PAULIANE PUCETTI

IMPACTS OF DIFFERENT HIGH-FORAGE BACKGROUNDING LENGTHS ON THE PERFORMANCE OF EARLY-MATURING NELLORE BULLS, AND EFFECTS OF DIFFERENT CONCENTRATE LEVELS IN AGRI-002E SORGHUM SILAGE-BASED DIETS ON THE METABOLISM OF BEEF CATTLE

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

Adviser: Sebastião de Campos Valadares Filho

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ABSTRACT

PUCETTI, Pauliane, D.Sc., Universidade Federal de Viçosa, March, 2024. **Impacts of different high-forage backgrounding lengths on the performance of early-maturing Nellore bulls, and effects of different concentrate levels in AGRI-002E sorghum silage-based diets on the metabolism of beef cattle.** Adviser: Sebastião de Campos Valadares Filho.

Our objectives with this study were 1) to evaluate the effects of implementing different high-forage backgrounding (HFB) in early-maturing Nellore bulls as an alternative strategy to conventional early mature bulls' system, and 2) to evaluate the effect of increasing concentrate levels in AGRI-002E sorghum silage (SS)-based diets on nutrient intake and digestibility, ruminal pH and ammonia concentration, Nitrogen (N) balance, efficiency of microbial protein synthesis, and in situ degradability of complete diets. For the first objective, forty young Nellore bulls, with an initial body weight (BW) of 265.25 ± 5 kg and an average age of 7 ± 1 month were used. The bulls were randomly allotted to four treatment groups with different HFB lengths (0, 28, 56, and 84 days), followed by a 14-day step-up adaptation period, and a high-concentrate finishing phase (140, 112, 84, and 56 days respectively). The HFB diet consisted of 800 g/kg of sorghum silage and 200 g/kg of concentrate, while the finishing diet contained 200 g/kg of corn silage and 800 g/kg of concentrate. Two digestibility trials were conducted for each treatment to estimate apparent digestibilities in both HFB and finishing phases. Bulls were weighed at the beginning and end of each phase to measure shrunk BW (SBW) and average daily gains (ADG). At the end of the experiment, all bulls were slaughtered to evaluate carcass characteristics. For the second objective, five rumen-cannulated Nellore bulls received five dietary treatments in a 5×5 Latin square experimental design, with diets containing 0, 200, 400, 600, and 800 g of concentrate/kg on a DM basis. Each period lasted 24 days, with 17 days for dietary adaptation and 7 days for data collection. In situ degradability assays, total feces and urine collection, and omasal and ruminal digesta collection were performed to estimate nutrient intake, digestibility, N balance, and ruminal parameters. In the first study, HFB did not influence nutrient intake, apparent digestibility, or performance during the backgrounding phase. However, during the finishing phase, increased HFB length improved the apparent digestibility of dry matter (DM), organic matter (OM), crude protein (CP), and non-fiber carbohydrates (NFC). The final SBW, carcass weight, and carcass gain were highest in the 0-day HFB group and lowest in the 84-day HFB group. Hot and cold carcass dressings were similar among the 0, 28, and 56-day HFB groups but lower in the 84-day HFB group. In conclusion, implementing HFB improves nutrient digestibility but does not affect nutrient intake, ADG, or gain-to-feed ratio during the finishing phase. While HFB may lead to a

decrease in overall performance, both 28 and 56-day HFB lengths yield similar results in SBW, carcass weight, and carcass gain, suggesting a compensatory mechanism. In the second study, increasing concentrate levels in SS-based diets led to linear increases in the intake of DM, OM, CP, and ether extract (EE), with neutral detergent fiber (NDF) and starch intake showing quadratic responses. Ruminal digestibility of DM, OM, and CP exhibited quadratic responses, while NDF digestibility decreased linearly. Apparent total-tract digestibility of DM, OM, and EE increased linearly, whereas NDF and CP digestibility decreased linearly. Increasing concentrate levels improved in situ ruminal degradation parameters and nitrogen utilization, with higher total volatile fatty acids and microbial protein synthesis efficiency. The findings indicate that AGRI-002E sorghum silage is effective as a fiber source for high-concentrate diets, but its effectiveness is limited without concentrate supplementation.

Keywords: Rearing phase, Nellore bulls, Concentrate levels, Roughage, Microbial protein synthesis, Total digestible nutrients.

PUCETTI, Pauliane, D.Sc., Universidade Federal de Viçosa, março de 2024. **Impactos de diferentes tempos de recria no desempenho de novilhos Nelore super precoces, e efeitos de diferentes níveis de concentrado em dietas à base de silagem de sorgo AGRI-002E no metabolismo de bovinos de corte.** Orientador: Sebastião de Campos Valadares Filho.

Os objetivos deste estudo foram 1) avaliar os efeitos da implementação de diferentes períodos de recria em novilhos Nelore super precoces como uma estratégia alternativa ao sistema convencional de novilhos super precoces, e 2) avaliar o efeito do aumento dos níveis de concentrado em dietas à base de silagem de sorgo AGRI-002E (SS) sobre a ingestão e digestibilidade de nutrientes, pH ruminal e concentração de amônia, balanço de nitrogênio, eficiência de síntese de proteína microbiana e degradabilidade *in situ* de dietas completas. Para o primeiro objetivo, foram utilizados quarenta novilhos Nelore recém desmamados, com peso corporal inicial (PC) de 265,25 ± 5 kg e idade média de 7 ± 1 mês. Os animais foram aleatoriamente distribuídos em quatro grupos de tratamento com diferentes períodos de recria (0, 28, 56 e 84 dias), seguidos de um período de adaptação de 14 dias e uma fase de terminação de alto concentrado (140, 112, 84 e 56 dias, respectivamente). A dieta de recria consistiu em 800 g/kg de silagem de sorgo e 200 g/kg de concentrado, enquanto a dieta de terminação continha 200 g/kg de silagem de milho e 800 g/kg de concentrado. Foram realizados dois ensaios de digestibilidade para cada tratamento para estimar as digestibilidades aparentes em ambas as fases de recria e terminação. Os novilhos foram pesados no início e no final de cada fase para medir o PC em jejum (PCJ) e os ganho médio diário (GMD). Ao final do experimento, todos os novilhos foram abatidos para avaliar as características de carcaça. Para o segundo objetivo, cinco novilhos Nelore com cânulas ruminais receberam cinco tratamentos dietéticos em um delineamento experimental de quadrado latino 5x5, com dietas contendo 0, 200, 400, 600 e 800 g de concentrado/kg na base de MS. Cada período teve duração de 24 dias, com 17 dias para adaptação dietética e 7 dias para coleta de dados. Foram realizados ensaios de degradabilidade *in situ*, coleta total de fezes e urina e coleta de digesta omasal e ruminal para estimar a ingestão de nutrientes, digestibilidade, balanço de N e parâmetros ruminais. No primeiro estudo, o tempo de recria não influenciou a ingestão de nutrientes, a digestibilidade aparente ou o desempenho durante a fase de recria. No entanto, durante a fase de terminação, o aumento do período do tempo de recria melhorou a digestibilidade aparente da matéria seca (MS), matéria orgânica (MO), proteína bruta (PB) e carboidratos não fibrosos (CNF). O PCE final, o peso da carcaça e o ganho de carcaça foram maiores para 0 dias de recria e menores no para 84 dias de recria. As carcaças quentes e frias foram semelhantes entre temos de recria de 0, 28 e 56 dias, mas menores para 84 dias de recria. Em conclusão, a implementação da recria melhora a digestibilidade de nutrientes, mas não afeta a ingestão de nutrientes, o GDM ou

eficiênci alimentar durante a fase de terminação. Embora a recria possa levar a uma diminuição no desempenho geral, os 28 e 56 dias de recria apresentaram resultados semelhantes em PCJ, peso de carcaça e ganho de carcaça, sugerindo um mecanismo compensatório. No segundo estudo, o aumento dos níveis de concentrado em dietas à base de SS resultou em aumentos lineares na ingestão de MS, MO, PB e extrato etéreo (EE), com a ingestão de fibra em detergente neutro (FDN) e amido mostrando respostas quadráticas. A digestibilidade ruminal de MS, MO e PB apresentou respostas quadráticas, enquanto a digestibilidade de FDN diminuiu linearmente. A digestibilidade aparente total de MS, MO e EE aumentou linearmente, enquanto a digestibilidade de FDN e PB diminuiu linearmente. O aumento dos níveis de concentrado melhorou os parâmetros de degradação ruminal *in situ* e a utilização de nitrogênio, produção ácidos graxos voláteis totais e eficiência de síntese de proteína microbiana. Os resultados indicam que a silagem de sorgo AGRI-002E é eficaz como fonte de fibra para dietas de alto concentrado, mas sua eficácia é limitada sem suplementação de concentrado.

Palavras-chave: Sequestro de bezerros, Novilhos Nelore, Sorgo boliviano gigante, Forragem, Síntese de proteína microbiana, Nutrientes digestíveis totais.

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INTRODUCTION

Since 2003, Brazil has held the title of the world's leading beef exporter, with the largest commercial cattle herd globally (USDA, 2019). Despite this success, a critical challenge facing the Brazilian beef industry revolves around the age at which cattle are slaughtered, significantly influencing the perceived quality of Brazilian meat. Although improvements have been observed in recent years, only seventeen percent of the total cattle slaughtered in Brazil in 2023 were under 24 months old (IBGE, 2024). To confront this challenge, a strategic shift towards an early-maturing bulls' system has gained prominence, which aims to finish animals at 12-14 months of age. Implementing this system requires the adoption of creep-feeding methods to nursing calves followed by high-grain diets from weaning to slaughter (Valadares Filho et al., 2018). However, these practices are costly, often leading to economic impracticality. Therefore, alternative nutritional strategies should be explored.

Incorporating periods of backgrounding or feed restriction into the growing phase can potentially reduce costs by reducing total dry matter (DM) intake and improving feed efficiency from compensatory growth as compared with ad libitum feeding (Knoblich et al. 1997). Compensatory growth has been associated with increases in average daily gain and DM intake, improvements in efficiency of gain, and alterations in composition of gain (Sainz et al. 1995; Muir et al. 2001) as compared to animals continuously fed on a higher plane of nutrition (Therkildsen et al. 2002).

In this context, introducing a high-forage backgrounding phase (HFB) in early-mature bulls' systems emerges as a strategic response to the economic challenges associated with the high costs of finishing diets. This method offers a cost-effective approach by relocating recently weaned calves to a confinement system, replicating the nutritional content of high-quality pastures. The focus is on cost-saving through the selection of low-cost forage crops, such as the AGRI-002E sorghum genotype, known for

its high dry matter (DM) production (Da Rosa et al., 2022). The focus is on cost-saving through the selection of these productive forage options and optimizing resource utilization in cattle management, while also incorporating a balanced approach between dietary restriction and the potential for compensatory growth during the finishing phase.

The AGRI-002E is a sorghum genotype with Bolivian genetics (AGRICOMSEEDS). Despite its high productivity, this variety demonstrates minimal grain yield and is utilized primarily for silage. Furthermore, the AGRI-002E sorghum variety can serve as a beneficial source of roughage for different activities, such as in high-forage diets or being utilized as a fiber source in high-grain finishing diets. However, it should be noted that the lower grain production of AGRI-002E variety results in lower starch and higher NDF concentrations, which in turn, lead to lower nutrient digestibility than whole-plant corn (Samarappuli and Berti, 2018). To overcome this issue, the inclusion of concentrate in the diet will increase the supply of digestible organic matter, leading to an increase in energy concentration and a decrease in the concentration of poorly digestible fiber, and consequently resulting in higher DM intake and digestibility (Pereira et al., 2007). There is a notable gap in the literature regarding the effectiveness and duration of HFB in early-mature bulls, as well as the effects of concentrate level in AGRI-002E sorghum silage-based diets on nutrient metabolism of beef cattle. Therefore, a comprehensive investigation of both aspects is essential to inform and enhance the sustainability and economic viability of such practices in the beef industry.

Thus the objectives of this study were 1) to evaluate the impact of different HFB lengths on nutrient intake, apparent digestibility, performance, and feed efficiency during the HFB and finishing phases, as well as on carcass characteristics and body composition in early-mature Nellore bull; 2) to evaluate the effect of increasing concentrate levels in AGRI-002E sorghum silage-based diets on nutrient intake and digestibility, ruminal pH

and ammonia concentration, Nitrogen balance, and the efficiency of microbial protein synthesis.

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CHAPTER 1

High-Forage Backgrounding Phase in Early-Maturing Nellore Bulls: Can Time-on-Feed Affect Finishing Performance?[1]

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ABSTRACT

The objectives of this study were to evaluate the effects of implementing different high-forage backgrounding (HFB) in early-maturing Nellore bulls as an alternative strategy to conventional early mature bulls' system. Forty young Nellore bulls, with an initial body weight (BW) of 265.25 ± 5 kg and an average age of 7 ± 1 month were used. Four animals were slaughtered to provide baseline empty body weight (EBW), and empty body composition at Day 0. The remaining animals were randomly allotted to four treatment groups (n = 9/group). The treatments consisted of four different high-forage backgrounding (HFB) lengths (0, 28, 56, and 84 days), followed by a 14-day step-up adaptation period, and a high-concentrate finishing phase (140, 112, 84, and 56 days respectively). The HFB diet consisted of 800 g/kg of sorghum silage and 200 g/kg of concentrate, while the finishing diet contained 200 g/kg of corn silage and 800 g/kg of concentrate. All animals were fed ad libitum in an electronic feeder (INTERGADO®, Intergado Ltd., Contagem, MG, Brazil), and individual daily feed intake was recorded. Two digestibility trials were conducted for each treatment to estimate apparent digestibilities in both HFB and finishing phases. The bulls were weighed at the beginning and the end of each phase (HFB and finishing) after undergoing 16 h of fasting to measure shrunk BW (SBW) and ADG of both periods. At the end of the experiment, all bulls were slaughtered to evaluate carcass characteristics. The HFB did not influence nutrient intake, apparent digestibility, and performance during the backgrounding phase ($P>0.05$). In the finishing phase, nutrient intake was not influenced by the HFB length ($P>0.05$), However, we observed an interaction ($P<0.01$) between HFB duration and days on feed was observed during the first 28 days of finishing phase. Increasing HFB length improved the apparent digestibility during the finish phase of DM, OM, CP, and NFC ($P<0.05$), with a tendency noted for fiber apparent digestibility and TDN intake during the finishing

phase was detected (P<0.10). The final SBW, carcass weight, and carcass gain were higher in the 0-day HFB group compared to the 28 and 56-day groups, while the 84-day HFB group exhibited the lowest values for these parameters (P<0.05). Hot and cold carcass dressings didn't differ among the 0, 28, and 56-day HFB lengths but were lower in the 84-day HFB group (P<0.05). In conclusion, implementing HFB improves nutrient digestibility but does not affect nutrient intake, ADG, or G:F ratio during the finishing phase. Although the implementation of HFB may lead to a decrease in overall performance, both 28 and 56-day HFB lengths yield similar results in SBW, carcass weight, and carcass gain, suggesting a compensatory mechanism.

Keywords: Rearing phase, Stocker operations, Compensatory growth, Zebu.

INTRODUCTION

Since 2003, Brazil has held the title of the world's leading beef exporter, with the largest commercial cattle herd globally (USDA, 2019). Despite this success, a critical challenge facing the Brazilian beef industry revolves around the age at which cattle are slaughtered, significantly influencing the perceived quality of Brazilian meat. Although improvements have been observed in recent years, only seventeen percent of the total cattle slaughtered in Brazil in 2023 were under 24 months old (IBGE, 2024). To confront this challenge, a strategic shift towards an early-maturing bulls' system has gained prominence, which aims to finish animals at 12-14 months of age. Implementing this system requires the adoption of creep-feeding methods to nursing calves followed by high-grain diets from weaning to slaughter (Valadares Filho et al., 2018). However, these practices are costly, often leading to economic impracticality. Therefore, alternative nutritional strategies should be explored.

Incorporating periods of backgrounding or feed restriction into the growing phase can potentially reduce costs by reducing total dry matter (DM) intake and improving feed efficiency from compensatory growth (Knoblich et al. 1997). Compensatory growth has been associated with increases in average daily gain and DM intake, improvements in efficiency of gain, and alterations in composition of gain (Sainz et al. 1995; Muir et al. 2001) as compared to animals continuously fed on a higher plane of nutrition (Therkildsen et al. 2002).

In this context, introducing a high-forage backgrounding (HFB) in early-mature bulls' systems emerges as a strategic response to the economic challenges associated with the high costs of finishing diets. This method offers a cost-effective approach by relocating recently weaned calves to a confinement system, replicating the nutritional content of high-quality pastures. The focus is on cost-saving through the selection of low-cost forage

crops and optimizing resource utilization in cattle management, while also incorporating a balanced approach between dietary restriction and the potential for compensatory growth during the finishing phase.

However, there is a notable gap in the literature regarding the effectiveness and duration of HFB in early-mature bulls. Therefore, a comprehensive investigation of the efficiency and duration of HFB in early-mature bulls' systems is essential to inform and enhance the sustainability and economic viability of such practices in the beef industry.

We hypothesized that the implementation of HFB in early-mature bulls' system would enhance nutrient intake, apparent digestibility, and overall performance during the finishing phase. Therefore, the objectives of this study were to evaluate the impact of different HFB lengths on nutrient intake, apparent digestibility, performance, and feed efficiency during the HFB and finishing phases, as well as on carcass characteristics and body composition in early-mature Nellore bulls.

MATERIALS AND METHODS

This study was conducted at the Experimental Feedlot of the Animal Science Department at the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. All procedures were previously approved by the Ethics Commission on the use of farm animals of Universidade Federal de Viçosa (#032/2021).

Animals, experimental design, and diets

Forty young Nellore bulls (initial BW of 265.25 ± 5 kg; age of 7 ± 1 mo) were used. Four animals were selected randomly to compose the reference group. This group was used as a baseline reference for initial carcass weight of remaining animals and were slaughtered at the beginning of the experiment. The remaining animals were randomly allocated into four groups (n = 9/group) and assigned to receive one of 4 treatments. The treatments consisted of different HFB lengths (0, 28, 56, and 84 days), followed by an

adaptation to the finishing diet over a 14-day period, and subsequently, the finishing phase (140, 112, 84, and 56 days, respectively; Figure 1). Backgrounding and finishing diets (Table 1) were formulated according to the BR-CORTE system (Valadares Filho et al., 2023) targeting an ADG of 0.4 kg/day and 1.2 kg/day, respectively.

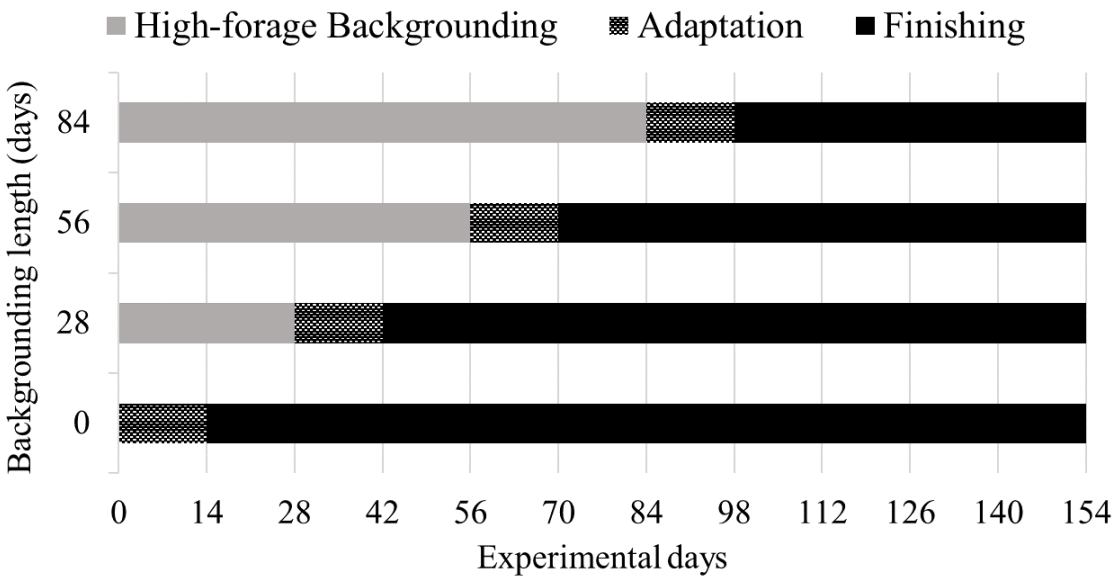

Figure 1. Experimental treatments

Table 1. Proportion of ingredients and nutrient composition of the experimental diets

Items	Diets	
	Backgrounding	Finishing
Ingredients, g/kg		
Corn Silage	-	200.0
Sorghum silage	800.0	-
Ground corn	136.6	740.4
Soybean Meal	28.4	23.4
Urea	4.5	4.5
Ammonium sulfate	0.5	0.5
Virginiamycin	-	1.3
Mineral mix[1]	30.0	30.0
Analyzed Composition, g/kg		
DM[2]	286.5	661.1
OM[3]	924.3	957.5
apNDF[4]	613.5	180.7
iNDF[5]	221.9	32.9
CP[6]	91.1	109.1
EE[7]	15.2	36.9
NFC[8]	212.6	638.8

[1]Mineral premix guarantees (per kg of DM): 190 - 240 g of Ca, 8.5 mg of Co (Min), 428 mg of Cu (Min),19 g of S (Min), 285 mg of Fe (Min), 10.3 g of P (Min), 21 mg of I (Min), 15 g of Mg (Min), 1285 mg of Mn (Min), 715 mg of monensin, 5.7 mg of Se (Min), 43 g of Na (Min), and 1714 mg of Zn (Min), 490g of protein nitrogen equivalent (Max). [2]Dry matter, [3]Organic matter, [4]Neutral detergent fiber corrected for ash and crude protein, [5]Indigestible neutral detergent fiber, [6]Crude protein, [7]Ether extract, [8]Non-fiber carbohydrates. In the "*Ingredients*" section, the unit g/kg represents grams of the ingredient per kilogram of dry matter in the diet. In the "*Analyzed composition*" section, the unit g/kg for dry matter represents grams of dry matter per kilogram of raw material, and for the other constituents represents grams of the constituent per kilogram of dry matter.

Measurements and sampling procedures

Each treatment was group-housed in a feedlot pen (48.0 m2) containing two electronic feeders (Model AF-1000 Master; Intergado, Contagem, MG, Brazil) and two waterers (ModelWD-1000 Master; Intergado, Contagem, MG, Brazil). Before the experiment, each bull was fitted with an ear tag (left ear) containing a unique radio frequency transponder (FDX-ISO 11784/11785; Allflex, Joinville, Santa Catarina, Brazil; Chizzotti et al., 2015). Bulls were allowed a 14-d adaptation period to the experimental conditions. The total mixed rations were provided twice a day, at 0700 and 1600 h. Feed delivery was adjusted daily to maintain minimum orts the next day and *ad libitum* intake. Daily feed intake was adjusted to maintain the orts within 2 to 5% of the amount offered (as-fed basis). The sorghum silage and corn silage were sampled daily and pooled weekly, oven-dried at 55°C for 72 h and ground in 1mm a knife mill (Willye mill, model TE-680, TECNAL, Brazil). Samples of each one of the concentrate ingredients were collected directly at the feed mill, and stored in a freezer at −20°C.

To assess apparent total-tract nutrient digestibility of the high-forage diet, fecal collections were carried out from day 14 to 18 for the groups that received the HFB for 28, 56 and 84 days. For the finishing diet, collections were performed 28 days into the finishing phase, specifically on days 42 to 46 for the treatment 0-d HFB, days 70 to 74 for the 28-d HFB, days 98 to 102 for the group with 56-d HFB, and days 126 to 130 for the group with 84-d HFB. Fecal samples were collected directly from the rectum of the animals. The collections were conducted at 1800 h on day 1, at 1500 h on day 2, at 1200 and on day 3, at 0900 h on day 4, and at 0600 h on day 5. The samples were immediately placed in a forced ventilation oven at 55°C for 72 h. Subsequently, they were ground using a 1-mm knife mill (Willye mill, model TE-680, TECNAL, Brazil). A composite sample (on an equal DM weight basis) from each animal was obtained and processed as

previously described for silage samples and stored for further chemical analyses. Indigestible neutral detergent fiber (iNDF) was used as a marker to estimate fecal DM excretion.

Body weight measurements were taken after 16 h of fasting to estimate shrunk body weight and average daily gain (ADG). Bulls were weighed at the beginning of the experiment, upon completion of the backgrounding phase, after the adaptation period and at the end of the trial, prior to slaughter (d 154). Animals were slaughtered via captive bolt stunning followed by exsanguination. Following slaughter, the gastrointestinal tract of each animal was emptied, washed, and weighed. The weight of the gastrointestinal tract was summed with the weight of heart, lungs, liver, spleen, kidneys, diaphragm, mesentery, tail, esophagus, trachea, pelvic and heart fat, head, hide, limbs, blood, and carcass for the determination of empty body weight (EBW). The cleaned organs and viscera were ground in an industrial cutter for 20 min to obtain a homogeneous sample of organs + viscera. The hide removed was manually chopped and sampled, and the head and limbs were ground in a bone grinder to obtain a homogeneous sample. The blood was quantified and subsampled. Samples of blood, head and limbs, organs and viscera, and hide, were lyophilized, processed, and grouped proportionally based on the dissection weights to compose a non-carcass sample.

After slaughter, the carcass of each animal was divided into two half-carcasses which were weighed and then cooled in a cold chamber at 4 °C for 18 h. Next, half-carcasses were removed from the cold chamber for weighing and the hot and cold carcass dressings were calculated. Subcutaneous fat thickness was measured using a digital caliper in the region between the 11th and 12th rib cut. The section between the 9th and 11th ribs was collected from the left half-carcass according to procedures described by Hankins and Howe (1946) to estimate the chemical composition of the carcass

components. This section was dissected into muscle, fat, and bone, and each portion was weighed separately. The muscle and fat from the section between the 9th and 11th ribs (HH section) of each bull were homogenized and ground to obtain a composite sample of muscle and fat. Bones from the same rib section were sliced with a band saw (Skymsen, model SFL-315HD, Santa Catarina, Brazil) in subsections of 1.5-cm length to obtain a representative sample of the bones. The composite sample of muscle and fat and the sample of rib bones were lyophilized and then ground in a knife mill (Fortinox, Piracicaba, São Paulo, Brazil) with a 1-mm mesh sieve. After processing samples of non-carcass and rib section were analyzed for DM, ash, CP and EE.

Laboratory analyses and calculations

Samples of feedstuffs, and feces were analyzed for DM (AOAC, 2012; method 934.01), organic matter (OM; AOAC, 2012; method 930.05), CP (AOAC, 2012; 981.10 method), and ether extract (EE; AOAC 2005; method 2003.05). The analysis of neutral detergent fiber (NDF) in feed and feces was performed according to the techniques described by Mertens (2002), without the addition of sodium sulphite, but with the addition of thermostable alpha-amylase (Ankom Tech. Corp., Fairport, NY, USA). The NDF concentration was corrected for residual ash and protein (apNDF). Estimations of neutral detergent insoluble nitrogen followed the technique described by Licitra et al. (1996). Indigestible NDF (iNDF) of fecal samples and feeds was determined according to Casali et al. (2008). Non-fiber carbohydrates (**NFC**) were calculated according to the following equation (Detmann and Valadares Filho., 2010): NFC (% DM) = 100 - [%CP - (%CP derived from urea +% urea) + % apNDF +% EE + %ash]. Fecal DM excretion was calculated by dividing iNDF intake by fecal iNDF concentration.

Carcass chemical composition was estimated using the equations described by Marcondes et al. (2010) for Nellore bulls, which were validated by Costa e Silva et al. (2013a) and recommended by BR-CORTE (2023):

$$\% \text{ EEcarc} = 4.31 + 0.31 \times \% \text{ EE}_{HH} + 1.37 \times \% \text{ VF}$$

$$\% \text{ CPcarc} = 17.92 + 0.60 \times \% \text{ CP}_{HH} - 0.17 \times \text{HCY}$$

$$\% \text{ Wcarc} = 48.74 + 0.28 \times \% \text{ W}_{HH} - 0.017 \times \text{EBW}$$

Where EEcarc = ether extract in the carcass, EE_{HH} = ether extract in the HH section; % VF = percentage of mesenteric fat plus renal, pelvic, and cardiac fat in the empty body; CPcarc = crude protein in the carcass; CP_{HH} = crude protein in the HH section; HCY = hot carcass yield (%); Wcarc = water in the carcass; W_{HH} = water in the HH section; EBW = empty body weight.

The composition of the empty body was estimated using values obtained from non-carcass analysis and carcass chemical composition estimated by equations described by BR-CORTE (2023).

Statistical analysis

Data were analyzed as a completely randomized design using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). Individual animals were treated as experimental units, and treatment was considered a fixed effect. The Tukey test was employed to compare means. Results were deemed significant when $P \leq 0.05$ and trending when $0.05 < P \leq 0.10$. The DMI over the first 28 days on finishing phase was analyzed as repeated measures, and was subjected to 8 covariance structures: unstructured, compound symmetric, heterogeneous compound symmetric, autoregressive of order one [AR(1)], heterogeneous first-order autoregressive [ARH(1)], toeplitz, heterogeneous toeplitz, and ante-dependence of order one [ANTE(1)]. The covariance structure that yielded the smaller Akaike and Schwarz's Bayesian criterion based on their −2 res log likelihood was considered to provide the best fit. Additionally, DMI variance was calculated for each bull from daily DMI data over the 28 days of the finishing phase after the 14-day step-up adaptation period.

RESULTS

Intake, digestibility and performance during high-forage backgrounding phase

The duration of the HFB had no impact on nutrient intake, apparent digestibility, or performance during the backgrounding phase ($P > 0.05$; Table 2).

Intake, digestibility, and performance during finishing phase

During the finishing phase, the HFB length had no impact on DM, OM, apNDF, CP, EE or NFC intake ($P>0.05$; Table 3). However, upon evaluating intake over the first 28 days of the finishing phase, it became apparent that despite the absence of disparities among the different HFB lengths ($P>0.05$), the interaction between HFB lengths and days in finishing phase was significant ($P<0.05$) (Figure 2).

Moreover, an increase in the HFB length resulted in a significant improvement in the apparent digestibility during the finish phase of DM, OM, CP, and NFC (P<0.05). Dry matter and OM digestibility were greater (P < 0.01) for 84-d and 56-d HFB compared to 0-d HFB. Further, 28-d HFB was similar to 56 and 0-d HFB. Crude protein digestibility was greatest (P < 0.05) for 56-d and lowest for 0-d and 28-d HFB, with 84-d HFB being intermediate. Non-fiber carbohydrate digestibility was greatest (P≤0.01) for 84-d and lowest for 0-d and 28-d HFB, with 56-d HFB being intermediate. Furthermore, there was a tendency in apNDF apparent digestibility and TDN intake during the finishing phase (P<0.10). ADG and feed efficiency were similar between all treatments (P > 0.05).

Table 2. Effects of high-forage backgrounding length on voluntary intake, total apparent digestibility, and performance of early-mature Nellore bulls during the backgrounding phase.

Items	High-forage backgrounding length (d)			SEM[1]	P-value
	28	56	84		
Intake, kg/d					
DM[2]	4.06	4.07	4.14	0.160	0.924
OM[3]	3.75	3.76	3.83	0.210	0.923
apNDF[4]	2.49	2.50	2.54	0.099	0.925
CP[5]	0.369	0.370	0.378	0.015	0.910
EE[6]	0.062	0.062	0.062	0.003	0.998
NFC[7]	0.86	0.87	0.88	0.03	0.930
TDN[8]	2.29	2.08	2.19	0.11	0.399
Intake, g/kg BW					
DM	1.50	1.47	1.48	0.056	0.921
Apparent digestibility g/kg					
DM	572.0	577.3	579.3	5.77	0.659
OM	600.2	605.6	606.4	6.02	0.738
apNDF	634.6	634.0	621.4	5.43	0.173
CP	686.0	688.6	704.2	7.71	0.219
EE	542.6	574.2	587.9	15.57	0.190
NFC	504.0	518.4	515.1	15.25	0.786

Performance					
ADG[9]	0.278	0.375	0.230	0.0524	0.401
G:F[10]	0.0688	0.0906	0.0678	0.011	0.299

[1]Standard error of mean, [2]Dry matter, [3]Organic matter, [4]Neutral detergent fiber corrected for residual ash and protein, [5]Crude Protein, [6]Ether extract, [7]Non-fiber carbohydrates, [8]Total digestible nutrients, [9]Average daily gain, [10]Gain-to-feed ratio.

Carcass Characteristics

The initial SBW was consistent for all treatments (P>0.05; Table 4). However, the final SBW, carcass weight, and carcass gain were greatest (P<0.05) for 0-d HFB and lowest for 84-d HFB, with intermediate values observed for 28-d and 56-d HFB. Hot and cold carcass dressings were similar between 0, 28, and 56-d HFB but were notably smaller for 84-d HFB (P<0.05).

The CP concentration on the final EBW did not differ among the treatments (P>0.05). However, retained CP was greater for 0 and 28-d HFB, with no significant difference between 28 and 56 days, while 84 days showed the lowest CP retention(P<0.05). Additionally, fat concentration on EBW was similar between 0-, 28-, and 56-d HFB, which were greater than 84-d HFB (P<0.05). No significant differences were detected in carcass length and backfat thickness among the different HFB lengths (P>0.05).

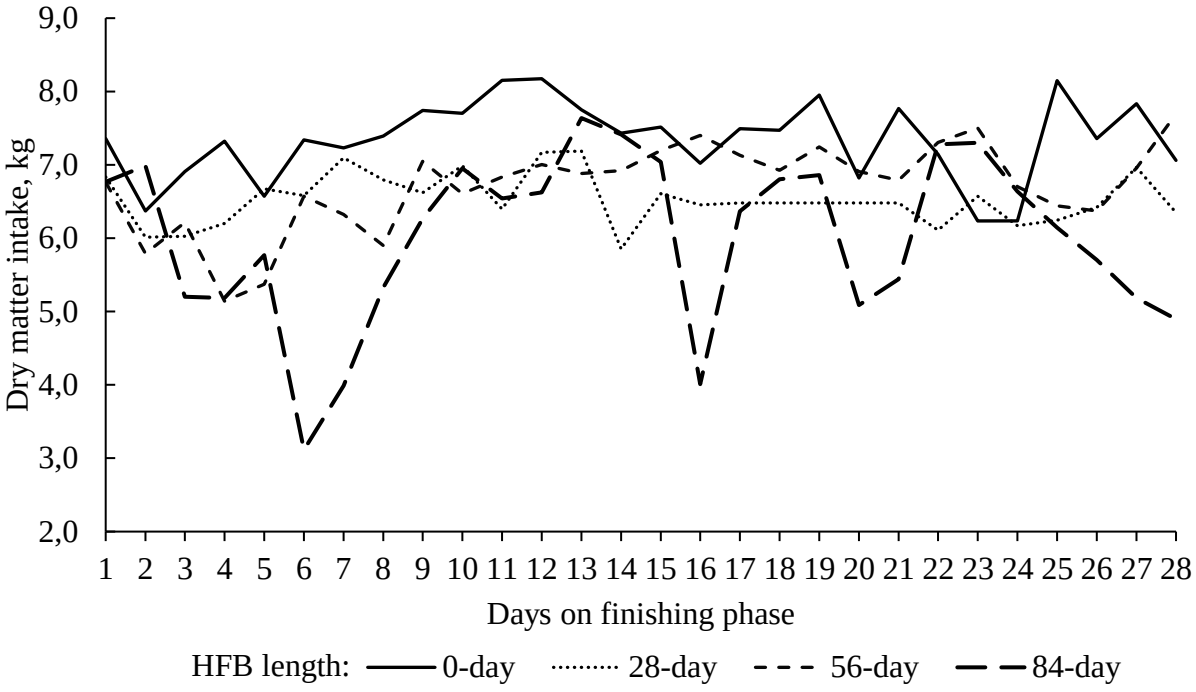

Figure 2. Effects of different high-forage backgrounding lengths (HFB) on subsequent dry matter intake, kg, of early-mature Nelore Bulls during the first 28 days of the finishing phase. Averages per treatment (P = 0.100; SEM = 0.363) – 0-day = 7.34, 28-day = 6.53, 56-day = 6.71, 84-day = 6.02. Significative interaction HFB*Days after adaptation was found (P <0.001).

Table 3. Effects of high-forage backgrounding length on voluntary intake, total apparent digestibility, and performance of early-mature Nellore bulls during the finishing phase.

Items	High-forage backgrounding length (d)				SEM[1]	P-value
	0	28	56	84		
Intake, kg/d						
DM[2]	6.63	6.44	7.09	6.18	0.305	0.212
OM[3]	6.35	6.16	6.79	5.92	0.292	0.211
apNDF[4]	1.2	1.16	1.28	1.12	0.055	0.215
CP[5]	0.724	0.702	0.774	0.674	0.0333	0.210
EE[6]	0.245	0.237	0.262	0.228	0.0113	0.210
NFC[7]	4.23	4.11	4.53	3.94	0.195	0.211
TDN[8]	5.31	5.01	6.05	5.32	0.257	0.062
Intake, g/kg BW						
DM	1.86	1.86	2.02	1.87	0.698	0.309
Apparent digestibility[9] g/kg						
DM	705.7^{c}	728.2^{bc}	752.8^{ab}	769.4^{a}	11.59	0.004
OM	723.2^{c}	746.7^{bc}	769.0^{ab}	787.4^{a}	10.93	0.002
apNDF	383.6	400.9	442.6	475.1	25.32	0.079
CP	691.9^{b}	699.3^{b}	741.5^{a}	730.5^{ab}	13.68	0.049
EE	809.2	836.6	860	853.9	17.94	0.238
NFC	820.6^{c}	853.5^{b}	871.6^{ab}	888.2^{a}	9.22	<0.001
Performance						
ADG[10]	1.15	1.14	1.24	1.05	0.082	0.442
G:F[11]	0.172	0.178	0.172	0.168	0.0087	0.873

[1]Standard error of mean, [2]Dry matter, [3]Organic matter, [4]Neutral detergent fiber corrected for residual ash and protein, [5]Crude Protein, [6]Ether extract, [7]Non-fiber carbohydrates, [8]Total digestible nutrients, [9]Aparent digestibility assessed 28 days into the finishing phase for each treatment, [10]Average daily gain, [11]Gain-to-feed ratio. [a,b]Means with different superscripts in the same row are different ($P < 0.05$).

Table 4. Effects of high-forage backgrounding length on body weight and carcass characteristics of early mature Nellore bulls.

Items	High-forage backgrounding length (d)				SEM[1]	P-value
	0	28	56	84		
Initial SBW[2], kg	265.8	265.8	265.7	263.8	9.87	0.998
Final SBW, kg	441.9[a]	416.8[b]	407.9[b]	363.3[c]	7.94	<0.001
Hot Carcass Weight, kg	274.2[a]	257.3[b]	249.0[b]	217.8[c]	5.40	<0.001
Cold Carcass Weight, kg	871.1[a]	254.3[b]	246.5[b]	215.3[c]	5.39	<0.001
Hot Carcass Dressing, %	62.0[a]	60.9[a]	61.0[a]	59.5[b]	0.470	0.007
Cold Carcass Dressing,%	61.3[a]	60.2[a]	60.3[a]	58.8[b]	0.460	0.006
Hot Carcass Gain, kg/day	0.729[a]	0.624[b]	0.567[b]	0.369[c]	0.0343	<0.001
Cold Carcass Gain, kg/day	0.723[a]	0.620[b]	0.565[b]	0.367[c]	0.0342	<0.001
CP on final EBW[3], g/kg	171	173	170	170	1.96	0.554
EE on final EBW, g/kg	147[a]	139[a]	145[a]	115[b]	5.37	<0.001
Retained CP, g/day	186.5[a]	171.0[ab]	148.5[b]	101.7[c]	9.12	<0.001
Retained fat, g/dia	277.2[a]	235.1[a]	241.1[a]	133.1[b]	18.03	<0.001
Carcass length, cm	121	119	112	116	1.40	0.165
Backfat thickness, mm	4.44	3.1	3.79	2.49	0.683	0.226

[1]Stantart error mean, [2]Shrunk body weight, [3]Empty body weight. [a, b] Means with different superscripts in the same row are different ($P < 0.05$).

Partial budgeting analysis

Considering the initial SBW, DMI, and ADG values from this study for backgrounding and finishing phases, a partial budget is presented in Table 5. The analysis assumes a constant DMI and ADG values throughout the entire finishing period, with all animals reaching a standardized weight of 450 kg, and a carcass dressing percentage equivalent to the treatment 0-d HFB. In the presented scenario, the partial economic analysis indicates that the treatments with 0-d and 56-d HFB presented the highest profitability.

Table 5. Partial economic analysis of different high-forage backgrounding lengths.

Items	High-forage backgrounding length (d)			
	0	28	56	84
Backgrounding phase costs				
Feed cost, USD	0.00	13.75	27.58	42.07
Operational cost	0.00	5.95	11.90	17.85
Finishing Phase costs				
Days on feed to acquire 450kg	147	141	118	139
Feed cost	213.16	198.71	182.85	187.24
Operational cost	44.65	42.85	35.82	42.08
Animal acquisition				
Initial body weight, kg	265.8	265.8	265.7	263.8
Cost of a young bull	548.13	548.13	547.92	544.00
Total Costs	805.93	809.40	806.06	833.25
Total revenue	883.69	883.69	883.69	883.69
Profit	77.75	74.29	77.62	50.44

[1]Feed prices and operational costs were based on February 2024 values in Campo Grande, Mato Grosso do Sul, Brazil. The cost per kg of DM for each feed is as follows: Corn silage (US$0.14), Sorghum silage (US$0.07), Ground corn (US$0.19), Soybean meal (US$0.52), Urea (US$0.53), Virginiamycin (US$10.08), Mineral mix (US$0.80). Additionally, the cost per kg of DM for each diet was calculated: Backgrounding diet (US$0.12) and Finishing diet (US$0.22). Operational costs per day for each phase were as follows: Backgrounding (US$0.21) and Finishing (US$0.30). The purchase price of the young bulls was US$2.06/kg of BW, and the selling price of the finished cattle was 3.17 US$/kg of carcass, both sourced from CEPEA (Center for Advanced Studies on Applied Economics – Luiz de Queiroz College of Agriculture/University of São Paulo), with data from February 27, 2024. These values are reflective of the specified time and location.

DISCUSSION

There was no effect on nutrient intake, apparent digestibility, ADG, and feed efficiency during the backgrounding phase. This absence of impact can be attributed to the fact that all animals had equal initial weights and received the same diet throughout this phase.

During the finishing phase, the different HFB lengths examined in this study demonstrated no significant impact on nutrient intake. However, upon evaluating the initial 28 days of the finishing phase, although there were no significant differences observed among different HFB lengths, the interaction between HFB lengths and days in

this phase proved to be significant. This interaction suggests that the impact of HFB length on daily DMI may vary over time, even if the overall means do not show clear differences between groups.

Furthermore, examining the daily DMI pattern of the 84-d HFB treatment revealed a greater fluctuation in daily DMI. As noted by Nagaraja and Titgemeyer (2007), cattle subjected to nutritional restriction before the adaptation period may face a higher risk of health issues due to potential challenges in ruminal adaptation to high-concentrate diets. This transition from a forage-based diet to high-concentrate diets is critical for feedlot cattle, as it involves significant changes in ruminal microbiota and fermentation processes, which can greatly affect animal performance. Considering this, the 14-day adaptation period utilized in this present study may not have been adequate to fully adapt the animals after 84 days of HFB.

Moreover, a pattern emerged in the apparent digestibility of key nutrients. Specifically, an increase in HFB length correlated with higher apparent digestibility of DM, OM, CP, and NFC, and exhibited a notable trend in enhancing the digestibility of apNDF as well. This observation suggests that an extended HFB positively influences the animals' ability to utilize these nutrients in the finishing phase. Furthermore, the noted tendency in TDN intake aligns with improved digestibility, providing a potential explanation for the observed trends in nutrient utilization. These findings underscore the nuanced relationship between HFB length, nutrient digestibility, and overall dietary efficiency during the finishing phase.

Despite the absence of significant effects of different HFB on ADG and G:F ratio, the final SBW, carcass weights, and carcass gain provide valuable insights into the overall performance. Notably, while the 0-day HFB length exhibited a higher final SBW, carcass weight, and carcass gain, there was no differences between the 28 and 56-day HFB

lengths, indicating a comparable performance between them. It's worth highlighting that, despite the 56-day HFB length having a shorter duration in the finishing diet (84 days in the finishing phase), the final SBW and carcass weights were comparable to the 28-day HFB length. This equivalence suggests that the animals undergoing the 56-day HFB length exhibited a compensatory mechanism, potentially optimizing nutrient utilization and body weight development during the finishing phase.

Considering that carcass gain reported in this study is relative to the overall experimental average and acknowledging the variable nature of carcass gain rates throughout the experiment, it can be inferred that, to achieve similar averages between the 28 and 56-day HFB, the carcass gain must have been higher during the finishing phase for the 56-day HFB. This is evident given the observed reduction in ADG during the HFB phase, resulting in a corresponding smaller carcass gain during the HFB phase. It's worth noting that the 84-day HFB treatment may also have an increased carcass gain during the finishing phase. However, the smaller carcass dressing observed for the 84-day HFB treatment, combined with the fact that all animals were slaughtered simultaneously, suggests that this treatment may not have had adequate time to fully express its impact. Additionally, the 84-day HFB treatment exhibited signs of inadequate adaptation to the diet at the first 28 days of the finishing phase, which could have also contributed to its failure to fully realize its potential.

Furthermore, the findings indicating no difference in carcass length among the treatments can provide valuable insights into the pattern of carcass gain in the animals. This pattern may explain the possible higher carcass gain in the animals with a 56-day HFB during the finishing phase. Understanding the physiology of animal growth reveals that bone tissue is described as an early developing tissue, while muscle tissue features intermediate growth, followed by fat tissue which reaches peak growth rates at the later

stages of cattle growth (Augustini, Branscheid, Schwarz, & Kirchgeßner, 1992; Berg & Butterfield, 1968; Owens et al., 1993). During the HFB phase, bone tissue deposition takes precedence, allowing for a larger 'frame' and facilitating increased carcass gain during the finishing phase. This suggests that the compensatory mechanism observed in the 56-day HFB length may be linked to the prioritization of bone tissue growth during the earlier phases, creating a foundation for enhanced carcass gain in the later stages of the experiment.

The concentration of CP in the final EBW showed no significant differences among treatments. The predominant component of the bulls' empty bodies is muscle tissue, and the proportion of CP within the muscle remained consistent across all treatments. Muscle growth initiates prenatally, characterized by an increase in the number of muscle fibers (hyperplasia), a process established before birth (Greenwood, Hunt, Hermanson, & Bell, 2000; Hocquette, 2010). Subsequent postnatal growth involves an increase in the size and diameter of muscle fibers (hypertrophy), accompanied by changes in fiber types (Picard & Gagaoua, 2020). Consequently, maintaining a constant protein content becomes essential to support muscle function and accommodate the frequent reorganization of muscle fibers throughout the bull's growth (Honig *et al.*, 2022). However, CP retention exhibited a distinct pattern, with higher values noted in the 0 and 28-d HFB treatments, showing no differences between 28 and 56-d HFB, and the 84-d HFB treatment presented the lowest CP retention. That is explained by different final SBW.

The proportion of fat in EBW has been reported to increase with the increasing of live weight of animals, as noted by Honig et al. (2022). In the present study, the concentration of EE in the EBW and retained fat showed no discernible differences among the 0, 28, and 56-d HFB treatments. However, these concentrations were notably

higher compared to the 84-d HFB. This observed distinction could be attributed to the shorter duration of finishing phase of the 84-d HFB treatment, potentially limiting the animals' time to reach the optimal weight for enhanced fat deposition.

Examining the partial budgeting analysis provides insights into potential profitability scenarios based on the initial SBW, DMI, and ADG values observed in this study during the backgrounding and finishing phases. Under the assumptions of constant DMI and ADG values, with all animals reaching a standardized weight of 450 kg, the results indicate that treatments with 0 and 56-d HFB demonstrated the highest profitability in the given scenario. However, the economic analysis presented in this study reflects a specific time and location, and results may vary under different conditions. This information serves as a guide for decision-making, emphasizing the importance of considering operational and feedstuff costs in the evaluation process.

However, it is imperative to interpret these findings within the context of the study's limitations. The assumptions of constant DMI and ADG throughout the finishing period, leading to a uniform weight endpoint, simplify a complex biological process. For better inferences, subsequent research should aim for a comprehensive understanding of carcass gain during the finishing phase. This involves conducting slaughter at the end of each HFB length and maintaining the animals in the finishing phase until they reach the slaughter weight. Furthermore, conducting experiments in commercial production settings would enable a complete economic analysis, considering all production factors influencing costs.

CONCLUSION

In conclusion, the implementation of high-forage backgrounding does not impact nutrient intake but enhances the apparent digestibility of DM, OM, CP, and NFC during the finishing phase. While this improvement suggests a potential increase in TDN intake, it does not affect ADG or G:F ratio during the finishing phase. Despite a possible decrease in overall performance with high-forage backgrounding implementation, both 28-d and 56-d high-forage backgrounding lengths reveal similar results on final SBW, carcass weight, and carcass gain, indicating a compensatory mechanism. The long-term effects of an 84-d high-forage backgrounding remain inconclusive, emphasizing the need for further research to enhance our understanding of factors influencing animal performance.

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CHAPTER 2

Effects of different concentrate levels in AGRI-002E sorghum silage-based diets on nutrient intake and digestibility, ruminal pH and ammonia concentration, ruminal degradability, and microbial efficiency in beef cattle [1]

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ABSTRACT

This study aimed to evaluate the effect of increasing concentrate levels in AGRI-002E sorghum silage (SS)-based diets on nutrient intake and digestibility, ruminal pH and ammonia concentration, Nitrogen (N) balance, efficiency of microbial protein synthesis, and *in situ* degradability of complete diets. Five rumen-cannulated Nellore bulls (age = 8 ± 1.0 months; initial BW = 242 ± 5 kg) received five dietary treatments in a 5×5 Latin square experimental design. The dietary treatments consisted of five concentrate levels (0, 200, 400, 600, and 800 g of concentrate/kg on a DM basis) in SS-based diets. The experiment lasted 120 d, with five periods of 24 d. Each period consisted of 17 d for dietary adaptation, and 7 d for data collection. *In* situ degradability assays were conducted to estimate ruminal degradability. Total feces and urine collection were performed to estimate nutrient intake and digestibility and estimate N balance. Omasal and ruminal digesta collection were performed to estimate ruminal digestibility and ruminal parameters. Increasing concentrate levels in SS-based diets led to linear increases ($P<0.001$) in the intake of dry matter (DM), organic matter (OM), crude protein (CP), and ether extract (EE). Neutral detergent fiber corrected for ash and protein contamination (apNDF) and starch intake showed quadratic responses ($P\leq0.008$). Ruminal digestibility of DM, OM and CP responded quadratically ($P\leq0.040$), while apNDF exhibited linear decrease ($P=0.003$). Starch ruminal digestibility exhibited a cubic effect ($P=0.016$). Apparent total-tract digestibility of DM, OM, and EE increased linearly ($P\leq0.001$), whereas apNDF, and CP digestibility decreased linearly ($P\leq0.012$), and starch presented a quadratic effect ($P=0.029$). *In situ* ruminal degradation parameters increased linearly with higher concentrate levels ($P<0.001$). Ruminal pH exhibited a quadratic pattern ($P=0.006$), ammonia concentration linearly decreased ($P=0.003$). Total volatile fatty acids, and butyrate showed linear increases ($P<0.001$), acetate and propionate had

quadratic effects (P<0.001), while the ratio Acetate to Propionate decreased linearly (P<0.001). N intake, fecal N excretion, retained N, total digestible nutrients, digestible organic matter, and microbial production increased linearly (P<0.001), although urine N excretion, urinary urea, and blood urea concentration decreased linearly (P<0.028). Therefore, increasing concentrate levels in AGRI-002E sorghum silage-based diets improve TDN intake, microbial protein synthesis efficiency, *in situ* ruminal degradability parameters, and nitrogen utilization. Moreover, our findings indicate that AGRI-002E sorghum silage demonstrates potential as a fiber source for high-concentrate diets. However, its effectiveness is limited without concentrate supplementation, emphasizing the importance of balanced dietary composition for optimal utilization in beef cattle.

Keywords: Roughage, Ruminal parameters, Microbial protein synthesis, Total digestible nutrients.

INTRODUCTION

The interest in sorghum silage as a feedstuff for livestock has been increasing in recent years, especially in regions with limited water availability for plant growth. Sorghum is highly tolerant to heat and drought stress (Rooney et al., 2007), requires low water input due to high-water use efficiency (Howell et al., 2008; McCollum et al., 2005), and can be grown on marginal land with lower fertilizer input (Ganyo et al., 2019; Maucieri et al., 2016).

Therefore, to meet the growing global demand for food production, plant breeders have focused on traits likely to affect its productivity, such as yield and/or forage quality. Yield depends on the potential of a plant to accumulate dry matter (DM; Miron et al., 2007). Consequently, developing new sorghum varieties with high DM yield at harvest is a desirable agronomic target.

The AGRI-002E is a sorghum genotype with Bolivian genetics (AGRICOMSEEDS) renowned for its high DM production (Da Rosa et al., 2022). Furthermore, characterized by minimal grain yield, it is predominantly cultivated for silage production. This heightened productivity significantly reduces overall production cost, making the AGRI-002E sorghum variety a valuable source of roughage for various purposes, such as high-forage diets or a fiber source in high-grain finishing diets.

However, it should be noted that the lower grain production results in lower starch and higher NDF concentrations, which in turn, lead to lower nutrient digestibility than whole-plant corn (Samarappuli and Berti, 2018). To overcome this issue, the inclusion of concentrate in the diet will increase the supply of digestible organic matter, leading to an increase in energy concentration and a decrease in the concentration of poorly digestible fiber, and consequently resulting in higher DM intake and digestibility (Pereira et al., 2007).

This way, it's crucial to emphasize the significance of assessing the AGRI-002E sorghum as a forage source in high-concentrate diets. Despite this, there is a notable gap in research regarding the effects of concentrate levels in AGRI-002E sorghum silage-based diets on beef cattle nutrient metabolism. Existing literature examining concentrate inclusion in diets based on other sorghum silage types often overlooks evaluations at higher levels of concentrate inclusion.

Thus, we hypothesized that increasing concentrate levels in AGRI-002E sorghum silage-based diets would improve intake, digestibility of nutrients and ruminal parameters. Additionally, we propose that AGRI-002E sorghum silage is a viable option for use in diets with high concentrate levels. The objective was to evaluate the effect of increasing concentrate levels in AGRI-002E sorghum silage-based diets on nutrient intake and digestibility, ruminal pH, ruminal ammonia nitrogen (NH_3-N) and volatile fatty acids (VFAs) concentration, N balance, and the efficiency of microbial protein synthesis.

MATERIALS AND METHODS

This study was conducted at the Experimental Feedlot of the Animal Science Department at the Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. All procedures were previously approved by the by the Ethics Commission on the use of farm animals of Universidade Federal de Viçosa (#032/2021).

Animals, experimental design, facilities, and diets

Five rumen-cannulated Nellore bulls (age = 8 ± 1.0 months; initial body weight = 242 ± 5 kg) were utilized in a 5 × 5 Latin square design. Bulls were treated for internal and external parasites before initiation of the experiment, were housed in a tie-stall barn with a concrete floor, and had free access to feed and water throughout the experiment.

The experiment was conducted using five 24-d periods. Each period consisted of 17-d adaptation to the experimental diets and 7-d for sample collection. The dietary treatments consisted of five concentrate levels (0, 200, 400, 600, and 800 g of concentrate/kg on a DM basis) in SS-based diets (Tables 1 and 2). During the adaptation, a step-up method was employed over the initial 6 days to gradually increase the concentrate level in the diets, particularly focusing on the 800 g/kg treatment, from a lower concentrate inclusion level to the target levels. The diets were formulated according to Valadares Filho et al. (2016) to provide approximately 120 g of crude protein per kg dietary DM. In the 0 g/kg concentrate diet, the crude protein level was adjusted with urea and ammonium sulfate. All diets contained the same inclusion rate of virginiamycin and mineral supplement (Table 2).

Total mixed rations (TMR) were provided twice a day, at 0700 h and 1600 h. Feed delivery was adjusted daily to maintain orts at approximately 50 g/kg of the amount offered on an as-fed basis. Once per week, SS was sampled and dried in a non-ventilated oven at 105˚C for 16 h to adjust dietary inclusion levels.

Table 1. Chemical composition of diet ingredients.

Ingredients	DM[1]	OM[2]	apNDF[3]	iNDF[4]	CP[5]	EE[6]	Starch
g/kg							
Sorghum silage	266.6	944.8	758.2	315.6	55.3	10.9	6.21
Ground corn	895.1	990.1	117.3	11.4	85.2	41.9	647.2
Soybean meal	907.6	943.5	140.1	12.5	484.3	24.7	22.5
Urea	995.8	1000.0	-	-	2792.1	-	-
Ammonium sulfate	992.5	989.4	-	-	1297.9	-	-
Virginiamicin	998.4	25.2	-	-	-	-	-
Mineral premix[7]	977.7	270.4	-	-	334.4	-	-

[1]Dry matter, [2]Organic matter, [3]Neutral detergent fiber corrected for ash and crude protein, [4]Indigestible neutral detergent fiber, [5]Crude protein, [6]Ether extract, [7]Mineral premix guarantees (per kg of DM): 190 - 240 g of Ca, 8.5 mg of Co (Min), 428 mg of Cu (Min),19 g of S (Min), 285 mg of Fe (Min), 10.3 g of P (Min), 21 mg of I (Min), 15 g of Mg (Min), 1285 mg of Mn (Min), 715 mg of monensin, 5.7 mg of Se (Min), 43 g of Na (Min), 1714 mg of Zn (Min), and 490g of protein nitrogen equivalent (Max). The unit g/kg for dry matter represents grams of dry matter per kilogram of raw material, and for the other constituents represents grams of the constituent per kilogram of dry matter.

In situ ruminal degradation procedures

From day 13 to 17 of each experimental period, *in situ* ruminal degradability of each TMR was evaluated. All ingredients of the diets were collected daily and stored at -20°C between day 2 and 8 of each experimental period, dried in a forced-air oven at 55°C for 72 h, and ground using a knife mill (model TE-680, Tecnal, Piracicaba, São Paulo, Brazil) with a 2-mm sieve. To compose the diet, SS and concentrate were weighed separately to provide the appropriate roughage:concentrate ratio on a DM basis.

Approximately 6 g of dried sample was weighed into nylon bags (Sefar Nitex, Switzerland; 50-μm porosity, 400 cm^2 surface area) and incubated in each animal. The incubation for each TMR was carried out in the same animal that was receiving the corresponding dietary treatment. The sample mass to bag surface area was 15 mg/cm^2. Incubation was performed to allow the following ruminal degradation times: 0, 2, 4, 6, 12, 24, 48, 72, and 96 h (Benedeti, *et al*., 2019; Silva *et al*., 2020). The number of bags varied as a function of the incubation time to guarantee enough residual samples after incubation, where more bags per sample were incubated for the longer (96, 72, and 48 h; n = 4) incubation times relative to the shorter (24, 12, 6, 4, 2, and 0 h; n = 2) incubation times. Thus, two blanks per incubation time and animal were incubated.

In situ bags were attached to a steel chain (90 × 2 cm) with a weight at the end, thus allowing for complete immersion within the ruminal fluid below the fiber mat. Bags were placed into the rumen in reverse order of incubation time such that all bags were removed at the same time for rinsing. After the incubation period, the bags were rinsed in running water followed by washing with cold tap water by hand by the same person. The endpoint for rinsing was when the rinse water appeared clear after flowing through the bags (Zanetti et al., 2017). The 0-h bags were not incubated in the rumen, but as with the incubated bags, they were rinsed in running water. Samples were oven-dried at 55°C for

72 h. In sequence, bags were placed in an oven at 105°C for 2 h and weighed. The residues of each diet were removed from the nylon bags, ground using a knife mill (Tecnal, Piracicaba, São Paulo, Brazil) with a 1-mm sieve.

Intake, total tract digestibility, and microbial efficiency

Feeds offered and orts from each animal were weighed daily during the collection period (from days 18 to 24). All diet ingredients and refusals were sampled daily and stored at –20˚C. At the end of each collection period, all samples were dried in a ventilated oven (55 ˚C) for 72 h and ground using a knife mill (model TE-680, Tecnal, Piracicaba, São Paulo, Brazil) with a 1-mm sieve. Daily samples of each diet ingredient were equally composited (DM basis) for each period. Also, daily refusal samples were composited for each animal within a period. The proportion of daily refusal in the composite sample was based on the amount of daily refusal by the total amount of refusal samples during the collection period, on a DM basis. The ingredient samples were analyzed individually and used to calculate dietary composition.

Daily dry matter intake was calculated by subtracting feed refusal DM from DM offered. To estimate nutrient digestibility, total feces were collected from each bull over four consecutive days, from days 18 to 21 of each experimental period. After 24 h of collection, the total fecal output was weighed, mixed, and approximately 250 g were oven-dried (55°C for 72 h). Fecal samples then were ground using a knife mill (model TE-680, Tecnal, Piracicaba, São Paulo, Brazil) with a 1-mm sieve. Based on fecal DM production of each day of the 4-d fecal collection period, a composite sample was constituded for each animal per experimental period.

Total urine output was collected from days 18 to 21 (over a 24 h period) using a collection funnel connected to tubing and a 20-L container with 200 mL of 50% sulfuric acid to prevent N volatilization. Total urine volume was measured daily using a 2-L

volumetric cylinder, mixed manually, subsampled at a constant percentage per day per animal, and stored frozen at -20˚C. Microbial efficiency was estimated as described by Barbosa et al. (2011) using daily purine derivative (allantoin and uric acid) excretion. Microbial efficiency was expressed as grams of microbial crude protein synthesized (gMCP) per kilogram of total digestible nutrient (TDN) intake and per kilogram of digestible OM (DOM) intake.

Table 2. Feedstuffs and composition of experimental diets.

Item	Concentrate level (g/kg)				
	0	200	400	600	800
Ingredients, g/kg					
Sorghum silage	950.1	800.0	600.0	400.0	200.0
Ground corn	0.0	89.2	304.3	519.4	734.5
Soybean meal	0.0	74.5	59.4	44.3	29.2
Urea	16.8	4.5	4.5	4.5	4.5
Ammonium sulfate	1.9	0.5	0.5	0.5	0.5
Virginiamycin	1.3	1.3	1.3	1.3	1.3
Mineral premix	30.0	30.0	30.0	30.0	30.0
Analyzed Composition, g/kg					
DM[1]	276.7	310.6	371.4	461.6	609.8
OM[2]	924.4	927.6	937.4	947.2	956.9
apNDF[3]	720.3	627.4	498.9	370.4	241.9
iNDF[4]	299.9	254.5	193.6	132.8	71.9
CP[5]	111.9	111.2	111.1	111.1	111.1
EE[6]	10.3	14.3	20.8	27.2	33.7
Starch	5.9	64.4	202.0	339.7	477.3

[1]Dry matter, [2]Organic matter, [3]Neutral detergent fiber corrected for ash and crude protein, [4] Indigestible neutral detergent fiber, [5]Crude protein, [6]Ether extract. In the "*Ingredients*" section, the unit g/kg represents grams of the ingredient per kilogram of dry matter in the diet. In the "*Analyzed composition*" section, the unit g/kg for dry matter represents grams of dry matter per kilogram of raw material, and for the other constituents represents grams of the constituent per kilogram of dry matter.

Marker infusion and Partial Digestibility estimation

Collections of omasal digesta were performed from days 22 to 24 of each experimental period to estimate ruminal degradability and ruminal flow. The digesta flow was estimated using a double marker (Rotta et al., 2014) approach, with Co-EDTA

associated with liquid and small particles, and iNDF associated with large particles, as described by Valadares Filho et al. (2011). From days 19 to 24 of each experimental period, a solution of 5 g/d of Co-EDTA (0.51 g cobalt/d) was continuously infused into the rumen of each bull via a peristaltic pump (model BP-600.4, Colombo, Parana, Brazil) and tubing connected to the ruminal fistula at a rate of 115 mL/h. A total of eight omasal samples per animal were collected over a 9-h interval for three days: day 1 at 0800 h and 1700 h; day 2 at 0200 h, 1100 h, and 2000 h; day 3 at 0500 h, 1400 h and 2300 h, simulating a 24-h collection with 3-h intervals. Omasal digesta samples were collected as described by Huhtanen et al. (1997) and adapted by Leão et al. (2004). At each collection time, 200 mL of omasal digesta simple was filtered (porosity of 100 μm, 44% of the surface, Sefar Nitex 100/44, Sefar, Thal, Switzerland). Solid and liquid phases were individually sampled and lyophilized (Liobras, São Carlos, São Paulo, Brazil). The solid phase samples were combined for each bull, weighed, and ground using a knife mill with a 1-mm sieve. The same process was used for samples from the liquid phase. The reconstituted omasal digesta was considered the sum of both solid and liquid phases (DM basis). Samples of ruminal contents (approximately 250 ml per collection time) were manually collected from the cranial, ventral, and caudal areas of the rumen, at the same times previously described for omasal collections. Samples were filtered through a 100-μm nylon filter (Sefar Nitex; Sefar, Thal, Switzerland; porosity of 100 μm), and the pH of the ruminal fluid was measured using a digital potentiometer (Kasvi, São José dos Pinhais, Paraná, Brazil). A 50-ml aliquot of ruminal fluid from each sample was stored at -80°C for further analysis of volatile fatty acids (VFA) and ammonia-N (NH3-N).

Laboratory analysis and calculations

Samples of feedstuffs, refusals, feces, and omasal digesta were analyzed for DM (method 934.01), N (method 981.10), and ash (method 930.05) according to AOAC

(2012) and EE (method 2003.05) according to AOAC (2005). Organic matter was considered the difference between DM and ash. Crude protein concentration was calculated by multiplying the total N concentration by 6.25. Neutral detergent fiber concentration was determined with the addition of thermostable α-amylase (Ankom Tech. Corp., Fairport, NY, USA), without the addition of sodium sulphite. The residue was corrected for ash (Mertens et al., 2002) and residual N (Licitra et al., 1996) (apNDF). The iNDF concentration was determined according to(Valente et al., 2011), through ruminal incubation of samples for 288-h using F57 filter bags (25 μm; ANKOM Technology, Macedon, NY). The starch concentration was quantified according to Silva et al. (2019). Samples of omasal digesta and ruminal fluid were analyzed for cobalt by atomic absorption spectrophotometry (Spctr AA-800; Varian spectrometer, Harbor City, CA, USA) according to Kimura and Miller (1957). Concentration of uric acid (automatic biochemical analyzer; autoanalyzer, model BS200E, Mark Mindray; Model BS200E; Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China) and allantoin in urine was determined using the methods described by Chen and Gomes (1992).

For VFA (acetate, butyrate, and propionate) analysis, ruminal fluid samples were centrifuged (12,000 × g, 10 min, 4◦C) and supernatants were treated as described by Siegfried et al. (1984). Ruminal VFA were analyzed via high performance liquid chromatography (HPLC) using a Dionex Ultimate 3000 Dual detector HPLC (Dionex Corporation, Sunnyvale, CA, USA) coupled to a refractive index Shodex RI-101 maintained at 40◦C using an ion exchange column Phenomenex Rezex ROA, 300 × 7.8 mm maintained at 45◦C. The mobile phase was prepared with 5 mmol/l sulfuric acid (H2SO4) and a flow of 0.7 ml/min. The concentration of NH3-N in ruminal and omasal fluids was determined according to the technique described by (Okuda et al., 1965).

In situ degradation parameters of DM were estimated by using the first-order asymptotic model described by Ørskov and McDonald (1979):

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

where Yt = degraded fraction of DM in time 't', (%); a = readily soluble fraction, (%); b = potentially degradable fraction in the rumen, (%); kd = rate constant for degradation of b, per h; and t = time, h.

The estimation of in vivo digestibility by the number of hours of *in situ* incubation was performed as described by (Alhadas et al., 2021). Briefly, for each in vivo digestibility of DM and OM, a confidence interval (1 - α= 0.95) was calculated based on the standard deviation. With the mean digestibility of diets, a higher value for digestibility (upper limit = mean + confidence interval) and a lower value for digestibility (lower limit = mean - confidence interval) were estimated. Then, estimated times for the *in situ* incubations to assess the in vivo digestibility of DM and OM were defined as the interval in which *in situ* degradation equaled in vivo digestibility (lower limit to upper limit), using the following equation:

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

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

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

Statistical analysis

The data were analyzed as a 5 × 5 Latin square design with PROC MIXED of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA) using the following statistical model: $Y_{ijk} = \mu + D_i + a_j + p_k + e_{ijk}$ where: Y_{ijk} = dependent variable, μ = general mean; D_i = fixed

effect of concentrate level i; a_j = random effect of animal j; p_k = random effect of experimental period k (random); and e_{ijk} = random error taken as normal and independently distributed (NID) (0, σ2e). Linear, quadratic, and cubic effects were evaluated using orthogonal contrasts obtained with PROC IML of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA), assuming their equidistance (0, 200, 400, 600, 800 g/kg). Ammonia-N concentrations and pH were analysed as repeated measures using the PROC MIXED procedure in SAS (version 9.4, SAS Institute Inc., Cary, NC, USA) using the REPEATED statement. The model tested was similar to that described above, including time as a fixed effect and all interactions. The unstructured covariance structure was used for random factors.

RESULTS

Intake and digestibility

Intake of DM (kg/d or g/kg of BW), OM, CP, and EE linearly increased ($P < 0.001$) with increasing concentrate level in the diet (Table 3). In contrast, apNDF and starch intake responded quadratically ($P \leq 0.008$), with the lowest observed value for apNDF occurring at a concentrate level of 800 g/kg, while starch intake was lowest in the no-concentrate diet.

Furthermore, alterations in the concentrate level not only affected intake but also influenced nutrient digestibility. Ruminal digestibility of DM, OM, and CP responded quadratically ($P \leq 0.040$) to the concentrate level. Conversely, ruminal digestibility of apNDF decreased linearly ($P = 0.003$). Moreover, a cubic effect resulting in a decreased starch ruminal digestibility was observed ($P = 0.016$). No significant differences were observed in the ruminal digestibility of EE ($P > 0.05$). Similar to ruminal digestibility, the apparent total-tract digestibility of DM, OM, and EE showed linear increases ($P < 0.001$).

In contrast, the apparent total-tract digestibility of apNDF, and CP decreased linearly (P < 0.012), and starch presented a quadratic pattern (P = 0.029).

Table 3 - Effects of increasing concentrate level in AGRI-002E sorghum silage-based diets on dry matter and nutrient intake, and ruminal and apparent total-tract digestibility.

Items	Concentrate level (g/kg)					P-value			SEM[1]
	0	200	400	600	800	L	Q	C	
Intake, kg/d									
DM[2]	2.70	3.33	4.86	5.48	5.90	< 0.001	0.357	0.360	0.37
OM[3]	2.52	3.11	4.58	5.23	5.68	< 0.001	0.423	0.346	0.35
apNDF[4]	1.93	2.01	2.38	1.98	1.45	0.090	0.008	0.466	0.17
CP[5]	0.36	0.45	0.63	0.70	0.76	< 0.001	0.337	0.507	0.04
EE[6]	0.03	0.05	0.11	0.16	0.21	< 0.001	0.107	0.307	0.01
Starch	0.10	0.26	1.04	1.94	2.86	< 0.001	0.001	0.090	0.11
Intake, g/kg BW									
DM	12.2	14.6	20.5	22.1	23.1	< 0.001	0.225	0.413	1.51
Ruminal digestibility, g/kg									
DM	193.9	238.5	293.5	370.3	485.8	< 0.001	0.040	0.647	21.42
OM	261.1	307.8	355.9	422.6	531.6	< 0.001	0.034	0.436	18.00
apNDF	409.1	374.0	325.5	336.1	302.9	0.003	0.465	0.599	23.54
CP	448.1	330.1	256.3	167.3	272.0	< 0.001	0.002	0.134	32.22
EE	161.6	257.9	214.6	210.3	233.0	0.730	0.741	0.550	108.34
Starch	1000.0	940.9	881.6	811.9	879.7	< 0.001	0.001	0.016	17.72
Apparent total-tract digestibility, g/kg									
DM	555.4	594.1	639.0	691.3	726.7	< 0.001	0.983	0.490	14.50
OM	574.0	612.5	660.4	705.3	739.8	< 0.001	0.773	0.537	14.07
apNDF	633.0	607.1	557.0	546.6	500.3	< 0.001	0.984	0.815	20.77
CP	797.9	797.6	781.4	763.5	776.4	0.012	0.442	0.101	11.44
EE	659.6	709.4	797.8	877.5	850.1	0.001	0.305	0.280	43.16
Starch	1000.0	1000.0	1000.0	994.2	973.0	0.003	0.029	0.366	5.25

[1]Standard error of mean, [2]Dry matter, [3]Organic matter, [4]Neutral detergent fiber corrected for residual ash and protein, [5]Crude Protein, [6]Ether extract. L, Q and C represent the effects of the linear, quadratic, and cubic contrasts, respectively.

Ruminal degradation

The increase in concentrate levels led to a rise in both the readily soluble fraction (a) and the potentially degradable fraction in the rumen (b), as well as in the rate constant for the degradation of b (kd; $P < 0.001$; Table 4). As a result, the ruminal degradability of dry matter at 24, 48, 72, and 96 hours also exhibited a linear increase with the increasing concentrate level ($P < 0.001$; Table 4). The *in situ* incubation times to access *in vivo* digestibility of DM and OM ranged between 36 and 72 hours (Fig. 1).

Table 4 - Effects of different concentrate levels in AGRI-002E sorghum silage-based diets on *in situ* degradation parameters of dry matter of the diets.

Items[1]	Concentrate level (g/kg)					P-value			SEM[2]
	0	200	400	600	800	L	Q	C	
Degradation parameters									
a	143	158	181	216	240	<0.001	0.245	0.345	6.34
b	452	482	542	565	580	<0.001	0.091	0.226	11.3
kd	0.0316	0.0380	0.0425	0.0411	0.0548	<0.001	0.365	0.057	0.00347
Ruminal degradation g/kg									
at 24h	382	441	527	563	662	<0.001	0.515	0.321	13.7
at 48h	494	554	652	692	776	<0.001	0.769	0.862	10.6
at 72h	546	601	697	743	807	<0.001	0.327	0.375	8.12
at 96h	571	621	713	764	816	<0.001	0.223	0.119	7.88

[1] a, readily soluble fraction (g/kg); b, potentially degradable fraction in the rumen (g/kg); kd, rate constant for degradation of b (per h); [2]Standard error of mean. L, Q and C represent the effects of the linear, quadratic, and cubic contrasts, respectively.

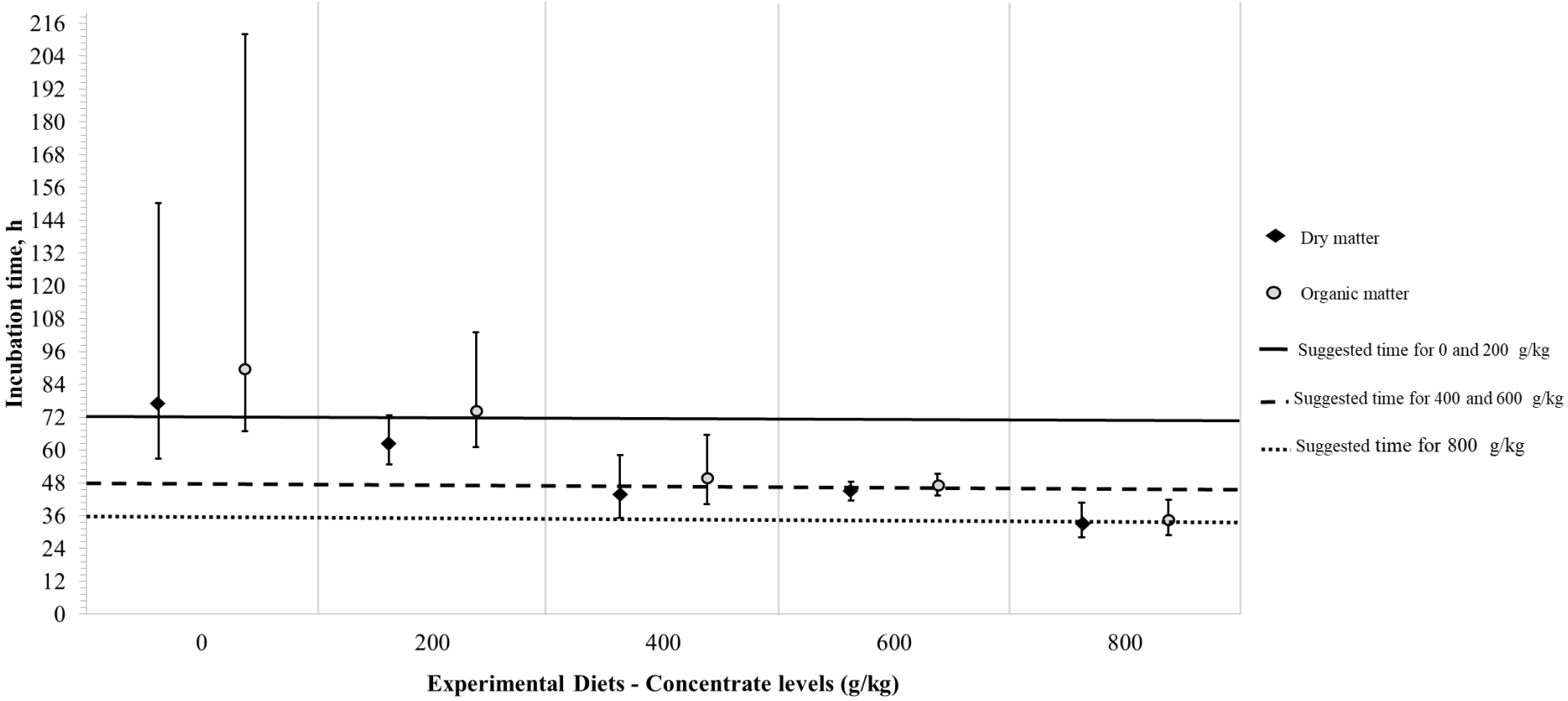

Fig. 1. Suggested incubation times for in situ procedure and incubation time intervals in which the *in situ* degradability predicts the in vivo digestibility of DM and OM of different concentrate levels in AGRI-002E sorghum silage-based diets.

Ruminal pH, ammonia and VFA

Ruminal pH showed a quadratic effect (P = 0.006) as the concentrate level in the diet increased, it also exhibited a quadratic effect depending on the sampling time (P = 0.002), and there was no interaction concentrate level x sampling time (P = 0.188; Fig. 2). Furthermore, the concentration of ruminal NH_3-N linearly decreased in response to the inclusion of concentrate levels (P = 0.003) and exhibited cubic variations throughout the day (P < 0.001; Fig. 3). Significative interaction concentrate level x sampling time was found (P < 0.001).

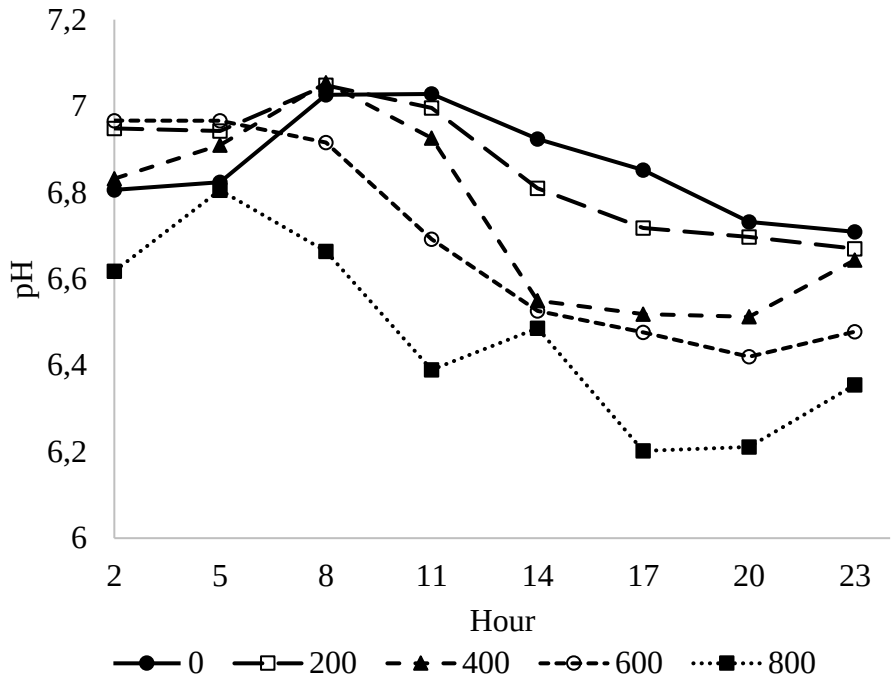

Fig. 2. Effects of different concentrate levels in AGRI-002E sorghum silage-based diets on ruminal pH. Averages per treatment (linear effect P < 0.001; quadratic effect, P = 0.006; SEM = 0.0164) - 0 g/kg = 6.86, 200 g/kg = 6.85, 400 g/kg = 6.74, 600 g/kg = 6.68, 800 g/kg = 6.47. Hourly averages (linear effect, P = 0.002; quadratic effect, P < 0.001; SEM = 0.0665) - 2h = 6.83, 5h = 6.89, 8h = 6.94, 11h = 6.81, 14h = 6.66, 17h = 6.55, 20h = 6.51, 23h = 6.57. There was no interaction Concentrate level * Sampling time (P = 0.188).

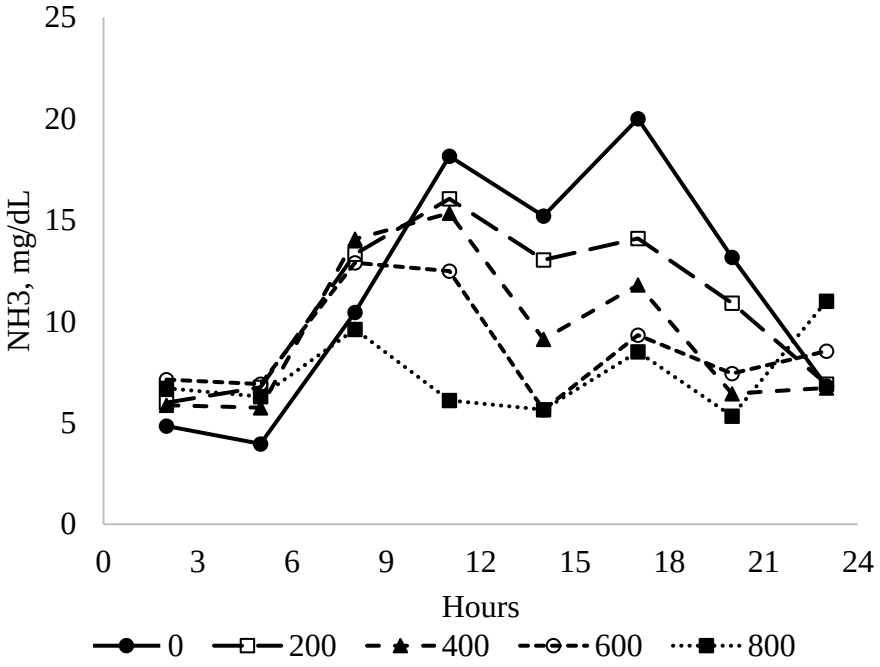

Fig. 3. Effects of different concentrate levels in AGRI-002E sorghum silage-based diets on ruminal ammonia concentration. Averages per treatment (linear effect P =0.003; SEM = 1.1619) - 0 g/kg = 11.58, 200 g/kg = 10.89, 400 g/kg = 9.39, 600 g/kg = 8.80, 800 g/kg = 7.41. Hourly averages (linear effect, P < 0.001; quadratic effect, P < 0.001; cubic effect, P < 0.001; SEM = 0.798) - 2h = 6.11, 5h = 5.94, 8h = 12.07, 11h = 13.63, 14h = 9.74, 17h = 12.75, 20h = 8.66, 23h = 8.00. Significative interaction Concentrate level * Sampling time was found (P <0.001).

In addition, the increase in concentrate level resulted in a linear increase (P = 0.001) in total VFA, and butyrate. A quadratic effect was found in acetate and proprionate concentrations (P ≤ 0.001), while it led to a linear decrease in the ratio Acetate:Propionate (P≤ 0.001; A:P; Table 5).

Nitrogen balance and microbial protein synthesis

The increase in concentrate level resulted in a linear increase in N, TDN, and DOM intake (Table 6). Furthermore, there was a linear increase ($P < 0.01$) observed in the excretion of N through feces and the retention of N (measured in g/d or g/kg of N intake). Additionally, the microbial N and microbial efficiency exhibited a linear increase ($P<0.01$). Conversely, the excretion of N through urine, the urinary urea, and the blood urea concentration demonstrated a linear decrease ($P<0.05$).

Table 5 - Effects of different concentrate levels in AGRI-002E sorghum silage-based diets on volatile fatty acids (VFA).

Items	Concentrate level (g/kg)					P-value			SEM[1]
	0	200	400	600	800	L	Q	C	
Total VFA, mmol/L	49.1	48.3	53.1	57.3	57.5	0.001	0.841	0.149	2.77
Individual, mmol/100mmol									
Acetate	70.6	66.9	63.8	57.3	47.81	<0.001	<0.001	0.235	1.01
Propionate	25.2	27.6	28.9	35.0	43.44	<0.001	<0.001	0.270	1.05
Butyrate	4.25	5.51	7.31	7.74	8.75	<0.001	0.372	0.986	0.617
Acetate to Propionate Ratio	2.83	2.42	2.22	1.66	1.10	<0.001	0.056	0.449	0.0954

[1]Standard error of mean. L, Q and C represent the effects of the linear, quadratic, and cubic contrasts, respectively.

Table 6 - Effects of different concentrate levels in AGRI-002E sorghum silage-based diets on Nitrogen (N) balance and microbial efficiency.

Items	Concentrate level (g/kg)					P-value			SEM[1]
	0	200	400	600	800	L	Q	C	
N intake, g/d	58.5	72.7	100.9	112.1	121.5	<0.001	0.337	0.498	7.14
Fecal N, g/d	11.7	14.6	22.4	26.6	27.3	<0.001	0.276	0.183	2.16
Urinary N, g/d	52.9	43.0	38.9	37.0	30.5	0.028	0.712	0.621	6.43
Urinary urea N, g/day	34.0	26.6	26.7	18.8	15.5	<0.001	0.982	0.750	2.83
Blood urea N, mg/dL	29.0	28.3	17.2	16.3	13.5	<0.001	0.274	0.082	1.88
Retained N, g/d	-6.19	15.05	39.60	48.56	63.66	<0.001	0.223	0.879	5.57
Retained N, g/kg N intake	-74.3	194.0	390.7	432.2	527.2	<0.001	0.074	0.575	75.9
TDN[2] intake, kg/day	1.61	2.03	3.21	3.97	4.55	<0.001	0.929	0.234	0.237
DOM[3] intake, kg/day	1.45	1.90	3.00	3.68	4.20	<0.001	0.734	0.271	0.224
Microbial N, g/day	21.4	24.7	53.1	53.7	82.5	<0.001	0.109	0.802	3.59
gMCP[4]/kg NDT intake	82.6	77.5	103.6	85.1	114.2	0.002	0.309	0.401	5.96
gMCP/lg DOM intake	91.8	82.5	110.8	91.8	123.9	0.003	0.166	0.523	6.52

[1]Standard error of mean, [2]Total digestible nutrients, [3]Digestible organic matter, [4]Microbial crude protein. L, Q and C represent the effects of the linear, quadratic, and cubic contrasts, respectively.

DISCUSSION

Intake and digestibility

The increased intake of DM, OM, CP, EE, and starch, with increasing inclusion of concentrate can be attributed to the higher supply of TDN in diets with greater concentrate content (Euclides Filho et al., 1997). Furthermore, Detmann et al. (2014) reported a linear negative relationship between dry matter intake and the content of undigested NDF in diets. The level of undigested NDF is influenced by the fiber content and the amount of iNDF in the diet, which decreases with the inclusion of concentrate. Additionally, the results support the theory that an increase in dietary concentrate enhances the degradation rate (kd), which in turn increases passage rate, thereby promoting increased intake (Van Soest, 1994).

With increasing concentrate levels in the diet, there is a simultaneous increase in starch concentration. This, combined with the linear increase in dry matter intake, provides an explanation for the quadratic pattern observed in starch intake. Regarding to ap NDF intake, the decrease with higher concentrate levels can be attributed to the dilution effect of concentrate intake on the overall dietary fiber content.

The linear increase in the total apparent digestibilities of DM and OM with increasing concentrate level is likely due to the increase in the intake of non- fiber carbohydrates as proposed by Valadares Filho (1985). This author attributed the increase in digestibility in diets with higher levels of concentrate to a larger concentration of non-structural carbohydrates in these diets, which are more digestible than structural carbohydrates. Linear increases for total apparent digestibilities of DM and OM, as a function of concentrate level, were also observed by Ítavo et al. (2002) and Pereira et al. (2007).

The linear decline in apparent total-tract NDF digestibility with increasing concentrate level may be because the existence of a competitive mechanism between amylolytic and fibrolytic microorganisms, given the accelerated growth of amylolytics because of increased efficiency in utilizing nitrogen available in the rumen (Olson et al., 1999). Consequently, diets with elevated concentrate levels could favor the expansion of amylolytic microorganisms at the expense of cellulolytic microorganisms and consequently decrease rate of fiber digestion. Similar results were found by Pereira et al. (2007) and Ítavo et al. (2002). Anyway, it has to be underlined the high fiber fractions digestibility of sorghum silage as reported also by Tudisco et al (2021) who attributed the results to the low lignin content.

Pereira et al. (2007) reported no effects of concentrate levels on ruminal digestibility of DM, OM, CP, and non-fiber carbohydrates, which contradicts the results in the present study where concentrate level resulted in a quadratic increase in ruminal digestibility of DM, OM, and starch, a quadratic decrease in ruminal digestibility of CP. The average ruminal apparent digestibility of CP obtained by Pereira et al. (2007) and Carvalho et al. (1997b) was 390 g/kg and 180 g/kg, respectively, whereas the current study found values ranging from 167 to 448 g/kg. Consistent with these authors' findings, positive values for ruminal CP digestibility may suggest ammonia absorption in the rumen, potentially leading to a loss of dietary protein. Given this observation, it is presumed that the dietary CP content exceeded the requirements of the ruminal microorganisms.

Similar to Pereira et al. (2007), who assessed the utilization of Volumax sorghum silage with varying levels of increasing concentrate, the present study revealed a linear decrease in the apparent total-tract digestibility of apNDF. Notably, no differences in ruminal digestibility were observed when expressed as a function of the total digestible.

However, the mean ruminal digestibility expressed as a function of the total digestible identified in the present study was 618.3 g/k, a value significantly lower than the 902 g/kg reported by Pereira (2007). Worth mentioning is that the highest value of apparent total-tract NDF digestibility recorded by Pereira et al. (2007) was 567.7 g/kg at a concentrate level of 200 g/kg of DM, whereas in the present research, at the same concentrate level, it was 607.1 g/kg.

The present study suggests no impact on ruminal digestibility of EE, which agrees with the findings of Carvalho et al. (1997b), Ítavo et al. (2002), and Pereira et al. (2007). However, these researchers reported ruminal digestibility of EE of 89 g/kg, 14 g/kg, and 107 g/kg, respectively, all of which are lower than the minimum value identified in this study, which was 162 g/kg.

An elevation in concentrate levels has been found to contribute to a decrease in the ruminal digestibility of starch. This reduction in starch ruminal digestibility can be attributed to an increased passage rate present in diets with elevated concentrate levels. This results aligns with the findings of Moharrery et al. (2014), who showed in their meta-analysis that increased starch intake, as occurred in higher concentrate level diets, reduced the starch ruminal digestibility with 14 g/kg per kg increase in starch intake.

Moreover, the relationship between concentrate levels and ruminal digestibility appears to display greater complexity, as quadratic and cubic effects were also observed. Apart from the linear and quadratic effects, the total-tract starch digestibility exhibited consistently high values, with the lowest recorded at 973 g/kg.

Ruminal degradation

In the present study, the *in vivo* apparent total-tract digestibility of DM was compared with DM *in situ* degradation. For the treatments with 0 and 200 g/kg of concentrate, the values for apparent total-tract DM digestibility were obtained through a 72-hour incubation period. For the treatments with 400 and 600 g/kg of concentrate, the corresponding digestibility values were determined based on a 48-hour incubation. In the case of the treatment with 800 g/kg of concentrate, the *in vivo* digestibility falls within the degradation values obtained for 24-hour and 48-hour incubations, approximately around 36 hours.

Several studies have demonstrated the utility of *in situ* degradability as a valuable tool for estimating *in vivo* digestibility of complete diets (Alhadas et al., 2021; Benedeti et al., 2019; Godoi et al., 2020; Silva et al., 2020, 2022). This approach provides valuable insights into nutrient degradation within the rumen, aiding in understanding feed utilization and digestion kinetics in ruminants while reducing cost, time, and labor. The choice of incubation hours varies based on factors such as forage type, quality, and concentrate levels in the diet.

Ruminal pH, ammonia and VFA

In contrast to the findings of this study, which revealed a linear increase in total VFA with increasing concentrate level, Kljak et al. (2017) did not identify significant differences. However, Kljak et al. (2007) reported that an increase in the concentrate level led to a linear decrease and increase in acetate and propionate, respectively. It is important to note that in this study, Kljak et al. (2007) evaluated concentrate levels ranging from 150g/kg to 450g/kg. In the current research, acetate and propionate presented quadratic patterns. Similar results were found for butyrate and ratio A:P, which exhibited linear increase and decrease, respectively, in both studies.

As expected, ruminal pH decreased with increasing concentrate in diets due to the increased amount of rapidly fermentable starch. Pereira et al. (2007) found no effects for concentrate levels in Volumax sorghum silage-based diets, which diverge from the results for AGRI002E sorghum silage-based diets in the present study. However, similar to the present research results, this author did not find effects for the interaction between concentrate level x sampling time and found a quadratic pattern for the hourly averages.

Notably, the lowest recorded pH value was 6.2, observed when the diet contained a concentrate level of 800 g/kg at the 17-h time point. This pH value is above the suggested inhibitory range of 5.0–5.5 for cellulolytic microorganism development, as proposed by Hoover (1986), and furthermore, exceeds the threshold below which fiber digestion becomes compromised. This observation underscores the suitability of AGRI-002E sorghum silage as an effective fiber source, capable of maintaining an adequate pH for ruminal fermentation in high-concentrate diets.

The present study found a linear decrease in the ruminal NH_3-N with inclusion of concentrate levels. This reduction may be due to the comparatively slower nitrogen usage efficiency by cellulolytic microorganisms (Olson *et al*., 1999), and the lower microbial growth in diets with lower concentrate level. Consequently, this reduces the need for ammonia and leads to the accumulation of this product within the rumen. In contrast to these findings, Pereira *et al*., (2007) did not observe any difference, and Kljak et al. (2017) identified a linear increase in ruminal NH_3-N attributed to concentrate levels in sorghum silage based-diets.

Moreover, while Pereira et al. (2007) detected a quadratic effect in Hourly averages of NH_3-N, the current study detected cubic effect. Additionally, a significant interaction effect between concentrate level and sampling time was found in the present study, which was not detected by Pereira et al., (2007).

Additionally, it is also evident that the highest values and a fast increase post-feeding (0700 h and 1600 h) in NH_3-N concentration, were prominent in the diet with a concentrate level of 0 g/kg. This observation can be partly explained by the fact that the nitrogen provided in this diet came from non-protein nitrogen sources. Moreover, in the no concentrate diet, there was insufficient energy for microbial growth, resulting in the accumulation of NH_3-N in the rumen.

Nitrogen balance and microbial protein synthesis

The increase in concentrate levels resulted in a linear rise in TDN and DOM intake. This corresponds with findings from Pereira et al. (2007), suggesting that diets with higher concentrate levels provide a greater supply of energy, which may affect overall nutritional quality.

A linear increase was observed in N intake and the amount of apparently digested N (g/d). This contrasts with Kljak et al., (2017) results, where they noted a linear decline as concentrate levels increased. Similarly, N retention, measured in both g/day and g/kg N intake, showed a parallel linear increase.

Conversely, some N-related parameters demonstrated inverse trends. The linear reduction in urinary N indicates greater utilization in the rumen and whole animal. This is echoed by the decreasing urinary urea and blood urea concentrations, which showed a parallel linear decrease. Collectively, these outcomes suggest enhanced N utilization efficiency with higher concentrate levels.

It's noteworthy that the retained nitrogen exhibited a negative value for the diet containing 0 g/kg of concentrate, indicating excessive nitrogen excretion through urine and hinting at potential muscle mass depletion. Moreover, the diet with 0 g/kg of concentrate showed significantly elevated concentrations of urea in both urine and blood, along with increased ruminal NH_3-N levels. These combined findings underscore notable

inefficiency in nitrogen utilization, likely due to the lack of readily available energy sources for ruminal microbial growth. This is likely because fast-digesting carbohydrates, such as starch, are more efficient at promoting microbial growth than fibrous carbohydrates (Stern and Hoover, 1979). Since the 0 g/kg concentrate diet primarily relies on non-protein nitrogen sources, such as urea and ammonium sulfate, it does compromise proper microbial protein synthesis due the asynchrony in the supply of N and energy to the ruminal microbes.

In the present research, a notable observation was the linear increase in microbial protein production and microbial efficiency with the elevation of concentrate levels. This outcome underscores the potential of higher concentrate diets to enhance microbial protein synthesis, contributing to improved nitrogen utilization and overall diet quality. Interestingly, this finding contrasts with the results of previous studies such as Pereira et al. (2007) and Kljak et al. (2017), both of which reported no significant differences in microbial protein production. It is worth speculating that, despite the numerical increase in microbial protein production in their studies, where concentrate levels ranged from 200 to 650 and 150 to 450 g/kg of DM, respectively, the absence of statistically significant linear or quadratic effects may be attributed to the specific concentrate range utilized by them.

CONCLUSION

The increase in concentrate levels in AGRI002E resulted in increased nutrient intake, except for apNDF. Additionally, increased ruminal degradation of DM, ruminal digestibility and apparent total-tract digestibility of DM, OM, and EE, were observed. However, there was a decrease in ruminal digestibility and apparent total-tract digestibility of apNDF, CP, and starch was found. Simultaneously, there was a decrease in ruminal pH; however, it remained within the appropriate range even in diets with higher

concentrate levels, indicating adequate ruminal fermentation. Furthermore, the increase in concentrate level resulted in greater N utilization, as evidenced by the decreased rumen NH_3-N concentration, increased retained N, and enhanced microbial protein synthesis efficiency.

These findings underscore the potential of AGRI-002E sorghum silage as a valuable fiber source for high-concentrate diets. However, the study highlights the necessity of concentrate supplementation for optimal utilization in beef cattle diets, emphasizing the importance of a balanced dietary composition.

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