveis de inclusão de HP-DDG, observou-se uma tendência linear de redução no consumo de matéria seca (CMS; P = 0,072), consumo de matéria orgânica (CMO; P = 0,083) e no consumo de proteína bruta (CPB; P = 0,063), além de reduzir significativamente, em comparação à dieta controle, o consumo de energia metabolizável (CEM; P < 0,05), carboidratos não fibrosos (CNF; P < 0,05) e proteína degradável no rúmen (PDR; P < 0,05). Observou-se aumento linear no consumo de extrato etéreo (EE; P < 0,05) e proteína não degradável no rúmen (PNDR; P < 0,05). Houve efeito significativo do tratamento (P = 0,007), com redução linear no consumo de NDT (P = 0,001) à medida que aumentou a inclusão de HP-DDG na dieta. Os valores variaram de 5,59 kg/d (0% HP-DDG) para 4,79 kg/d (12% HP-DDG), indicando redução na ingestão energética conforme aumentou-se a inclusão do HP-DDG. Não houve efeito sobre os consumos de FDNcp, proteína metabolizável e a relação entre o CMS e o peso corporal (CMS/PC; P > 0,10). A inclusão de HP-DDG influenciou significativamente (P < 0,05) a digestibilidade da matéria seca (MS), matéria orgânica (MO), proteína bruta (PB) e carboidratos não fibrosos (CNF), com redução dos valores nos tratamentos com HP-DDG em comparação ao controle (0% HP-DDG). Em contrapartida, o extrato etéreo (EE) apresentou aumento (P < 0,05) na digestibilidade com a inclusão de HP-DDG, especialmente até o nível de 8%. O consumo de nitrogênio (N), a excreção urinária de N e o N absorvido também foram reduzidos (P < 0,05) e a eficiência de utilização do nitrogênio (N retido/N absorvido) foi aumentada (P = 0,026) especialmente até o nível de 8%, enquanto o N fecal e o N retido não diferiram entre os tratamentos (P > 0,10). A produção de proteína microbiana (PBMic) e sua eficiência (g PBMic/kg NDT e g PBMic/kg MO digestível) não foram afetadas pelos tratamentos (P > 0,10). A inclusão de HP-DDG não resultou em efeitos significativos (P > 0,10) sobre a produção absoluta ou relativa de metano (CH4) e dióxido de carbono (CO2). O ganho de carcaça quente foi reduzido (P < 0,05), e o ganho de peso corporal vazio apresentou tendência à redução (P = 0,052) nos tratamentos com HP-DDG em comparação ao controle (0% HP-DDG). Em relação à expressão gênica nos tecidos de fígado e músculo, observou-se resposta quadrática para os genes glutamato-oxaloacetato transaminase 1 (GOT1; P = 0,022) e Forkhead box O1 (FOXO1; P = 0,016), enquanto os genes fator de crescimento semelhante à insulina 1 (IGF1) e subunidade catalítica alfa 1 da proteína quinase ativada por AMP (PRKAA1) não foram influenciados pelos tratamentos (P > 0,10).A substituição do FSU por HP-DDG promoveu ajustes nutricionais e metabólicos sem comprometer o desempenho animal, sustentando seu uso como fonte de proteína bypass.

Palavras-chave: aminoácidos; proteína bypass; digestibilidade de nutrientes; balanço de nitrogênio; zebuínos

SUMÁRIO

GENERAL INTRODUCTION

The growing global demand for renewable energy sources has driven the development of the biofuels industry, particularly the production of ethanol from grains such as corn (Buenavista et al., 2021; Zhou and Yan, 2024). This activity generates a significant amount of co-products with high potential for use in animal nutrition (Malik et al., 2024), with distillers' dried grains (DDG) being one of the most relevant (Buenavista et al., 2021; Malik et al., 2024). The DDG is the solid residue resulting from the fermentation and distillation of grains and concentrates the non-fermentable nutrients of corn, presenting high levels of protein, fiber, and lipids (Klopfenstein et al., 2008). Thus, its use in ruminant feeding has proven promising from both nutritional and economic perspectives (Stalker et al., 2009; Espinosa and Stein, 2018).

In recent years, the ethanol industry has adopted technologies to optimize the quality of the generated co-products (Hart et al., 2019). One of these innovations is front-end fractionation, which removes the fibrous fraction before enzymatic processing, resulting in a DDG with higher crude protein (CP) concentration, essential amino acids, and rumen undegradable protein (RUP), known as high-protein DDG (HP-DDG; Rosentrater and Muthukumarappan, 2006; Liu and Rosentrater, 2012; Malik et al., 2024). In addition, HP-DDG presents lower starch and lignin content and greater digestibility of residual fiber, which can improve animal performance and feed efficiency.

In beef cattle production systems, the choice of protein source is strategic, especially during physiologically demanding phases such as growing and finishing (Greenwood, 2021). Protein not only meets maintenance requirements but also directly influences muscle tissue deposition, nitrogen (N) utilization efficiency, and productive performance. Sources with higher RUP content and balanced essential amino acid profiles can optimize protein synthesis and reduce urinary N losses (Schingoethe et al., 2009; Dias et al., 2024).

DDG, in turn, has a variable composition depending on the raw material and industrial processing, with CP contents ranging from 25 to 50% of DM, and high levels of amino acids such as leucine, glutamate, valine, and methionine (Espinosa and Stein, 2018). HP-DDG, in particular, also contains a significant amount of sulfur, due to the use of sulfuric acid in the industrial process, which may affect ruminal microbiota and fermentative processes (Uwituze et al., 2011). Despite this, its inclusion in ruminant diets has shown positive effects on N utilization efficiency, reduction of urinary excretion, and stimulation of molecular pathways related to anabolism, such as IGF1–mTOR–FOXO1 signaling (Rehman et al., 2023; Fernandes et al., 2024).

In the finishing phase, partial or total replacement of soybean meal and urea with HP-DDG in high-concentrate diets has proven effective in maintaining animal performance while promoting relevant metabolic adjustments (Dias et al., 2024). These adjustments include reduced urinary N excretion, decreased methane and CO_2 emissions, and changes in hepatic and muscle gene expression, such as *GOT1* and *FOXO1*, related to amino acid and protein metabolism (Rosa e Silva et al., 2024; Fernandes et al., 2024). A study conducted by Rosa e Silva et al. (2022) showed that increasing DDG inclusion improved fiber digestibility, nitrogen retention, and productive performance, as a result of greater RUP supply.

Therefore, our study aimed to evaluate: 1 - the effects of supplementation with distillers' dried grains (DDG) containing different amino acid concentrations (AA(+); higher density of essential and non-essential amino acids, and AA(-); lower density of essential and non-essential amino acids) on the productive performance of Nellore bulls during the backgrounding phase in an intensive feedlot system; 2 - evaluate the effects of partially or totally replacing soybean meal plus urea (SMU) with high-protein dried distillers' grains (HP-DDG) on animal performance, nutrient digestibility, microbial efficiency, nitrogen balance, carcass traits, greenhouse gas emissions, and the expression of genes related to protein metabolism in Nellore bulls during the finishing phase.

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CHAPTER 1 - Effects of supplementation with distillers' dried grains containing different amino acid concentrations: impacts on cattle performance during the backgrounding phase

ABSTRACT

This study evaluated the effects of supplementation with distillers dried grains (DDG) containing different amino acid concentrations (AA(+), higher density of essential and non-essential amino acids; AA(−), lower density of essential and non-essential amino acids on the productive performance of Nellore bulls during the backgrounding phase in an intensive feedlot system. Forty-eight Nellore steers (8 ± 1.0 months of age; 219.74 ± 24.70 kg body weight) were assigned to two dietary treatments: 1.00 kg/animal/day of AA(+) DDG, or 1.00 kg/animal/day of AA(−) DDG plus 0.110 kg/animal/day of urea to ensure isonitrogenous diets. The experimental period lasted 68 days (12 days of adaptation, 56 days of data collection). Nutrient intake did not differ ($P > 0.05$) between treatments, except for amino acids, which were greater ($P < 0.05$) in animals fed AA(+) DDG. Digestibility of dry matter, ash- and protein-free neutral detergent fiber, and organic matter was higher ($P < 0.05$) with AA(+) DDG, while non-fibrous carbohydrates, crude protein, and ether extract digestibility were unaffected. Urinary and fecal nitrogen were lower ($P < 0.05$), and nitrogen retention and retained-to-consumed nitrogen ratio were higher ($P < 0.05$) with AA(+) DDG, whereas nitrogen intake and absorbed nitrogen were unchanged. Microbial efficiency was not affected ($P > 0.05$). Animals fed AA(+) DDG had greater ($P < 0.05$) final shrunk body weight, total shrunk weight gain, and average daily gain compared with those fed AA(−) DDG, indicating improved productive performance and supporting the potential of AA(+) DDG as a viable alternative to conventional protein sources in intensive backgrounding systems.

Keywords: beef, DDG, feed efficiency, nutrient utilization, zebu.

INTRODUCTION

In recent years, feedlot systems in tropical regions have increasingly adopted the practice of transitioning weaned calves directly into feedlot systems without an intermediate stocking or backgrounding phase in pasture (Nascimento, 2021). This approach serves as a nutritional strategy aimed at shortening the production cycle and enhancing profitability by accelerating growth and optimizing feed efficiency. Within this system, post-weaned calves are initially placed on a high-forage diet for approximately 50 to 70 days (Pucetti, 2024). By implementing this strategy, producers seek to improve feed conversion efficiency, reduce overall time to market, and maximize carcass quality, making it an increasingly popular approach in intensive beef production systems in tropical climates (Costa et al., 2018; Roth et al., 2019).

Physiologically, the backgrounding phase is characterized by high protein requirements, mainly due to rapid muscle growth, making the use of high-quality protein sources essential to maximize productive efficiency. In this context, maintaining a nutrient balance, especially of amino acids (AAs), is crucial (Chen et al., 2021). The ethanol industry has evolved over time and now produces several types of distillers' dried grains (DDG; Espinosa and Stein, 2018), including those obtained through "front-end" fractionation, a technology that removes the fibrous portion of the grain prior to the fermentation process. This separation increases the concentration of starch and protein in the remaining fraction, which, after fermentation and drying, results in a high-protein DDG, commonly referred to as high protein DDG, with greater concentrations of essential and non-essential amino acids (AA(+) DDG) and a higher proportion of rumen-undegradable protein (RUP), which improves nitrogen utilization and microbial protein synthesis (Liu and Rosentrater, 2012; Chen et al., 2021).

These characteristics make AA(+) DDG a promising ingredient for backgrounding diets, which typically contain lower proportions of concentrate and require high-quality protein sources to support optimal growth performance (Batistelli et al., 2022). In contrast, low-protein DDG, resulting from less selective fractionation or standard ethanol production, remains widely available and economically attractive. However, its lower amino acid and RUP content may limit its effectiveness unless combined with non-protein nitrogen sources, such as urea, to formulate isonitrogenous diets. Therefore, evaluating both AA(+) and AA(−) DDG in balanced diets becomes essential not only to optimize animal performance and nitrogen efficiency but also to promote the sustainable use of agro-industrial byproducts in intensive beef production systems.

Despite *front-end* DDG's nutritional potential, there are still uncertainties regarding its actual impact on animal performance, particularly the influence of fiber pre-fractionation and protein composition compared to DDG without the same processing.

Thus, we hypothesized that supplementing 1 kg/animal/day of AA(+) DDG (obtained through front-end processing) promotes greater growth performance and better nitrogen use efficiency compared to DDG without front-end processing (AA(−) DDG) in Nellore cattle during the backgrounding phase. Therefore, this study aimed to evaluate the effects of supplementation with AA(+) DDG or AA(−) DDG on productive performance and nitrogen utilization in Nellore bulls during the backgrounding phase in an intensive feedlot system.

MATERIALS AND METHODS

Animal care

The Ethics Committee on the Use of Production Animals at the Universidade Federal de Viçosa approved all animal-related procedures (protocol N° 27/2024).

Animal handling, experimental design, and diets

The experiment was conducted at the Experimental Feedlot of the Department of Animal Science at the Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais, Brazil.

Forty-eight Nellore bulls with an average age of 8 ± 1.0 months and an average body weight (BW) of 219.74 ± 24.70 kg were utilized. The experiment was conducted as a completely randomized design and evaluated two types of supplemented dried distiller's grains (DDG) as a concentrated source containing different amino acid concentrations: AA(+) DDG; higher density of essential and non-essential amino acids, and AA(-) DDG; lower density of essential and non-essential amino acids, due to the difference in crude protein (CP) content between the two DDG types, urea was added as a protein source to standardize the dietary protein level. Consequently, the amount of each DDG supplied on an as-fed basis differed in absolute terms between treatments; however, the diets remained isoproteic. The treatments consisted of: group 1 received 1 kg/animal/day of AA(+) DDG, and group 2 received 1.00 kg/animal/day of AA(-) DDG plus 0.110 kg/animal/day urea to balance protein content, both on an as-fed basis. The study lasted 68 days, including a 12-day adaptation period for the bulls to the environment and diets, followed by 56 days of data collection (Pucetti, 2024).

Bulls were identified, weighed, treated for endo and ectoparasites, and housed in a 75 m² collective pen with concrete floors per group. Each pen was equipped with three electronic feeders and three electronic drinkers (model AF-1000 Master; Intergado Ltda., Contagem, Minas Gerais, Brazil; Chizzotti et al., 2015). Animals were randomly assigned to two groups: Group 1 (24 animals, AA(+) DDG) and Group 2 (24 animals, AA(-) DDG).

The animals were adapted for 12 days, during which all bulls were gradually acclimated to the experimental facilities, social interactions within their respective groups, and management routines. From the first day of adaptation, the total amount of concentrate (1 kg of DDG/animal/day), corresponding to each treatment, was provided in full, without progressive increases. Forage (corn silage) was offered *ad libitum* throughout the experimental period. At the end of the adaptation period, the bulls were weighed after a 16-hour solid fasting period to obtain shrunk body weight (SBW).

The diets were formulated to meet animals' requirements according to the BR-CORTE system (Valadares Filho et al., 2023), targeting an average daily gain of 1.30 kg/d (Table 2). Corn silage and DDG were weighed separately and then mechanically mixed at the time of feeding. Such a total mixed ration was provided ad libitum twice a day (07:00 and 16:00 hours), and the bulls had free access to water. The diet was adjusted daily to allow approximately 5% refusal of the offered total on an as-fed basis. Refusals were mixed with the feeds that were offered the next day.

The bulls were weighed every 28 days, without fasting, to monitor the average daily gain (ADG). To quantify their total shrunk body weight gain (TSBWG), ADG, and performance, the first (iSBW) and last (fSBW) body weights (Days 1 and 56, respectively) were used, which were preceded by a 16-h solid fasting period.

Sampling and digestibility assays

Corn silage was sampled daily and stored at –20°C. Corn silage samples were combined weekly (percent as-fed basis), dried in a forced-air oven (55°C) for 72 h, and ground through a 2- and 1-mm screen (Fortinox, Piracicaba, São Paulo, Brazil) and stored at room temperature for further analyses. Next, the total DM of these samples was evaluated according to AOAC (AOAC, 2012) by using method 934.01. Regarding the corn silage samples, composite samples were prepared proportionally for each experimental period. The DDG used as the concentrate source for each treatment was sampled directly from the feed mill silos on the days the DDG was transported to the feedlot. These ingredients were analyzed and used to calculate the diet composition.

To evaluate the total apparent digestibility of nutrients, N balance, and microbial synthesis, spot fecal and urine samples were collected from all bulls on d 41 to 45. Fecal samples were collected during five consecutive days at 18:00 on d 1, at 15:00 on d 2, at 12:00 on d 3, at 09:00 on d 4, and 06:00 on d 5 (Zanetti et al., 2020; Andrade et al., 2023). Urine samples were collected at 12:00 on d 3 and at 06:00 on d 5. Fecal samples were obtained directly from the rectum of the animals. Approximately 200 g of feces were collected per sampling time and oven-dried (55 °C for 72 h). Subsequently, a composite sample was performed for each animal in each collection period. The indigestible neutral detergent fiber (iNDF) was used as a marker to estimate the fecal DM production and then calculate the apparent digestibility of nutrients.

The urine was collected using collection cups. At the end of each collection, two urine samples were taken. We obtained a 10-mL sample that was diluted with 40 mL of 0.036 N H_2SO_4 to avoid losses of purine derivatives. Another 20-mL sample was collected without dilution to quantify the total N content. Subsequently, for both cases, a composite was performed per animal and collection period. Urine composite samples were stored at -20°C until further analysis.

Laboratory analysis and calculations

The samples of corn silage, DDG, and feces were analyzed for their contents of DM (AOAC, 2012;method 934.01), organic matter (OM; AOAC, 2012; method 930.05), total N (AOAC, 2012; method 981.10), ethereal extract (EE; AOAC, 2006; method 945.16), ashes (MM; AOAC, 1990; method 924.05), and neutral detergent fiber (NDF; Mertens et al., 2002). The NDF quantification was performed without the addition of sodium sulfite but with the addition of thermostable alpha-amylase to neutral detergent (Ankom Tech. Corp., Fairport, NY). The NDF concentrations were corrected for ash (Mertens et al., 2002)and residual N compounds (Licitra et al., 1996). We calculated crude protein (CP) content as the product of total N content and the factor 6.25. The iNDF content was determined according to Valente et al. (2011). Non-fibrous carbohydrates (NFC) were calculated according to Detmann and Valadares Filho (2010) using the equation: NFC = 100 – [(% CP diet - % CP derived from urea + % urea) + % NDF + % EE + % MM]. The contents of total digestible nutrients (TDN) were estimated through the sum of the digestible nutrients, where TDN = digestible CP + 2.25 × digestible EE + digestible NDF + digestible NFC (NRC, 2001). For the amino acid composition of corn silage, tabulated data were obtained from the Feedipedia platform – AFROS/FAO, an international database on feed ingredients for animal nutrition.

Urine samples were analyzed for creatinine, uric acid, and allantoin. The analyses of uric acid and creatinine were performed by using an automatic biochemical analyzer (Minudray/model BS200E, Shenzhen, China), whereas the allantoin analysis was performed according to the colorimetric method described by Chen and Gomes (1992).

The daily excretion of creatinine (EC; g/d) was calculated based on the following equation EC (g/d) = 0.0345 × $EBW^{0.9491}$ × 1000, where EBW = empty body weight; (Costa and Silva et al., 2012).Then, the daily urinary volume (L/d) was estimated from the ratio between the estimated EC (g/d) and creatinine concentrations in the spot urine sample (g/L).

The total excretion of purine derivatives was calculated by the sum of the amounts of allantoin and uric acid excreted in the urine, which were obtained by the product between their concentration in the urine and the urinary volume. Absorbed purines and ruminal synthesis of nitrogen compounds were calculated according to Barbosa et al. (2011).

Amino acid and sulfuric acid analyses were performed in a private laboratory. The amino acid composition, except for tryptophan, was determined by high-performance liquid chromatography (HPLC) after pre-column derivatization with phenylisothiocyanate (PITC), according to the methods described by White et al. (1986) and Hagen et al. (1989). This technique allows for the separation and quantification of amino acids through spectrophotometric detection of phenylthiocarbamyl derivatives. The tryptophan content was determined using a colorimetric method adapted from Lucas and Sotelo (1980) , which involves alkaline hydrolysis followed by reaction with specific reagents and spectrophotometric quantification (Figures 1 and 2).

Total sulfur content was analyzed by combustion in a high-temperature tube furnace, following the ASTM D4239-18e1 (2018) standard. The method involves complete oxidation of sulfur in the sample and quantification by infrared detection (Figure 3)

The crude microbial protein (MCP; g/d) was calculated by the product of the ruminal synthesis of nitrogen compounds and factor 6.25. The microbial efficiency was calculated by the ratio between the MCP and TDN and digested OM (DOMI) intakes expressed in g MCP/kg TDN and g MCP/kg DOMI.

The N balance of the animals was also calculated. For this purpose, we calculated the intake and excretion of fecal N (g/day) as the ratio of N content in the diets (by DM basis) and fecal DM excretion (g/day), respectively. In addition, urinary N excretion (g/day) was estimated based on chemical analysis, with values multiplied by the estimated urinary volume (Costa e Silva et al., 2012). Retained N was calculated as the difference between N intake and the sum of fecal N and urinary N. Absorbed N was calculated as N intake minus fecal N.

Statistical analysis

Data were analyzed in a completely randomized design by PROC MIXED of SAS (version 9.4):

$$Y_{ij} = \mu + T_i + e_{ij}$$

where: $\boldsymbol{\mu}$ = general constant; $\mathbf{T_i}$ = fixed effect of diet; and $\mathbf{e_{ij}}$ = random error with zero mean and variance σ^2. Outliers were identified in this study; the criterion adopted for identifying outliers was based on the normal distribution curve, in which values of Student's standardized residuals greater than |3| were considered as outliers and, therefore, removed from the respective database. Effects were considered significant when $P \leq 0.05$.

RESULTS AND DISCUSSION

The amino acid and sulfur composition of AA(+) DDG and AA(-) DDG

Figures 1,2, and 3 present the amino acid composition and sulfur content (%) of the DDG samples and corn silage: AA(+) DDG and AA(-) DDG. The AA(+) DDG showed higher levels of all analyzed amino acids compared to the AA(–) DDG. The greatest numeric differences were observed for glutamic acid (9.83 vs. 3.14%), aspartic acid (3.34 vs. 1.39%), alanine (3.94 vs. 1.37%), and proline (4.89 vs. 1.44%). Leucine also presented notably high values in both DDG types, with 6.86% in AA(+) DDG and 1.91% in AA(–) DDG (Figures 1 and 2), which is expected due to the naturally high leucine content in corn grain, the primary raw material for DDG production (Penz Júnior and Gianfelice, 2008). Leucine plays a key role in growing beef cattle, particularly by regulating muscle growth and stimulating protein synthesis through the activation of the mTOR pathway (Rehman et al., 2023) and is essential for lean tissue deposition.

The total amino acid content was nearly three times greater in the AA(+) DDG (51.41%) compared to the AA(–) DDG (17.70%). This difference was also reflected in total sulfur content (0.77 % vs. 0.54 %, Figure 3). These values are attributed to the use of sulfuric acid during the industrial processing of DDG (Uwituze et al., 2011). However, sulfur presence is also essential for the microbial synthesis of sulfur-containing amino acids such as methionine and cystine. According to the National Research Council (NRC, 1996), the recommended sulfur concentration in beef cattle diets is 0.15% of dry matter (DM), while levels above the tolerance threshold of 0.4% may negatively affect animal performance. In the present study, the sulfur content did not exceed 0.22% of DM, remaining well within the recommended tolerance limits.

Intake and apparent digestibility of nutrients

The observed forage-to-concentrate (F:C) ratio was 83:17 for bulls receiving the AA(+) DDG and 82:18 for those fed the AA(–) DDG (Table 2). These differences were attributed primarily to the variation in dry matter content between the two DDG sources (Table 1).

The DM and nutrient intake were not influenced (P > 0.05) by the type of DDG (Table 3). However, due to the differences in amino acid concentration between the DDG sources, the amino acid intake differed between treatments, with a higher amino acid supply observed in the AA(+) DDG group (P < 0.05). The primary objective of using DDG in cattle diets is to provide an alternative protein source in the concentrate (Renesto, 2021) depending on the type of processing, variations in composition, digestibility, and palatability can be observed (Koreleski, 2008; Nuez Ortín and Yu, 2009 and Santos, 2023) which directly affects intake and, consequently, animal performance. Additionally, the DM intake relative to shrunk body weight (SBW) of the animals in the treatments was similar, at 1.97% and 1.90%, respectively. Other studies using DDG during the growing phase also found no variation in DM intake (Griffin et al., 2012; Renesto, 2021).

The apparent digestibility of DM, OM, and apNDF was influenced (P < 0.05; Table 4) by the type of DDG supplemented. For DM, OM, and apNDF digestibility, the AA(+) DDG presented higher values (685.80, 700.30, and 711.50 kg/d, respectively) than the AA(-) DDG (654.30, 670.10, and 694.80 kg/d). In a pasture study, the inclusion of DDG at levels between 150 and 200 g/kg in the dry matter of the diet of cattle improved the digestibility of nutrients, mainly the digestibility of DM, OM, and apNDF (Picanço et al., 2024), corroborating the results found in our study. This may be related to nutritional profile and greater degradability, especially of NDF, which may have increased the passage rate and consequently greater digestibility for AA(+) DDG. In addition, it is worth mentioning that the use of corn silage as roughage is another factor that may have influenced the higher digestibility values, especially of DM (69% and 65%, respectively), since digestibility is dependent on the quality of the roughage used, and generally high-quality roughages such as corn silage tend to have greater digestibility (Cabral et al., 2006). The digestibility of apNDF may vary according to the composition of DDG, which is influenced by the production process. As digestibility is an intrinsic characteristic of the feed, factors such as chemical composition and physical structure are decisive. In the present study, greater apNDF digestibility (P = 0.006) was observed for the AA(+) DDG compared to the AA(-) DDG. This effect may be related to the additional fractionation steps used in the technology known as FST™ (Fiber Separation Technology™), which involves fiber separation before fermentation (Hoffmann, 2019). The use of this technology concentrates protein content, potentially reducing lignin and/or improving the proportion of digestible fiber (AA(+) DDG). In contrast, the AA(-) DDG (removed during this pre-fermentation step) may retain more fibrous residues with higher lignin content, which could have negatively impacted apNDF digestibility.

The type of DDG had no effect (P > 0.05) on the apparent digestibility of CP, EE and NFC (Table 4). A difference in crude protein (CP) and non-fibrous carbohydrates (NFC) digestibility was expected between treatments, considering the differences in the nutritional composition of the DDG sources used (Table 1).

The AA(+) DDG presented a greater concentration of total amino acids (Figure 3), which would theoretically favor CP digestibility and the supply of metabolizable protein (Schingoethe et al., 2009). On the other hand, the inclusion of urea in the diet containing AA(-) DDG + urea could stimulate microbial protein synthesis in the rumen, provided there was adequate synchronization with the availability of fermentable energy (Satter and Roffler, 1975; Hoover and Stokes, 1991). Therefore, these two factors, greater supply of amino acids in the intestine and enhanced microbial protein synthesis in the rumen, may have contributed to the similar digestibility coefficients observed between the CP treatments.

In the case of NFC, although the AA(-) DDG presented a higher total content of non-fibrous carbohydrates (NFC), the AA(+) DDG, due to its processing method (FST™), tends to contain more fermentable fractions of these carbohydrates. This is particularly true for DDG products that undergo front-end fractionation technologies such as FST™, which result in lower concentrations of insoluble fiber and greater availability of digestible nutrients (Christen et al., 2010). Theoretically, these compositional differences could positively influence NFC digestibility. However, the inclusion of urea in the diet containing AA(-) DDG may have promoted a favorable ruminal environment for amylolytic and saccharolytic bacterial activity, provided that fermentable energy and available nitrogen were adequately synchronized (Hoover and Stokes, 1991), thus resulting in no significant differences in NFC digestibility between treatments.

For ether extract (EE) digestibility, the lack of treatment effect may be attributed to the similar EE content between the DDG sources (Table 1) and across the experimental diets (Table 2). According to Li et al. (2011), when EE levels in the diets are comparable, differences in the digestibility of this nutrient tend to be minimal.

Nitrogen balance and microbial efficiency

Supplementation with different types of DDG did not affect nitrogen intake, and absorption of nitrogen (g/d) ($P > 0.05$; Table 4). Similar results were reported by Rosa e Silva et al. (2024) and are consistent with the findings of the present study. The authors partially attributed the absence of effects on nitrogen intake and absorption to the composition of the DDG used, particularly the high proportion of RUP (rumen undegradable protein), which provided greater amino acid supply to the small intestine without necessarily increasing the rumen-degradable fraction (RDP), essential for microbial protein synthesis. Additionally, the urea present in the AA(-) DDG diet contributed non-protein nitrogen (NPN), which, when properly synchronized with fermentable energy, can be efficiently utilized.

However, urinary N, fecal N, retained N, and the ratio between retained N and ingested N were affected by the type of DDG supplemented ($P \leq 0.05$). Although the diets were isoproteic, the biological value of the protein in each type of DDG can influence these variables. AA(+) DDG has a higher total amino acid content (Figure 3), making it more efficiently used by the animal for body protein synthesis. In contrast, AA(-) DDG has a lower amount of amino acids and a lower concentration of protein that is not fully utilized (resulting in lower N retention). This unused protein can be deaminated and converted to urea, increasing N excretion in the urine (Van Soest, 1994; Caldas Neto et al., 2008).

The AA(+) DDG diet resulted in lower fecal and urinary N excretion compared to the AA(-) DDG diet ($P \leq 0.05$). This reduction in N excretion suggests a more efficient utilization of ingested N, possibly due to the greater availability of absorbable amino acids from the AA(+) DDG (Figure 3), which reduces the need for deamination and subsequent excretion of N in the urine and feces (Rosa e Silva et al., 2024). The findings of Qin et al. (2025) are consistent with our results. The authors evaluated three diets with different CP levels, with or without supplementation of rumen-protected amino acids (isoleucine, leucine, methionine, and threonine) in lactating dairy cows. They observed that the diet with the lowest CP level supplemented with protected amino acids maintained milk production while drastically reducing nitrogen excretion, indicating greater nitrogen utilization efficiency compared to diets with higher CP levels without amino acid supplementation.

In addition, N retention and the ratio of retained N to ingested N were higher in the AA(+) DDG diets ($P < 0.05$; Table 4). This indicates that a greater proportion of ingested N was used for body protein synthesis, reflecting a better efficiency in the utilization of dietary

N. The presence of RUP in AA(+) DDG provides directly absorbable amino acids in the small intestine, which may improve N retention in body tissues (Souza et al., 2021).

The production of microbial crude protein (MCP) and the microbial efficiency, both expressed to TDN (MCPtdn) and DOMI (MCPdomi) intakes, were not influenced (P > 0.05) by supplementation with DDG sources with different protein levels in the backgrounding phase (Table 4). The results suggest that, despite the increased amino acid flow due to the composition of the AA(+) DDG, there was no increase in microbial nitrogen flow or microbial efficiency (g of microbial N/kg of digested OM and kg of TDN remained unchanged; P > 0.05). In other words, the AA(+) DDG primarily contributed *bypass* protein and rumen-undegraded nitrogen, reducing the proportion of degraded N that was effectively incorporated into microbial protein. Conversely, the urea content in the AA(–) DDG was sufficient to equalize microbial efficiencies between the two treatments. Similarly, (Montano et al., 2023), evaluating the partial replacement of steam-flaked corn (SFC) with increasing levels of dried distillers' grains with solubles (DDGS) in growing and finishing diets for Holstein steers, also reported no treatment effect on ruminal microbial efficiency (P = 0.79).

Animal performance

fSBW, TSBWG, and ADG were significantly influenced by DDG type (P ≤ 0.05), and were higher in animals supplemented with AA(+) DDG compared to animals supplemented with AA(-) DDG (Table 5). The supplementation with two types of DDG with different amino acid concentrations did not influence the DMI of the animals. However, the AA(+) DDG allowed for an increase in weight gain of approximately 19 kg compared to animals supplemented with the AA(-) DDG. The diets were isoproteic, which implies that the amino acid profile was important for the gain increase. Furthermore, it is important to emphasize that the different amino acid concentrations of essential amino acids, especially lysine, methionine, threonine, leucine, valine, and isoleucine, are stimulants of muscle synthesis, optimizing weight gain.

Leucine, for example, plays a significant role in stimulating protein synthesis through the signaling pathway of the mammalian target of rapamycin complex 1 (mTORC1), one of the main cellular regulators of growth, protein synthesis, cell proliferation, and energy metabolism(Duan et al., 2016; Li et al., 2011; Rehman et al., 2023). It acts by stimulating the activation of mTORC1, facilitating its translocation to the lysosomal surface, where factors such as insulin and IGF-1 further enhance its activation. This leads to increased myofibrillar protein synthesis, enhanced ribosome biogenesis (which is essential for expanding translational capacity), and promotes muscle cell differentiation and hypertrophy (Liu and Sabatini, 2020). Practically, by directly stimulating muscle protein synthesis, leucine contributes to greater lean tissue deposition, improved feed efficiency, and a potential increase in average daily gain (ADG), particularly during periods of rapid growth such as backgrounding and finishing phases, ultimately resulting in greater final body weight gain. Thus, an additional daily gain of 300 g/day is a crucial factor to consider when using this type of product, as this gain can result in a shorter feedlot while producing high-quality meat (Griffin et al., 2012).

CONCLUSION

Overall, supplementation with AA(+) DDG improved the productive performance of Nellore bulls during the backgrounding phase in an intensive feedlot system, while also increasing nitrogen use efficiency, resulting in lower fecal and urinary excretion and greater nitrogen retention. These results indicate that the use of AA(+) DDG contributes to more efficient and sustainable production systems, with reduced nutrient waste and lower potential environmental impact.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

The authors declare that they did not use any generative AI and AI-assisted technologies in the writing process.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Table 1 - Chemical composition of ingredients used in experimental diets

Ingredients	DM[1]	OM[2]	CP[3]	EE[4]	apNDF[5]	NFC[6]	iNDF[7]
		g/Kg DM					
Corn silage	265.33	944.17	77.10	27.20	589.50	289.65	140.40
AA(+) DGG[9]	922.97	976.46	507.56	88.38	297.41	83.11	10.46
AA(-) DGG[9]	906.86	966.94	215.22	83.77	511.99	155.95	41.95
Ureia + AS[8]	976.18	997.55	2782.22	-	-	-	-
Mineral premix[10]	983.24	84.93	-	-	-	-	-

[1]Dry matter; [2]Organic matter; [3]Crude protein; [4]Ethereal extract; [5]Neutral detergent fiber corrected for ash and crude protein; [6]Non-fibrous carbohydrates; [7]Indigestible neutral detergent fiber; [8]Urea/ammonium sulfate (AS) ratio of 9:1.

[9]The treatments were: AA(+) DDG = supplementation with 1 kg of AA(+) DDG, which is a higher density of essential and non-essential amino acids; AA(-) DDG = supplementation with 1.00 kg/animal/day of AA(-) DDG which is a lower density of essential and non-essential amino acids + 0.110 kg/animal/day urea/AS, for the diets to be isoproteic.

[10]Basic composition of the product: Calcitic limestone; Sodium chloride (common salt); Ventilated sulfur; Monodicalcium phosphate; Autolyzed yeast from sugar cane; Magnesium oxide; Raspberry aroma; Mixed herbal aroma; Silicon dioxide; Onion extract; Grape seed extract; Calcium iodate; Narasina; Manganese oxide; Zinc oxide; Chromium propionate; Sodium Selenite; Cobalt sulfate; Copper sulfate; Iron sulfate; Vitamin A; Vitamin D3; Vitamin E.

[10]Guarantee levels per kg of product: Mixed herbal aroma (min) 19.00MG; Onion extract (min) 34.00MG; Grape seed extract (min) 6.00MG; Narasina 417.00MG; Crude Protein (min) 0.00MG; Calcium (min) 162.00G; Calcium (max) 300.00G; Sulfur (min) 15.00G; Ethereal extract (min) 0.00MG; FDA (max) 0.00MG; Crude fiber (max) 45.00MG; Fluoride (max) 457.49MG; Phosphorus (min) 23.00G; Magnesium (min) 15.00G; Mineral matter (max) 1395.53G; Humidity (max) 24.46G; Sodium (min) 55.00G; Cobalt (min) 15.00MG; Copper (min) 335.00MG; Chromium (min) 11.10MG, Iron (min) 560.00MG; Iodine (min) 16.70MG; Manganese (min) 667.00MG; Selenium (min) 3.35MG; Vitamin A (min) 73,340.00 IU; Vitamin D3 (min) 9,200.00 IU; Vitamin E (min) 500.00 IU; Zinc (min) 1000.00MG.

Table 2 - Proportions of ingredients and chemical composition of nutrients in experimental diets

Item	Concentrate[2]		Diets[2]	
	AA(+) DDG	AA(-) DDG	AA(+) DDG	AA(-) DDG
	Proportion of ingredients (g/kg DM)			
Corn silage	-	-	829.72	816.15
AA(+) DDG	921.70	-	156.95	-
AA(-) DDG	-	827.76	-	152.18
Ureia + AS[3]	-	99.72	-	18.33
Mineral premix[45]	78.30	72.52	13.33	13.33
	Chemical composition (g/kg DM)			
DM[1]	927.43	918.54	301.98	304.28
OM[1]	906.66	906.05	937.80	937.31
CP[1]	467.81	455.55	147.42	149.08
EE[1]	81.46	69.35	36.42	34.79
apNDF[1]	274.13	423.83	535.89	559.68
NFC[1]	76.60	129.10	253.43	260.75

[1]DM = Dry matter; OM = Organic matter; CP = Crude protein; EE = Ether extract; apNDF = Neutral detergent fiber corrected for ash and crude protein; NFC = Non-fibrous carbohydrates.

[2]The treatments were : AA(+) DDG = supplementation with 1 kg of AA(+) DDG, which is a higher density of essential and non-essential amino acids; AA(-) DDG = supplementation with 1.00 kg/animal/day of AA(-) DDG which is a lower density of essential and non-essential amino acids + 0.110 kg/animal/day urea/AS, for the diets to be isoproteic.

[3]Urea/ammonium sulfate (AS) ratio of 9:1. To correct the crude protein in the AA(-) DDG treatment, 110g/d of urea with ammonium sulfate was added.

[4]Basic composition of the product: Calcitic limestone; Sodium chloride (common salt); Ventilated sulfur; Monodicalcium phosphate; Autolyzed yeast from sugar cane; Magnesium oxide; Raspberry aroma; Mixed herbal aroma; Silicon dioxide; Onion extract; Grape seed extract; Calcium iodate; Narasina; Manganese oxide; Zinc oxide; Chromium propionate; Sodium Selenite; Cobalt sulfate; Copper sulfate; Iron sulfate; Vitamin A; Vitamin D3; Vitamin E.

[5]Guarantee levels per kg of product: Mixed herbal aroma (min) 19.00MG; Onion extract (min) 34.00MG; Grape seed extract (min) 6.00MG; Narasina 417.00MG; Crude Protein (min) 0.00MG; Calcium (min) 162.00G; Calcium (max) 300.00G; Sulfur (min) 15.00G; Ethereal extract (min) 0.00MG; FDA (max) 0.00MG; Crude fiber (max) 45.00MG; Fluoride (max) 457.49MG; Phosphorus (min) 23.00G; Magnesium (min) 15.00G; Mineral matter (max) 1395.53G; Humidity (max) 24.46G; Sodium (min) 55.00G; Cobalt (min) 15.00MG; Copper (min) 335.00MG; Chromium (min) 11.10MG, Iron (min) 560.00MG; Iodine (min) 16.70MG; Manganese (min) 667.00MG; Selenium (min) 3.35MG; Vitamin A (min) 73,340.00 IU; Vitamin D3 (min) 9,200.00 IU; Vitamin E (min) 500.00 IU; Zinc (min) 1000.00MG.

Table 3 - Dry matter, nutrient, and amino acids intake of Nelore bulls supplemented with AA(+) and AA(-) DDG in the backgrounding phase

Item[1]	Treatments[2]		SEM	P-Valor
	AA(+) DDG	AA(-) DDG[3]		
DM. kg/day	5.07	4.71	0.165	0.125
OM. kg/day	4.75	4.41	0.155	
CP. kg/day	0.75	0.70	0.024	
EE. kg/day	0.18	0.17	0.006	
apNDF. kg/day	2.78	2.58	0.090	
NFC. kg/day	1.30	1.21	0.042	
TDN. kg/day	3.92	3.60	0.128	0.082
DM/SBW. %	1.97	1.90	0.061	0.438
Essential amino acid, g/day				
Arginine	26.09	15.36	0.607	0.001
Histidine	21.71	10.67	0.454	0.001
Isoleucine	29.81	16.05	0.657	0.001
Leucine	92.08	43.44	1.883	0.001
Lysine	20.20	13.43	0.510	0.001
Methionine	12.74	6.95	0.283	0.001
Phenylalanine	37.17	19.05	0.796	0.001
Threonine	29.02	17.33	0.682	0.001
Tryptophan	2.37	1.38	0.055	0.001
Valine	40.21	22.64	0.910	0.001
Non-essential amino acid, g/day				
Alanine	61.96	35.45	1.417	0.001
Aspartic Acid	55.14	34.50	1.336	0.001
Cystine	11.24	6.27	0.253	0.001
Glutamic Acid	132.00	65.95	2.785	0.001
Glycine	28.92	19.07	0.726	0.001
Proline	65.11	31.27	1.344	0.001
Serine	35.94	19.76	0.802	0.001
Tyrosine	31.64	14.92	0.647	0.001
Total Amino Acids, g/day	730.76	392.22	16.068	0.001

[1]DM = Dry matter; OM = Organic matter; CP = Crude protein; EE = Ethereal extract; apNDF = Neutral detergent fiber corrected for ash and crude protein; NFC = Non-fibrous carbohydrates; TDN = Total Digestible Nutrients; DOMI = Intake of digested organic matter; DM/SBW = Dry matter intake according to average shrunk body weight. %.

[2]The treatments were: AA(+) DDG = supplementation with 1 kg of AA(+) DDG, which is a higher density of essential and non-essential amino acids; AA(-) DDG = supplementation with 1.00 kg/animal/day of AA(-) DDG which is a lower density of essential and non-essential amino acids + 0.110 kg/animal/day urea/AS, for the diets to be isoproteic.

[3]Urea/ammonium sulfate (AS) ratio of 9:1. To correct the crude protein in the AA(-) DDG treatment. 110g/d of urea with ammonium sulfate was added.

Table 4 - Digestibility of dry matter and nutrients, nitrogen balance, and microbial production in Nelore bulls supplemented with AA(+) and AA(-) DDG in the backgrounding phase

Item[1]	Treatments[2]		SEM	P-Valor
	AA(+) DDG	AA(-) DDG[3]		
Digestibility of nutrients (g/kg)				
DM	685.80	654.30	0.005	< .0001
OM	700.30	670.10	0.004	< .0001
CP	777.90	763.60	0.005	0.056
EE	886.40	879.60	0.005	0.322
apNDF	711.50	694.80	0.004	0.006
NFC	762.80	781.00	0.008	0.112
Nitrogen balance				
N intake (g/day)	121.72	122.96	3.105	0.780
N feces (g/day)	26.63	29.42	0.976	0.050
N urine (g/day)	59.60	68.17	2.834	0.038
N absorbed (g/day)	95.09	93.53	2.437	0.653
N retained (g/day)	35.50	25.36	2.594	0.008
Nr:Ni	29.22	20.36	2.026	0.003
Microbial production				
MCP (g/day)	422.46	400.37	15.816	0.328
MCPtdn (MCP g/ TDN kg)	108.53	114.78	5.052	0.386
MCPdomi. (MCP g/ DOM kg)	127.73	139.74	6.109	0.171

[1]DM = Dry matter; OM = Organic matter; CP = Crude protein; EE = Ethereal extract; apNDF = Neutral detergent fiber corrected for ash and crude protein; NFC = Non-fibrous carbohydrates; N intake = Nitrogen intake ; N feces = Nitrogen excretion via feces; N urine = Nitrogen excretion via urine; N absorbed = Nitrogen absorbed; N retained = Nitrogen retained in the animal's body; Nr:Ni = ration of retained nitrogen to absorbed nitrogen; MCP = microbial crude protein; Mic. Effi. (MCP g/ TDN kg) = microbial efficiency in grams of microbial crude protein per kilogram of total digestible nutrients; Mic. Effi. (MCP g/ DOM kg) = microbial efficiency in grams of microbial crude protein per kilogram of digestible organic matter intake.

[2]The treatments were: AA(+) DDG = supplementation with 1 kg of AA(+) DDG, which is a higher density of essential and non-essential amino acids; AA(-) DDG = supplementation with 1.00 kg/animal/day of AA(-) DDG which is a lower density of essential and non-essential amino acids + 0.110 kg/animal/day urea/AS, for the diets to be isoproteic.

[3]Urea/ammonium sulfate (AS) ratio of 9:1. To correct the crude protein in the AA(-) DDG treatment. 110g/d of urea with ammonium sulfate was added.

Table 5 - Effect of supplementation with AA(+) and AA(-) DDG on animal performance of Nellore bulls in the backgrounding phase

Item[1]	Treatments[2]		SEM	P-Valor
	AA(+) DDG	AA(-) DDG[3]		
iSBW (kg)	220.44	219.04	5.095	0.847
fSBW (Kg)	297.15	278.21	5.956	0.029
TSBWG(Kg)	76.71	59.17	2.452	<.0001
ADG (Kg/day)	1.37	1.06	0.044	<.0001

[1]iSBW = Initial shrunk average body weight; fSBW = Final shrunk average body weight; TSBWG = Total shrunk body weight gain; ADG = Average daily gain

[2]The treatments were: AA(+) DDG = supplementation with 1 kg of AA(+) DDG, which is a higher density of essential and non-essential amino acids; AA(-) DDG = supplementation with 1.00 kg/animal/day of AA(-) DDG which is a lower density of essential and non-essential amino acids + 0.110 kg/animal/day urea/AS, for the diets to be isoproteic.

[3]Urea/ammonium sulfate (AS) ratio of 9:1. To correct the crude protein in the AA(-) DDG treatment. 110g/d of urea with ammonium sulfate was added.

Figure 1. – Essential amino acid composition of AA(+) DDG,AA(-) DDG and Corn Silage

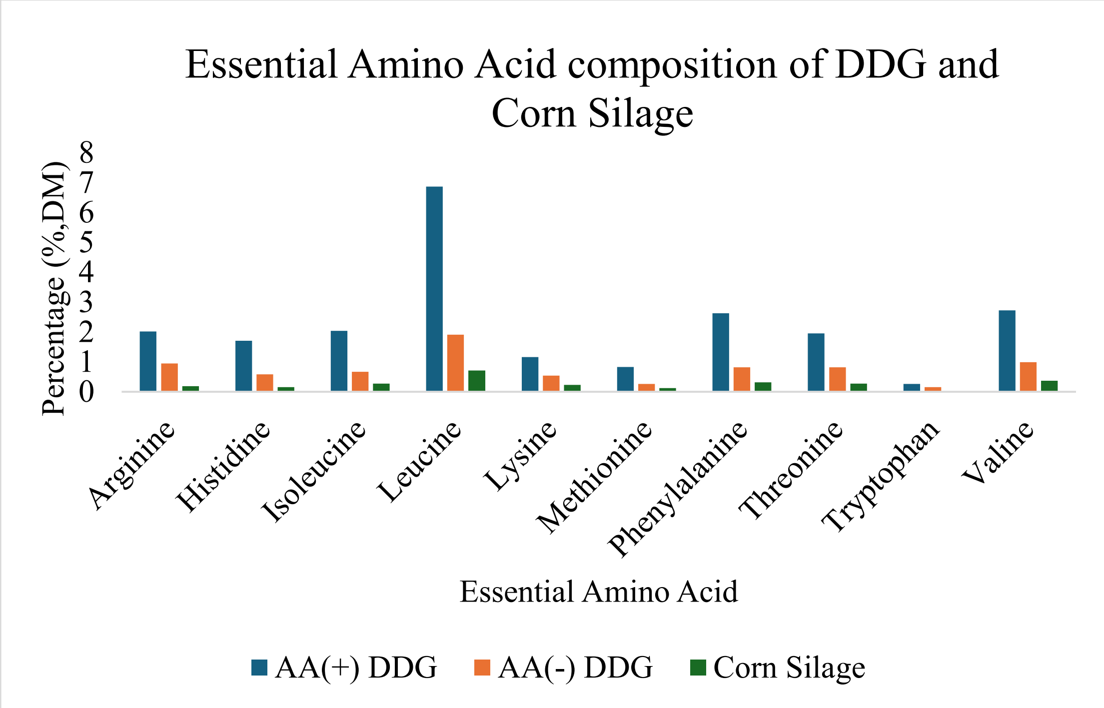

Figure 1. Essential amino acid composition (% of dry matter) of AA(+) DDG, AA(−) DDG, and corn silage: arginine – AA(+) DDG: 2.02, AA(−) DDG: 0.95, corn silage: 0.19; histidine – AA(+) DDG: 1.71, AA(−) DDG: 0.58, corn silage: 0.15; isoleucine – AA(+) DDG: 2.04, AA(−) DDG: 0.67, corn silage: 0.27; leucine – AA(+) DDG: 6.86, AA(−) DDG: 1.91, corn silage: 0.71; lysine – AA(+) DDG: 1.16, AA(−) DDG: 0.54, corn silage: 0.23; methionine – AA(+) DDG: 0.83, AA(−) DDG: 0.26, corn silage: 0.12; phenylalanine – AA(+) DDG: 2.63, AA(−) DDG: 0.82, corn silage: 0.32; threonine – AA(+) DDG: 1.95, AA(−) DDG: 0.82, corn silage: 0.27; tryptophan – AA(+) DDG: 0.26, AA(−) DDG: 0.15, corn silage: not detected; valine – AA(+) DDG: 2.72, AA(−) DDG: 0.99, corn silage: 0.37.

Figure 2 – Non-essential amino acid composition of AA(+) DDG,AA(-) DDG and Corn Silage

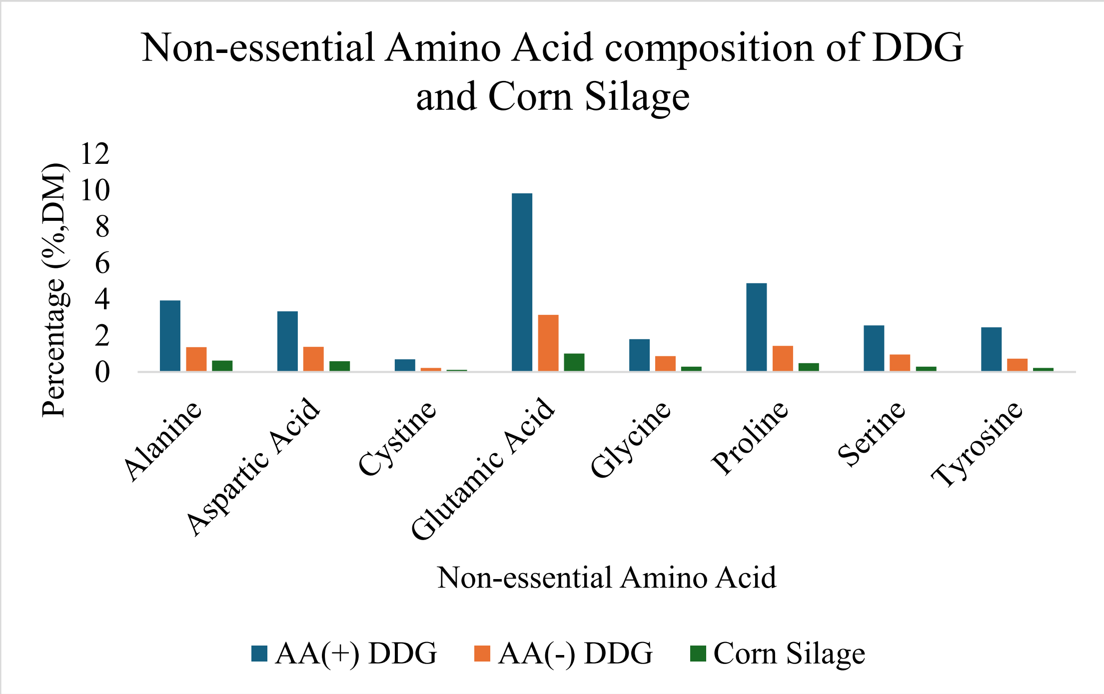

Figure 2. Non-essential amino acid composition (% of dry matter) of AA(+) DDG, AA(−) DDG, and corn silage: alanine – AA(+) DDG: 3.94, AA(−) DDG: 1.37, corn silage: 0.62; aspartic acid – AA(+) DDG: 3.34, AA(−) DDG: 1.39, corn silage: 0.59; cystine – AA(+) DDG: 0.70, AA(−) DDG: 0.22, corn silage: 0.12; glutamic acid – AA(+) DDG: 9.83, AA(−) DDG: 3.14, corn silage: 1.02; glycine – AA(+) DDG: 1.80, AA(−) DDG: 0.88, corn silage: 0.30; proline – AA(+) DDG: 4.89, AA(−) DDG: 1.44, corn silage: 0.49; serine – AA(+) DDG: 2.57, AA(−) DDG: 0.96, corn silage: 0.30; tyrosine – AA(+) DDG: 2.45, AA(−) DDG: 0.74, corn silage: 0.22; taurine and hydroxyproline were not detected in any of the ingredients.

Figure 3 – The total amino acid composition and sulfur content (%, DM basis) of the AA(+) DDG, AA(-) DDG, and Corn Silage

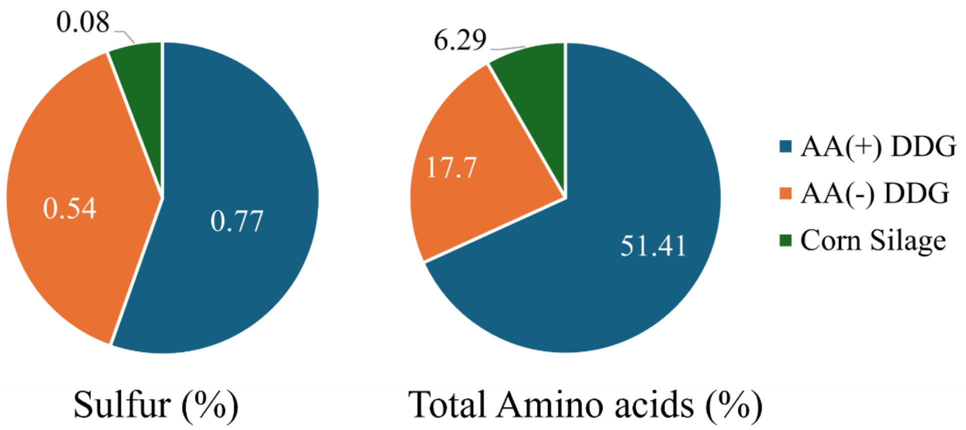

Figure 3. Total amino acid and sulfur content (% of DM basis) in AA(+) DDG, AA(−) DDG, and corn silage. The AA(+) DDG showed the highest total amino acid content (51.41%), followed by AA(−) DDG (17.70%) and corn silage (6.29%). Similarly, sulfur content was greater in AA(+) DDG (0.77%) compared to AA(−) DDG (0.54%) and corn silage (0.08%).

CHAPTER 2 - Effects of dietary inclusion of high-protein distillers dried grains as a replacement for soybean meal–urea on performance, greenhouse gas emissions, and protein metabolism–related gene expression in finishing young Nellore bulls

ABSTRACT

This study evaluated the effects of dietary inclusion of high-protein distillers dried grains (HP-DDG) replacing soybean meal–urea on performance, digestibility, nitrogen balance, microbial efficiency, carcass traits, greenhouse-gas emissions, and protein–metabolism gene expression in finishing Nellore bulls. Forty-four steers (301.3 ± 34.3 kg; 10 ± 1 months) were assigned to diets with 0, 4, 8, or 12% HP-DDG (dry matter (DM), basis) in a completely randomized design for 100 d after a 14 d adaptation. Increasing HP-DDG tended to reduce DM intake (DMI), organic matter (OM), and crude protein (CP) intakes ($P = 0.072, 0.083, 0.063$) and reduced metabolizable energy (MEI), non-fibrous carbohydrate (NFC), and rumen-degradable protein (RDP) intakes, while increasing ether extract (EE) and rumen-undegradable protein (RUP; $P < 0.05$). The apNDF intake, metabolizable protein intake, or DMI as a percentage of shrunk body weight were unaffected (SBW; $P > 0.10$). Increasing HP-DDG inclusion decreased the digestibilities of DM, OM, CP, and NFC, while increasing EE digestibility ($P < 0.05$). N intake, urinary N, and N absorption decreased, improving N-use efficiency ($P < 0.05$), with no effects on fecal N or N retention ($P > 0.10$). Microbial protein synthesis, CH_4, and CO_2 emissions were unaffected ($P > 0.10$) by treatments. Hot carcass gain decreased ($P < 0.05$), and empty body weight gain tended to decrease ($P = 0.052$) with the inclusion of HP-DDG. Gene expression showed quadratic responses for glutamate-oxaloacetate transaminase 1 ($P = 0.022$) and Forkhead box O1 ($P = 0.016$), with no changes in insulin-like growth factor 1 or AMP-activated protein kinase catalytic subunit alpha 1 ($P > 0.10$). Overall, HP-DDG altered nutrient utilization without negatively affecting performance at inclusion levels of up to 8% and is therefore recommended as a source of protein in feedlot cattle diets.

Keywords: amino acid, bypass protein, nutrient digestibility, nitrogen balance, zebu.

INTRODUCTION

In recent years, co-products from the corn-based ethanol industry, particularly dried distillers grains (DDG), have gained prominence in ruminant nutrition due to their availability, competitive cost, and nutritional value(Klopfenstein et al., 2008; Corassa et al., 2022; Parsons et al., 2023). Within this category, high-protein DDG (HP-DDG) emerges from a processing technique known as *front-end fractionation*, in which the fibrous fraction (hull) of ground corn is mostly removed before fermentation (Rosentrater and Muthukumarappan, 2006b). This process results in a co-product with higher crude protein content, most of which is rumen-undegradable protein (RUP), lower starch levels, and a greater proportion of residual lipids (Espinosa and Stein, 2018). Moreover, due to the use of sulfuric acid in the processing, the final product presents a high sulfur content, which may impact rumen microbiota and fermentation dynamics (McAllister and Newbold, 2008; Van Zijderveld et al., 2010), especially about greenhouse gas (GHG) production (Van Zijderveld et al., 2010).

These changes make HP-DDG a nutritionally distinct feed ingredient. However, its amino acid profile, characterized by high leucine levels and an imbalanced branched-chain amino acid (BCAA) ratio, may lead to nutritional imbalances, reducing protein utilization efficiency and negatively affecting feed intake and performance (D'mello, 2003; Morales et al., 2020; Hong et al., 2023).

The nutritional composition of HP-DDG, which includes high RUP content, fermentable residual fiber, and residual lipids, may significantly alter dry matter intake (DMI), nutrient digestibility, and nitrogen balance, especially when urea is removed from the diet (Espinosa and Stein, 2018; Rosa e Silva et al., 2024). Increased RUP supply favors the delivery of amino acids to the small intestine, but its efficiency depends on synchronization with available energy and an adequate amino acid profile (Allen, 2000; Benchaar et al., 2013).

In addition to nutritional and productive effects, there is growing interest in the potential environmental impact of HP-DDG use, particularly concerning greenhouse gas emissions. Studies have indicated that including DDG in ruminant diets may reduce enteric methane (CH_4) emissions by modulating the rumen microbiota and replacing fermentable carbohydrates with lipids and RUP(McGinn et al., 2009; Benchaar et al., 2013).

Moreover, evaluating gene expression of metabolic biomarkers in specific tissues can provide a more sensitive insight into diet-induced physiological adaptations, particularly those related to protein profile and supply (Abdelrahman et al., 2022; Fernandes et al., 2024). Genes such as *GOT1* (glutamate oxaloacetate transaminase 1), which is involved in hepatic gluconeogenesis, and *FOXO1* (Forkhead Box O1), a key regulator of muscle proteolysis, are influenced by variations in nitrogen intake and essential amino acid availability. Likewise, the *PRKAA1* gene, which encodes the α1 catalytic subunit of AMP-activated protein kinase (AMPK), functions as a cellular energy sensor and is modulated by changes in energy and nutrient availability (Winder, 2001; Suryawan et al., 2013).

Thus, we hypothesized that the dietary inclusion of high-protein distillers dried grains (HP-DDG) replacing soybean meal and sulfated urea (SMU) would improve animal performance, carcass characteristics, expression of genes related to protein metabolism, and reduce greenhouse gas emissions. Therefore, the objective of this study was to evaluate the effects of dietary inclusion of HP-DDG (0, 4, 8, and 12% of total dietary DM) replacing SMU on nutrient intake and digestibility, microbial efficiency, nitrogen balance, carcass traits, enteric methane (CH_4) and carbon dioxide (CO_2) emissions, and the expression of protein metabolism–related genes (*IGF1*, *FOXO1*, *PRKAA1*, and *GOT1*) in young Nellore bulls finished under feedlot conditions.

MATERIALS AND METHODS

Animal care

The Ethics Committee on the Use of Production Animals of the Universidade Federal de Viçosa approved all animal-related procedures (protocol N° 27/2024).

Animal handling, experimental design, and diets

The experiment was conducted at the Experimental Feedlot of the Department of Animal Science at the Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais, Brazil.

Forty-nine Nellore bulls with an average age of 10 ± 1.0 months and an average body weight (BW) of 301.33 ± 34.31 kg were utilized. The experiment was conducted as a completely randomized design scheme.

Generally, in Brazil, feedlots use 1% urea/AS on a DM basis to balance their diets. Sulfated urea was included to reflect the typical use of non-protein nitrogen in commercial feedlot diets for finishing cattle in Brazil (Sinclair et al., 2012). Thus, in the present experiment, a practical or conventional diet consisting of soybean meal and 1% urea/AS was used as the control treatment.

The treatments consisted of four levels of inclusion of HP-DDG based on total dietary DM, replacing soybean meal and sulfated urea (SMU). Thus, the evaluated treatments consisted of a diet without inclusion of HP-DDG (0% DDG, Control) replacing SMU, a diet with 4% inclusion of HP-DDG (4% DDG) replacing SMU, a diet with 8% inclusion of HP-DDG (8% DDG) replacing SMU, and a diet replacing the entirety of SMU, with 12% inclusion of HP-DDG (12% DDG). The experiment lasted 114 days (d), with 14 days for the bulls to adapt to the location and diets and 100 days for data collection.

Bulls were identified, weighed, treated against endo, and ectoparasites, and housed in 50 m² collective pens with concrete floors, which were equipped with electronic feeders and drinkers (model AF-1000 Master; Intergado Ltda., Contagem, Minas Gerais, Brazil;Chizzotti et al., 2015). To avoid ruminal problems, the bulls were adapted for 14 days to the final forage-to-concentrate ratio as follows: they received 50% of the total concentrate for the first 5 days, which was then increased to 65% for the next 5 days, and to 80% for the final 4 days of the adaptation period. At the end of the adaptation period, the bulls were weighed after a 16-hour solid fasting period and were randomized into two groups: baseline (5 bulls) and experimental (44 bulls). The five bulls in the baseline group (BW = 287 ± 45.9 kg) were slaughtered at the beginning of the experiment to measure empty BW (EBW) and to estimate the initial EBW of the remaining experimental bulls. The 44 bulls intended to evaluate the partial and total replacement of SMU by HP-DDG were randomly distributed into four groups, 11 animals each, which were randomly assigned to one of the four experimental diets.

The roughage: concentrate ratio was 20:80 (DM basis; Table 1), and diets were formulated to meet animals' requirements according to the BR-CORTE system (Valadares Filho et al., 2023), targeting an average daily gain of 1.2 kg/day (Table 2). Corn silage and concentrate were weighed separately and then mechanically mixed at the time of feeding. Such a total mixed ration was provided ad libitum twice a day (07:00 and 16:00 hours), and the bulls had free access to water. The diet was adjusted daily to allow approximately 5% refusal of the offered total on an as-fed basis. Refusals were mixed with the new feed that was offered the next day.

The bulls were weighed every 25 days to monitor the average daily gain (ADG). For quantification of their performance, the first and last weights (Days 1 and 100, respectively) were used, which were preceded by a 16-h solid fasting period.

Collections, digestibility assays, and slaughter procedures

Corn silage was sampled daily and stored at –20°C until further analysis. Forage samples were combined weekly (percent as-fed basis), dried in a forced-air oven (55°C) for 72 h, and ground through a 2- and 1-mm screen (Fortinox, Piracicaba, São Paulo, Brazil). Next, the total DM of these samples was evaluated according to (AOAC, 2012) by using method 934.01. Based on the DM content of corn silage, composite samples were assembled proportionally to the amount of roughage offered for 4 weeks. The individual ingredients used to make the concentrate were sampled directly from the feed mill silos on the days the concentrate was mixed. These ingredients were analyzed individually and used to calculate the diet composition.

Two digestibility trials were conducted to evaluate the total apparent digestibility of nutrients, N balance, and microbial efficiency. Spot fecal and urine samples were collected from all bulls on d 35 to 39 (period 2) and d 84 to 88 (period 4). Fecal samples were collected during five consecutive days at 18:00 on d 1, at 15:00 on d 2, at 12:00 on d 3, at 09:00 on d 4, and 06:00 on d 5. Urine samples were collected at 12:00 on d 3 and at 06:00 on d 5. Fecal samples were obtained directly from the rectum of the animals. Approximately 200 g of feces were collected per sampling time and oven-dried (55 °C for 72 h). Subsequently, a composite sample was performed for each animal in each collection period. The indigestible neutral detergent fiber (iNDF) was used as a marker to estimate the fecal DM production and then calculate the apparent digestibility of nutrients. Mean digestibility values from periods 2 and 4 were used for each dietary constituent.

The urine was collected using collection cups. At the end of each collection, two urine samples were taken. We obtained a 10-mL sample that was diluted with 40 mL of 0.036 N H_2SO_4 to avoid losses of allantoin and uric acid. Another 20-mL sample was collected without dilution to quantify the total N content. Subsequently, for both cases, a composite was performed per animal and collection period. Urine composite samples were stored at -20°C until further analysis.

At the end of the experiment, all bulls were slaughtered at the UFV after a 16-hour solid fasting period. The slaughter was performed via stunning and severing of the jugular vein for total bleeding. The digestive tract of each bull was emptied and washed, and each organ was weighed separately. The weight of the non-carcass components (NCC) was composed of the sum of the weights of the heart, lungs, liver, spleen, kidneys, internal fat, diaphragm, mesentery, tail, tongue, trachea, esophagus, reproductive tract, washed gastrointestinal tract, head (no leather), leather (body, head, and limbs), hooves and blood. The NCC was added to the carcass weight to determine the EBW. The carcass of each bull was divided into two halves that were weighed (hot carcass weight; HCW) to evaluate the hot carcass yield (HCY) and cooled at 4°C for 24h. After this time, carcasses were weighed (cold carcass weight; CCW) to evaluate the cold carcass yield (CCY). The carcass length (CL) was measured as the distance from the cranial edge of the ischiopubic symphysis to the medial cranial edge of the first rib. To determine body chemical composition, samples from the NCC and the section between the 9th and 11th rib (Hankins and Howe, 1946; HH section) of the left carcass of each bull were collected, weighed, dissected, and lyophilized (AOAC, 2012; method 934.01). After the samples were processed, chemical analyses of the HH section and NCC were carried out.

Methane and carbon dioxide measurements

Daily enteric methane (CH_4) and carbon dioxide (CO_2) emissions, expressed in grams per day, were quantified using the GreenFeed system (C-Lock Inc., Rapid City, South Dakota, USA). This portable feeding station facilitates spot sampling of exhaled air during eructation and respiration while animals consume feed throughout the day. During the experiment, animals from each treatment group had access to the GreenFeed system for 7 consecutive days every 28 days. During these periods, small portions of the experimental concentrate diet were offered, limited to a maximum of 5 drops per portion (50 g per drop), to encourage voluntary visits. Each visit lasted between 3 to 7 minutes, during which the system identified individual animals via electronic ear tags (radio frequency, RFID) and activated a ventilation system that directed exhaled air to non-dispersive infrared sensors. These sensors measured real-time concentrations of CH_4 and CO_2, while an airflow meter quantified the volume of air sampled. The mass of gas emitted was calculated based on the difference between measured concentrations and background levels, multiplied by the airflow rate, with corrections applied using the ideal gas law and capture factors determined by tracer methods. Weekly calibrations were performed using standard gas cylinders (zero and span) to ensure measurement accuracy. Data collected were automatically stored and processed, with results made available in Excel spreadsheets for subsequent analysis.

Gene expression involved in the regulation of protein synthesis

At slaughter, after stunning and cutting the jugular vein for complete bleeding, tissue samples of the *Longissimus dorsi* muscle and liver were collected in triplicate per animal (approximately 30–50 mg per sample) from seven randomly selected animals per treatment group (n = 7 animals/treatment). The samples were immediately macerated and snap-frozen in liquid nitrogen, then stored at −80 °C until further processing. Pulverized frozen tissues were used for total RNA extraction and subsequent quantification of mRNA abundance for *IGF1*, *GOT1*, *FOXO1*, and *PRKAA1* genes.

Total RNA was extracted from 0.1 g of tissue using the MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit (Thermo Fisher Scientific) on the KingFisher Flex Purification System, following the manufacturer's instructions. Total RNA was quantified using a NanoDrop spectrophotometer (GE Healthcare Life Sciences Inc.) and integrity was assessed using a 1% agarose gel. RNA samples were reverse transcribed into cDNA using the GoScript™ Reverse Transcription kit. The cDNA was then diluted to a concentration of 5 ng/µL and stored at -20°C until PCR analysis.

Real-time PCR analysis was performed with duplicate samples using a CFX96 Real-Time System (Bio-Rad) thermal cycler. SyBR Green GoTaq qPCR Master Mix Reagent (Promega Corporation) was used according to the manufacturer’s protocol. The efficiency was tested for each gene with serial dilutions of cDNA at concentrations of 5, 15 and 45 ng/µL and different concentrations of master mix for the reactions of 100, 200 and 400 nM. The efficiency was considered acceptable when the amplification of the target gene was comparable to that to the control gene, resulting in an efficiency between 80-100%. This value was determined based on calculations using the Ct values of the PCR instruments. The amplification protocol for all reactions started with a first step at 95º C for 10 minutes, followed by 40 cycles at 95º C for 15 seconds, and ended with a final step at 60º C for 60 seconds.

The expression of each target gene for each animal was calculated by subtracting the cycle threshold (Ct) of the control gene (endogenous gene) from that of the target gene (Ct of target gene – Ct endogenous gene). The results were expressed relative to GAPDH using the formula 2 – ΔCt, where ΔCt = (Ct target gene – Ct endogenous gene). Target genes evaluated in muscle tissue included Forkhead Box O1 (FOXO1) and Protein Kinase AMP-Activated Catalytic Subunit Alpha 1 (PRKAA1); and in the liver, insulin-like growth factor (IGF-1) and glutamate transaminase-oxaloacetic acid (GOT1, Table 9).

Laboratory analyses and calculations

The samples of corn silage, concentrate ingredients, and feces were analyzed for their contents of DM (AOAC, 2012; method 934.01), organic matter (OM; AOAC, 2012); method 930.05), total N (AOAC, 2012; method 981.10), ethereal extract (EE; AOAC, 2006; method 945.16), ashes (MM; (AOAC, 1990; method 924.05) and neutral detergent fiber (NDF; Mertens et al., 2002). The NDF quantification was performed without the addition of sodium sulfite but with the addition of thermostable alpha-amylase to neutral detergent (Ankom Tech. Corp., Fairport, NY). The NDF concentrations were corrected for ash (Mertens et al., 2002) and residual N compounds (Licitra et al., 1996). We calculated crude protein (CP) content as the product of total N content and the factor 6.25. The iNDF content was determined according to Valente et al. (2011). Non-fibrous carbohydrates (NFC) were calculated as proposed by

Detmann & Valadares Filho, (2010) from the equation: NFC = 100 – [(%CP diet - %CP derived from urea+ % urea) + %apNDF + %EE + %MM], where NFC = non-fibrous carbohydrates; %CP = crude dietary protein; apNDF = dietary neutral detergent fiber corrected by nitrogen and ashes; EE = dietary ether extract content; MM = ash content of the diet. The contents of total digestible nutrients (TDN) were estimated through the sum of the digestible nutrients, where: TDN = digestible CP + 2.25 × digestible EE + digestible NDF + digestible NFC (NRC, 2001).

Amino acid and sulfuric acid analyses were performed in a private laboratory. The amino acid composition, except for tryptophan, was determined by high-performance liquid chromatography (HPLC) after pre-column derivatization with phenylisothiocyanate (PITC), according to the methods described by White et al. (1986) and Hagen et al. (1989). This technique allows for the separation and quantification of amino acids through spectrophotometric detection of phenylthiocarbamyl derivatives. The tryptophan content was determined using a colorimetric method adapted from Lucas and Sotelo (1980) , which involves alkaline hydrolysis followed by reaction with specific reagents and spectrophotometric quantification (Figures 1 and 2).

Total sulfur content was analyzed by combustion in a high-temperature tube furnace, following the ASTM D4239-18e1 (2018) standard. The method involves complete oxidation of sulfur in the sample and quantification by infrared detection (Figure 3)

Daily CH_4 and CO_2 emissions were calculated relative to dry matter intake (g/kg DMI) and average daily gain (g/kg ADG). Urine samples were analyzed for creatinine, uric acid, and allantoin. The analyses of uric acid and creatinine were performed by using an automatic biochemical analyzer (Mindray/model BS200E, Shenzhen, China), whereas the allantoin analysis was performed according to the colorimetric method described by Chen and Gomes, (1992).

The daily excretion of creatinine (EC; g/d) was calculated based on the following equation (EC (g/d) = 0.0345 × $SBW^{0.9491}$ × 1000, where SBW = shrunk body weight; Costa e Silva et al., 2012). Then, the daily urinary volume (L/d) was estimated from the ratio between the estimated EC (g/d) and creatinine concentrations in the spot urine sample (g/L).

The total excretion of purine derivatives was calculated by the sum of the amounts of allantoin and uric acid excreted in the urine, which was obtained by the product between their concentration in the urine and the urinary volume. Absorbed purines and ruminal synthesis of nitrogen compounds were calculated according to Barbosa et al. (2011).

The crude microbial protein (MCP; g/d) was calculated by the product of the ruminal synthesis of nitrogen compounds and factor 6.25. The microbial efficiency was calculated by the ratio between the MCP and TDN, and digestible OM (DOMI) intakes expressed in g MCP/kg TDN and g MCP/kg DOMI.

The N balance of the animals was also calculated. For this purpose, we calculated the intake and excretion of fecal N (g/day) as the ratio of N content in the diets (by DM bases) and feces excretion in DM bases (g/day), respectively. In addition, we calculated N urinary excretion (g/day) as the difference between N consumed less N fecal and N retained as described by Cole et al. (2003), which we obtained through comparative slaughter, whose procedures we described in the slaughter procedures. Daily retained N was obtained from the difference between total nitrogen in final shrunk body weight minus total nitrogen in initial shrunk body weight divided by the number days in the experiment.

Statistical analysis

All data were analyzed in a completely randomized design using the GLIMMIX procedure of SAS (version 9.4; SAS Institute Inc., 2013, Cary, NC, EUA). The statistical model included the fixed effect of treatment, and degrees of freedom were adjusted using the

Kenward-Roger approximation to improve estimation accuracy. The normal distribution was assumed for all dependent variables. The general model used was:

$$Yij = \mu + Ti + eij$$

where: **μ** = general constant; **Ti** = fixed effect of diet; and **eij** = residual error associated with each observation. Polynomial contrasts (linear and quadratic) were used to assess trends related to increasing levels of high-protein DDG inclusion. Additionally, Dunnett's multiple comparisons test was performed for all parameters to compare each treatment with the control (0% HP-DDG). Effects were considered significant when $P < 0.05$, and trends were discussed when $0.05 \leq P \leq 0.10$. The symbol (*) denotes a significant difference ($P < 0.05$, and trends were discussed when $0.05 \leq P \leq 0.10$.) compared with the control treatment, according to Dunnett's test.

The criterion adopted for identifying outliers was based on the normal distribution curve. Values of Student's standardized residuals greater than $|3|$ were considered outliers and, therefore, removed from the respective database.

RESULTS

The amino acid and sulfur composition of DDG High Protein

Figure 1 presents the amino acid and sulfur composition (as a percentage of dry matter) of the high-protein dried distillers' grains (HP-DDG) sample. A rich protein profile was observed, with predominance of glutamic acid (9.83%), aspartic acid (3.34%), alanine (3.94%), proline (4.89%), and leucine (6.86%), the latter being naturally abundant in corn-based ingredients, the main substrate used in DDG production. Among the essential amino acids, notable concentrations were observed for valine (2.72%), isoleucine (2.04%), threonine (1.95%), methionine (0.83%), and lysine (1.16%), indicating the ingredients' potential to meet the nutritional requirements of growing cattle. Tryptophan content was 0.26%, which, although relatively low, falls within the range commonly reported for corn byproducts. The total amino acid content was 51.41%. Total sulfur content was 0.77% a high value yet typical of DDG due to the use of sulfuric acid during the industrial ethanol production process.

Intake, apparent digestibility of nutrients, and microbial efficiency

A trend toward a linear effect was observed for the DMI (P = 0.072), OM (P = 0.083), and CP intake (P = 0.063), indicating a gradual reduction in the intake of these components as DDG inclusion in the diet increased. No significant effects (P > 0.10) were observed for apNDF and metabolizable protein intake (Table 3).

On the other hand, ether extract (EE) intake was significantly influenced by the treatments (P = 0.006; Table 3), showing a positive linear effect (P < 0.001), indicating that EE intake increased proportionally with the level of DDG inclusion in the diet. Non-fibrous carbohydrate (NFC) intake was also affected by the treatments (P = 0.003), presenting a negative linear effect (P < 0.001), with a progressive reduction in intake as DDG levels increased. A similar effect was observed for total digestible nutrients (TDN) intake, which showed a significant linear reduction (P < 0.001).

Metabolizable energy intake (MEI) decreased with increasing HP-DDG inclusion in the diet (P = 0.008), showing a significant linear effect (P < 0.001), indicating a lower total energy supply in diets with higher DDG levels. However, DMI relative to shrunk body weight (SBW) was not affected by the treatments (P = 0.475), indicating a consistent intake proportional to body weight regardless of the diet (Table 3).

Ruminal degradable protein (RDP) intake was significantly reduced as HP-DDG inclusion increased (P < 0.001), presenting a linear decrease (P < 0.001). Conversely, ruminal undegradable protein (RUP) intake increased linearly with increasing levels of HP-DDG in the diet (P < 0.001), providing a greater supply of bypass protein, which is characteristic of high-protein DDG. However, metabolizable protein intake did not differ among treatments (P >

0.10), suggesting that the reduction in RDP supply was compensated for by the increase in RUP, resulting in a relatively constant supply of metabolizable protein across the diets (Table 3).

According to the Dunnett test, animals fed the 12% HP-DDG diet had lower intakes of NFC, TDN, MEI, and RUP (P = 0.003, 0.007, 0.008 and <0.001, respectively), and higher intakes of EE and RDP (P = 0.006 and <0.001, respectively) compared to the control group (0% HP-DDG; Table 3).

The apparent digestibility of DM, OM, CP, and NFC was significantly reduced with increasing DDG inclusion in the diets ($P < 0.05$), with a linear decreasing effect ($P < 0.05$; Table 4). The apparent digestibility of EE showed a trend toward treatment effect ($P = 0.052$), with a significant linear increase ($P < 0.001$) as DDG levels in the diets increased. However, the apparent digestibility of apNDF was not influenced by the level of HP-DDG in the diet (P > 0.10; Table 4).

Compared with the control, 0% HP-DDG, treatment (Dunnett test), the 12% HP-DDG diet resulted in lower digestibilities of DM, OM, CP, and NFC ($P = \leq 0.001$), and higher digestibilities of EE ($P = 0.052$), with no effect on apNDF ($P = 0.289$; Table 4).

Microbial crude protein (MCP) production and microbial efficiency, both expressed relative to TDN and digestible organic matter (DOM), were not influenced (P > 0.10) by the inclusion of HP-DDG replacement of soybean meal and urea in the diets (Table 4).

Nitrogen balance, animal performance, carcass traits, and methane production

N intake, N urinary, N absorbed (g/day), and the ratio of retained to intake nitrogen were significantly influenced ($P < 0.05$) by the inclusion of HP-DDG replacement levels of soybean meal and urea (SMU), showing a linear decrease for nitrogen intake ($P < 0.001$), urinary nitrogen ($P < 0.001$), and absorbed nitrogen ($P < 0.001$), and a quadratic effect for the retained: intake nitrogen ratio ($P = 0.029$). A quadratic trend was observed for fecal nitrogen excretion

(*P* = 0.099), while retained nitrogen did not differ significantly among treatments (*P* = 0.629; Table 4).

According to the Dunnett test, N intake was lower (*P* < 0.001) in the 8% and 12% HP-DDG diets compared with the control diet (0% HP-DDG). Urinary N excretion and N absorbed were reduced (*P* < 0.001) in all HP-DDG diets (4%, 8% and 12%), whereas no differences were observed for N retained or fecal N excretion (Table 4; *P* > 0.10).

Among the animal performance and carcass traits evaluated, hot carcass gain (HCG) was significantly affected (P = 0.040), and a linear trend was observed for empty body weight gain (EBWG; P = 0.052). Other variables such as initial SBW (iSBW), final SBW (fSBW), initial EBW (iEBW), final EBW (fEBW), weight of non-carcass components (Ncarc), cold carcass weight (CCW), hot carcass weight (HCW), average daily gain (ADG), carcass length (CL), ribeye area (REA), hot carcass yield (HCY) were not significantly influenced (P > 0.10) by inclusion of HP-DDG replacement of soybean meal and urea in the diets. According to the Dunnett test, no variables related to animal performance differed from the control treatment (Table 5; *P* > 0.10).

In table 6, the daily production of methane (CH_4) and carbon dioxide (CO_2) did not differ significantly among treatments (*P* = 0.153 and *P* = 0.226, respectively). However, a decreasing linear trend was observed for absolute CO_2 emissions with increasing dietary levels of HP-DDG (*P* = 0.057), which may reflect alterations in energy metabolism or improvements in ruminal fermentation efficiency.

No significant differences were observed in the relative emissions of CH_4 or CO_2 when expressed per unit of dry matter intake (CH_4/DMI, CO_2/DMI), average daily gain (CH_4/ADG, CO_2/ADG), or carcass gain (CH_4/CARC, CO_2/CARC; *P* > 0.05). Additionally, no linear or quadratic trends were detected for these variables.

The Dunnett test showed no differences in gas production variables compared with the control treatment (Table 6; $P > 0.10$).

Gene expression involved in the regulation of protein synthesis

The expression of the IGF1gene in the liver was not significantly affected by the treatments ($P = 0.975$; Figure 2), and no linear or quadratic trends were observed ($P > 0.10$). However, a quadratic effect was observed for GOT1 expression ($P = 0.022$), indicating a possible fluctuation in gene expression according to HP-DDG inclusion levels, with the lowest expression observed in the 4% HP-DDG group and the highest in the 12% HP-DDG group. The Dunnett test indicated a trend for treatment effect on GOT1 expression ($P = 0.092$).

In muscle tissue, PRKAA1 expression was not significantly influenced by the treatments (P = 0.337), nor were linear or quadratic effects detected ($P > 0.10$). For FOXO1, a quadratic effect was observed ($P = 0.016$), indicating reduced expression at intermediate inclusion levels (4 and 8% HP-DDG), with recovery at 12%. According to the Dunnett test, there was a trend toward a treatment effect on FOXO1 ($P = 0.098$).

DISCUSSION

Intake and digestibility of nutrients and microbial production

Variations in dry matter intake (DMI) and nutrient digestibility in response to DDG inclusion in ruminant diets have been widely reported in the literature and are influenced by several factors, including the type of DDG used (wet DDG, DDG with solubles, high- or low-protein DDG, or DDG derived from different grains such as corn or sorghum), experimental conditions, inclusion level, animal growth stage, production system, basal diet composition, and feeding method (Stelzleni et al., 2016). DMI and nutrient digestibility are key determinants of animal performance (Cabral et al., 2006; Andrade et al., 2023), with approximately 70% of the variation in digestible energy intake across diets and animals is explained by DMI, while the remaining 30% is attributed to digestibility differences (Lima et al., 2015; Fonseca et al., 2021).

In the present study, replacing soybean meal–urea (SMU) with high-protein DDG (HP-DDG) tended to decrease linearly the intake of DM, OM, and CP as DDG inclusion increased, with no consistent effects on apNDF or metabolizable protein intake. Similar variability in DMI responses to HP-DDG has been reported. In grazing cattle, Dias et al. (2024) observed no significant effect when replacing SMU with HP-DDG at various inclusion levels, whereas Chesini et al. (2023) reported a linear increase in DM and OM intake in lactating dairy cows with HP-DDG inclusion. In feedlot-finished Nellore cattle, Rosa e Silva et al. (2022) found that including DDG (0, 10, 20, and 30% of DM) as a replacement for corn meal increased DMI during the first 35 days, but effects disappeared thereafter. Additionally, animals fed higher DDG levels showed greater CP and EE intake and lower NFC intake across all evaluation periods. In the present study, increasing DDG levels in the diet altered nutrient intake profile, likely due to the chemical composition of HP-DDG and the overall diet (Table 2).

The reduction in NFC, TDN, and metabolizable energy (ME) intake in our study appears directly associated with decreased DM intake, a pattern also observed by Stalker et al. (2009) in beef cattle supplemented with DDGS at different intervals. The lack of effect of DDG on DMI has been reported in several studies (Corrigan et al., 2008; Depenbusch et al., 2008; Leupp et al., 2009; Felix et al., 2012; Schoonmaker et al., 2013; Stotzer et al., 2022; Rosa e Silva et al., 2024) although some have found negative (Hünerberg et al., 2013) or positive effects (Alhadas et al., 2021; Rosa e Silva et al., 2022).

As expected from its composition, EE intake increased linearly with HP-DDG inclusion due to the residual lipid content of the co-product, which typically ranges from 8% to 12% (Kerr et al., 2013; U.S. Grains Council, 2012) and contributes to dietary energy density (NASEM, 2016). Nevertheless, caution is warranted during diet formulation, as excessive EE levels may impair fiber digestion and ruminal fermentation (Oldick and Firkins, 2000).

Rumen-degradable protein (RDP) intake decreased with increasing inclusion levels of HP-DDG, whereas rumen-undegradable protein (RUP) intake increased. HP-DDG derived from *front-end* fiber fractionation contains higher RUP concentrations (Lee et al., 2016). According to the authors, the co-product has a higher proportion of rumen-insoluble protein (fraction C: 41.2% of CP) and lower effective CP degradability compared with conventional corn DDGS and wheat DDGS, reinforcing its potential as a bypass protein source for ruminants.

A reduction in nutrient digestibility with increasing dietary DDG levels has been previously reported (Benchaar et al., 2013; Judy et al., 2019). In the present study, the digestibility of DM, OM, and CP decreased linearly with greater substitution of SMU by HP-DDG. HP-DDG produced via front-end fractionation typically contains lower starch and higher NDF concentrations compared with corn or soybean meal (Palowski et al., 2021; Rosa e Silva et al., 2024) and is characterized by higher EE and CP levels, particularly in the RUP form (Lee et al., 2016). In this study, differences in nutrient digestibility among treatments may be attributed to three main factors: (1) reduced DMI (Cabral et al., 2006), (2) the forage-to-concentrate ratio (Picanço et al., 2024; Rosa e Silva et al., 2024), and (3) the high RUP content of HP-DDG (Allen, 2000; Stalker et al., 2009).

Apparent EE digestibility increased linearly with HP-DDG inclusion, likely due to its elevated EE concentration from industrial processing, as DDG-rich diets tend to offer greater EE availability. In this study, EE levels did not exceed 3.6% of dietary DM, remaining below 6 - 7% DM threshold (Patra, 2013).

Microbial protein synthesis and efficiency were unaffected, indicating that the diets maintained adequate rumen-degradable nitrogen and fermentable energy supply, despite the high RUP content of HP-DDG and the removal of urea. This suggests that HP-DDG can be included in finishing diets without impairing microbial activity, provided nitrogen and energy are properly synchronized. However, Allen (2000) concluded that high-RUP diets reduced microbial protein synthesis, indicating that this response may be context-dependent.

Nitrogen (N) balance, animal performance, carcass traits, and methane production

Urinary nitrogen (N) excretion and absorbed N decreased linearly with increasing HP-DDG levels, reflecting lower N intake. At 12% HP-DDG, urinary N was 32% lower and absorbed N was 23% lower than the control treatment (0% HP-DDG), which aligns with the findings of Dias et al. (2024). In contrast, Rosa e Silva et al. (2024) reported a 334% increase in urinary N excretion with the highest DDG inclusion, accounting for over 50% of total N excretion. These authors attributed the increase to dietary urea, suggesting that in diets with > 20% DDG, removing urea may reduce urinary N losses. In our study, HP-DDG inclusion was accompanied by proportional urea reduction, which likely contributed to the observed decrease in urinary N. However, it is unclear whether this reduction was driven by the lower N intake itself or by the absence of urea, since retained N remained unchanged, indicating that reduced absorption was offset by reduced excretion.

In addition, the protein profile, particularly the high rumen-undegradable protein (RUP) content of HP-DDG, may have influenced these results. While RUP increases the supply of protein to the small intestine, its effective utilization depends on an adequate profile of essential amino acids (D'mello, 2003; Kamalak et al., 2005). A reduction in absorbed N may indicate limited post-ruminal availability of digestible amino acids, either due to deficiencies in DDG amino acid profile or reduced absorptive efficiency. Even so, the lower urinary N excretion suggests improved efficiency of absorbed N utilization for metabolic purposes, possibly prioritizing maintenance functions.

A phenomenon known as the *leucine antagonism effect*, often described in diets containing HP-DDG derived from corn-based ethanol production, may also contribute to the lack of improvement in nitrogen retention. Leucine, abundant in corn, may compete for intestinal absorption with other branched-chain amino acids (BCAA), particularly valine and isoleucine (Smith and Austic, 1978; May et al., 1991), reducing muscle availability and potentially impairing protein synthesis.

Fecal nitrogen excretion exhibited a quadratic trend, with the lowest values observed at the 8% HP-DDG inclusion level. The initial reduction may reflect improved dietary protein digestibility, while the increase at 12% could result from excess RUP with an imbalanced essential amino acid profile. Overall, these results indicate that while HP-DDG can reduce urinary N losses, excessive inclusion may increase fecal losses due to amino acid profile limitations, potentially constraining post-ruminal protein utilization efficiency (D'mello, 2003).

Animal performance and carcass traits were generally maintained across treatments. However, hot carcass gain (HCG) decreased significantly, and empty body weight gain (EBWG) tended to decrease with increasing HP-DDG inclusion, consistent with Schoonmaker et al. (2013). These reductions may relate to lower dietary energy supply and imbalances in protein and amino acid profiles, as previously discussed. Alhadas et al. (2021), evaluating different inclusion levels of low-fat, de-solubilized DDG, also observed decreasing trends in dressing percentage and average daily gain, whereas Klopfenstein et al. (2008), through a meta-analysis of nine studies, reported a quadratic response, with average daily gain of finishing bulls maximized at about 30% DDGS inclusion.

Although differences in most performance variables were not significant, the inclusion of HP-DDG at 12% of dietary DM was associated with reduced TDN and MEI intake, which in turn coincided with approximately 13 kg (≈ 1 arroba) lower CCW compared with lower inclusion levels. This pattern suggests that, under the conditions of the present study, HP-DDG levels above 8% may not be optimal for maximizing carcass yield in finishing young bulls in feedlot conditions.

Several studies have demonstrated that DDG inclusion in ruminant diets can reduce enteric methane emissions, as observed in dairy cattle (Birkelo et al., 2004; Benchaar et al., 2013; Foth et al., 2015), beef cattle (McGinn et al., 2009; Hünerberg et al., 2013), and sheep (Ma et al., 2019). These reductions are generally attributed to decreased ruminal availability of fermentable substrate, redirection of hydrogen flow, increased dietary fat content, and modulation of ruminal microbiota due to higher RUP levels.

In the present study, methane production (g/day) showed a quadratic trend, increasing up to the 8% HP-DDG inclusion level and decreasing at 12%. This initial increase may be related to the presence of urea, which could stimulate fiber-fermenting microorganisms and acetate production, thereby enhancing methanogenesis (Van Soest, 1994). Reductions observed at higher inclusion levels may be associated with decreased ruminal fermentation due to higher RUP, lowering hydrogen availability for methanogens.

Methane mitigation from DDG inclusion is often attributed to its higher fat content, which can suppress methanogens and promote biohydrogenation of fatty acids (McGinn et al., 2009; Benchaar et al., 2013; Foth et al., 2015). However, a meta-analysis by Malik et al. (2024), found no consistent effect of ether extract on methane production, consistent with the present study. Another possible mechanism is dietary sulfur, as sulfate reduction is thermodynamically favored over CO_2 reduction (Mcallister et al., 1995). In this experiment, HP-DDG increased dietary sulfur but remained below the NRC (2001) maximum tolerable level (0.4% DM), with no effect on methane output. Similar results were reported by Castillo-Lopez et al. (2017). In contrast, Judy et al. (2019) observed a tendency toward reduced methane emissions when calcium sulfate was added to DDG-based diets in dairy cows. Van Zijderveld et al. (2010) reported a 16% reduction in sheep fed sulfur-supplemented diets, but no effect on dairy cows at lower inclusion rates.

Beyond methane, carbon dioxide (CO_2) is another major byproduct of ruminal carbohydrate fermentation, linked to fiber degradation (Janssen, 2010). Diets high in protein and low in fiber, such as HP-DDG, may lower CO_2 output by promoting propionate formation, a pathway that consumes hydrogen and produces less CO_2 (Ungerfeld, 2013). In this study, CO_2 production (g/day) tended to decrease linearly with HP-DDG inclusion, suggesting a potential environmental benefit of its use in finishing diets.

Gene expression involved in the regulation of protein synthesis

GOT1 (glutamate oxaloacetate transaminase 1) is involved in hepatic amino acid metabolism and gluconeogenesis and displays a non-linear expression pattern, which may reflect hepatic adjustments to nitrogen availability and dietary protein quality, particularly in response to changes in RUP supply. HP-DDG is characterized by high digestibility of essential amino acids, which may influence the regulation of hepatic metabolic genes (Dias et al., 2024).

In muscle tissue, no linear effect was detected in FOXO1 or PRKAA1 expression. However, FOXO1 showed a significant quadratic response. As a key regulator of muscle protein catabolism, modulated by insulin, IGF-1, and cellular energy status, fluctuations in FOXO1 expression may represent an adaptive mechanism to variations in amino acid supply from DDG, potentially impacting proteolysis and maintenance pathways. Conversely, the stable expression of PRKAA1, which encodes the catalytic α1 subunit of AMP-activated protein kinase (AMPK), suggests that the energy status of muscle tissue was not substantially altered by DDG inclusion.

These findings suggest that DDG inclusion can trigger subtle metabolic adaptations at the molecular level in hepatic and muscle tissues, even in the absence of major changes in productive performance, highlighting the sensitivity of molecular biomarkers to dietary composition and reinforcing the value of omics-based approaches in ruminant nutrition research.

CONCLUSION

Our findings indicate that the inclusion of high-protein distiller dried grains (HP-DDG) replacing soybean meal and sulfated urea (SMU) altered the intake and digestibility of specific nutrients, including DM, OM, CP, NFC, EE, RUP, and RDP, without impairing animal performance. Moreover, HP-DDG reduced urinary nitrogen excretion, indicating a potential environmental benefit by decreasing nitrogen losses from the production system. Therefore, HP-DDG can partially-(up to 8% of the diet, DM) replace soybean meal and urea, representing a viable alternative protein source in bypass protein-based formulations.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

The authors declare that they did not use any generative AI and AI-assisted technologies in the writing process.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Table 1 - Chemical composition of ingredients used in experimental diets

Ingredients	DM[1]	OM[2]	CP[3]	EE[4]	apNDF[5]	NFC[6]	iNDF[7]
		g/Kg DM					
Corn silage	283.09	939.65	77.60	29.75	565.58	285.38	161.63
Ground corn	885.90	986.78	78.00	32.00	142.25	732.75	16.00
HP-DDG	924.36	975.36	510.64	79.10	320.63	64.98	10.05
Soybean meal	883.46	920.31	505.00	15.75	150.50	275.00	22.00
Ureia + AS[8]	985.06	986.35	2577.71	0.00	0.000	0.00	0.00
Mineral premix	985.30	66.68	0.00	0.00	0.000	0.00	0.00

[1]Dry matter; [2]Organic matter; [3]Crude protein; [4]Ethereal extract; [5]Neutral detergent fiber corrected for ash and crude protein; [6]Non-fibrous carbohydrates; [7]Indigestible neutral detergent fiber; [8]Urea/ammonium sulfate (AS) ratio of 9:1.

Table 2 - Proportions of ingredients and chemical composition of nutrients in experimental diets

Item	Concentrate				Diets			
	0% HP-DDG	4% HP-DDG	8% HP-DGG	12% HP-DDG	0% HP-DDG	4% HP-DDG	8% HP-DGG	12% HP-DDG
	Proportion of ingredients (g/kg DM)							
Corn silage	-	-	-	-	200.0	200.0	200.0	200.0
Ground corn	874.4	853.8	833.1	812.5	699.5	683.0	666.5	650.0
HP-DDG	-	50.0	100.0	150.0	0.0	40.0	80.0	120.0
Soybean meal	74.9	49.9	25.0	0.0	59.9	40.0	20.0	0.0
Ureia + AS[2]	13.2	8.8	4.4	0.0	10.6	7.1	3.5	0.0
Mineral premix[34]	37.5	37.5	37.5	37.5	30.0	30.0	30.0	30.0
	Chemical composition (g/kg DM)							
DM[1]	890.25	891.78	894.57	896.75	622.57	623.21	624.25	625.10
OM[1]	947.29	948.39	949.47	950.56	945.76	946.64	947.51	948.38
CP[1]	140.37	140.01	140.01	139.97	127.81	127.53	127.53	127.50
EE[1]	29.16	32.06	34.96	37.87	29.28	31.60	33.92	36.24
apNDF[1]	135.66	144.99	154.33	163.67	221.64	229.11	236.58	244.05
NFC[1]	655.16	647.92	640.79	605.11	581.20	575.41	569.71	541.16

[1]DM = Dry matter; OM = Organic Matter; CP = Crude Protein; EE = Ethereal Extract; apNDF = Neutral detergent fiber corrected for ash and crude protein; NFC = Non-fibrous carbohydrates.

[2]The treatments are: 0% HP-DDG = diet with no substitution of soybean meal and urea by high-protein DDG; 4% HP-DDG = diet with 4% substitution of soybean meal and urea by high-protein DDG; 8% HP-DDG = diet with 8% substitution of soybean meal and urea by high-protein DDG; 12% HP-DDG = diet with 12% substitution of soybean meal and urea by high-protein DDG. Urea/ammonium sulfate (AS) ratio of 9:1rea/ammonium sulfate (AS) ratio of 9:1.

[3]Basic composition of the product: Calcitic limestone; Sodium chloride (Common salt); Ventilated sulfur; Monodicalcium phosphate; Magnesium oxide; Probiotic additive; Raspberry aroma; Mixed herbal aroma; Sodium bicarbonate; Silicon dioxide; Onion extract; Grape seed extract; Calcium iodate; Monensin sodium; Manganese oxide; Zinc oxide; Sodium Selenite; Cobalt sulfate; Copper sulfate; Iron sulfate; Vehicle; Vitamin A; Vitamin D3; Vitamin E.

[4]Guarantee levels per kg of product: Mixed Herbal Flavor (min) 5.00MG; Onion extract (min) 9.00MG; Grape seed extract (min) 1.60MG; Monensin 1038.00MG; Saccharomyces cerevisiae (min) 100X 10E9 UFC, Calcium (min) 198.00G; Calcium (max) 290.00G; Sulfur (min) 24.50G; Fluoride (max) 222.75MG; Phosphorus (min) 11.00G; Magnesium (min) 19.00G; Sodium (min) 61.50G; Cobalt (min) 11.10MG; Copper (min) 335.00MG; Iron (min) 370.00MG; Iodine (min) 27.70MG; Manganese (min) 1668.00MG; Selenium (min) 7.40MG; Vitamin A (min) 112000.00UI; Vitamin D3 (min) 14900.00UI; Vitamin E (min) 136.00UI; Zinc (min) 2223.00MG.

Table 3 - Effect of inclusion of high-protein distillers grains (HP-DDG) replacing soybean meal and sulfated urea (SMU) based on total dietary on dry matter and nutrient intake of Nelore bulls during the finishing phase[1]

Item	Treatments[2]				P-Valor		
	0% HP-DDG	4% HP-DDG	8% HP-DGG	12% HP-DDG	TRAT	LIN	QUA
DM , kg/d	6.95±0.194	6.64±0.194	6.77±0.194	6.38±0.194	0.214	0.072	0.850
OM, kg/d	6.58±0.183	6.28±0.183	6.41±0.183	6.05±0.183	0.236	0.083	0.849
CP, kg/d	0.88±0.025	0.85±0.025	0.86±0.025	0.81±0.025	0.194	0.063	0.878
EE, kg/d	0.20±0.006	0.21±0.006	0.23*±0.006	0.23*±0.006	0.006	<0.001	0.722
apNDF, kg/d	1.54±0.045	1.52±0.045	1.60±0.045	1.56±0.045	0.641	0.536	0.791
NFC, kg/d	4.06±0.110	3.83±0.110	3.87±0.110	3.45*±0.110	0.003	<0.001	0.381
TDN, kg/d	5.59±0.161	5.30±0.161	5.42±0.161	4.79*±0.161	0.007	<0.001	0.310
DM/SBW, %	1.85±0.046	1.77±0.046	1.82±0.046	1.76±0.046	0.475	0.276	0.781
MEI, Mcal/d	19.04±0.680	18.11±0.680	17.18±0.680	15.69*±0.680	0.008	<0.001	0.685
RDP, kg/d	0.56±0.013	0.46*±0.013	0.39*±0.013	0.29*±0.013	<0.001	<0.001	0.779
RUP, kg/d	0.32±0.013	0.39*±0.013	0.48*±0.013	0.53*±0.013	<0.001	<0.001	0.569
Met. Protein, kg/d	0.62±0.018	0.60±0.018	0.63±0.018	0.60±0.018	0.707	0.776	0.840

[1]DM = Dry matter; OM = Organic Matter; CP = Crude Protein; EE = Ethereal Extract; apNDF = Neutral detergent fiber corrected for ash and crude protein; NFC = Non-fibrous carbohydrates; TDN = Total Digestible Nutrients; DM/SBW = Dry matter intake according to average shrunk body weight, %; MEI = Metabolizable energy intake; RDP = Rumen digestible protein; RUP = Rumen undigestible protein; Met. Protein = Metabolizable protein intake; TRAT = Differences of TRAT least squares mean; LIN = linear effect; and QUA = quadratic effect;

[2]The treatments are: 0%DDG = control diet without inclusion of high-protein DDG, containing soybean meal and urea as protein sources; 4%DDG = diet with 4% inclusion of high-protein DDG, with partial reduction of soybean meal and urea; 8%DDG = diet with 8% inclusion of high-protein DDG, with further partial reduction of soybean meal and urea; 12%DDG = diet with 12% inclusion of high-protein DDG, in which soybean meal and urea were completely removed. Urea/ammonium sulfate (AS) ratio of 9:1;

[3]Analysis was performed using Dunnett's multiple comparisons test for all parameters, and orthogonal polynomial contrasts were used to assess linear, quadratic, and cubic effects associated with increasing levels of HP-DDG inclusion;

[4]The symbol (*) denotes a significant difference ($P \leq 0.05$) compared with the control diet (0% HP-DDG), according to Dunnett's test.

Table 4 - Effect of inclusion of high-protein distillers grains (HP-DDG) replacing soybean meal and sulfated urea (SMU) based on total dietary on dry matter and nutrient digestibility, microbial production, and nitrogen balance in Nellore bulls during the finishing phase[1]

Item	Treatments[2]				P-Valor[34]		
	0% HP-DDG	4% HP-DDG	8% HP-DGG	12% HP-DDG	TRAT	LIN	QUA
		Digestibility of nutrients (g/day)					
DM	756.8±8.85	748.8±8.85	740.7±8.85	702.3*±8.85	<0.001	<0.001	0.093
OM	773.2±8.48	766.1±8.48	756±8.48	722.2*±8.48	<0.001	<0.001	0.123
CP	711.4±12.31	696.6±12.31	696.3±12.31	641.9*±12.31	0.001	<0.001	0.116
EE	796.8±11.59	819.5±11.59	836.7*±11.59	838.5*±11.59	0.052	<0.001	0.372
apNDF	619.2±14.80	628.1±14.80	614.6±14.80	588.9±14.80	0.289	0.122	0.249
NFC	898±7.32	878.6±7.32	881.3±7.32	843.0*±7.32	<0.001	<0.001	0.203
		Microbial production					
MCP (g/day)	423.16±23.371	370.43±23.371	377.51±23.371	364.25±23.371	0.283	0.112	0.403
Mic. Effi. (MCP g/ TDN kg)	85.99±5.372	76.37±5.372	84.02±5.372	84.82±5.372	0.755	0.929	0.437
Mic. Effi. (MCP g/ dOM kg)	94.69±5.858	86.06±5.858	93.52±5.858	94.42±5.858	0.690	0.801	0.420
		Nitrogen balance					
N intake (g/day)	145.40±4.476	134.01±4.476	123.78*±4.476	126.61*±4.476	<0.001	0.001	0.119
N feces (g/day)	42.09±2.373	41.47±2.373	39.25±2.373	46.64±2.373	0.180	0.287	0.099
N urine (g/day)	68.35±2.920	56.61*±2.920	49.73*±2.920	46.47*±2.920	<0.001	<0.001	0.154
N absorbed (g/day)	103.31±3.149	92.53*±3.149	84.52*±3.149	79.97*±3.149	<0.001	<0.001	0.329
N retained (g/day)	34.96±1.308	35.92±1.308	34.79±1.308	33.49±1.308	0.629	0.349	0.393
Nr:Ni	24.23±1.008	26.88±1.008	28.41*±1.008	26.53±1.008	0.044	0.068	0.029

[1]DM = Dry matter; OM = Organic Matter; CP = Crude Protein; EE = Ethereal Extract; apNDF = Neutral detergent fiber corrected for ash and crude protein; NFC = Non-fibrous carbohydrates; MCP = microbial crude protein; Mic. Effi. (MCP g/ TDN kg) = microbial efficiency in grams of microbial crude protein per kilogram of total digestible nutrients; Mic. Effi. (MCP g/ dOM kg) = microbial efficiency in grams of microbial crude protein per kilogram of digestible organic matter; N intake = Nitrogen intake ; N feces = Nitrogen excretion via feces; N urine = Nitrogen excretion via urine; N absorbed = Nitrogen absorbed; N retained = Nitrogen retained in the animal's body; Nr:Ni = ration of retained nitrogen to absorbed nitrogen; TRAT = Differences of TRAT least squares mean; LIN = linear effect; and QUA = quadratic effect;
[2]The treatments are: 0%DDG = control diet without inclusion of high-protein DDG, containing soybean meal and urea as protein sources; 4%DDG = diet with 4% inclusion of high-protein DDG, with partial reduction of soybean meal and urea; 8%DDG = diet with 8% inclusion of high-protein DDG, with further partial reduction of soybean meal and urea; 12%DDG = diet with 12% inclusion of high-protein DDG, in which soybean meal and urea were completely removed. Urea/ammonium sulfate (AS) ratio of 9:1;
[3]Analysis was performed using Dunnett's multiple comparisons test for all parameters, and orthogonal polynomial contrasts were used to assess linear, quadratic, and cubic effects associated with increasing levels of HP-DDG inclusion
[4]The symbol (*) denotes a significant difference ($P \leq 0.05$) compared with the control diet (0% HP-DDG), according to Dunnett's test.

Table 5 - Effect of inclusion of high-protein distillers grains (HP-DDG) replacing soybean meal and sulfated urea (SMU) based on total dietary on animal performance and carcass characteristics in Nellore bulls during the finishing phase[1]

Item	Treatments[2]				P-Valor[34]		
	0% HP-DDG	4% HP-DDG	8% HP-DGG	12% HP-DDG	TRAT	LIN	QUA
iSBW (kg)	303.41±9.220	302.77±9.220	301.82±9.220	297.32±9.220	0.965	0.643	0.835
fSBW (kg)	447.27±10.529	446.27±10.529	446.09±10.529	427.36±10.529	0.484	0.210	0.404
iEBW (kg)	262.45±7.975	261.90±7.975	261.08±7.975	257.18±7.975	0.965	0.643	0.835
fEBW (kg)	403.56±9.654	400.48±9.654	400.45±9.654	383.75±9.654	0.465	0.176	0.484
CCW (kg)	259.13±6.722	257.15±6.722	257.13±6.722	244.49±6.722	0.402	0.151	0.432
HCW (kg)	264.61±6.632	263.61±6.632	264.48±6.632	251.11±6.632	0.410	0.188	0.356
ADG (kg/day)	1.44±0.060	1.44±0.060	1.44±0.060	1.30±0.060	0.271	0.134	0.251
EBWG (kg/day)	1.31±0.048	1.28±0.048	1.28±0.048	1.16±0.048	0.163	0.052	0.329
HCG (kg/day)	0.87±0.030	0.86±0.030	0.87±0.030	0.77±0.030	0.067	0.040	0.126
REA (cm^2)	81.00±2.524	77.51±2.524	80.55±2.524	74.77±2.524	0.301	0.182	0.656
HCY (%)	58.64±0.450	58.53±0.450	59.43±0.450	59.09±0.450	0.467	0.264	0.795

[1]iSBW = Initial shrunk average body weight; fSBW = Final shrunk average body weight; iEBW = Initial empty body weight; fEBW = Final empty body weight; CCW = Cold carcass weight; HCW = Hot carcass weight; ADG = Average daily gain; EBWG = Empty body weight gain; HCG = Hot carcass gain; REA = Ribeye area; HCY = Hot carcass yield; TRAT = Differences of TRAT least squares mean; SEM = standard error of the mean; LIN = linear effect; and QUA = quadratic effect;

[2]The treatments are: 0%DDG = control diet without inclusion of high-protein DDG, containing soybean meal and urea as protein sources; 4%DDG = diet with 4% inclusion of high-protein DDG, with partial reduction of soybean meal and urea; 8%DDG = diet with 8% inclusion of high-protein DDG, with further partial reduction of soybean meal and urea; 12%DDG = diet with 12% inclusion of high-protein DDG, in which soybean meal and urea were completely removed.Urea/ammonium sulfate (AS) ratio of 9:1;

[3]Analysis was performed using Dunnett's multiple comparisons test for all parameters, and orthogonal polynomial contrasts were used to assess linear and quadratic effects associated with increasing levels of HP-DDG inclusion;

[4]The symbol (*) denotes a significant difference (P ≤ 0.05) compared with the control diet (0% HP-DDG), according to Dunnett's test.

Table 6 - Effect of inclusion of high-protein distillers grains (HP-DDG) replacing soybean meal and sulfated urea (SMU) based on total dietary on methane and carbon dioxide production in Nellore bulls during the finishing phase[1]

Item	Treatments[2]				P-Valor[3]		
	0% HP-DDG±SEM	4% HP-DDG±SEM	8% HP-DGG±SEM	12% HP-DDG±SEM	TRAT	LIN	QUA
CH_4, g/day‡	160.62±13.835	168.24±14.583	177.14±16.5358	131.13±13.835	0.153	0.213	0.078
CO_2, g/day‡	6966.57±189.380	6882.21±199.620	6795.04±226.350	6432.15±189.380	0.226	0.057	0.495
CH4/DMI g/kg‡	23.07±2.304	26.36±2.429	25.58±2.754	20.98±2.304	0.386	0.504	0.117
CH4/ADG g/kg‡	113.37±12.022	124.36±12.672	126.29±14.369	105.29±12.022	0.622	0.685	0.220
CH4/CARC g/kg‡	185.32±19.036	209.39±20.066	211.39±22.753	174.13±19.036	0.491	0.716	0.140
CO2/DMI g/kg‡	997.63±28.878	1064.85±30.440	979.38±34.516	1017.42±28.878	0.269	0.843	0.638
CO2/ADG g/kg‡	4862.55±224.280	4967.02±236.410	4921.75±268.06	5110.32±224.280	0.883	0.497	0.861
CO2/CARC g/kg‡	7984.01±299.810	8403.56±316.03	8137.48±358.35	8474.16±299.810	0.645	0.382	0.897

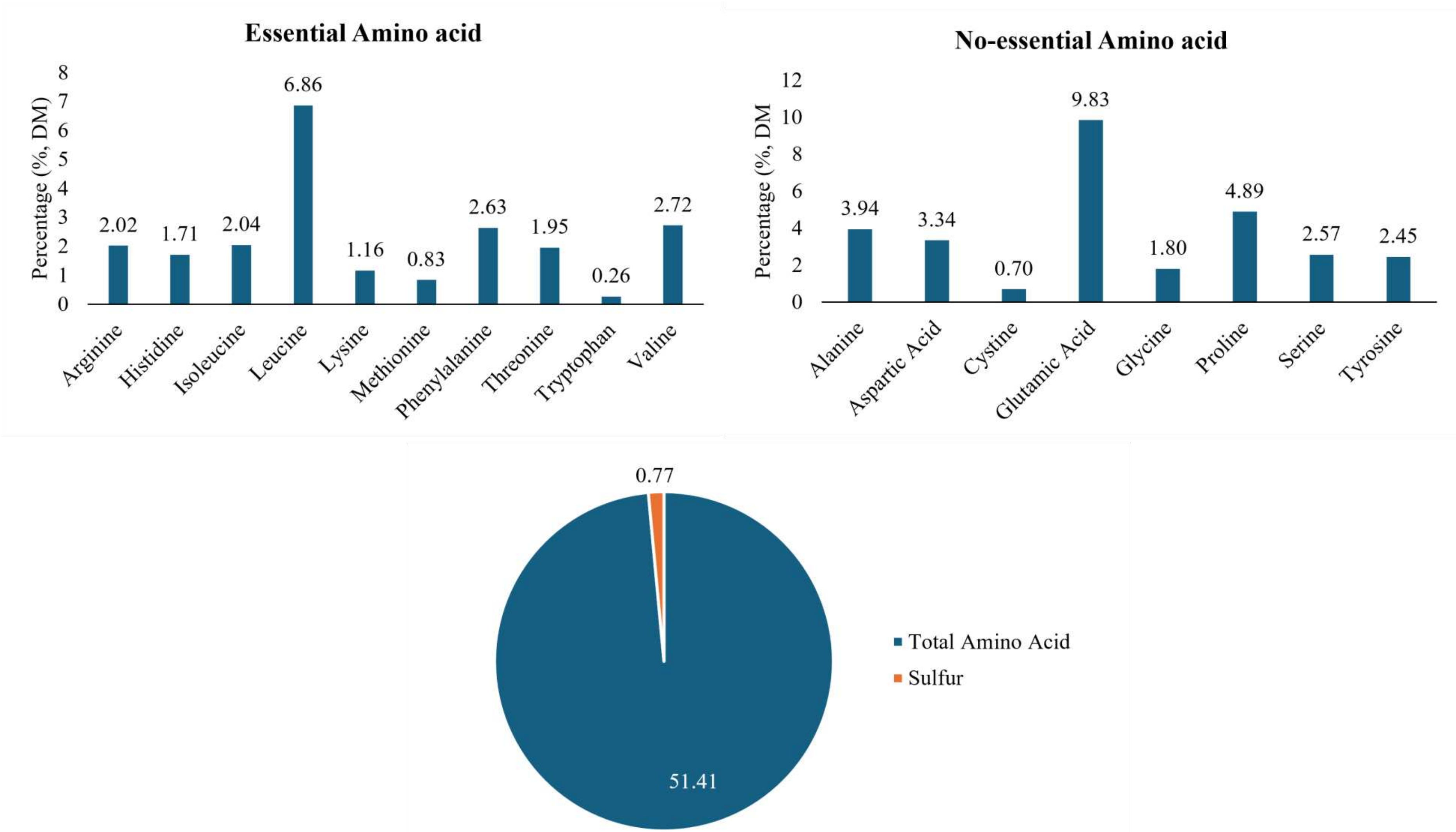
[1]CH4 = Methane; CO2 = Carbon dioxide; CH4/DMI = Methane production expressed as grams of CH4 per kilogram of dry matter intake (DMI); CH4/ADG = Methane production expressed as grams of CH4 per kilogram of average daily gain (ADG); CH4/CARC = Methane production expressed as grams of CH4 per kilogram of carcass gain; CO2/DMI = Carbon dioxide production expressed as grams of CO2 per kilogram of dry matter intake (DMI); CO2/ADG = Carbon dioxide production expressed as grams of CO2 per kilogram of average daily gain (ADG); CO2/CARC = Carbon dioxide production expressed as grams of CO2 per kilogram of carcass gain; TRAT = Differences of TRAT least squares mean; SEM = standard error of the mean; LIN = linear effect; QUA = quadratic effect.

[2]The treatments are: 0%DDG = control diet without inclusion of high-protein DDG, containing soybean meal and urea as protein sources; 4%DDG = diet with 4% inclusion of high-protein DDG, with partial reduction of soybean meal and urea; 8%DDG = diet with 8% inclusion of high-protein DDG, with further partial reduction of soybean meal and urea; 12%DDG = diet with 12% inclusion of high-protein DDG, in which soybean meal and urea were completely removed. Urea/ammonium sulfate (AS) ratio of 9:1.

[3]Analysis was performed using Dunnett's multiple comparisons test for all parameters, and orthogonal polynomial contrasts were used to assess linear and quadratic effects associated with increasing levels of HP-DDG inclusion.

‡ For methane and carbon dioxide data, records were available for only 36 animals.

Figure 1 – The total amino acid composition and sulfur content (%, DM) of the HP-DDG



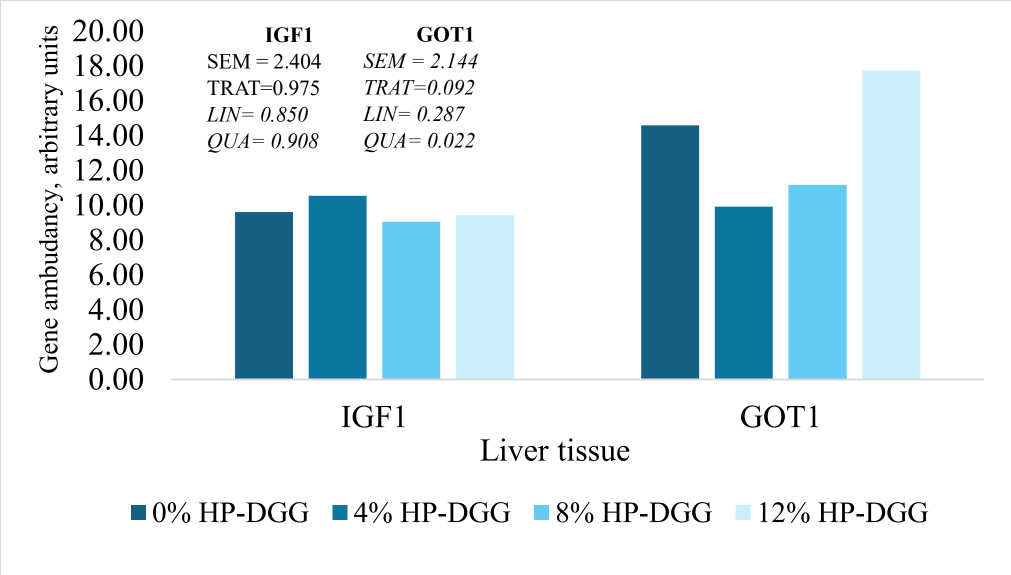


Figure 2 - Effect of inclusion of high-protein distiller's grains (HP-DDG) replacing soybean meal and sulfated urea (SMU) based on total dietary on gene expression in liver tissues of Nelore bulls during the finishing phase. Treatments = 0%DDG = control diet without inclusion of high-protein DDG, containing soybean meal and urea as protein sources; 4%DDG = diet with 4% inclusion of high-protein DDG, with partial reduction of soybean meal and urea; 8%DDG = diet with 8% inclusion of high-protein DDG, with further partial reduction of soybean meal and urea; 12%DDG = diet with 12% inclusion of high-protein DDG, in which soybean meal and urea were completely removed. Urea/ammonium sulfate (AS) ratio of 9:1; IGF1 = Insulin-like growth factor 1; GOT1 = Glutamic-Oxaloacetic Transaminase 1; TRAT = Differences of TRAT least squares mean; SEM = standard error of the mean; LIN = linear effect; QUA = quadratic effect.

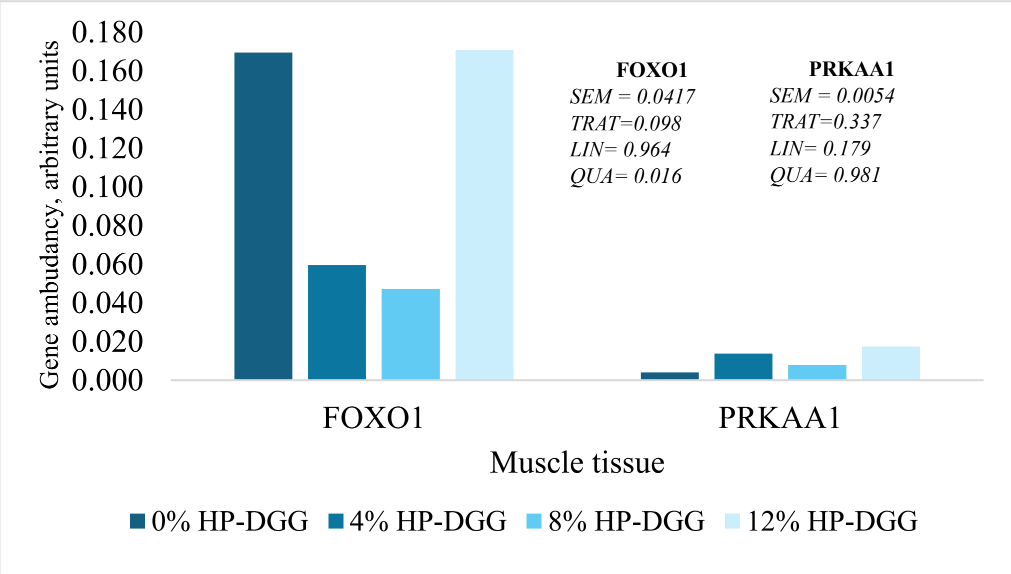


Figure 3 - Effect of inclusion of high-protein distiller's grains (HP-DDG) replacing soybean meal and sulfated urea (SMU) based on total dietary gene expression in muscle tissues of Nelore bulls during the finishing phase. Treatments = 0%DDG = control diet without inclusion of high-protein DDG, containing soybean meal and urea as protein sources; 4%DDG = diet with 4% inclusion of high-protein DDG, with partial reduction of soybean meal and urea; 8%DDG = diet with 8% inclusion of high-protein DDG, with further partial reduction of soybean meal and urea; 12%DDG = diet with 12% inclusion of high-protein DDG, in which soybean meal and urea were completely removed. Urea/ammonium sulfate (AS) ratio of 9:1; FOXO1 = Forkhead Box O1; PRKAA1 = Protein Kinase AMP - Actived Catalytic Subunit Alpha 1; TRAT least squares mean; SEM = standard error of the mean; LIN = linear effect; QUA = quadratic effect.