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Effect of concentrate level on digestive processes, nutrient flow, nitrogen utilization, and microbial protein synthesis in beef cattle

Felipe Pedrosa Melgaço
Doctor Scientiae

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FELIPE PEDROSA MELGAÇO

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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: Sebastiao de C. V. Filho

Co-adviser: Luciana Navajas Renno

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“[...]sem Mim nada podeis fazer.” (João 15, 5).

ABSTRACT

MELGAÇO, Felipe Pedrosa, D.Sc., Universidade Federal de Viçosa, September, 2025. **Effect of concentrate level on digestive processes, nutrient flow, nitrogen utilization, and microbial protein synthesis in beef cattle**. Adviser: Sebastiao de Campos Valadares Filho. Co-adviser: Luciana Navajas Renno.

Optimize concentrate levels in beef cattle diets is essential to improve nutrient utilization, microbial protein synthesis, and animal performance while preventing ruminal disorders. The hypothesis of this study was that increasing dietary concentrate levels would enhance intake, digestibility, and microbial protein synthesis, but could reduce ruminal fiber degradation. The objective was to evaluate the effects of increasing dietary concentrate levels on intake, digestibility, ruminal kinetics, nitrogen metabolism, and microbial protein synthesis in F1 (Red Angus × Nellore) cattle. Four rumen- and ileal-cannulated bulls (average initial body weight = 471 ± 51.88 kg; average age = 18 months) were allocated in a 4 × 4 Latin square design and fed corn silage–based diets containing 0, 27.5, 55.0, or 82.5% concentrate. Each experimental period lasted 24 days, including 14 days of adaptation and 10 days of sampling. Dry matter intake ($P = 0.0115$), organic matter intake ($P = 0.0361$), and total digestible nutrient intake ($P = 0.0179$) showed quadratic responses, with maximum values between 55.0 and 82.5% concentrate. Physically effective neutral detergent fiber decreased linearly ($P < 0.0001$), reaching 3.98% of dietary dry matter in the highest-concentrate diet. Ruminal digestibility of dry matter, organic matter, non-fibrous carbohydrates, and starch increased with increasing concentrate levels, whereas ruminal crude protein digestibility was unaffected. Total tract digestibility of dry matter and organic matter increased linearly, while potentially digestible neutral detergent fiber digestibility showed a quadratic response. In situ degradability of dry matter, organic matter, and crude protein increased with dietary energy density, whereas neutral detergent fiber degradation was reduced at the highest concentrate level. Nitrogen intake and nitrogen retention increased linearly, whereas microbial protein synthesis and its efficiency showed quadratic responses, with estimated maximum values at approximately 48% concentrate inclusion. Ruminal pH was not affected by treatment ($P = 0.1827$). These results demonstrate that moderate-to-high concentrate inclusion, with adequate physically effective neutral detergent fiber (peNDF), optimizes energy and nitrogen utilization, enhances microbial protein synthesis, and ensures efficient nutrient use without compromising fiber digestion in feedlot beef cattle.

Keywords: ruminant nutrition; ruminal kinetics; microbial nitrogen flow

RESUMO

MELGAÇO, Felipe Pedrosa, D.Sc., Universidade Federal de Viçosa, setembro de 2025. **Efeito do nível de concentrado sobre os processos digestivos, fluxo de nutrientes, utilização de nitrogênio e síntese de proteína microbiana em bovinos de corte**. Orientador: Sebastiao de Campos Valadares Filho. Coorientadora: Luciana Navajas Renno.

Otimizar os níveis de concentrado nas dietas de bovinos de corte é essencial para melhorar o aproveitamento de nutrientes, a síntese de proteína microbiana e o desempenho animal, ao mesmo tempo em que se previnem distúrbios ruminais. A hipótese deste trabalho foi que o aumento do teor de concentrado na dieta aumentaria o consumo, a digestibilidade e a síntese de proteína microbiana, mas poderia reduzir a degradação de fibra ruminal. O objetivo foi avaliar os efeitos de níveis crescentes de concentrado sobre o consumo, a digestibilidade, a cinética ruminal, o metabolismo do nitrogênio e a síntese de proteína microbiana em bovinos F1 (Red Angus × Nelore). Quatro bovinos canulados no rúmen e íleo (Peso corporal inicial médio = 471 ± 51.88 kg, idade média de 18 meses) foram alocados em um delineamento quadrado latino 4×4 e alimentados com dietas à base de silagem de milho contendo 0, 27.5, 55.0 ou 82.5% de concentrado. Cada período experimental durou 24 dias, incluindo 14 dias de adaptação e 10 dias de coleta de amostras. O consumo de matéria seca ($P = 0.0115$), matéria orgânica ($P = 0.0361$) e nutrientes digestíveis totais ($P = 0.0179$) apresentou resposta quadrática, com valores máximos entre 55.0 e 82.5% de concentrado. O teor de FDN fisicamente efetiva (FDNfe) reduziu linearmente ($P < 0.0001$), atingindo 3.98% da MS na dieta com maior teor de concentrado. A digestibilidade ruminal da matéria seca, matéria orgânica, carboidratos não fibrosos e amido aumentou com o aumento do concentrado, enquanto a digestibilidade ruminal da proteína bruta não foi alterada. A digestibilidade total da matéria seca e matéria orgânica aumentou linearmente, enquanto a digestibilidade da fibra potencialmente digestível apresentou resposta quadrática. A degradabilidade in situ da matéria seca, matéria orgânica e proteína bruta aumentou com a densidade energética da dieta, enquanto a degradação da FDN foi reduzida no maior nível de concentrado. O consumo e a retenção de nitrogênio aumentaram linearmente, enquanto a síntese de proteína microbiana e sua eficiência apresentaram resposta quadrática, com máximo estimado próximo de 48% de concentrado. O pH ruminal não foi afetado pelos tratamentos ($P = 0.1827$). Esses resultados demonstram que a inclusão moderada a alta de concentrado, com adequado teor de FDNfe, otimiza a utilização de energia e nitrogênio, aumenta a síntese de proteína microbiana e assegura o uso eficiente dos nutrientes sem comprometer a digestão da fibra em bovinos de corte confinados.

Palavras-chave: nutrição de ruminantes; cinética ruminal; fluxo de nitrogênio microbiano

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Effect of concentrate level on beef cattle nutrient degradability, nitrogen utilization, and microbial protein synthesis

SUMMARY

1 GENERAL INTRODUCTION

A persistent challenge in beef cattle production is to maximize nutrient intake and animal performance while maintaining rumen health. This balance largely depends on the forage-to-concentrate ratio. High-concentrate diets increase the availability of fermentable energy, enhancing microbial protein synthesis (MPS), which is the main source of metabolizable protein for ruminants (NASEM, 2016; BR-CORTE, 2023). However, rapid starch (ST) fermentation can lower ruminal pH, impair fibrolytic microbial activity, and decline fiber degradation, creating a delicate trade-off between energy supply and rumen stability. Understanding how these dynamics interact is essential, particularly in tropical feedlot systems where high ST diets are widely used, and the risk of ruminal acidosis is elevated.

Moderate concentrate inclusion generally improves digestibility of dry matter, organic matter, and nitrogen utilization while maintaining ruminal function and microbial efficiency. Beyond certain point, excessive concentrate (>80%) can disrupt fiber digestion, shift microbial populations, and reduce MPS efficiency (Hackmann & Firkins, 2015). These responses highlight the importance of synchronizing fermentable energy with physically effective fiber, not only to sustain rumen stability but also to optimize nitrogen utilization and prevent metabolic disorders. By integrating assessments of *in situ* degradability, ruminal kinetics, nitrogen balance, and microbial protein synthesis, it is possible to capture a comprehensive picture of how diet composition drives nutrient utilization.

This work addresses these gaps by evaluating the effects of increasing dietary concentrate on intake, digestibility, ruminal kinetics, nitrogen utilization, and microbial protein synthesis. By integrating ruminal physiology with practical feeding strategies, it provides insights to improve nutritional efficiency and reduce nitrogen losses in beef cattle systems.

It is hypothesized that increasing dietary concentrate levels would enhance feed intake, nutrient digestibility, and microbial protein synthesis, but may impair fiber degradation if peNDF is insufficient and nitrogen utilization efficiency as a result of reduced ruminal pH. The objectives of this work are to evaluate the effects of concentrate levels on intake and total and partial apparent and digestibility, ruminal passage and degradation kinetics, ruminal pH, *in situ* degradability parameters, nitrogen balance, and microbial protein synthesis under tropical production conditions.

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2 EFFECT OF INCREASING CONCENTRATE LEVELS ON INTAKE, DIGESTIBILITY, RUMEN KINETICS, AND RUMEN PH IN BEEF CATTLE FED CORN SILAGE-BASED DIETS

2.1 ABSTRACT

The optimization of dietary concentrate levels in beef cattle diets is critical to maximize nutrient utilization and animal performance, while avoiding ruminal disturbances such as acidosis. However, the effects of increasing concentrate inclusion on intake, digestibility, and nutrient flow dynamics, especially under tropical conditions, require further elucidation. It is hypothesized that the increase of concentrate levels improves feed intake and digestibility of nutrients. However, reductions in roughage neutral detergent fiber physically effective (peNDF) in the diet may compromise rumen health by decreasing ruminal buffering capacity and altering ruminal pH dynamics. The objective of this study was to evaluate the effects of increasing dietary concentrate levels on intake, ruminal, intestinal, and total tract digestibility, as well as nutrient flow kinetics in beef cattle fed corn silage-based diets. Four rumen- and ileal-cannulated F1 (Red Angus × Nellore) bulls (initial BW = 471 kg ± 51.88; 18 months) were allocated in a 4×4 Latin square design and fed diets with 0, 27.5, 55.0, or 82.5% concentrate. Each experimental period lasted 24 days, including 14 days of adaptation and 10 days of sampling. Intake, digestibility, digesta flow, and ruminal kinetics were assessed using a dual-marker system (Co-EDTA and indigestible neutral detergent fiber). Dry matter (DM) (P = 0.0115), organic matter (OM) (P = 0.0361), and total digestible nutrient (TDN) (P = 0.0179) intakes exhibited quadratic responses, with maximum estimated values between 55 and 82.5% concentrate, while intake of potentially digestible neutral detergent fiber (apNDF) (P = 0.0014) had maximum estimated value at 10.35% concentrate. Crude protein (CP) (P = 0.0028), ether extract (EE) (P = 0.0001), non-fibrous carbohydrates (NFC) ($P < 0.0001$), and starch (ST) ($P < 0.0001$) intakes increased linearly with concentrate levels. Ruminal digestibility of DM (P = 0.0098), OM (P = 0.0282), EE (P = 0.0063), NFC (P = 0.0086), and ST (P = 0.0338) increased linearly, while CP ruminal digestibility was unaffected. Small intestinal digestibility of DM and OM remained stable ($P > 0.05$), but NFC (P = 0.0307) and ST (P = 0.0147) digestibility declined linearly. Total tract digestibility of DM ($P < 0.0001$) and OM (P = 0.0001) increased linearly; apNDF (P = 0.0297) and NFC (P = 0.0313) showed quadratic patterns, and ST digestibility remained consistently high (97.04% on average). Degradation and intake rates of DM ($P < 0.0001$), OM ($P < 0.0001$), NFC ($P < 0.0001$), and ST ($P = 0.0134$) increased linearly, while passage rate increased only for OM ($P = 0.0260$) with increasing concentrate level. Ruminal pH was not affected by concentrate level ($P = 0.1827$), remained stable across all treatments with a mean value of 6.48. However, it was observed a cubic pattern in function of measurement time ($P < 0.0001$). Nonetheless, 17.5% forage inclusion in the 82.5% concentrate diet maintained ruminal pH above critical levels, preventing acidosis, at physically effective NDF (peNDF) level of 3.98%. These findings demonstrate that increasing dietary concentrate levels enhanced nutrient intake and overall tract digestibility. However, intakes of DM and OM, as well as the total tract digestibility of apNDF, followed a quadratic pattern, indicating an optimal level for nutrient utilization. This highlights the importance of balancing concentrate with adequate roughage peNDF to maximize energy intake and nutrient use without compromising fiber digestion.

Key words: Ruminant nutrition, Forage-to-concentrate interaction, Fermentation acidity

2.2 INTRODUCTION

Dry matter intake (DMI) by beef cattle is modulated by a complex relationship of individual, environmental, dietary, and management factors. Individual determinants include genetics, body weight, body composition, and sex class, while environmental aspects involve temperature, humidity, and seasonality (NASEM, 2016; BR-CORTE, 2023). Dietary variables, particularly forage-to-concentrate ratio, fiber quality, particle size, and grain processing, are well-established modulators of DMI. Additionally, feeding frequency and use of total mixed rations (TMR) also contribute to intake regulation (Ferreira and Mertens, 2005; Caetano *et al*., 2015; Goulart *et al*., 2020; Godoi *et al*., 2021).

Increasing dietary concentrate levels is a commonly adopted strategy in feedlot systems to enhance dietary energy density and improve growth performance. Higher concentrate inclusion often promotes greater intake and digestibility of dry matter (DM) and organic matter (OM) (Chizzotti *et al*., 2012; Detmann *et al*., 2014; Rotta *et al*., 2014; Barbizan, 2020; Detmann *et al*., 2024). However, intake may decrease once animals fulfill their energy requirements (Mertens, 1987; Forbes, 2003; NASEM, 2016; BR-CORTE, 2023).

Beyond metabolic regulation, high-starch diets pose challenges to ruminal stability. Excessive concentrate inclusion leads to rapid fermentation, reducing ruminal pH and impairing the activity of key cellulolytic species (Russell & Dombrowski, 1980; Calsamiglia *et al*., 2002). When pH drops below 5.8, fibrolytic bacteria exhibit drastic reductions in adhesion and enzyme production, compromising neutral detergent fiber (NDF) digestibility and altering volatile fatty acid profiles (Roger *et al*., 1990; Pucetti *et al*., 2024).

Maintaining ruminal pH within a physiologically safe range (5.8–6.5) is therefore critical to sustain microbial efficiency (BR-CORTE, 2023). This stability is largely dependent on the inclusion of physically effective fiber (peNDF) to stimulate salivary buffering and support microbial adhesion. However, despite its importance, the minimum peNDF requirement that preserves ruminal health while maximizing energy intake remains poorly defined for tropical feedlot systems using corn silage-based diets.

While significant progress has been made in understanding the effects of concentrate inclusion, there remains a lack of integrative evaluations that simultaneously examine intake dynamics, digestibility, ruminal fermentation kinetics, and pH regulation under practical feeding conditions. Addressing these knowledge gaps is essential to develop nutritional strategies that balance energy intake, fiber utilization, and ruminal health. Such information is critical for optimizing feeding programs that enhance production efficiency while minimizing the risk of metabolic disorders, thereby supporting sustainable beef cattle production under tropical conditions.

It is hypothesized that increasing dietary concentrate levels will enhance feed intake and nutrient digestibility; however, reductions in peNDF may compromise rumen health by decreasing ruminal buffering capacity and altering ruminal pH dynamics. Thus, the balance between dietary energy density and peNDF is expected to play a key role in determining ruminal fermentation patterns and degradation kinetics. The objective of the present study was to evaluate how different concentrate levels and the associated changes in peNDF in the diets of beef cattle affect total and partial apparent intake and digestibility of dietary components, ruminal passage and degradation kinetics, and ruminal pH values.

2.3 MATERIAL AND METHODS

2.3.1 ANIMALS, DIETS, AND EXPERIMENTAL DESIGN

The experiment was conducted at the Ruminant Nutrition Laboratory (LabNUR) of the Department of Animal Science at the Federal University of Viçosa (UFV), in Viçosa, Minas Gerais, Brazil. The project was approved by the Ethics Committee on the Use of Production Animals (CEUAP) of the Federal University of Viçosa (Protocol no. 26/2024).

Four rumen and ileal-fistulated F1 bulls (Red Angus x Nellore) were used, with an average initial body weight (BW) of 471 kg ± 51.88 and an average age of 18 months. A 4×4 Latin square design was used, involving four animals, four experimental periods, and four treatments, ensuring each animal received all treatments. The treatments consisted of diets with increasing levels of concentrate inclusion: 0% (1.89% urea/ammonium sulfate and 0.98% mineral mix, on average), 27.5%, 55%, and 82.5%. The diets were composed of corn silage, ground corn, soybean meal, urea:ammonium sulfate, a vitamin–mineral premix, and virginiamycin (Table 1). All diets were formulated to be isoproteic (110g/kg of crude protein), according to the BR-CORTE system (2023).

Table 1 - Chemical composition of diet ingredients

Items	Ingredients					
	Corn silage	Corn meal	Soybean meal	Urea/A.S.[14]	Premix[15]	Virginiamycin[16]
DM[1]	31.41 ±1.464	88.35	89.58	95.85	100.00	100.00
OM[2]	90.03 ±4.296	98.73	93.09	99.72	-	-
CP[3]	5.96 ±0.885	7.82	48.18	263.49	-	-
EE[4]	1.97 ±0.597	3.55	2.23	-	-	-
NDF[5]	46.35 ±2.150	9.93	12.47	-	-	-
peNDF (%NDF)[6]	49.11 ±2.937	-	-	-	-	-
peNDF	22.76 ±1.056	-	-	-	-	-
apNDF7	41.21 ±2.380	8.93	10.28	-	-	-
iNDF8	17.59 ±3.660	2.07	1.77	-	-	-
pdNDF9	23.63 ±6.520	6.86	8.51	-	-	-
ADF[10]	25.70 ±2.492	2.52	5.96	-	-	-
NFC[11]	40.89 ±2.350	78.43	32.40	-	-	-
ST[12]	24.69 ±1.820	71.43	5.13	-	-	-
ROM[13]	16.20 ±0.529	7.00	27.27	-	-	-

Source: elaborated by the author. [1]Dry matter, [2]Organic matter, [3]Crude protein, [4]Ether extract, [5]Neutral detergent fiber, [6]Physically effective neutral detergent fiber, [7]Neutral detergent fiber corrected for ash and protein, [8]Indigestible neutral detergent fiber, [9]Potencially degradable neutral detergent fiber, [10]Acid detergent fiber, [11]Non fibrous carbohydrates, [12]Starch, [13]Residual organic matter, [14]Urea and ammonium sulfate (9:1), [15]Premix guarantees (Per kg of DM): 242.00-300.00 g of Ca, 11.10 mg of Co (Min), 556.00 mg of Cu (Min), 24.50 g of S (Min), 370.00 mg of Fe (Min), 13.50 g of P (Min), 27.70 mg of I (Min), 19.00 g of Mg (Min), 1668.00 mg of Mn (Min), 928.00 mg of monensina, 7.40 mg of Se (Min), 61.50 g of Na (Min), [16]V-max® 2 (20.000g/kg, Phibro Animal Health).

The animals were housed in individual tie-stall pens, each measuring 6m² (2 x 3m), with concrete flooring and individual feed bunks and water troughs. All animals were treated for endo and ectoparasites. A 30-day adaptation period to the diets and the experimental environment was carried out prior to data collection.

2.3.2 EXPERIMENTAL PERIOD

After the adaptation period, the animals were subjected to experimental diets for 96 days, divided into four 24-day periods. During the first 14 days of each experimental period, the animals were adapted to the diets to allow stabilization of nutrient flow throughout the gastrointestinal tract. The dietary transition was carried out gradually, with 25% of the new diet being introduced every three days.

Diets were offered *ad libitum*, allowing for a 5% refusal rate on a fresh matter basis. Animals were fed twice daily at 08:00 and 16:00. To ensure accurate intake, the amount of feed and orts were weighed daily, with adjustments made for the next day's supply. The corn silage was collected directly from the silo each morning and mixed with the concentrate component. From the 15th to the 23rd day of each experimental period, samples of the diets and orts (approximately 5% of the total) were collected, placed in plastic bags, and stored in a freezer at -20°C. At the end of each period, a composite sample was prepared for each animal and dried in a forced-air oven at 55°C for 72h (Method G-001/2; Detmann *et al.*, 2025). The samples were then ground in a knife mill using a 1 mm screen and subjected to final drying in an oven at 105°C at 16h (Method G-003/1; Detmann *et al.*, 2025), after which they were stored in plastic containers for subsequent analyses.

2.3.3 FECAL COLLECTION

In each experimental period, total fecal collection was carried out from the 15th to the 18th day. Feces were collected directly from the concrete floor and stored in pre-weighed plastic buckets with lids. Every 24 hours, the total feces were weighed, thoroughly homogenized, and a subsample (approximately 250g) was collected and dried in a forced-air oven at 55°C for 72h using pre-weighed aluminum trays. The dried samples were then ground in a knife mill using 1mm and 2mm sieves. At the end of each period, a composite sample was prepared for each animal, proportional to the pre-dried fecal weight.

Subsequently, laboratory analyses of the feces were performed. These data, together with the feed intake measurements, were used to determine the total *in vivo* digestibility of diets.

2.3.4 COLLECTIONS OF RUMINAL, OMASAL, AND ILEAL DIGESTA

Eight digesta collections were carried out at 9-hour intervals from the 19th to the 21st day of each experimental period. Samples of ruminal, omasal, and ileal digesta were collected at 08:00, 17:00, 02:00, 11:00, 20:00, 05:00, 14:00, and 23:00, following the methodology described by Huhtanen *et al.* (1997), adapted by Leão (2002). A continuous infusion of the Co-EDTA marker (5 g/d) diluted in 4 liters of water was administered via the ruminal fistula using peristaltic pumps from day 16 to day 21.

Table 2 - Proportion of ingredients and composition of experimental diets

Diets	0	27.5	55	82.5
Ingredients			%	
Corn silage	97.13	72.50	45.00	17.50
Corn meal	0.00	20.36	48.56	76.70
Soybean meal	0.00	5.10	4.51	3.97
Urea/A.S.[1]	1.89	0.94	0.83	0.73
Premix	0.98	0.98	0.98	0.98
Virginiamycin[2]	0.00	0.13	0.13	0.13
Composition			%	
DM[3]	32.03	38.21	48.70	67.15
OM[4]	89.58	91.05	93.48	95.91
CP[5]	10.78	10.85	10.83	10.85
EE[6]	1.91	2.27	2.72	3.16
apNDF[7]	40.03	32.11	23.21	14.40
iNDF[8]	17.26	13.37	9.06	4.75
pdNDF[9]	22.77	18.74	14.16	9.64
ADF[10]	24.96	19.40	12.99	6.63
Corn silage apNDF[11]	40.03	29.87	18.54	7.21
Corn silage peNDF[12]	22.11	16.50	10.24	3.98
NFC[13]	37.62	47.53	58.54	69.42
ST[14]	23.98	32.80	46.17	59.40
ROM[15]	13.64	14.73	12.36	10.02

Source: elaborated by the author. [1]Urea and ammonium sulfate (9:1), [2]V-max® 2 (20.000g/kg, Phibro Animal Health). [3]Dry matter, [4]Organic matter, [5]Crude protein, [6]Ether extract, [7]Neutral detergent fiber corrected for ash and proteins, [8]Indigestible neutral detergent fiber, [9]Potentially degradable neutral detergent fiber, [10]Acid detergent fiber, [11] Corn silage neutral detergent fiber corrected for ash and protein, [12] Corn silage physically effective neutral detergent fiber, [13]Non fibrous carbohydrates, [14]Starch, [15]Residual organic matter.

From the filtered ruminal fluid (100mL), pH was measured immediately after sampling and phase separation using a digital pH meter. Omasal digesta was collected by inserting a sampling tube toward the reticule-omasal orifice. Once the tip of the tube was correctly positioned in the orifice, digesta was aspirated using a vacuum pump. In the omasal digesta samples, solid and liquid phases with small particles were separated using a 100µm nylon filter (Sefar Nitex 100/44, Sefar, Thal, Switzerland). Initially, each omasal digesta sample was divided into two aliquots: 200mL were used for phase separation, and another 200 mL represented the whole digesta.

Ileal digesta was collected through the ileum fistula, with approximately 200mL of material obtained per sampling, representing the whole digesta. All omasal and ileal digesta samples were freeze-dried and ground in a knife mill with a 1 mm sieve.

2.3.5 DIGESTA FLOW

DM and other dietary component flows were estimated using the dual marker system described by France and Siddons (1986). Cobalt-EDTA (Co-EDTA) was used as the marker for the liquid and small particle phases, while indigestible neutral detergent fiber (iNDF) was used as the internal marker for the solid phase.

To estimate digesta flow, cobalt concentrations in the omasal digesta were determined via atomic absorption spectrophotometry after sample digestion with nitric-perchloric acid (INCT method M-004/2; Detmann *et al.*, 2025). Omasal digesta flow was estimated using the reconstitution technique described by Faichney (1975). The iNDF concentrations was used as the solid-phase marker in omasal digesta, while Co-EDTA was used as the liquid and small particles phase marker. For ileal digesta, only iNDF concentration was used as the marker. The markers were quantified in digesta samples from the respective gastrointestinal compartments, as well as in diet and refusal samples.

Rumen content evacuation and sampling were performed on the 21st day of the experimental period, 4 hours after the morning feeding, and again on the 24th day, prior to the next feeding. Rumen nutrient pools were calculated according to the methodology described by Allen and Linton (2007).

Rumen contents were manually collected through the ruminal fistula. The material was weighed and filtered through cotton cloth to separate it into solid and liquid phases, which were weighed individually. The liquid phase was sampled into four pre-weighed 500mL plastic containers, each containing approximately 200g of liquid digesta. Similarly, the solid phase was sampled into two plastic containers, with approximately 300g in each. All samples were freeze-dried and subsequently ground in a knife mill fitted with a 1mm sieve.

Ruminal, small intestinal, large intestinal, and total apparent digestibility of DM, OM, crude protein (CP), ether extract (EE), ash- and protein-free neutral detergent fiber (apNDF), non-fibrous carbohydrates (NFC), and ST were determined, all expressed relative to total intake.

The rates of intake (ki), passage (kp), and digestion (kd) were estimated according to the methodology proposed by Allen and Linton (2007), as described in the following equations:

$$ki = (\frac{intake}{ruminal\, pool}) \times 100$$

$$kp = (\frac{ruminal\, flow}{ruminal\, pool}) \times 100$$

$$kd = ki - kd$$

2.3.6 LABORATORY ANALYSES

The following analyses were performed on samples of corn silage, concentrate, refusals, feces, and ruminal, omasal, and ileal digesta: DM, using INCT method G-003/1; ash (mineral matter, MM), using INCT method M-001/3; CP, using INCT method N-001/3; EE, using INCT methods G-004/1; NDFap, using INCT method F-001/3; corrections for ash and nitrogen in NDF, using INCT methods N-004/2 and M-002/2; iNDF, using INCT method F-008/2; acid detergent fiber (ADF), using INCT method F-003/3 (Detmann *et al.*, 2025); ST, according to Silva *et al.* (2019); and NFC.

The pdNDF fraction was calculated by subtracting iNDF from ap NDF:

$$pdNDF = \mathrm{apNDF} - \mathrm{iNDF}$$

where apNDF = neutral detergent fiber corrected for ash and protein content; iNDF = indigestible neutral detergent fiber, determined after 288 h of *in situ* ruminal incubation; pdNDF = potentially degradable NDF fraction, representing the fiber fraction available for ruminal fermentation.

To determine the physically effective fiber fraction of the corn silage, the Penn State Particle Separator (PSPS) was used, consisting of sieves with apertures of 19, 8, and 4 mm. The material retained on the 4-mm sieve and on the sieves above was considered physically effective. The NDF concentration of this retained material was analyzed and multiplied by the proportion of material remaining on the sieves of 4 mm and larger, resulting in the peNDF content.

The NFC content was calculated according to the following equation proposed by Detmann and Valadares Filho (2010):

$$NFC = 1000 - [(\mathrm{CP}\frac{\mathrm{g}}{\mathrm{kg}} - \mathrm{urea\ CP}\frac{\mathrm{g}}{\mathrm{kg}} + \mathrm{urea}\frac{\mathrm{g}}{\mathrm{kg}}) + \mathrm{apNDF}\frac{\mathrm{g}}{\mathrm{kg}} + \mathrm{EE}\frac{\mathrm{g}}{\mathrm{kg}} + \mathrm{ash}\frac{\mathrm{g}}{\mathrm{kg}}]$$

where: NFC = non-fibrous carbohydrates (g/kg); CP = crude protein; apNDF = neutral detergent fiber corrected for ash and protein; EE = ether extract.

The total digestible nutrients (TDN) of the diets were calculated using the following equation:

$$TDN = \mathrm{CP} + \mathrm{dNFC} + \mathrm{dapNDF} + 2{,}25 \times \mathrm{dEE}$$

where: NDT = TDN = total digestible nutrients (%); dCP = digestible crude protein (%); dNFC = digestible non-fibrous carbohydrates ;(%); dapNDF = digestible neutral detergent fiber corrected for ash and protein (%); dEEd= digestible ether extract (%).

Residual organic matter (ROM) was calculated by subtracting the ST content from the non-fibrous carbohydrates.

2.3.7 STATISTICAL ANALYSES

The statistical analysis was conducted using R Studio software (version 4.4.1, Posit PBC, 2024). To evaluate the effect of the treatments on the studied variables, a mixed model was fitted using the lme() function from the nlme package, adopting a Latin square design. The model considered the concentrate level in the diet as a fixed effect (treatment) and the factors period and animal as random effects, according to the following statistical model:

$$\mathrm{Yijk} = \mu + \mathrm{di} + \mathrm{aj} + \mathrm{pk} + \mathrm{eijk}$$

where: μ = overall constant; di = effect of diet i (fixed); aj = effect of animal j (random); pk = effect of experimental period k (random); eijk= residual random effect of diet i, animal j, and experimental period k.

The significance of the effects was evaluated using analysis of variance (ANOVA) for linear, quadratic, and cubic effects, adopting a significance level of 5% (Type I error). The adjusted coefficient of determination (R^2) was calculated to assess the model fit quality. The model adequacy was verified by analyzing the residuals, applying the Shapiro-Wilk test to assess normality and the Bartlett test to check for homogeneity of variances. To identify the point of maximum or minimum for the fitted quadratic equations, the critical value of concentrate content was calculated by deriving the regression equation.

To evaluate the effects of dietary concentrate level and measurement time on ruminal pH, mixed linear models with repeated measures were fitted using the lme() function from the nlme package:

$$\mathrm{Yijkl} = \mu + \mathrm{di} + \mathrm{hj} + (\mathrm{d} \times \mathrm{h})\mathrm{ij} + \mathrm{pk} + \mathrm{al} + \mathrm{eijkl}$$

where: μ = overall constant; d_i = effect of diet i (fixed); h_j = effect of measurement time j (fixed); $(d \times h)_{ij}$ interaction between concentrate level i and measurement time j; p_k = effect of experimental period k (random); a_l = effect of animal l (random); e_{ijkl}= residual error associated with repeated measures over time

Ruminal pH data, originally recorded in wide format for each experimental unit defined by period (PER), replication (REP), and dietary concentrate level, were measured repeatedly at eight post-feeding times (2, 5, 8, 11, 14, 17, 20, and 23 h). All categorical variables were treated as factors. Ruminal pH was analyzed using linear mixed-effects models in the nlme package, including fixed effects of concentrate level, time (h), and their interaction, with REP nested within PER as a random effect (~1 | PER/REP). Repeated measurements within each animal and experimental period were modeled using a compound symmetry covariance structure (corCompSymm) to account for within-animal correlations over time. Model parameters were estimated by REML, and significance was tested using anova(), with polynomial contrasts performed to evaluate linear, quadratic, and cubic effects of time (h).

2.4 RESULTS

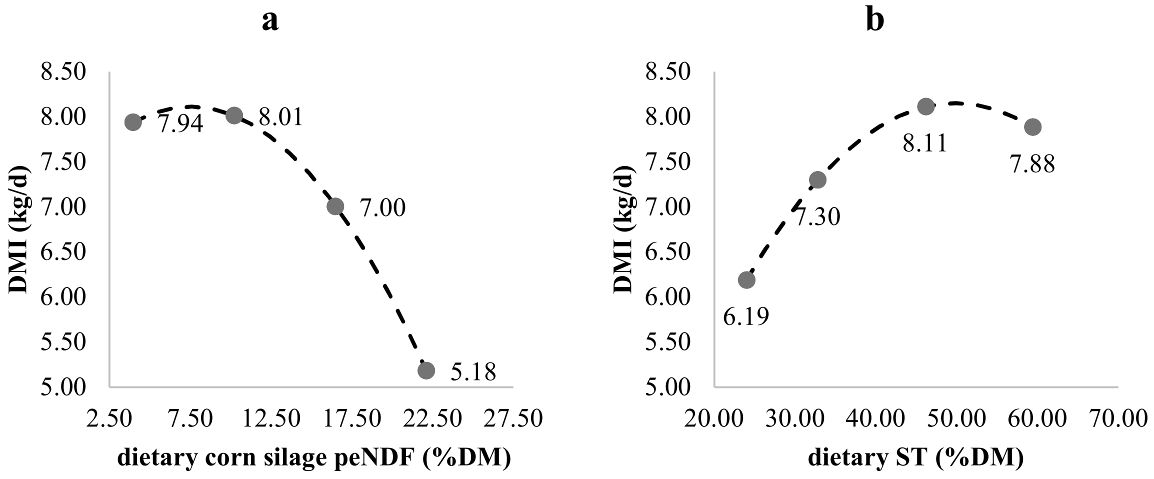
2.4.1 INTAKE AND DIGESTIBILITY

2.4.2 DRY MATTER

The intake of DM expressed in kg/d exhibited a quadratic response (*P* = 0.0115), with a maximum estimated intake point (8.08kg/d) at 63.41% of concentrate level (Table 3). In contrast, DMI expressed as a percentage of BW increased linearly (*P* = 0.0104) by 0.0041% for each 1% increase in the dietary concentrate level. Apparent ruminal, large intestine, and total digestibility of DM increased linearly with increasing dietary concentrate levels (Table 4). No effect of concentrate level was observed on small intestine digestibility of DM (*P* = 0.1082), with an average value of 23.98% (Table 4).

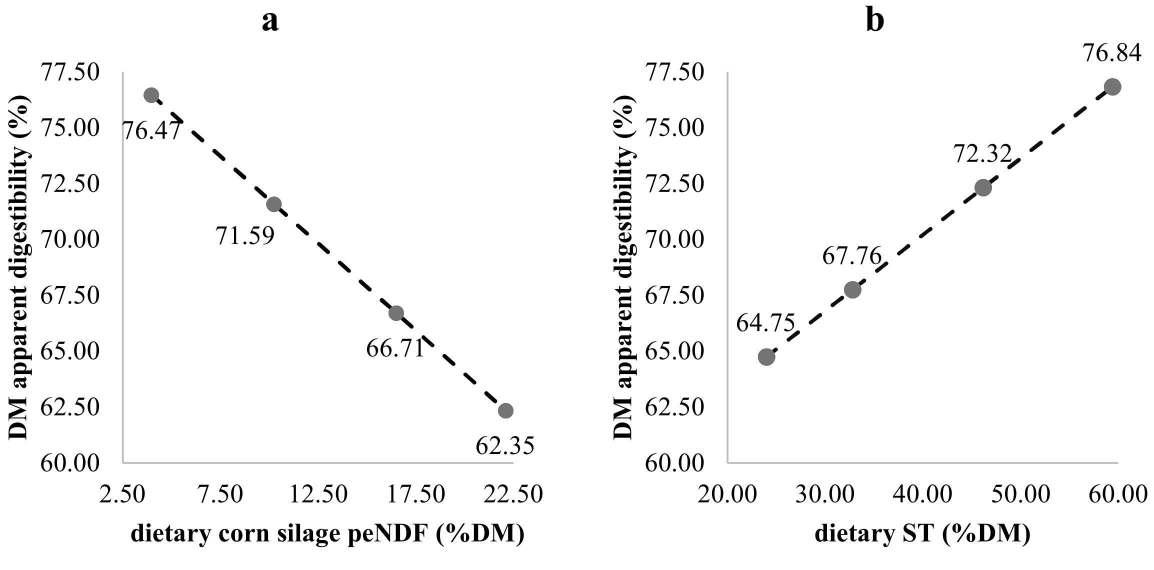
The total amount of DM digested followed the intake pattern (Table 6), with maximum value estimated (6.03kg/d) at 75.36% of concentrate, whereas the amount of DM digested in the rumen increased linearly (*P* = 0.0004) as concentrate level increased.

Dietary corn silage peNDF (%DM) level influenced quadratically DMI, with a maximum estimated value (8.11 kg/d) at 7.54% of peNDF (Figure 1a). The same patter was observed for the effect of ST level on DMI, with a maximum estimated value (8.15 kg/d) at 49.84% of ST (Figure 1b).



Source: elaborated by the author.

Figure 1 – Effect of dietary physically effective neutral detergent fiber (peNDF) and dietary starch (ST) on dry matter intake (DMI) (a: $DMI\ (kg/d) = 7.3263 + 0.2084 \times dietary\ corn\ silage\ peNDF - 0.0138 \times dietary\ corn\ silage\ peNDF^2;\ P = 0.0084,\ R^2 = 0.84$); (b: $DMI\ (kg/d) = 0.858 + 0.2926 \times dietary\ ST - 0.0029 \times dietary\ ST^2;\ P = 0.012,\ R^2 = 0.81$).



Source: elaborated by the author.

Figure 2 – Effect of dietary physically effective neutral detergent fiber (peNDF) from corn silage and dietary starch (ST) on dry matter apparent digestibility (DMD) (a: $DMD\ (\%) = 79.565 – 0.7788 \times dietary\ corn\ silage\ peNDF;\ P < 0.0001,\ R^2 = 0.83$); (b: $DMD\ (\%) = 56.563 + 0.3414 \times dietary\ ST;\ P < 0.0001,\ R^2 = 0.79$).

2.4.3 ORGANIC MATTER

The intake of OM. expressed in kg/d, followed the same pattern as DM, exhibiting a quadratic response ($P = 0.0361$) (Table 3). Maximum estimated intake (7.66kg/d) was reached at 70.92% of concentrate. Apparent ruminal, large intestine, and total digestibility of OM increased linearly with increasing concentrate (Table 4). Similar to DM, no effect of concentrate level was observed on small intestine digestibility of OM ($P = 0.1341$), which showed an average value of 19.12%.

Total amount of OM digested followed the intake pattern (Table 6), with maximum value estimated (5.88kg/d) at 80.37% of concentrate. The amount of OM digested in the rumen increased linearly ($P = 0.0004$) as concentrate level increased.

2.4.4 NEUTRAL DETERGENT FIBER

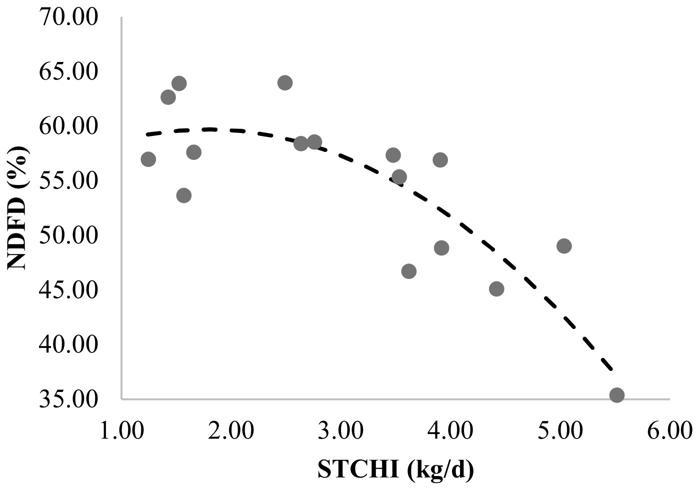
The intake of apNDF in kg/d and as a percentage of BW both exhibited quadratic responses ($P = 0.0014$ and $P = 0.0133$, respectively), with the identification of maximum intake points (2.54kg/d and 0.49%BW) at low concentrate levels (10.35% and 8.52% concentrate level) (Table 3). No significant effect of dietary concentrate level was observed on ruminal ($P = 0.4452$) and small intestine ($P = 0.5593$) apparent digestibility of apNDF (Table 5), with average values of 80.19% and 11.29%, respectively. However, large intestine digestibility decreased linearly ($P = 0.0039$), and total digestibility exhibited a quadratic response ($P = 0.0297$). The estimated maximum total apNDF digestibility (60.10%) occurred at a concentrate level of 16.21%, beyond which apNDF digestibility declined.

peNDF from corn silage decreased linearly with increasing concentrate level ($R^2 = 0.99$, $P = <0.0001$), as follow:

$$Corn\ silage\ peNDF = 19.832 - 0.1961 \times \%C$$

where: peNDF = physically effective neutral detergent fiber (dry matter basis); %C = concentrate level.

Furthermore, ST intake resulted in a quadratic response in the apparent digestibility of apNDF ($P = 0.0346$), with the maximum estimated apNDF digestibility (59.50%) observed at ST intake of 1.80kg/d (Figure 2).



Source: elaborated by the author.

Figure 3 – Effect of starch intake (STI) on neutral detergent fiber (NDFD) digestibility ($NDFD (\%) = 54.2959 + 5.7703 \times STI - 1.5988 \times STI^2; P = 0.0346, R^2 = 0.67$).

Both the amount of apNDF digested in the rumen (P = 0.0001) and in the total digestive tract (P < 0.0001) showed a quadratic pattern with maximum value estimated (2.07kg/d and 1.51kg/d) at 10.10% and 9.46% of concentrate, respectively.

2.4.5 NON-FIBROUS CARBOHYDRATES

The intake of NFC expressed in kg/d, increased linearly (P < 0.0001) by 0.039kg/d for each 1% increase in the concentrate level (Table 3). Ruminal NFC digestibility increased 0.26% (P = 0.0086) in the rumen and decreased 0.21% (P = 0.0307) in the small intestine for each 1% increase in the dietary concentrate level (Table 5). No effect was observed on large intestine digestibility of NFC, with an average value of -2.25% (P = 0.0612). Total NFC digestibility followed a quadratic pattern with the maximum estimated value (86.56%) observed at 76.06% of concentrate. The amount of NFC digested in the rumen (P < 0.0001) and in the total digestive tract (P < 0.0001) increased linearly as concentrate level increased.

2.4.6 STARCH

ST intake, expressed in kg/d, increased linearly (P < 0.0001) by 0.040kg/d for each 1% increase in the concentrate (Table 3). ST digestibility increased 0.11% in the rumen (P = 0.0338) and decreased 0.10% in the small intestine (P = 0.0147) for each 1% increase in concentrate (Table 5). However, large intestine (P = 0.43) and total ST digestibility (P = 0.1748) were not affected, showing average values of 1.41% and 97.03%, respectively. The amount of ST digested in the rumen ($P < 0.0001$) and in the total digestive tract ($P < 0.0001$) increased linearly as concentrate level increased.

2.4.7 CRUDE PROTEIN

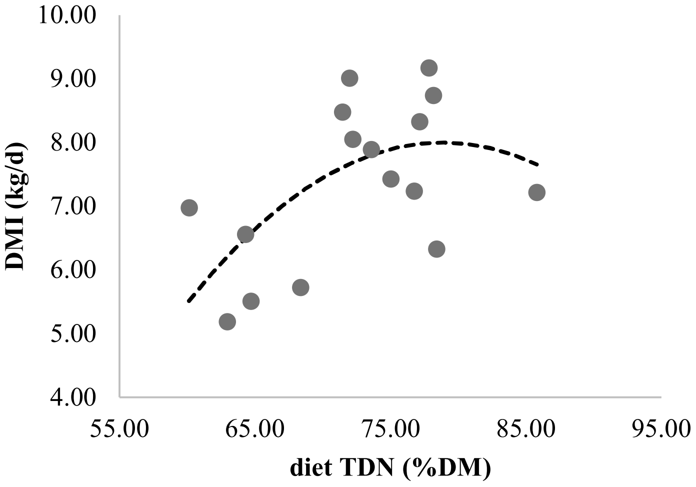
The intake of CP, expressed in kg/d increased linearly ($P = 0.0028$) by 2.27g/d for each 1% increase in the concentrate (Table 3). No effect of concentrate level was observed on ruminal ($P = 0.5522$), small intestine ($P = 0.5522$), large intestine ($P = 0.1313$), or total apparent digestibility *(*$P = 0.785$) of CP. Average digestibility values were -2.82% in the rumen and 72.15% for the total tract.

2.4.8 ETHER EXTRACT

The intake of EE, expressed in kg/d, increased linearly ($P = 0.0001$) by 0.0016kg/d for each 1% increase in the concentrate (Table 3). Ruminal and small intestine apparent digestibility of EE increased linearly. However, large intestine ($P = 0.5683$) and total digestibility ($P = 0.6138$) were not affected, showing average values of -14.46% and 74.84%, respectively.

2.4.9 TOTAL DIGESTIBLE NUTRIENTS

The TDN content of the diets increased linearly by 0.19%, while TDN showed a quadratic response ($P = 0.0179$) (Table 3). The relationship between DMI (kg/d) and dietary TDN content revealed a quadratic response ($P = 0.050$), with the maximum intake estimated at 8.00kg/d when TDN content of the diet reached 78.85% (Figure 3).



Source: elaborated by the author.

Figure 4 – Effect of diet total digestible nutrients (TDN) content on dry matter intake (DMI) ($DMI\ (kg/d) = -36.07621 + 1.11803 \times TDN(\%) - 0.00709 \times TDN(\%)^2;\ P = 0.05,\ R^2 = 0.73$).

Table 3 - Effect of concentrate level on dry matter (DM), organic matter (OM), crude protein (CP), extract (EE), neutral detergent fiber corrected for ash and protein (apNDF), non-fibrous carbohydrates (NFC), starch, residual organic matter (ROM), and total digestible nutrients (TDN) daily intakes (kg/d)

Items	0	27.5	55	82.5	SEM[1]	L (*P*-value)	R^2	Q (*P*-value)	R^2	Equation	x max.	y max.
	kg/d											
DM	6.12	7.33	8.16	7.87	0.313	0.0015	0.71	0.0115	0.84	$6.08 + 0.0632\ x\ \%C - 0.0005 \times \%C^2$	63.41	8.08
OM	5.55	6.77	7.64	7.58	0.315	0.0005	0.73	0.0361	0.81	$5.52 + 0.0604\ x\ \%C - 0.0005 \times \%C^2$	70.92	7.66
CP	0.68	0.80	0.88	0.86	0.033	0.0028	0.67	0.0748	0.74	$713.09 + 2.2678 \times \%C$	-	-
EE	0.11	0.16	0.21	0.24	0.016	0.0001	0.70	0.5139	0.70	-	-	-
apNDF	2.51	2.48	2.02	1.22	0.151	< 0.0001	0.77	0.0014	0.91	$2.51 + 0.0053\ x\ \%C - 0.0003 \times \%C^2$	10.35	2.54
NFC	2.30	3.48	4.70	5.43	0.339	< 0.0001	0.91	0.2636	0.92	$2.39 + 0.0386 \times \%C$		
ST	1.43	2.39	3.71	4.64	0.336	< 0.0001	0.94	0.9699	0.93	$1.40 + 0.0398 \times \%C$	-	-
ROM	0.87	1.10	1.00	0.79	0.059	0.5345	0.01	0.0541	0.30	-	-	-
TDN	3.90	5.25	6.07	6.28	0.302	< 0.0001	0.81	0.0179	0.88	$3.90 + 0.0601\ x\ \%C - 0.0004 \times \%C^2$	79.64	6.29
	%BW											
DM	1.19	1.41	1.58	1.50	0.050	0.0104	0.37	0.0639	0.55	$1.25 + 0.0041 \times \%C$	-	-
apNDF	0.49	0.47	0.39	0.23	0.030	< 0.0001	0.71	0.0133	0.84	$0.49 + 0.0008\ x\ \%C - 0.0001 \times \%C^2$	8.52	0.49
	% DM											
TDN	63.93	71.18	74.52	80.04	1.720	< 0.0001	0.77	0.6218	0.77	-	-	-

Source: elaborated by the author. 0 = 0% of concentrate, 27.5 = 27.5% of concentrate, 55 = 55% of concentrate, 82.5 = 82.5% of concentrate on DM basis; [1]standard error of the mean. L and Q refer to linear or quadratic effects, respectively. R^2 refers to adjusted determination coefficient.

Table 4 - Effect of concentrate level on ruminal, small intestine, large intestine, and total dry matter (DM), organic matter (OM), crude protein (CP), and ether extract (EE) apparent digestibility

Items	0	27.5	55	82.5	SEM	L (*P-value*)	R^2	Q (*P-value*)	R^2	Equation
DM (%)										
ruminal	49.89	53.34	58.08	63.05	2.073	0.0098	0.41	0.8235	0.39	49.45 + 0.1608 × %C
small intestine	28.55	25.93	21.63	19.83	2.078	0.1082	0.12	0.9200	0.05	-
large intestine	-15.16	-9.82	-7.03	-6.61	1.421	0.0084	0.50	0.2309	0.54	-13.9178 + 0.1034 × %C
Total	63.28	69.45	72.68	76.27	1.369	< 0.0001	0.84	0.2504	0.85	64.0891 + 0.1535 × %C
OM (%)										
ruminal	60.21	63.67	66.02	70.49	1.767	0.0282	0.31	0.8735	0.29	60.12 + 0.1207 × %C
small intestine	22.87	20.48	17.85	15.26	1.852	0.1341	0.10	0.9798	0.10	-
large intestine	-15.58	-11.50	-9.30	-8.16	1.147	0.0090	0.43	0.4224	0.44	-14.803 + 0.089 × %C
total	67.50	72.65	74.57	77.59	1.132	0.0001	0.72	0.4002	0.73	68.25 + 0.1170 × %C
CP (%)										
ruminal	-0.20	-10.40	-1.64	0.98	3.322	0.5522	0.52	0.1632	0.57	-
small intestine	56.59	71.19	63.36	68.54	3.091	0.5522	0.00	0.4660	-0.03	-
large intestine	17.17	10.79	8.33	3.87	3.106	0.1313	0.09	0.8779	0.03	-
total	73.56	71.58	70.05	73.39	0.799	0.7850	-0.07	0.1160	0.13	-
EE (%)										
ruminal	-6.96	-7.20	33.50	54.95	11.296	0.0063	0.55	0.4857	0.54	-15.13 + 0.8194 × %C
small intestine	87.91	98.78	59.82	36.40	12.671	0.0428	0.44	0.3821	0.48	99.54 - 0.7003 × %C
large intestine	-11.97	-12.62	-14.56	-18.70	4.388	0.5683	0.08	0.8467	0.04	-
total	68.98	78.96	78.76	72.64	2.267	0.6138	0.05	0.0893	0.17	-

Source: elaborated by the author. 0 = 0% of concentrate, 27.5 = 27.5% of concentrate, 55 = 55% of concentrate, 82.5 = 82.5% of concentrate on DM basis; [1]standard error of the mean. L and Q refer to linear or quadratic effects, respectively. R^2 refers to adjusted determination coefficient.

Table 5 - Effect of concentrate level on ruminal, small intestine, large intestine, and total neutral detergent fiber corrected for ash and protein (apNDF), non-fibrous carbohydrates (NFC), and starch (ST) apparent digestibility

Items	0	27.5	55	82.5	SEM	L (*P-value*)	R^2	Q (*P-value*)	R^2	Equation	x max.	y max.
apNDF (%)												
ruminal	81.00	82.63	79.07	78.05	1.792	0.4452	0.04	0.7249	0.03	-	-	-
small intestine	10.01	8.82	13.76	12.56	2.288	0.5593	-0.04	0.6730	-0.06	-	-	-
large intestine	-31.88	-31.82	-38.23	-46.58	2.094	0.0039	0.45	0.1927	0.52	-	-	-
total	59.14	59.63	54.60	44.03	1.911	0.0011	0.55	0.0297	0.70	$59.14 +0.1183 \times \%C - 0.0037 \times \%C^2$	16.21	60.1
NFC (%)												
ruminal	58.69	71.80	75.94	81.55	3.477	0.0086	0.46	0.4882	0.46	$61.09 + 0.2644 \times \%C$	-	-
small intestine	23.83	12.72	8.33	5.92	3.247	0.0307	0.03	0.4365	0.35	$21.42 - 0.2113 \times \%C$	-	-
large intestine	-7.33	-1.50	0.50	-0.66	1.342	0.0612	0.23	0.1460	0.35	-	-	-
total	75.20	83.02	84.77	86.81	1.319	0.0001	0.71	0.0313	0.81	$75.51 + 0.2906 \times \%C - 0.0019 \times \%C^2$	76.06	86.56
ST (%)												
ruminal	86.66	89.40	90.82	96.45	1.635	0.0338	0.25	0.6321	0.26	$86.21 + 0.1120 \times \%C$	-	-
small intestine	7.69	8.98	1.68	0.81	1.320	0.0147	0.33	0.6373	0.34	$8.9828 - 0.1016 \times \%C$	-	-
large intestine	3.12	-0.47	4.05	-1.06	1.079	0.4300	-0.02	0.7390	-0.09	-	-	-
total	97.47	97.91	96.56	96.20	0.455	0.1748	0.23	0.6330	0.21	-	-	-

Source: elaborated by the author. 0 = 0% of concentrate, 27.5 = 27.5% of concentrate, 55 = 55% of concentrate, 82.5 = 82.5% of concentrate on DM basis; [1]standard error of the mean. L and Q refer to linear or quadratic effects, respectively. R^2 refers to adjusted determination coefficient.

Table 6 - Effect of concentrate level on ruminal, small intestine, and total digested dry matter (DM), organic matter (OM), neutral detergent fiber corrected for ash and protein (apNDF), non-fibrous carbohydrates (NFC), and starch (ST)

Items	0	27.5	55	82.5	SEM	L (*P-value*)	R^2	Q (*P-value*)	R^2	Equation	x max.	y max.
DM (%)												
ruminal	3.04	3.84	4.72	4.97	0.245	0.0004	0.63	0.3764	0.65	$3.1455 + 0.0242 \times \%C$	-	-
small intestine	1.77	1.93	1.78	1.51	0.156	0.4782	0.15	0.4740	0.15	-	-	-
Total	3.88	5.10	5.92	5.99	0.282	< 0.0001	0.80	0.0095	0.89	$3.8672 + 0.0573 \times \%C - 0.0004 \times \%C^2$	75.36	6.03
OM (%)												
ruminal	3.34	4.26	5.03	5.35	0.254	0.0004	0.65	0.3400	0.66	$3.475 + 0.0247 \times \%C$	-	-
small intestine	1.28	1.42	1.38	1.12	0.121	0.6243	0.07	0.4166	0.10	-	-	-
total	3.75	4.94	5.68	5.88	0.275	< 0.0001	0.81	0.0253	0.87	$3.7425 + 0.0532 \times \%C - 0.0003 \times \%C^2$	80.37	5.88
apNDF (%)												
ruminal	2.032	2.04	1.59	0.96	0.129	0.0001	0.737	0.0055	0.87	$2.0439 + 0.0043 \times \%C - 0.0002 \times \%C^2$	10.10	2.07
small intestine	0.27	0.22	0.29	0.15	0.046	0.5138	-0.038	0.6354	-0.02	-	-	-
total	1.48	1.48	1.10	0.54	0.109	< 0.0001	0.755	0.0025	0.90	$1.4936 + 0.0035 \times \%C - 0.0002 \times \%C^2$	9.46	1.51
NFC (%)												
ruminal	1.36	2.47	3.57	4.43	0.323	< 0.0001	0.86	0.6286	0.86	$1.41 + 0.0375 \times \%C$	-	-
small intestine	0.54	0.48	0.40	0.31	0.082	0.2486	0.29	0.9058	0.25	-	-	-
total	1.74	2.91	3.98	4.72	0.316	< 0.0001	0.93	0.2090	0.93	$1.8322 + 0.0364 \times \%C$	-	-
ST (%)												
ruminal	1.24	2.13	3.37	4.48	0.336	< 0.0001	0.93	0.5185	0.93	$1.1605 + 0.0399 \times \%C$	-	-
small intestine	0.11	0.22	0.06	0.04	0.029	0.1478	0.09	0.2995	0.16	-	-	-
total	1.40	2.34	3.58	4.47	0.321	< 0.0001	0.94	0.8717	0.94	$1.3805 + 0.0380 \times \%C$	-	-

Source: elaborated by the author. 0 = 0% of concentrate, 27.5 = 27.5% of concentrate, 55 = 55% of concentrate, 82.5 = 82.5% of concentrate on DM basis; [1]standard error of the mean. L and Q refer to linear or quadratic effects, respectively. R^2 refers to adjusted determination coefficient.

2.5 INTAKE, PASSAGE AND DEGRADATION RATES

A linear increase in ki was observed for DM, OM, NFC, and ST with increasing dietary concentrate levels (Table 7), with estimated increases of 0.024% ($P < 0.0001$), 0.030% ($P < 0.0001$), 0.011% ($P < 0.0001$), and 0.20% ($P = 0.0314$). In contrast, apNDF exhibited a quadratic response ($P = 0.0367$). Among the passage rates, only the kp of OM was affected by the concentrate level, showing a linear increase ($P = 0.0261$). The average kp values for DM, apNDF, NFC, and ST were 1.77% ($P = 0.11$), 0.44% ($P = 0.771$), 2.66% ($P = 0.22$), and 2.18% ($P = 0.638$), respectively.

A linear increase in kd was observed for DM, OM, NFC, and ST, with increases of 0.020% ($P < 0.0001$), 0.025% ($P < 0.0001$), 0.11% ($P < 0.0001$), and 0.20% ($P < 0.0134$), respectively, for each 1% increase in dietary concentrate. The kd of apNDF exhibited a quadratic response ($P = 0.0263$), with a maximum estimated value of 2.05% at a dietary concentrate level of 23.30%.

2.6 RUMINAL pH

The analysis of variance showed that dietary concentrate level did not affect ruminal pH ($P = 0.1827$), nor was there a significant interaction between concentrate level and measurement time ($P = 0.2132$). However, measurement time had a cubic effect on ruminal pH ($P < 0.0001$). The temporal variation in ruminal pH for the different concentrate levels is presented in Figure 4, while the average ruminal pH for each dietary treatment is shown in Figure 5a. A cubic pattern of ruminal pH over measurement time (h) was detected (Figure 5b), which can be described by the regression equation:

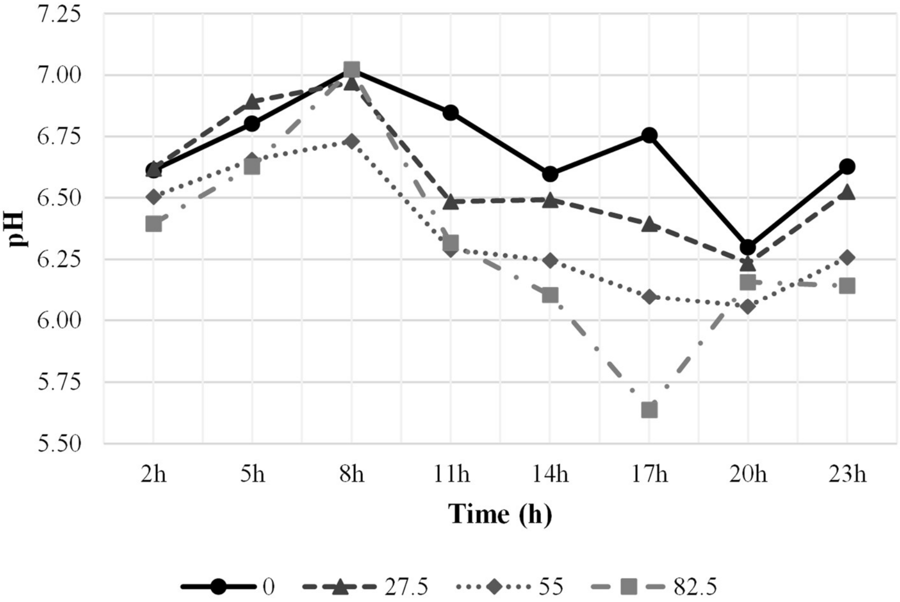
$$\text{Rumen pH} = 6.2213 + 0.2204 \times \text{time (h)} - 0.0201 \times \text{time (h)}^2 + 0.000488 \times \text{time (h)}^3$$

According to this model, the estimated maximum pH of 6.95 occurred at 7:32 h, and the minimum pH of 6.48 occurred at 19:58 h.

Table 7 - Effect of concentrate level on dry matter (DM), organic matter (OM), neutral detergent fiber (apNDF), non-fibrous carbohydrates (NFC), and starch (ST) intake (ki), passage (kp), and digestion (kd) rates

Items	0	27.5	55	82.5	SEM[1]	L (*P-value*)	R^2	Q (*P-value*)	R^2	Equation	x max	y max
DM (% per h)												
ki	3.12	3.68	4.69	4.94	0.21	< 0.0001	0.88	0.2766	0.89	$3.15 + 0.0236 \times \%C$	-	-
kp	1.56	1.73	1.97	1.83	0.09	0.1102	0.34	0.2654	0.38	-		
kd	1.56	1.96	2.71	3.11	0.17	< 0.0001	0.83	0.9876	0.83	$1.53 + 0.0197 \times \%C$	-	-
OM (% per h)												
ki	3.31	3.96	5.15	5.68	0.25	< 0.0001	0.90	0.7218	0.90	$3.28 + 0.0303 \times \%C$	-	-
kp	1.30	1.45	1.75	1.68	0.08	0.0261	0.37	0.3995	0.39	$1.33 + 0.0052 \times \%C$	-	-
kd	2.00	2.50	3.41	4.01	0.21	< 0.0001	0.86	0.7710	0.85	$1.94 + 0.0251 \times \%C$	-	-
apNDF (% per h)												
ki	2.42	2.40	2.41	1.71	0.10	0.0139	0.33	0.0367	0.54	$2.39 + 0.0107 \times \%C - 0.0002 \times \%C^2$	23.96	2.51
kp	0.46	0.42	0.50	0.39	0.04	0.7714	-0.10	0.7455	-0.10	-		
kd	1.96	1.98	1.91	1.32	0.09	0.0065	0.38	0.0263	0.59	$1.94 + 0.0095 \times \%C - 0.0002 \times \%C^2$	23.30	2.05
NFC (% per h)												
ki	6.02	9.24	12.30	15.30	1.03	< 0.0001	0.79	0.9096	0.78	$6.08 + 0.1124 \times \%C$	-	-
kp	2.34	2.57	2.97	2.75	0.20	0.2196	0.50	0.4422	0.49	-		
kd	3.67	6.67	9.34	12.60	0.99	< 0.0001	0.73	0.9193	0.72	$3.67 + 0.1065 \times \%C$	-	-
ST (% per h)												
ki	15.30	25.40	31.7	31.20	2.84	0.0314	0.25	0.2992	0.32	$17.77 + 0.1968 \times \%C$	-	-
kp	1.75	2.92	2.97	1.08	0.44	0.6376	-0.10	0.0908	0.10	-		
kd	13.5	22.50	28.7	30.10	2.69	0.0134	0.32	0.4150	0.35	$15.30 + 0.2039 \times \%C$	-	-

Source: elaborated by the author. 0 = 0% of concentrate, 27.5 = 27.5% of concentrate, 55 = 55% of concentrate, 82.5 = 82.5% of concentrate on DM basis; [1]standard error of the mean. L and Q refer to linear or quadratic effects, respectively. R^2 refers to adjusted determination coefficient.



Source: elaborated by the author.

Figure 5 – Ruminal pH variation over time (h) among concentrate levels.

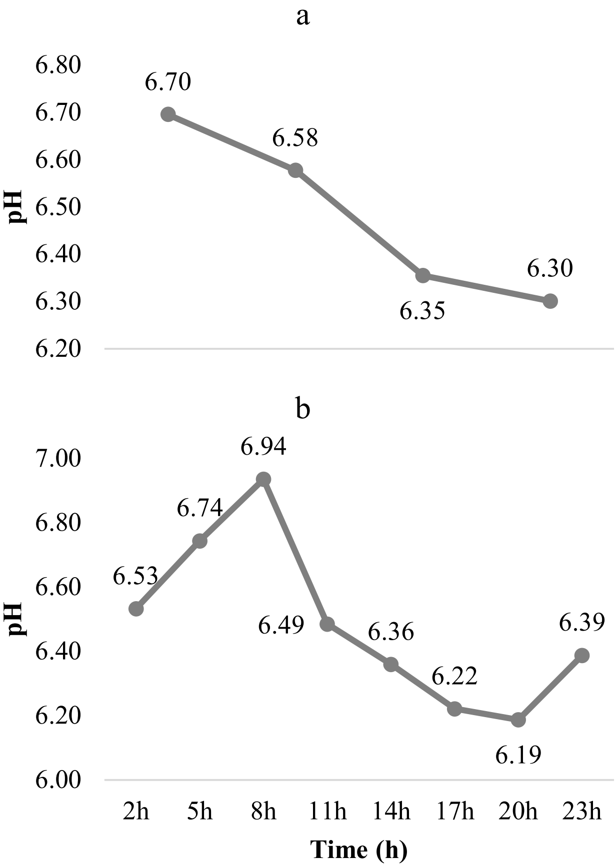


Figure 6 - Effect of concentrate level (a) and measurement time (b) on ruminal pH.

Source: elaborated by the author.

2.7 DISCUSSION

In the present study, dietary concentrate levels ranged from 0% to 82.5%, with the highest level being similar to those used in commercial Brazilian feedlots (Silvestre and Millen, 2021). This wide range allowed a significant variation in fiber and energy content of diets. As the concentrate proportion increased, the dietary energy concentration (TDN) increased, primarily due to higher NFC content, while apNDF, iNDF, and peNDF decreased. This shift in diet composition explains the quadratic pattern observed for DM and OM intake. At lower concentrate levels, intake was likely constrained by the physical fill effect of the fiber fraction (Allen, 1996). Conversely, as energy density increased, the animals reached their metabolic set point, where intake is mainly regulated by energy demand (Forbes, 2003; NASEM, 2016; BR-CORTE, 2023).

Consistent with this, according to Detmann *et al.* (2014), feed intake in cattle is directly related to digestible OM content and inversely related to undigested apNDF. Increasing dietary concentrate raises digestible OM and reduces the fraction of undigested apNDF. Thus, higher dietary energy density reduces the effect of physical fill in the reticulorumen on intake (Allen, 1996), potentially increasing both intake and apparent digestibility of DM and OM (Chizzotti *et al*., 2012; Rotta *et al*., 2014).

Regarding the digestive processes, dry matter disappearance from the rumen occurs via two main pathways: ruminal fermentation and passage of undegraded particles to the omasum (Mertens and Grant, 2020). Although increased intake typically elevates kp, which could reduce digestibility by shortening retention time (Mertens, 2005; Allen *et al*., 2014; Goulart *et al*., 2020), the linear increase observed in DM and OM digestibility suggests that the improvement in dietary fermentability, by increasing concentrate level, compensated for any increase in passage rate. This indicates faster microbial turnover and improved efficiency in the ruminal digestion of the potentially digestible fractions.

However, the utilization of the fibrous fraction followed a different pattern. The quadratic response observed in apNDF kd and total tract digestibility may be attributed to changes in microbial population dynamics. Interestingly, the decline in fiber utilization at high concentrate levels occurred despite the maintenance of a relatively stable ruminal pH. This suggests a carbohydrate effect, where the presence of rapidly fermentable starch leads to a competitive inhibition of fibrolytic species (Mertens and Grant, 2020). Furthermore, different types of microorganisms specialize in fermenting specific components, and changes in diet composition favor fast-growing amylolytic groups at the expense of slower-growing fibrolytic species (Hungate, 1966; Russell & Rychlik, 2001).

Considering the ruminal environment, when acid production exceeds the ruminal absorption capacity, ruminal pH decreases, predisposing animals to subacute (pH 5.5–5.0) or acute (<5.0) acidosis, with fibrolytic activity already impaired at pH below 5.8 (Hungate, 1966; Calsamiglia *et al.*, 2002; Nagaraja & Titgemeyer, 2007). Increased hydrogen ion concentration forces acid-sensitive fibrolytic bacteria to expend energy maintaining proton gradients, compromising adhesion, enzyme production, fiber degradation, and microbial growth (Russell & Wilson, 1996; Hiltner & Dehority, 1983; Roger *et al.*, 1990; Hackman & Firkins, 2015; Goulart *et al.*, 2020; Pucetti *et al.*, 2024). Prolonged low pH reduces fiber digestion by inhibiting acid-sensitive microbes (Russell & Dombrowski, 1980; Calsamiglia *et al.*, 2002), decreasing fiber degradation and apNDF utilization efficiency (Firkins, 2021), which can limit the benefits of high-concentrate feeding if adequate fiber is not provided.

Interestingly, in this study, ruminal pH remained above the critical threshold of 5.8 for most of the time, even with 82.5% concentrate. This stability is remarkable considering that peNDF from corn silage was only 3.98%. This result is particularly relevant given that in Brazil, the proportion of forage included in finishing diets has decreased over the years. Silvestre and Millen (2021) reported that nutritionists have recommended an average forage inclusion of 16.75% (DM basis) in finishing diets for beef cattle, with values ranging from 7.17% to 35%. The recommended total NDF content for such diets averaged 21.8%. In the present study, the diet containing 82.5% of concentrate, had a total apNDF content of 14.40% (DM basis), of which 7.21% originated from corn silage. Furthermore, peNDF content from corn silage was 3.98% (DM basis). Notably, despite the high ST content (59.40% on DM basis) and low corn silage peNDF level, this physically effective fiber inclusion was sufficient to prevent ruminal acidosis.

The maintenance of ruminal pH above 5.8, even at the 82.5% concentrate level with only 3.98% peNDF, reinforces the paradigm established by Armentano and Pereira (1997), which defines fiber effectiveness as a dynamic animal response rather than a static chemical property. This scenario suggests that the physical structure of corn silage, although minimal, reached a critical threshold capable of stimulating the insalivation and endogenous buffering required to neutralize rapid starch fermentation. Such stability aligns with the findings of Alhadas *et al.* (2021), who demonstrated that strategic levels of peNDF in high-grain diets are sufficient to preserve ruminal health and microbial efficiency. However, the observed decline in apNDF digestibility at higher inclusion levels indicates that while the peNDF was sufficient to prevent acidosis, it could not mitigate the 'carbohydrate effect' and the competitive inhibition of fibrolytic bacteria, highlighting the metabolic trade-off inherent in high-energy density systems. The maintenance of the ruminal environment can be further explained by genetic factors. Ruminants naturally consume predominantly fibrous feeds, especially grasses; however, high-concentrate finishing diets in feedlots significantly alter ruminal physiology by increasing short-chain fatty acids and lactic acid, lowering pH, and modifying microbial populations (Pinto, 2022; Pucetti *et al.*, 2024). Studies comparing genetic groups have shown that *Bos taurus taurus*, (e.g. Angus) and *Bos taurus indicus* animals (e.g. Nellore) respond differently to these challenges. In this context, Pinto (2022) observed that Nellore cattle tend to maintain a higher and more stable ruminal pH compared to Angus when fed high-energy diets, a fact attributed to the greater microbial diversity found in Nellore animals. Nevertheless, despite experiencing lower ruminal pH for longer periods, no breed effect was observed on dry matter intake in the study by Pinto (2022). This lack of difference in intake, even under more acidic conditions, suggests that taurine breeds may exhibit greater metabolic tolerance and a higher threshold for the physiological signals that typically trigger intake depression during ruminal acidosis.

This adaptation allows *Bos taurus taurus* to maintain performance in high-starch systems where a more specialized and homogeneous microbiota handles the fermentative challenge without compromising feed ingestion. In the present study, crossbred animals (Red Angus x Nellore) were used, and remarkably, no statistical differences were observed in ruminal pH even when the concentrate level was increased to 82.5%. This stability suggests that the F1 progeny may benefit from a synergistic combination of traits: the inherent ruminal resilience and greater microbial diversity of the Nellore, coupled with the metabolic tolerance to high-starch diets characteristic of the Angus. Furthermore, the maintenance of ruminal pH within physiological limits, despite the high energy density, indicates that the crossbred animal possesses an efficient buffering capacity and a balanced fermentative rate, which broadens the safety margin for the inclusion of high concentrate levels in corn silage-based diets.

In addition to the effect of genetics and peNDF, virginiamycin supplementation may also prevent ruminal acidosis, inhibiting Gram-positive lactic acid-producing bacteria (Coe *et al.*, 1999). This stability in the ruminal environment of crossbred animals (Red Angus x Nellore) contrasts with the findings of Pinto (2022) for purebred Angus, which experienced more pronounced drops in pH. This suggests that the crossbred animal might benefit from a combination of the Angus's metabolic adaptability to high-starch diets, characterized by a more homogeneous and specialized microbiota.

Finally, the results obtained in this study show significant practical applicability for diet formulation across different beef cattle production systems. The higher energy content (%TDN on DM basis) and the greater apparent digestibility of DM and OM with the 82.5% concentrate diet demonstrate that maximum energetic efficiency can be achieved without compromising ruminal health when fiber inclusion is properly managed. These findings highlight the importance of balancing energy density with effective fiber sources, especially peNDF, to stimulate chewing, salivation, and buffering, thereby preventing acidosis even in highly fermentable diets. In practical terms, feedlot diets with concentrate levels as high as 82.5% can be used safely, provided that minimum levels of physically effective fiber and ionophore are maintained.

The intermediate concentrate level (27.5%) also showed adequate responses in terms of intake, digestibility, and ruminal pH stability, indicating its suitability as a nutritional strategy for semi-intensive systems, such as pasture-based growing diets with supplementation or transitional diets during the dry-to-rainy season. This inclusion level helps maintain energy supply while minimizing the risk of metabolic disturbances such as ruminal acidosis. Together, these findings provide a technical reference for minimum forage and fiber requirements in high-concentrate beef cattle diets and support flexible nutritional planning across production phases. The quadratic responses observed for intake and digestibility indicate that concentrate levels can be tailored according to animals' nutritional requirements, forage availability and quality, and economic or technical objectives.

2.8 CONCLUSION

Increasing concentrate in corn silage–based diets enhanced energy intake and NFC digestibility; however, it led to lower fiber utilization while maintaining ruminal pH within a safe physiological range. Even at the highest concentrate level (82.5%) and high ST inclusion (59.4% on DM basis), the inclusion of adequate roughage fiber, specifically 3.98% peNDF from corn silage, combined with virginiamycin supplementation, effectively prevented ruminal acidosis and maintained proper rumen function. While the 82.5% concentrate diet supported greater nutrient use, the intermediate level (27.5%) provided satisfactory intake, digestibility, and ruminal stability, making it a practical option for semi-intensive production systems. Overall, these results underscore the importance of balancing concentrate inclusion with sufficient physically effective fiber and strategic feed additives to optimize productivity while preserving ruminal health.

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3 EFFECT OF CONCENTRATE LEVEL ON BEEF CATTLE NUTRIENT DEGRADABILITY, NITROGEN UTILIZATION, AND MICROBIAL PROTEIN SYNTHESIS

3.1 ABSTRACT

Efficient nitrogen utilization and microbial protein synthesis (MPS) are essential for improving ruminant productivity and reducing environmental nitrogen losses. High-concentrate diets increase fermentable energy supply for ruminal microbes; however, excessive concentrate inclusion may negatively affect fiber degradability and microbial efficiency. It was hypothesized that increasing dietary concentrate would enhance MPS while reducing ruminal fiber degradation, thereby influencing nitrogen utilization efficiency. This study evaluated the effects of increasing concentrate levels on *in situ* degradability, nitrogen metabolism, and MPS in beef cattle, as well as the predictive performance of BR-CORTE and NASEM equations. Four rumen-cannulated F1 (Red Angus × Nellore) bulls (average initial BW = 472 ± 8.5 kg) were assigned to a 4×4 Latin square design and fed corn silage-based diets containing 0, 27.5, 55.0, or 82.5% concentrate. *In situ* degradability of dry matter (DM), organic matter (OM), crude protein (CP), and neutral detergent fiber (NDF) was evaluated using the nylon-bag technique, and MPS was estimated using urinary purine derivatives. Increasing concentrate levels reduced the soluble fraction (a) of DM and OM but increased the potentially degradable fraction (b) and effective degradability. The estimated ruminal retention times required to equate *in situ* and *in vivo* data (target times) for DM and OM ranged from 13.68 to 19.74 h, showing an increasing trend as energy density rose. For CP, a drastic shift was observed: in the 0% concentrate diet (urea-supplemented), the target time exceeded the experimental window (> 96 h), whereas in high-starch diets, it decreased and stabilized at an average of 7.38 h, indicating higher precision for true protein sources. NDF degradation exhibited a quadratic response, with maximal effective degradability at moderate concentrate inclusion; however, at 82.5% concentrate, NDF target times exceeded 96 h, reflecting a severe depression in fibrolytic activity. Nitrogen intake, absorbed nitrogen, and nitrogen retention increased linearly, whereas MPS and its efficiency showed quadratic patterns, peaking at approximately 48% concentrate inclusion. The reduction in urinary nitrogen excretion at higher concentrate levels, alongside the stabilization of CP target times, suggests optimized nitrogen capture and ruminal synchrony. In conclusion, moderate concentrate inclusion (~48%) optimizes ruminal degradation of non-fiber carbohydrates and MPS without severely compromising fiber digestion, although current prediction models show limited ability to estimate MPS under isonitrogenous tropical beef cattle diets.

Key words: *in situ* degradability, nitrogen metabolism, microbial protein synthesis.

3.2 INTRODUCTION

The nutritional efficiency of ruminants is closely linked to the dynamics of feed degradation in the rumen, which is influenced by diet composition, particularly the ratio of concentrate to forage. The *in situ* degradability technique is widely used to evaluate the ruminal disappearance of dry matter (DM), organic matter (OM), crude protein (CP), and neutral detergent fiber (NDF), allowing estimation of key kinetic parameters such as the soluble fraction (a), potentially degradable fraction (b), undegradable fraction (c), and degradation rate (kd) (Ørskov & McDonald, 1979). These parameters provide critical insights into the fermentative behavior of feeds and their contribution to nutrient availability.

Increasing dietary concentrate levels typically enhance the availability of rapidly fermentable carbohydrates, accelerating degradation rates of non-structural components (Zhao *et al.*, 2016; Alhadas *et al.*, 2021). However, this often occurs at the expense of fiber degradability due to ruminal pH reduction and shifts in microbial populations (Zhao *et al.*, 2016). Such changes influence the delicate balance between degradation kinetics and passage rates, ultimately affecting nutrient digestibility and energy supply to the animal. Despite the importance of these interactions, the quantitative relationship between dietary concentrate levels and specific degradability parameters remains underexplored in tropical beef cattle systems.

Concomitantly, microbial protein synthesis (MPS) represents the primary source of metabolizable protein for ruminants and depends on the synchronized availability of rumen-degradable nitrogen and fermentable energy (Dewhurst *et al.*, 2000; Hackmann & Firkins, 2015). Diets with higher concentrate proportions tend to improve microbial protein yield by increasing ATP availability, although excessive concentrate inclusion can impair microbial efficiency if ruminal pH falls below optimal levels (Hackmann & Firkins, 2015; Prendergast & Gibbs, 2015). Therefore, understanding how concentrate inclusion affects nitrogen intake, excretion, retention, and microbial protein flow is essential for optimizing nitrogen utilization and minimizing environmental nitrogen losses.

Feeding systems such as BR-CORTE (2023) and NASEM (2016) provide equations to predict MPS; however, empirical validations indicate these models may have limited accuracy under specific nutritional conditions typical of tropical beef production (Galyean & Tedeschi, 2014; Benedeti *et al.*, 2020). Integrating direct measurements of nitrogen balance and microbial efficiency with detailed feed degradation assessments can thus enhance model calibration and improve diet formulation strategies.

Improving the understanding of optimal concentrate inclusion in tropical beef cattle diets is essential for balancing feed efficiency and ruminal health. Enhanced knowledge of feed degradation kinetics and MPS under tropical conditions can contribute to refining protein evaluation models and feeding strategies, with potential benefits for animal productivity and environmental sustainability in tropical production systems.

It is hypothesized that increasing dietary concentrate levels enhances MPS while reducing ruminal fiber degradation, thereby negatively affecting nitrogen utilization efficiency. Thus, the objective of this study was to evaluate the effects of increasing dietary concentrate levels on *in situ* degradability parameters, nitrogen balance, and MPS in beef cattle.

3.3 MATERIAL AND METHODS

3.3.1 ANIMALS, DIETS, AND EXPERIMENTAL DESIGN

The experiment was conducted at the Ruminant Nutrition Laboratory (LabNUR) within Department of Animal Science at the Federal University of Viçosa (UFV), in Viçosa, Minas Gerais, Brazil. The project was approved by the Ethics Committee on the Use of Production Animals (CEUAP) of the Federal University of Viçosa (Protocol no. 26/2024).

Four rumen and ileum-fistulated F1 (Red Angus × Nellore) bulls were used, with an average initial body weight (BW) of 471 kg ± 51.8 and an average age of 18 months. The experiment followed a 4×4 Latin square design, involving four animals, four experimental periods, and four treatments. The diets were composed of corn silage, ground corn, soybean meal, urea:ammonium sulfate, a vitamin–mineral premix, and virginiamycin (Table 1). All diets were formulated to be isoproteic (110 g/kg of crude protein), according to the BR-CORTE (2023) system. The four concentrate levels evaluated were 0%, 27.5%, 55%, and 82.5%. The 0% treatment consisted of non-organic supplementation, which included 1.89% urea/ammonium sulfate and 0.98% mineral mix on average. The complete composition of experimental diets is shown in Table 2.

The animals were housed in individual tie-stall pens, each measuring 6 m^2 (2 x 3 m), with concrete floor and individual feed bunks and water troughs. All animals were treated for endo- and ectoparasites. A 30-day adaptation period to the diets and the experimental environment was carried out prior to data collection.

3.3.2 EXPERIMENTAL PERIOD

After the adaptation period, the animals were subjected to experimental diets for 96 days, divided into four 24-day periods. During the first 14 days of each experimental period, the animals were adapted to the diets to allow stabilization of nutrient flow throughout the gastrointestinal tract. The dietary transition was carried out gradually, with 25% of the new diet being introduced every three days.

Diets were offered *ad libitum*, allowing for a 5% refusal rate on a fresh matter basis. Animals were fed twice daily at 08:00 and 16:00. To ensure accurate intake, the amount of feed and orts were weighed daily, with adjustments made for the next day's supply. The corn silage was collected directly from the silo each morning and mixed with the concentrate component. From the 15th to the 23rd day of each experimental period, samples of the diets and orts (approximately 5% of the total) were collected, placed in plastic bags, and stored in a freezer at -20°C. At the end of each period, a composite sample was prepared for each animal and dried in a forced-air oven at 55°C for 72h (Method G-001/2; Detmann *et al.*, 2025). The samples were then ground in a knife mill using a 1 mm screen and subjected to final drying in an oven at 105°C at 16h (Method G-003/1; Detmann *et al.*, 2025), after which they were stored in plastic containers for subsequent analyses.

3.3.3 FECAL AND URINE COLLECTION

In each experimental period, a total collection of feces and urine was carried out. from the 15th to the 18th day. Feces were collected directly from the concrete floor and stored in pre-weighed covered buckets. Every 24 hours during the collection, the feces were weighed, homogenized, and a sample (approximately 250 g) was taken. These subsamples were dried in forced-air ovens at 55ºC for 72 hours in previously weighed aluminum trays. Then, the samples were ground using knife mills with 1 and 2 mm sieve screens. A proportional composite fecal sample was prepared for each animal at the end of the collection period. These samples, combined with intake data, were used to determine the total *in vivo* digestibility of the diets.

Total urine collection was conducted using collection funnels attached to the animals, with hoses leading to plastic containers. To prevent volatilization of nitrogen compounds, 200 mL of 20% sulfuric acid solution was added to each container.

Every 24 hours, the urine containers were weighed to determine the daily urine volume. The urine was homogenized, and two samples were collected and stored in separate plastic containers: one concentrated sample and one diluted sample (in 0.036 N sulfuric acid).

Table 1 - Chemical composition of diet ingredients

Items	Ingredients					
	Corn silage	Corn meal	Soybean meal	Urea/A.S.[14]	Premix[15]	Virginiamycin[16]
DM[1]	31.41 $_{\pm1.464}$	88.35	89.58	95.85	100.00	100.00
OM[2]	90.03 $_{\pm4.296}$	98.73	93.09	99.72	-	-
CP[3]	5.96 $_{\pm0.885}$	7.82	48.18	263.49	-	-
EE[4]	1.97 $_{\pm0.597}$	3.55	2.23	-	-	-
NDF[5]	46.35 $_{\pm2.150}$	9.93	12.47	-	-	-
peNDF (%NDF)[6]	49.11 $_{\pm2.937}$	-	-	-	-	-
peNDF	22.76 $_{\pm1.056}$	-	-	-	-	-
apNDF7	41.21 $_{\pm2.380}$	8.93	10.28	-	-	-
iNDF8	17.59 $_{\pm3.660}$	2.07	1.77	-	-	-
pdNDF9	23.63 $_{\pm6.520}$	6.86	8.51	-	-	-
ADF[10]	25.70 $_{\pm2.492}$	2.52	5.96	-	-	-
NFC[11]	40.89 $_{\pm2.350}$	78.43	32.40	-	-	-
ST[12]	24.69 $_{\pm1.820}$	71.43	5.13	-	-	-
ROM[13]	16.20 $_{\pm0.529}$	7.00	27.27	-	-	-

Source: elaborated by the author. [1]Dry matter, [2]Organic matter, [3]Crude protein, [4]Ether extract, [5]Neutral detergent fiber, [6]Physically effective neutral detergent fiber, [7]Neutral detergent fiber corrected for ash and protein, [8]Indigestible neutral detergent fiber, [9]Potencially degradable neutral detergent fiber, [10]Acid detergent fiber, [11]Non fibrous carbohydrates, [12]Starch, [13]Residual organic matter, [14]Urea and ammonium sulfate (9:1), [15]Premix guarantees (Per kg of DM): 242.00-300.00 g of Ca, 11.10 mg of Co (Min), 556.00 mg of Cu (Min), 24.50 g of S (Min), 370.00 mg of Fe (Min), 13.50 g of P (Min), 27.70 mg of I (Min), 19.00 g of Mg (Min), 1668.00 mg of Mn (Min), 928.00 mg of monensina, 7.40 mg of Se (Min), 61.50 g of Na (Min), [16]V-max® 2 (20.000g/kg, Phibro Animal Health).

For the diluted sample, the daily urine volume was estimated based on the relationship between the weight of the container holding the total urine and the weight of the graduated cylinder containing the subsample. The formula used in the spreadsheet was:

$$Daily\ urine\ volume\ (L) = 2 \times (A - B)/(C - D)$$

where:

A = weight of the container + urine (kg)

B = tare weight of the container (kg)

C = weight of the graduated cylinder + urine subsample (kg)

D = tare weight of the graduated cylinder (kg)

First, the actual mass of urine in the container was calculated:

$$A - B$$

Next, the mass of the urine subsample was determined:

$$C - D$$

Because the subsample represents half of the final dilution (1 part urine + 1 part acid solution), the total daily urine volume was estimated by multiplying the ratio between the total urine mass and the subsample mass by 2.

A correction Factor (F) was then applied, calculated as:

$$F = Urine\ volume\ sampled\ on\ day\ 1/Urine\ volume\ of\ day\ 1$$

This factor remained constant for all subsequent days.

The urine volume for diluted samples was daily determined as:

$$Urine\ volume\ to\ be\ sampled = F \times daily\ urine\ volume\ (L)$$

All samples were stored in a freezer at -20ºC.
Nitrogen concentration in the urine was determined following the Method N-001/2 from Detmann *et al.* (2025). The nitrogen balance estimate (N retained, g/d), from the nitrogen consumed by the animals (g/d), was obtained by subtracting the nitrogen lost via feces and urine (g/d), according to the following equation:

$$Retained\ N\ (g) = N\ intake(g) - (Fecal\ N\ (g) + Urine\ N\ (g))$$

3.3.4 COLLECTIONS OF RUMINAL AND OMASAL DIGESTA

Digesta samples from the rumen, omasum, and ileum were collected at 9-hour intervals over three days (from day 19 to 21 of each experimental period). The collection times were 08:00, 17:00, 02:00, 11:00, 20:00, 05:00, 14:00, and 23:00. The procedure followed the methodology described by Huhtanen *et al.* (1997) and adapted by Leão (2002).

A continuous infusion of the Co-EDTA marker (5 g/d) diluted in 4 liters of water was administered via the ruminal fistula using peristaltic pumps from day 16 to day 21. To collect omasal digesta, a sampling tube was inserted toward the reticulo-omasal orifice, and the digesta was aspirated using a vacuum pump once the tube was correctly positioned.

Omasal digesta samples were separated into solid and liquid phases with small particles were separated using a 100 µm nylon filter (Sefar Nitex 100/44, Sefar, Thal, Switzerland). Each omasal digesta sample was initially divided into two aliquots: 200 mL were used for phase separation and another 200 mL representing the whole digesta.

During the omasal digesta collection, a sampling tube was introduced toward the reticule-omasal orifice, and digesta was aspirated via a vacuum pump. Each omasal digesta sample was divided into three parts: 500 mL were allocated for bacterial isolation, 200 mL were allocated for separation into two phases (solid and liquid with small particles), and another 200 mL represented the whole digesta. The samples designated for phase separation and bacterial isolation were frozen at -20ºC. The whole digesta samples were dried in a forced-air oven at 55ºC for 72 hours.

Bacteria associated with particles (BAP) and bacteria associated with the liquid phase (BAL) were isolated. The methodology used was that described by Reynal *et al.* (2005), adapted by Krizsan *et al.* (2010). The omasal digesta samples were filtered through a 100 µm nylon filter with 44% surface pore area (Sefar Nitex 100/44, Sefar, Thal, Switzerland). The material retained on the filter was washed with saline solution (NaCl 9 g/L), and from this fraction, the BAP isolation was performed. The BAL isolation was performed from the filtrate, which was centrifuged at 1000 × g for 10 minutes at 5ºC. The resulting pellet was separated for BAP isolation, and the supernatant was centrifuged at 11,250 × g for 30 minutes at 5ºC. To the pellet resulting from this second centrifugation, 200 mL of saline solution were added. The material was centrifuged again at 16,500 × g for 20 minutes at 5ºC, and the resulting pellet was lyophilized for subsequent BAL analysis.

Table 2 - Proportion of ingredients and composition of experimental diets

Diets	0	27.5	55	82.5
Ingredients			%	
Corn silage	97.13	72.50	45.00	17.50
Corn meal	0.00	20.36	48.56	76.70
Soybean meal	0.00	5.10	4.51	3.97
Urea/A.S.[1]	1.89	0.94	0.83	0.73
Premix	0.98	0.98	0.98	0.98
Virginiamycin[2]	0.00	0.13	0.13	0.13
Composition			%	
DM[3]	32.03	38.21	48.70	67.15
OM[4]	89.58	91.05	93.48	95.91
CP[5]	10.78	10.85	10.83	10.85
EE[6]	1.91	2.27	2.72	3.16
apNDF[7]	40.03	32.11	23.21	14.40
iNDF[8]	17.26	13.37	9.06	4.75
pdNDF[9]	22.77	18.74	14.16	9.64
ADF[10]	24.96	19.40	12.99	6.63
Corn silage apNDF[11]	40.03	29.87	18.54	7.21
Corn silage peNDF[12]	22.11	16.50	10.24	3.98
NFC[13]	37.62	47.53	58.54	69.42
ST[14]	23.98	32.80	46.17	59.40
ROM[15]	13.64	14.73	12.36	10.02

Source: elaborated by the author. [1]Urea and ammonium sulfate (9:1), [2]V-max® 2 (20.000g/kg, Phibro Animal Health). [3]Dry matter, [4]Organic matter, [5]Crude protein, [6]Ether extract, [7]Neutral detergent fiber corrected for ash and proteins, [8]Indigestible neutral detergent fiber, [9]Potentially degradable neutral detergent fiber, [10]Acid detergent fiber, [11] Corn silage neutral detergent fiber corrected for ash and protein, [12] Corn silage physically effective neutral detergent fiber, [13]Non fibrous carbohydrates, [14]Starch, [15]Residual organic matter.

For BAP isolation, 700 mL of 0.90% saline solution (weight/volume) with 0.10% Tween (volume/volume) were added to the flasks containing the samples, which were homogenized and stored at 4ºC for 12 hours. After this period, the samples were filtered through a 100 µm nylon filter with 44% surface pore area (Sefar Nitex 100/44, Sefar, Thal, Switzerland). The filtrate was centrifuged at 11,250 × g for 30 minutes at 5ºC. To the resulting pellet, 200 mL of saline solution were added, followed by a new centrifugation at 16,250 × g for 20 minutes at 5ºC. The resulting pellet was lyophilized. The samples for BAP and BAL isolation were stored, and later analyses of dry matter, mineral matter, total nitrogen, and RNA were performed.

The pH of 100 mL of filtered ruminal fluid was measured immediately after sampling and phase separation using a digital pH meter.

3.3.5 DIGESTA FLOW

The flow of DM and other dietary component was estimated using the dual marker system (France and Siddons,1986). Cobalt-EDTA (Co-EDTA) was used as the marker for the liquid and small particle phases, while indigestible neutral detergent fiber (iNDF) was used as the internal marker for the solid phase.

To estimate digesta flow, cobalt concentrations were determined in the omasal digesta via atomic absorption spectrophotometry after sample digestion with nitric-perchloric acid (INCT method M-004/2; Detmann *et al.*, 2025). Omasal digesta flow was estimated using the reconstitution technique described by Faichney (1975). Indigestible neutral detergent fiber was quantified in digesta samples from their respective gastrointestinal compartments, as well as in feed and orts samples.

3.3.6 MICROBIAL PROTEIN SYNTHESIS AND MICROBIAL PROTEIN SYNTHESIS EFFICIENCY

Microbial protein synthesis was estimated through purine bases analysis, according to Zinn and Owens (1980) and modified by Ushida *et al.* (1985). The analysis was conducted using spectrophotometry (high-performance liquid chromatography). The synthesis of microbial nitrogen and CP was estimated. The efficiency of microbial synthesis was expressed as grams of microbial crude protein in relation to the intake of total digestible nutrients (TDN), ingested organic matter (IOM), rumen degraded NDF corrected for ash and protein (RDapNDF), and rumen degraded starch (RDST).

3.3.7 *IN SITU* DEGRADATION

The nutritional composition of the ingredients and diets used in the *in situ* degradability assay is presented in Tables 4 and 5. Figure 1 shows the experimental framework. The procedures for *in situ* assay followed the recommendation of Detmann *et al.* (2025). The *in situ* degradability of DM, OM, CP of the diets was determined using the first-order kinetics model proposed by Ørskov and McDonald (1979):

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

where: Y(t) = the fraction of DM, OM, CP degraded at time t (%); a = the readily fermentable fraction (%); b = the potentially degradable fraction; kd = the degradation rate of b per hour; t = time (hours).

The effective degradation of DM, OM, CP and NDF was calculated considering the *in vivo* passage rate (Table 3), based on the following equation:

$$ED = a + (b \times kd)/(kp + kd)$$

where ED represents the effective degradability; a = the readily fermentable fraction (%); b = the potentially degradable fraction (%); kp = the passage rate of b per hour; kd = the degradation rate for b per hour.

Rumen undegradable protein (RUP) was calculated based on the following equation:

$$RUP = 100 - ED$$

where RUP represents the rumen undegradable protein fraction (%); ED = effective degradability (%).

The *in situ* degradability of NDF was determined using the model proposed by Mertens and Loften (1980):

$$Y(t) = b \times (e^{-k \ \times t}) + I$$

where Y(t) represents the non-degraded NDF residue at a time t (%); b is the potentially degradable fraction in the rumen (%); kd = the degradation rate of b per hour; t is the time-independent variable (h); and I is the undegradable fraction (%).

The NDF degradation equation was adjusted, and the fractions were standardized according to Waldo *et al*. (1972), using the following equations:

$$PD = 100 - b$$

where PD represents the standardized potentially degradable fraction (%); b the potentially degradable fraction (%).

The effective degradability of NDF was determined using the *in vivo* passage rate, based on the following equation:

$$ED = (b \times kd)/(kp + kd)$$

where ED represents the effective degradability; b = the potentially degradable fraction (%); kp = the passage rate of b per hour; kd = the degradation rate for b per hour.

Table 3 – Dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), and crude protein (CP) passage (kp), and digestion (kd) rates

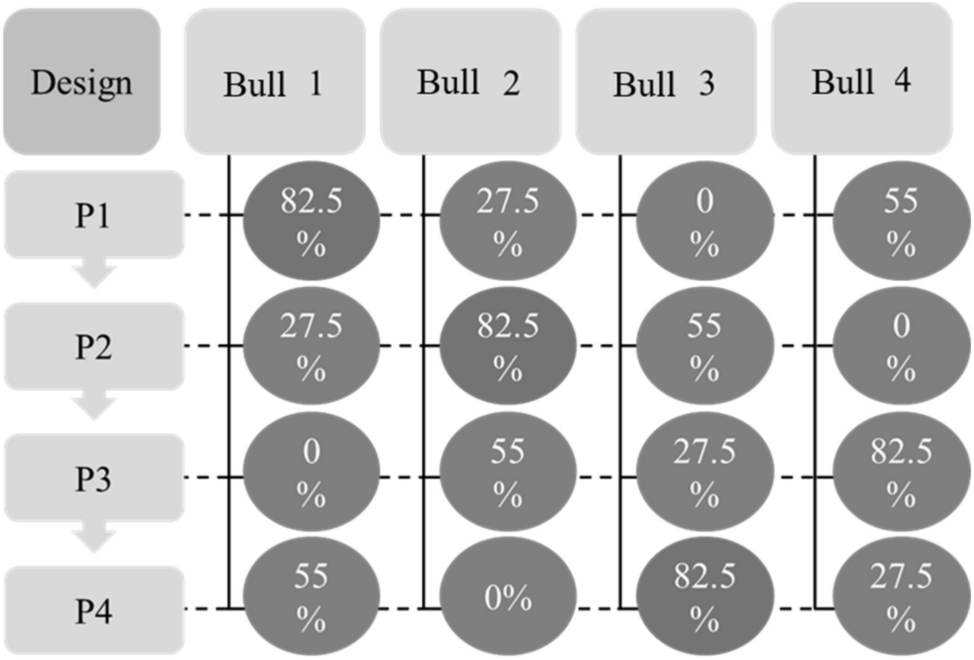
Diets	0	27.5	55	82.5
DM (% per h)				
kp	1.555	1.727	1.974	1.828
OM (% per h)				
kp	1.302	1.453	1.748	1.678
NDF (% per h)				
kp	0.460	0.420	0.500	0.390
CP (% per h)				
kp	3.357	3.914	4.244	4.464

Source: elaborated by the author. 0 = 0% of concentrate on DM basis, 27.5 = 27.5% of concentrate on DM basis, 55 = 55% of concentrate on DM basis, 82.5 = 82.5% of concentrate on DM basis.

Table 4 - Chemical composition of diet ingredients for *in situ* trial

	Ingredients					
Items	Corn silage	Corn meal	Soybean meal	Urea/AS[5]	Premix[6]	Virginiamycin[7]
			%			
DM[1]	27.28	90.03	89.15	95.85	98.13	99.00
OM[2]	94.04	98.85	92.97	99.72	-	-
CP[3]	5.68	8.42	48.09	263.49	-	-
NDF[4]	45.02	9.28	14.18	-	-	-

Source: elaborated by the author. [1]Dry matter, [2]Organic matter, [3]Crude protein, [4]Neutral detergent fiber, [5]Urea and ammonium sulfate (9:1), [6]Premix guarantees (Per kg of DM): 242.00-300.00 g of Ca, 11.10 mg of Co (Min), 556.00 mg of Cu (Min), 24.50 g of S (Min), 370.00 mg of Fe (Min), 13.50 g of P (Min), 27.70 mg of I (Min), 19.00 g of Mg (Min), 1668.00 mg of Mn (Min), 928.00 mg of monensina, 7.40 mg of Se (Min), 61.50 g of Na (Min), [7]V-max® 2 (20.000g/kg, Phibro Animal Health)



Source: elaborated by the author.

Figure 1. The experimental framework was structured to investigate the effects of varying concentrate proportions on the *in situ* degradability of diets in beef cattle. In this design, columns correspond to individual animals, and rows indicate distinct experimental periods. Each cell, where a row and column intersect, identifies a unique experimental unit. For example, the first animal (first column) was fed a diet containing 0% concentrate during the first period, then 27.5% concentrate in the second period, followed by 55% and 82.5% concentrate in the third and fourth periods, respectively. The abbreviations inside each cell specify the concentrate level of the diet administered or incubated at that particular animal-period combination. This crossover design allows every animal to receive all treatments in different periods, which facilitates the control of variability attributed to individual animals and temporal effects. The *in situ* degradability of each diet was assessed in the same animal that received that specific diet during the corresponding experimental period, that is, ruminal incubation of each treatment was performed in animals consuming the respective diets. The degradation was assessed at intervals of 0, 3, 6, 12, 18, 24, 48, 72, and 96 hours.

Table 5 - Proportion of ingredients and composition of experimental diets for *in situ* trial

Diets	0	27.5	55	82.5
Ingredients		%		
Corn silage	97.13	72.50	45.00	17.50
Corn meal	0.00	20.36	48.56	76.70
Soybean meal	0.00	5.10	4.51	3.97
Urea/A.S.[1]	1.89	0.94	0.83	0.73
Premix[2]	0.98	0.98	0.98	0.98
Virginiamycin[3]	0.00	0.13	0.13	0.13
Composition		%		
DM[4]	27.93	34.80	46.45	66.80
OM[5]	93.11	94.01	95.47	96.80
CP[6]	11.16	11.28	11.29	11.34
NDF[7]	43.54	33.99	23.87	14.70

Source: elaborated by the author. [1]Urea and ammonium sulfate (9:1), [2]Premix guarantees (Per kg of DM): 242.00-300.00 g of Ca, 11.10 mg of Co (Min), 556.00 mg of Cu (Min), 24.50 g of S (Min), 370.00 mg of Fe (Min), 13.50 g of P (Min), 27.70 mg of I (Min), 19.00 g of Mg (Min), 1668.00 mg of Mn (Min), 928.00 mg of monensina, 7.40 mg of Se (Min), 61.50 g of Na (Min), [3]V-max® 2 (20.000g/kg, Phibro Animal Health), [4]Dry matter, [5]Organic matter, [6]Crude protein, [7]Neutral detergent fiber.

3.3.8 LABORATORY ANALYSES

The following analyses were performed on samples of corn silage, concentrate, refusals, feces, and ruminal digesta: DM, using INCT method G-003/1; ash (mineral matter, MM), using INCT method M-001/3; CP, using INCT method N-001/3; EE, using INCT methods G-004/1; neutral detergent fiber corrected for ash and protein (apNDF), using INCT method F-001/3; corrections for ash and nitrogen in NDF, using INCT methods N-004/2 and M-002/2; iNDF, using INCT method F-008/2; acid detergent fiber (ADF), using INCT method F-003/3 (Detmann *et al.*, 2025); starch (ST), according to Silva *et al.* (2019); and non-fibrous carbohydrates (NFC).

The potentially degradable neutral detergent fiber (PdNDF) fraction was calculated by subtracting iNDF from apNDF:

$$pdNDF = \mathrm{apNDF} - \mathrm{iNDF}$$

where apNDF = neutral detergent fiber corrected for ash and protein content; iNDF = indigestible neutral detergent fiber, determined after 288 h of in situ ruminal incubation; pdNDF

= potentially degradable NDF fraction, representing the fiber fraction available for ruminal fermentation.

The non-fibrous carbohydrate (NFC) content was calculated according to the following equation proposed by Detmann and Valadares Filho (2010):

$$NFC = 1000 - [(\mathrm{CP}\frac{\mathrm{g}}{\mathrm{kg}} - \mathrm{urea\ CP}\frac{\mathrm{g}}{\mathrm{kg}} + \mathrm{urea}\frac{\mathrm{g}}{\mathrm{kg}}) + \mathrm{apNDF}\frac{\mathrm{g}}{\mathrm{kg}} + \mathrm{EE}\frac{\mathrm{g}}{\mathrm{kg}} + \mathrm{ash}\frac{\mathrm{g}}{\mathrm{kg}}]$$

where: NFC = non-fibrous carbohydrates (g/kg); CP = crude protein; apNDF = neutral detergent fiber corrected for ash and protein; EE = ether extract.

The TDN of the diets was calculated using the following equation:

$$TDN = \mathrm{CP} + \mathrm{dNFC} + \mathrm{dapNDF} + 2{,}25 \times \mathrm{dEE}$$

where: TDN = total digestible nutrients (%); dCP = digestible crude protein (%); dNFC = digestible non-fibrous carbohydrates ;(%); dapNDF = digestible neutral detergent fiber corrected for ash and protein (%); dEEd= digestible ether extract (%).

3.3.9 STATISTICAL ANALYSES

The statistical analysis was conducted using R Studio software (version 4.4.1, Posit PBC, 2024). To evaluate the effect of the treatments on the studied variables, a mixed model was fitted using the lme() function from the nlme package, adopting a Latin square design. The model considered the concentrate level in the diet as a fixed effect (treatment) and the factors period and animal as random effects, according to the following statistical model:

$$\mathrm{Yijk} = \mu + \mathrm{di} + \mathrm{aj} + \mathrm{pk} + \mathrm{eijk}$$

where: μ = overall constant; d_i = effect of diet i (fixed); a_j = effect of animal j (random); p_k = effect of experimental period k (random); e_{ijk}= residual random effect of diet i, animal j, and experimental period k.

The significance of the effects was evaluated using analysis of variance (ANOVA), adopting a significance level of 5% (Type I error). The adjusted coefficient of determination (R^2) was calculated to assess the model fit quality. The model adequacy was verified by analyzing the residuals, applying the Shapiro-Wilk test to assess normality and the Bartlett test to check for homogeneity of variances. To identify the point of maximum or minimum for the fitted quadratic equations, the critical value of concentrate content was calculated by deriving the regression equation.

To assess the effect of ruminal pH on MPS and its efficiency, regression analyses were performed using MPS expressed relative to IOM, RDapNDF, and RDST. The models tested linear relationships between ruminal pH and MPS or MPS efficiency.

The estimation of ruminal retention time (T (h)) and the integration of *in vivo* and in situ data were performed using the tidyverse environment. For each nutrient fraction (DM, OM, CP, and NDF), the ruminal retention time was determined by solving the non-linear degradation equations for the time variable, where the *in situ* degradability was mathematically equal to the *in vivo* digestibility.

For DM, OM, and CP, the analytical solution followed the model:

$$T = -[\ln(1 - ((\mathrm{Dinvivo} - \mathrm{a})/\mathrm{b}))]/\mathrm{kd}$$

where: T = ruminal retention time (h); Dinvivo = *in vivo* digestibility mean (%); a = rapidly soluble fraction (%); b = potentially degradable fraction (%); kd = fractional degradation rate (h^{-1}).

For NDF, the solution was based on the residue model:

$$T = -[\ln(1 - (\mathrm{Dinvivo}/\mathrm{b})]/\mathrm{kd}$$

where: T = ruminal retention time (h); Dinvivo = *in vivo* digestibility mean (%); b = potentially degradable fraction (%); kd = fractional degradation rate (h^{-1}).

To account for biological variation, 95% Confidence Intervals (CI) for the *in vivo* digestibility means were calculated using the qt() function. These intervals allowed for the estimation of minimum (Tmin) and maximum (Tmax) retention times for each treatment. A conditional logic was implemented to handle instances where *in vivo* digestibility exceeded the in situ degradability potential (Dinvivo≥b); in such cases, the retention time was classified as mathematically undetermined and reported as exceeding the maximum incubation timeframe (> 120h). Visual validation of the ruminal clearance rates and the intersection between in situ disappearance curves and *in vivo* digestibility thresholds was conducted using the ggplot2 package.

3.4 RESULTS

3.4.1 *IN SITU* DEGRADATION

Table 6 presents the observed *in situ* degradation of DM, OM, CP, and NDF residue in cattle. These descriptive values were used to fit equations for effective degradability.

Increasing dietary concentrate levels induced significant changes in degradation parameters, showing both linear and quadratic effects. For DM and OM, a fraction decreased linearly by 0.07% ($P = 0.0003$) and 0.05% ($P = 0.0029$), respectively, for each 1% increase in concentrate level (Table 7). However, DM and OM b fraction increased linearly by 0.1391% ($P < 0.0001$) and 0.1157% ($P = 0.0001$), respectively, for each 1% increase in concentrate level. The same pattern was observed for DM and OM kd. Effective degradability of DM and OM also rose linearly, by 0.08% ($P = 0.0003$) and 0.07% ($P = 0.0006$) for each 1% increase in dietary concentrate level.

For CP, a ($P = 0.017$) and b ($P = 0.0026$) fractions exhibited quadratic responses (Table 8). Fraction a reached a minimum estimated value of 57.60% at 90.56% of dietary concentrate, whereas fraction b reached a maximum of 40.08% at 93.66% concentrate.

The kd of CP did not respond to concentrate level ($P = 0.591$), with an average value of 0.065, while RUP increased linearly and ED decreased linearly by 0.12% ($P = 0.0002$) for each 1% increase in dietary concentrate level.

Regarding NDF, fractions b, showed a quadratic effect ($P = 0.0038$) with maximum estimated value (70.54%) at 27.88% concentrate level (Table 8), whereas kd remained unaffected ($P = 0.2109$), with an average value of 0.0575. I fraction also had a quadratic effect ($P = 0.0068$), however with a minimum estimated value (26.25%) at 28.94% concentrate level. Similarly, PD fraction showed a quadratic effect ($P = 0.0038$), with a minimum estimated value (19.46%) at 27.88% concentrate level. ED of NDF showed a quadratic effect ($P = 0.0031$) with a maximum estimated value (62.16) at %) at 26.11% concentrate level.

Table 6 - Observed *in situ* degradation of dry matter (DM), organic matter (OM), and crude protein (CP) and neutral detergent fiber (NDF) residue for each concentrate level (%C)

Time (h)	0	3	6	12	18	24	48	72	96
				0 %C					
DM	49.95	51.79	54.38	60.90	64.12	68.85	76.88	79.74	81.33
OM	53.23	55.15	57.43	63.67	66.82	71.33	78.99	81.71	83.20
NDF residue	100.00	86.73	81.53	72.96	67.57	62.65	42.26	40.31	34.33
CP	85.58	87.19	87.93	89.52	90.05	90.62	91.81	92.95	93.45
				27.5 %C					
DM	48.01	52.19	55.37	65.96	70.54	75.91	83.09	85.32	87.00
OM	52.58	55.84	58.90	68.92	73.25	78.32	85.11	87.25	88.84
NDF residue	100.00	87.29	80.97	68.53	61.51	53.19	38.14	33.88	29.33
CP	69.43	73.11	76.06	85.48	86.86	88.83	92.83	94.50	95.57
				55 %C					
DM	45.83	50.97	54.80	66.51	72.00	74.47	84.25	87.71	88.71
OM	50.06	54.97	58.96	69.65	74.73	77.14	86.16	89.41	90.16
NDF residue	100.00	85.38	81.53	74.20	67.94	65.16	48.36	39.22	34.59
CP	64.38	67.25	69.92	79.49	83.24	85.37	92.86	95.80	96.38
				82.50 %C					
DM	43.26	53.00	59.72	70.24	77.35	82.21	86.96	88.47	89.83
OM	48.09	57.08	63.21	72.94	79.55	84.03	88.37	89.84	90.99
NDF residue	100.00	87.56	82.48	76.89	69.72	68.77	66.35	56.87	53.61
CP	58.01	64.39	68.60	77.99	85.31	89.41	95.01	95.90	97.27

Source: elaborated by the author.

Table 7 – Effect of concentrate level (%C) on in situ degradation parameters of dry matter (DM) and organic matter (OM)

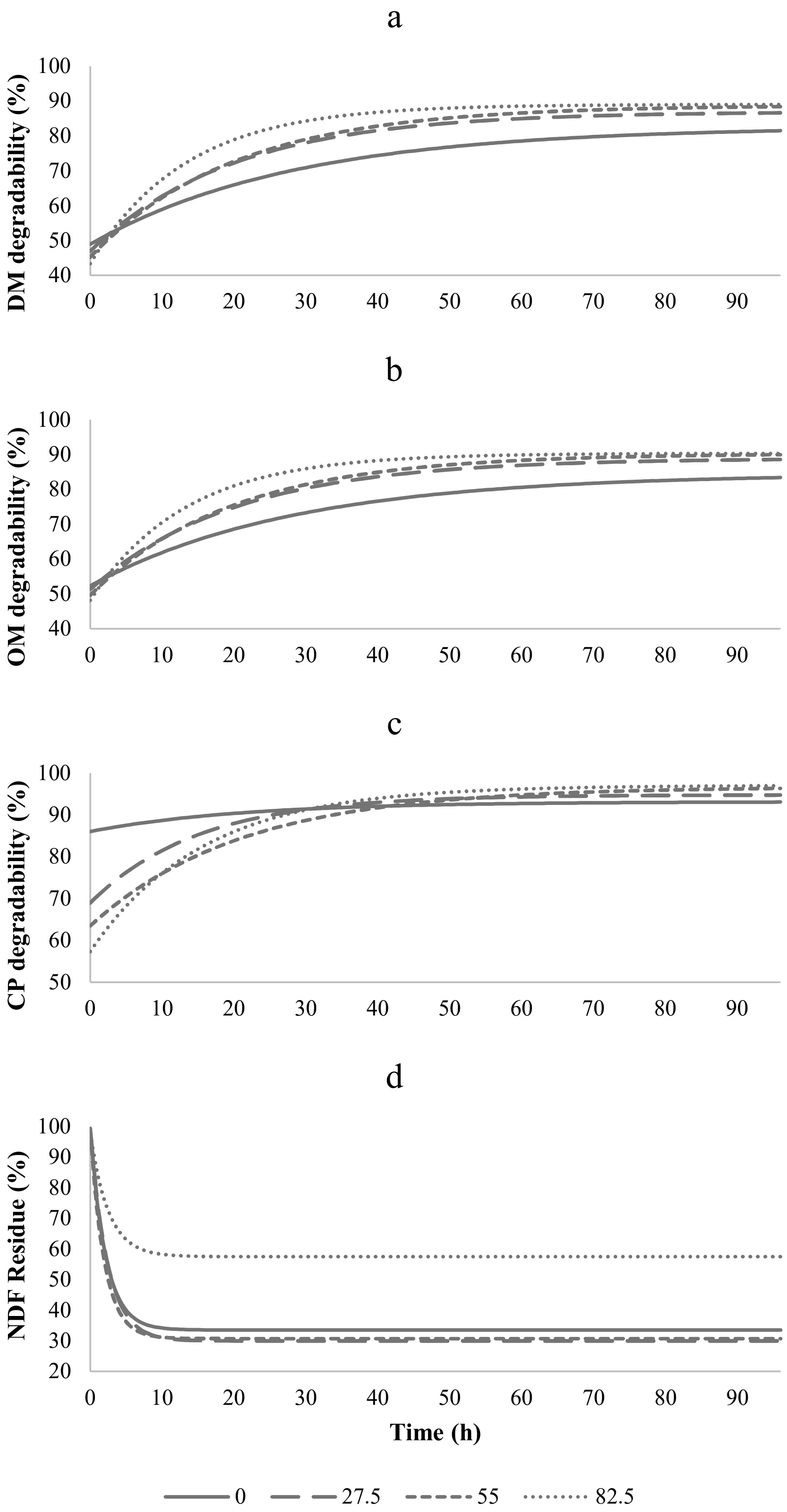
Items	0	27.5	55	82.5	SEM[5]	L (*P*-value)	R^2	Q (*P*-value)	R^2	Equation	x max	y max	x min	y min
DM														
a[1]	48.76	46.92	45.15	43.22	0.733	0.0003	0.49	0.9677	0.49	48.7705 - 0.0669 × %C	-	-	-	-
b[2]	34.46	40.05	43.89	45.93	1.302	> 0.0001	0.68	0.2313	0.46	35.3423 + 0.1391 × %C	-	-	-	-
kd[3]	0.04	0.05	0.05	0.08	0.005	0.0010	0.55	0.5842	0.56	0.0353 + 0.00046 × %C	-	-	-	-
ED[4]	72.37	77.49	78.44	81.23	0.955	0.0003	0.67	0.7613	0.65	72.9650 + 0. 0835 × %C				
OM														
a	52.12	51.27	49.50	48.03	0.661	0.0029	0.3	0.7273	0.29	52.3363 - 0.0511 × %C	-	-	-	-
b	32.89	37.64	40.98	42.39	1.149	0.0001	0.59	0.2645	0.65	33.7028 + 0.1157 × %C	-	-	-	-
kd	0.04	0.05	0.05	0.08	0.005	0.0010	0.56	0.4362	0.58	0.037 + 0.00044 × %C	-	-	-	-
ED	74.67	79.85	80.73	83.11	0.910	0.0006	0.62	0.4056	0.63	76.68775 + 0.07215 × %C	-	-	-	-

Source: elaborated by the author. [1]soluble fraction, [2]potentially degradable fraction, [3]degradability rate, [4]effective degradability, [5]standard error of the mean. L and Q refer to linear or quadratic effects, respectively. R^2 refers to adjusted determination coefficient.

Table 8 – Effect of concentrate level (%C) on in situ degradation parameters of crude protein (CP) and neutral detergent fiber (NDF)

Items	0	27.5	55	82.5	SEM[8]	L (*P*-value)	R^2	Q (*P*-value)	R^2	Equation	x max	y max	x min	y min
CP														
a[1]	84.89	68.90	63.37	57.23	2.753	>0.0001	0.84	0.0170	0.91	$84.3371 - 0.5905 \times \%C + 0.0033 \times \%C^2$	-	-	90.56	57.6
b[2]	8.69	25.93	33.50	40.07	3.100	>0.0001	0.89	0.0026	0.95	$9.12 + 0.6611 \times \%C - 0.0035 \times \%C^2$	93.66	40.08	-	-
kd[3]	0.07	0.07	0.05	0.07	0.010	0.5910	0.07	0.5180	0.08	-				
RUP[4]	8.78	10.11	11.50	10.71	1.113	0.0002	0.66	0.0987	0.73	$11.0270 + 0.1158 \times \%C$	-	-	-	-
ED[5]	89.91	85.20	81.00	80.69	1.113	0.0002	0.66	0.0987	0.73	$11.0270 - 0.1158 \times \%C$	-	-	-	-
NDF														
b	63.74	68.13	65.76	40.54	3.367	0.0136	0.32	0.0038	0.65	$62.9316 + 0.5458 \times \%C - 0.0098 \times \%C^2$	27.88	70.54	-	-
kd	0.04	0.05	0.03	0.11	0.016	0.2109	0.12	0.2563	0.21	-				
I[6]	33.49	29.91	29.48	56.46	3.502	0.0266	0.27	0.0068	0.57	$34.7065 - 0.5845 \times \%C + 0.0101 \times \%C^2$	-	-	28.94	26.25
PD[7]	36.26	31.87	34.24	59.46	3.367	0.0136	0.36	0.0038	0.67	$37.0675 - 0.5458 \times \%C + 0.0098 \times \%C^2$	-	-	27.88	19.46
ED	46.65	50.68	42.01	31.28	3.116	0.0062	0.39	0.0031	0.73	$56.8868 + 0.4041 x \%C - 0.0077 \times \%C^2$	26.11	62.16	-	-

Source: elaborated by the author. [1]soluble fraction, [2]potentially degradable fraction, [3]undegradable fraction, [4]rumen-undegraded protein, [5]effective degradability, [6]indigestible fraction, [7]potentially degradable fraction, [8]standard error of the mean. L and Q refer to linear or quadratic effects, respectively. R^2 refers to adjusted determination coefficient.



Source: elaborated by the author.

Figure 2 – *In situ* degradation curves of dry matter (DM), organic matter (OM), crude protein (CP), and residue curve of neutral detergent fiber (NDF).

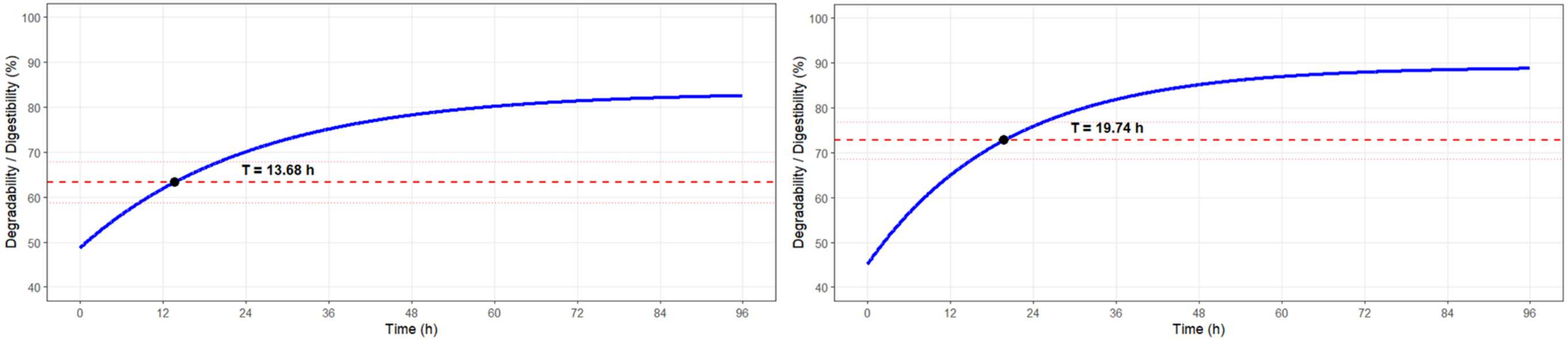
For DM and OM, higher concentrate levels resulted in greater effective degradability over time, particularly in treatments with 55% and 82.5% concentrate, which showed faster and more extensive degradation (Figure 2). For NDF, an opposite trend was observed, with increased concentrate levels leading to lower degradation.

The estimated ruminal retention times for the different nutritional fractions, determined by the intersection of *in situ* and *in vivo* data, were significantly influenced by the dietary concentrate levels (%C) (Table 9; Figures 3, 4, 5, and 6). In the diet with 0% of concentrate, the estimated times for CP exceeded 96 h. For DM and OM, the target retention times reached their peak at the 55% of concentrate level (19.74 h and 18.92 h, respectively) before slightly decreasing at the 82.5% level (Figures 3 and 4). Regarding CP, a progressive increase in the target retention time was observed with concentrate inclusion, rising from 1.56 h at the 27.5% %C level to 7.38 h at the 82.5% level (Figure 5). In contrast, the retention times for NDF were considerably higher, with values exceeding the 96-hour evaluation period at the highest concentrate level (Figure 6).

Table 9 -Estimated ruminal retention time (h) of dry matter (DM), organic matter (OM), crude protein (CP), and neutral detergent fiber (NDF) according to the dietary concentrate level (%C), based on the intersection of in situ degradation curves and *in vivo* digestibility coefficients

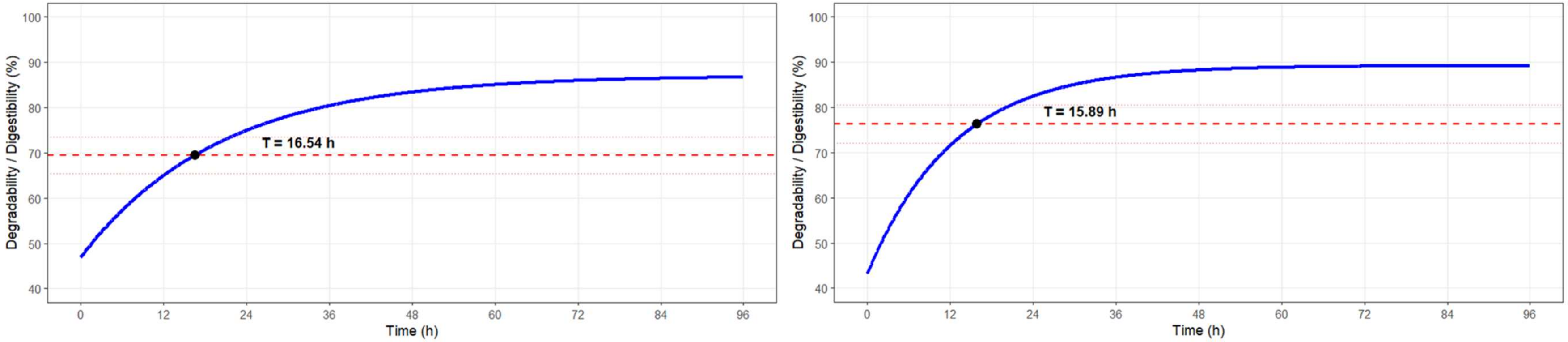
Items	%C	Min. Time (h)	Target time (h)	Max. Time (h)
DM	0	8.47	13.68	20.26
	27.5	12.37	16.54	21.80
	55	15.31	19.74	25.44
	82.5	12.40	15.89	20.76
OM	0	10.59	15.76	22.29
	27.5	12.21	16.79	22.74
	55	13.75	18.92	25.92
	82.5	11.04	14.94	20.63
CP	0	-[1]	-	-
	27.5	> 96.00	1.56	3.91
	55	> 96.00	4.45	11.1
	82.5	6.23	7.38	8.63
NDF	0	41.35	65.72	> 96 .00
	27.5	32.88	41.63	57.53
	55	44.29	59.12	86.52
	82.5	17.33	> 96.00	> 96.00

Source: elaborated by the author. Min. Time (h), Target time (h), and Max. Time (h) represent the lower bound of the 95% confidence interval, the mean, and the upper bound of the 95% confidence interval of *in vivo* digestibility, respectively. Values reported as > 96.00 h indicate that the nutrient digestibility exceeded the degradation potential within the evaluated incubation period. [1]Not estimated: for treatment with 0% concentrate, the CP target time could not be calculated because the protein degradation was instantaneous, preventing the intersection of *in situ* and *in vivo* disappearance curves.



0% concentrate level

55% concentrate level

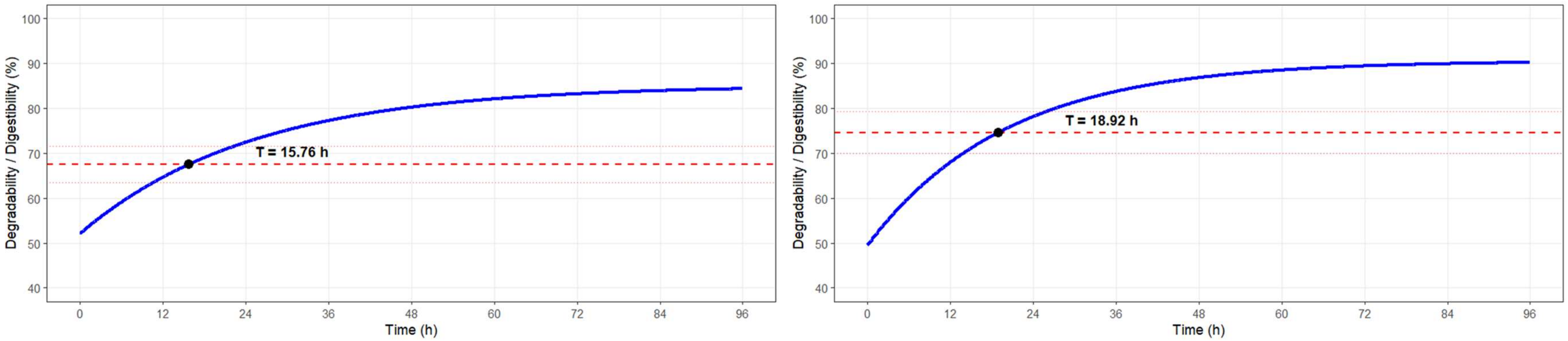


27.5% concentrate level

82.5% concentrate level

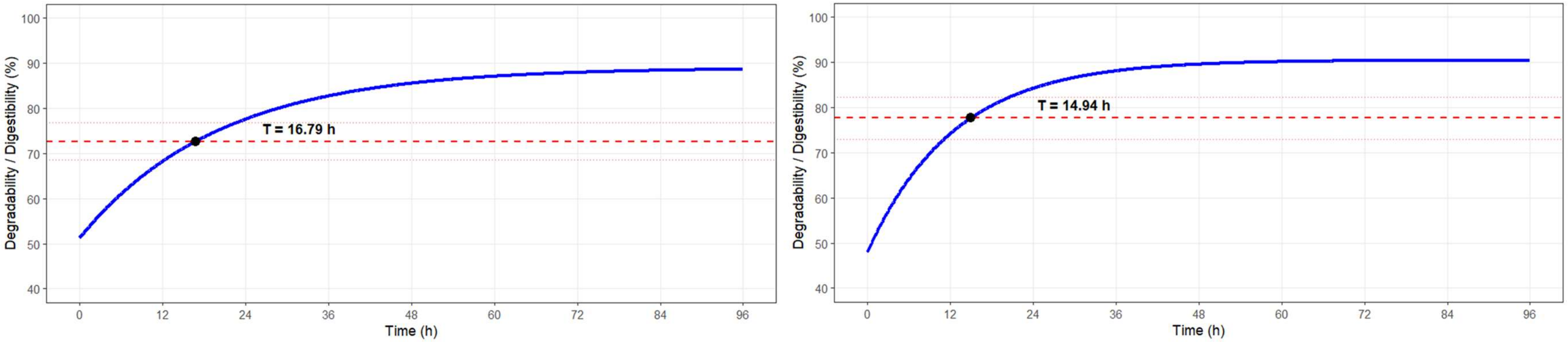
Source: elaborated by the author.

Figure 3 – Graphical representation of the equivalence between *in situ* degradation and *in vivo* digestibility to estimate ruminal retention time for dry matter (DM). The intersection points between the *in situ* kinetic curve and the *in vivo* digestibility thresholds (mean and 95% confidence interval) define the minimum, target, and maximum ruminal residence times.



0% concentrate level

55% concentrate level



27.5% concentrate level

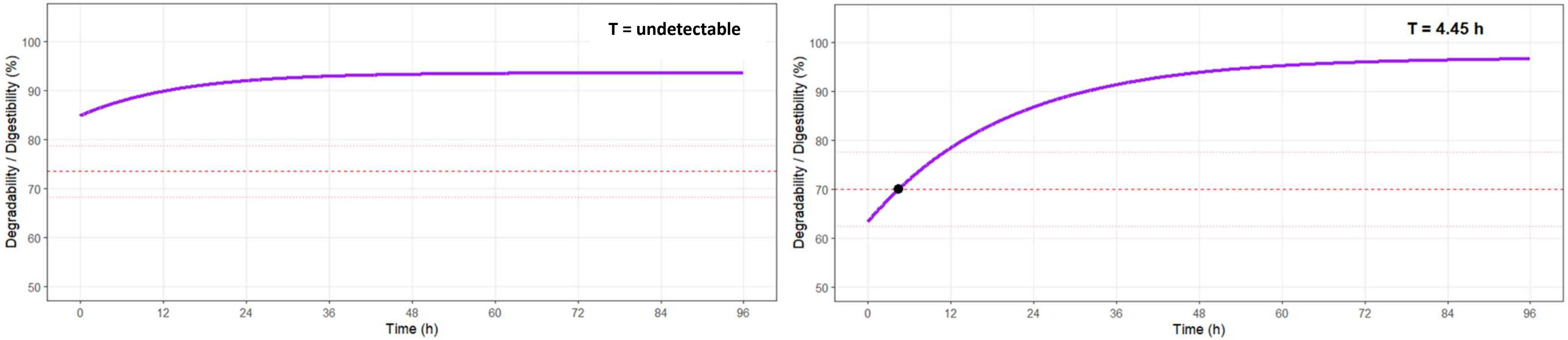
82.5% concentrate level

Source: elaborated by the author.

Figure 4 – Graphical representation of the equivalence between *in situ* degradation and *in vivo* digestibility to estimate ruminal retention time for organic matter (OM). The intersection points between the *in situ* kinetic curve and the *in vivo* digestibility thresholds (mean and 95% confidence interval) define the minimum, target, and maximum ruminal residence times.

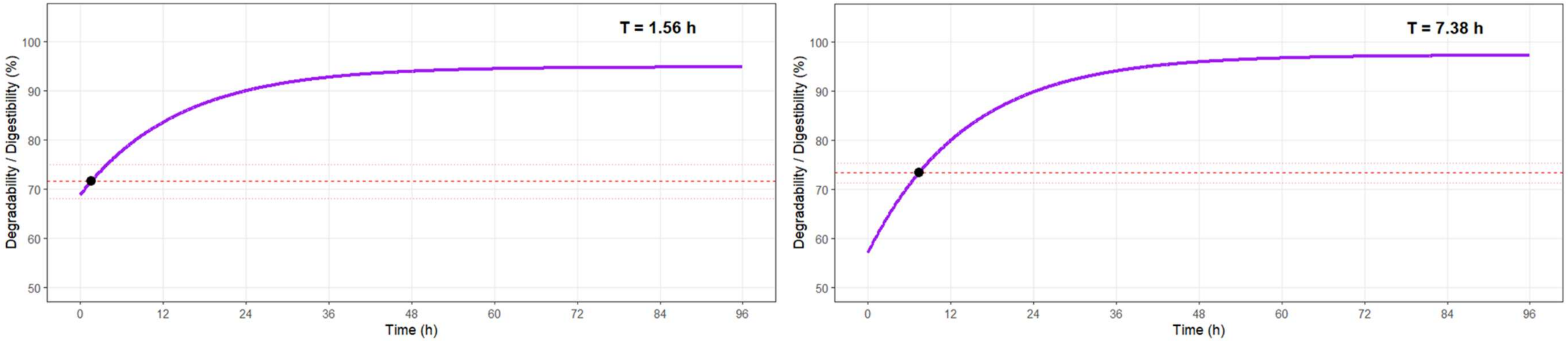
0% concentrate level

55% concentrate level



27.5% concentrate level

82.5% concentrate level

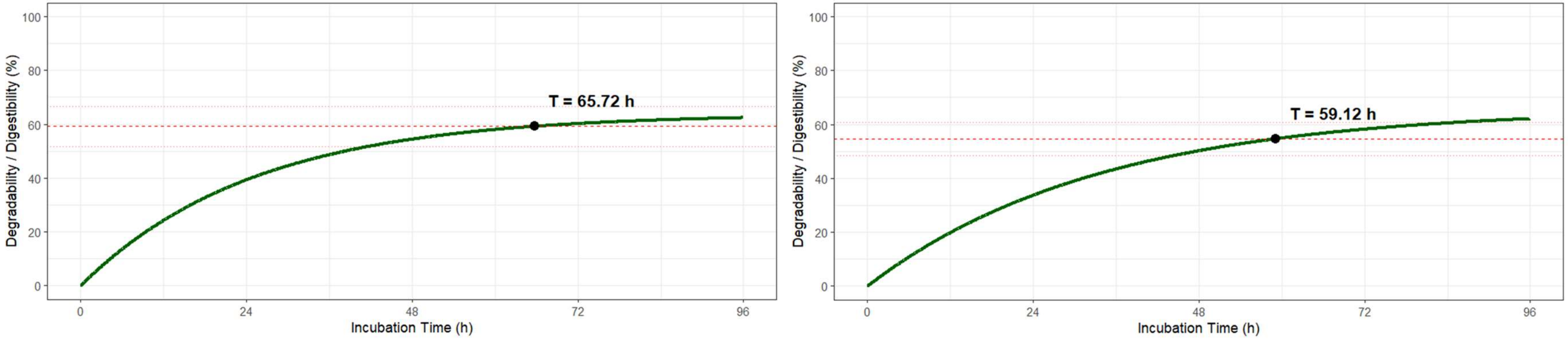


Source: elaborated by the author.

Figure 5 – Graphical representation of the equivalence between *in situ* degradation and *in vivo* digestibility to estimate ruminal retention time for crude protein (CP). The intersection points between the *in situ* kinetic curve and the *in vivo* digestibility thresholds (mean and 95% confidence interval) define the minimum, target, and maximum ruminal residence times.

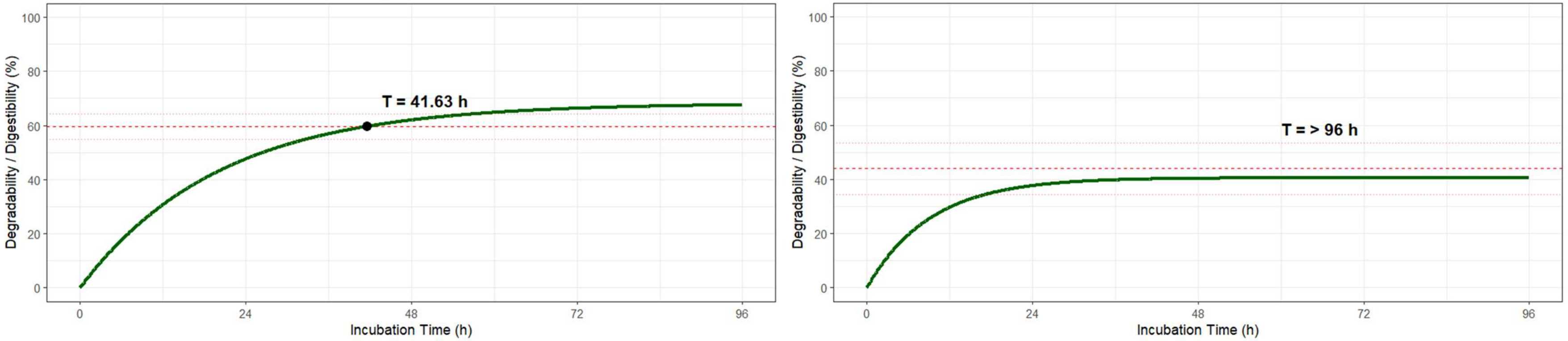
0% concentrate level

55% concentrate level



27.5% concentrate level

82.5% concentrate level



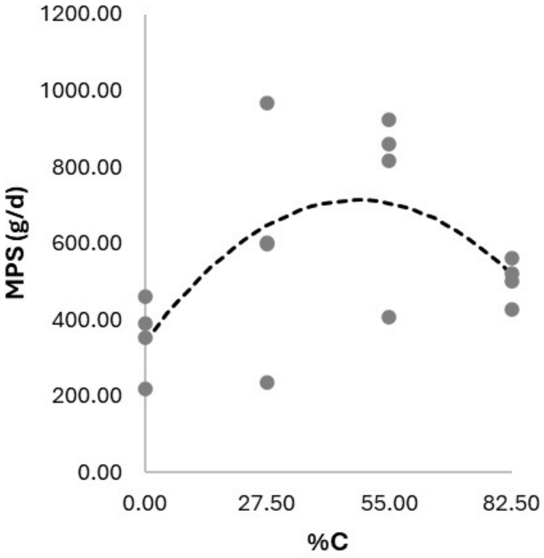
Source: elaborated by the author.

Figure 6 – Graphical representation of the equivalence between *in situ* degradation and *in vivo* digestibility to estimate ruminal retention time for neutral detergent fiber (NDF). The intersection points between the *in situ* kinetic curve and the *in vivo* digestibility thresholds (mean and 95% confidence interval) define the minimum, target, and maximum ruminal residence times.

3.4.2 NITROGEN BALANCE AND MICROBIAL PROTEIN SYNTHESIS

Nitrogen intake increased linearly by 0.36 g/d (*P* = 0.0028) for each 1% increase in dietary concentrate level (Table 10). Fecal nitrogen excretion followed a quadratic pattern (*P* = 0.0157), with a maximum estimated value of 40.77 g/d at 54.08% concentrate. Urinary nitrogen excretion decreased linearly by 0.21 g/d (*P* = 0.0156) per 1% increase in concentrate level, while nitrogen retention increased by 0.46 g/d (*P* = 0.0013).

Microbial protein synthesis (MPS), which showed a quadratic response (*P* = 0.0243), with a maximum estimated value of 713.45 g/d at 47.84% of dietary concentrate (Figure 7).



Source: elaborated by the author.

Figure 7 – Effect of concentrate level on microbial protein synthesis (MPS) in the rumen (*MPS (g/d) = 340.8926 + 15.576 × %C - 0.1628 × %C²; P = 0.0243, R² = 0.39*).

Table 10 - Effect of concentrate level (%C) on nitrogen balance, microbial protein synthesis, and efficiency of microbial protein synthesis

Items	0	27.5	55	82.5	SEM[8]	L (*P*-value)	R^2	Q (*P*-value)	R^2	Equation	x max	y max
N intake, g/d	109.47	127.66	140.73	138.38	5.040	0.0028	0.67	0.0748	0.74	$114.0937 + 0.3629 \times \%C$	-	-
CP[1] intake, g/d	684.21	797.88	879.59	864.85	31.500	0.0028	0.67	0.0748	0.74	-	-	-
Fecal N, g/d	28.68	36.30	42.21	36.82	1.849	0.0391	0.41	0.0157	0.65	$28.2034 + 0.4649 \times \%C - 0.0043 \times \%C^2$	54.08	40.77
Urine N, g/d	66.31	50.30	53.70	46.14	3.340	0.0156	0.56	0.3693	0.57	$62.6788 - 0.2077 \times \%C$	-	-
Retained N, g/d	14.48	41.06	44.83	55.41	5.100	0.0013	0.60	0.2445	0.63	$19.9599 + 0.4603 \times \%C$	-	-
MPS[2], g/d	356.25	600.01	751.04	502.20	56.114	0.2753	0.02	0.0243	0.39	$340.8926 + 15.576 \times \%C - 0.1628 \times \%C^2$	47.84	713.45
RUP[3] (g/d)	321.08	256.14	216.89	114.60	53.690	0.9322	0.33	0.0335	0.54	$340.0901 – 9.8061 \times \%C + 0.1204 \times \%C^2$	40.7	140.42
MPS (g/kg TDN[4])	92.33	120.20	125.76	80.71	10.123	0.7383	0.16	0.0507	0.39	$90.9205 + 1.882 \times \%C - 0.0241 \times \%C^2$	39.05	127.66
MPS (g/kg IOM[5])	64.35	92.49	100.94	67.09	8.287	0.8184	0.01	0.0438	0.21	$63.2168 + 1.7514 \times \%C - 0.0205 \times \%C^2$	42.74	100.67
MPS (g/kg RDapNDF[6])	175.38	305.02	489.09	533.54	45.576	0.0005	0.62	0.4865	0.63	$186.9738 + 4.5766 \times \%C$	-	-
MPS (g/kg RDST[7])	297.71	301.11	228.05	113.88	30.725	0.0169	0.39	0.2547	0.44	$328.8692 - 2.2711 \times \%C$	-	-

Source: elaborated by the author. [1]crude protein, [2]microbial protein synthesis, [3]rumen undegradable protein, [4]total digestible nutrients, [5]ingested organic matter, [6]rumen degraded neutral detergent fiber corrected for ash and protein, [76]rumen degraded starch, [8]standard error of the mean. L and Q refer to linear or quadratic effects, respectively. R^2 refers to adjusted determination coefficient.

Microbial protein synthesis efficiency, expressed relative to TDN, showed a quadratic trend in response to dietary concentrate level, with maximum estimated value of 127.66 g/kg TDN, when expressed relative to OM intake (OMI) it was observed a quadratic response ($P = 0.0438$) with maximum estimated value of 100.67 g/kg IOM observed at 39.05% and 42.74% concentrate, respectively. Regarding RDapNDF and RDST, MPS efficiency increased linearly by 4.58 g/kg RDapNDF and decreased by 2.27 g/kg RDST for each 1% increase in concentrate level.

Rumen pH did not affect microbial protein synthesis or its efficiency when expressed in terms of rumen-degraded OM, or ST ($P > 0.05$). However, a linear reduction in MPS efficiency was observed as a function of rumen-degraded NDF:

$$\text{MPS (g/kg RDapNDF)} = 2829.1946 - 378.5075 \times \text{pH} \quad (P = 0.046,\ R^2 = 0.27)$$

where MPS = microbial protein synthesis; RDapNDF = rumen-degradable neutral detergent fiber corrected for ash and protein.

3.5 DISCUSSION

3.5.1 *IN SITU* DEGRADATION

The increase of dietary concentrate level changed the fermentation pattern, likely due to the greater availability of rapidly fermentable carbohydrates and the reduction of structural components from forage sources (Pucetti *et al*., 2024; Zhao *et al*., 2016). A linear reduction in the fraction a of DM and OM may reflect the dilution of readily degradable compounds from corn silage as concentrate replaces forage. This pattern was also reported in DDG-based diets (Alhadas *et al*., 2021), which showed a lower a fraction due to smaller proportions of water-soluble nutrients.

The dynamic nature of these changes is further evidenced by the estimated ruminal retention times required to equate *in situ* and *in vivo* data (target times). As concentrate inclusion increased, the time needed for DM and OM degradation to represent the real digestive process also rose. It is crucial to clarify that this increase in target time does not imply slower dietary degradation; rather, it reflects the enhanced digestive efficiency of the animal under high-energy diets. Due to physical constraints of the nylon bag technique, such as limited pore surface area and reduced turnover of contents within the bag, the *in situ* disappearance rate may not proportionally match the increased aggressiveness of the open ruminal environment in high-concentrate systems.

Furthermore, while undigested residues *in vivo* are subject to ruminal escape and passage to the omasum, the *in situ* technique keeps these residues within the bags, preventing any disappearance other than that achieved through actual solubilization or degradation. Consequently, as *in vivo* digestibility rises, a longer *in situ* residence period is required for the substrate disappearance to reach the superior coefficients of actual digestion.

This shift demonstrates that conventional fixed incubation periods, such as the 24h recommendation proposed by Silva *et al.* (2020) for standardized grain-based diets, are insufficient to characterize diverse dietary matrices. While Silva *et al.* (2020) prioritized laboratory practicality and standardization, the findings of this study corroborate the perspective of Alhadas *et al.* (2021), reinforcing that equivalence time is not a static parameter but a variable that adapts to dietary energy density and the host's biological response.

In contrast, the b fraction increased with higher concentrate levels. This shift does not necessarily imply a slower overall degradation but rather reflects the replacement of a fraction by b fraction that require microbial colonization. Despite the larger b fraction, ED increased linearly, indicating that the high fermentability of the starch-rich concentrate compensated for the reduction in the soluble fraction. This suggests that the ruminal environment, stimulated by greater fermentable energy, maintained high kd that effectively increased the extent of degradation within the residence time. These results highlight that in high-concentrate diets, the ED is driven more by the rapid fermentation of the b fraction than by the initial solubility of the ingredients.

Regarding CP, both the a and b fractions exhibited quadratic responses, in which a fraction decreased and reached an estimated minimum value (57.60% at 90.56% of concentrate level) and b fraction increasing until reaching an estimated maximum value (40.08% at 93.66% of concentrate level) before declining at the highest concentrate level. These patterns are consistent with changes in ruminal retention time and nitrogen availability under increasing concentrate levels (Pucetti *et al.*, 2024; Zhao *et al.*, 2016).

Specifically, in the diet with 0% concentrate, a fraction is primarily attributed to the inclusion of only urea to correct the crude protein content of the corn silage. Unlike soybean meal, urea is characterized by instantaneous ruminal solubilization. However, this distinct degradation dynamic posed a challenge for the methodology of equivalence times. While the *in situ* kinetic parameters for the diet with 0% of concentrate were estimated, it was not possible to determine the CP target time for this treatment. The *in situ* method may struggle to accurately represent nitrogenous sources with extremely high solubility (Broderick, 1994). In this study, the instantaneous disappearance of urea probably prevented a mathematical intersection between the in situ degradation curve and the in vivo passage data. When the digestion rate is essentially instantaneous, the competition between digestion and passage becomes unbalanced, often precluding the determination of a discrete intersection point between these two distinct biological processes (Mertens, 2020).

The increase in true protein sources, coupled with increasing energy availability, likely optimized the metabolic status of the ruminal environment. The observed reduction in urinary nitrogen excretion, and increase in nitrogen retention, as concentrate levels rose reinforces this efficiency, indicating that the surplus of ammonia was more effectively captured for microbial protein synthesis. Such findings align with the principle that protein value is maximized when there is a tight coupling between the degradation rates of dietary nitrogen and the supply of fermentable carbohydrates (Broderick, 1994). The kd of CP remained unchanged, while RUP increased linearly with concentrate level inclusion. This indicates that the greater protein escape was due to the reduction in the soluble fraction and a likely increase in ruminal passage rate. As a result, CP effective degradability decreased at higher concentrate levels, reflecting changes in dietary protein partitioning and ruminal dynamics.

NDF degradation was negatively affected by increasing dietary concentrate level, exhibiting a quadratic pattern in which ED of NDF reached its maximum estimated value at intermediate concentrate inclusion, decreasing beyond this point. These results are consistent with the fact that highly fermentable grain sources may reduce fiber digestion, particularly under conditions of rapid fermentation and limited buffering capacity (Zhao *et al.*, 2016).

The importance of this impact is highlighted by the estimated retention times for NDF, which frequently exceeded the total experimental window. This indicates that under high-starch challenges, the degradation rate of fiber within the nylon bags is so severely depressed by the 'carbohydrate effect' and localized pH drops that the intersection with *in vivo* data becomes mathematically unreachable within standard timeframes. This discrepancy, surpassing even the equivalence times reported by Alhadas *et al.* (2021), confirms that in high-concentrate systems, the *in situ* methodology may fail to reach a point of intersection with *in vivo* fiber digestibility due to a profound depression in fibrolytic activity.

Evidence from other studies evaluating different fiber sources suggests that the impact of concentrate level on *in situ* degradation can be modulated by the physicochemical characteristics of the roughage (Goulart *et al.* 2020; Pucetti *et al.*, 2024). For example, diets containing more digestible sources of NDF, such as soybean hulls or low-lignin cottonseed hulls, exhibit greater effective degradability of DM and NDF compared to those using more lignified or less accessible sources like sugarcane bagasse. The observed changes in degradation kinetics reflect a complex interplay between diet composition, ruminal microbial ecology, and fermentation dynamics.

While increasing concentrate enhances the overall degradability of DM and OM, its effects on CP were more complex, leading to reduced CP degradability at higher concentrate levels. These findings are consistent with studies that emphasize the need for synchronized energy and nitrogen release to avoid the overuse of highly fermentable concentrates in fiber-dependent systems (Benedeti *et al*., 2020; Firkins *et al*., 1996).

3.5.2 NITROGEN BALANCE AND MICROBIAL PROTEIN SYNTHESIS

The linear increase in nitrogen retention with increasing dietary concentrate suggests greater efficiency in nitrogen utilization. This is likely due to the higher availability of fermentable energy, which favors MPS in the rumen and reduces urinary nitrogen losses. Increasing dietary energy stimulates microbial ATP production, promoting cell growth and enhancing nitrogen incorporation into microbial biomass (Hackmann & Firkins, 2015). According to Martineau *et al*. (2011), both TDN (positively) and indigestible NDF (negatively) affect portal absorption of nitrogen derived from amino acids. This supports the concept that an adequate energy supply promotes microbial multiplication and nitrogen capture, ultimately improving intestinal amino acid absorption. However, the positive effect of dietary energy on nitrogen use efficiency persists only while ruminal conditions remain stable.

In the present study, MPS exhibited a quadratic response, with an estimated maximum value at 47.84% of concentrate in dietary DM. Beyond this level, MPS decreased, indicating that additional increases in concentrate compromised the efficient use of available ruminal nitrogen. This pattern was reflected in the quadratic behavior of MPS efficiency relative to TDN and IOM, demonstrating that synchrony between energy and nitrogen release was maximized at intermediate concentrate levels.

Millen *et al*. (2020) demonstrated that high-concentrate diets reduce microbial diversity and impair the abundance of fibrolytic species. Similarly, Cooper *et al.* (2002) reported that increasing starch availability improves MPS only up to a certain point, after which fermentation disturbances restrict microbial growth. These findings support the observation that excessive concentrate levels may exceed the adaptive capacity of the rumen ecosystem.

The nonlinear responses of both MPS and MPSE highlight that microbial growth is not driven by nitrogen or energy availability alone, but by their synchrony. According to Firkins *et al.* (1996), the efficiency of microbial protein synthesis is maximized when the supply of fermentable energy coincides with rumen-degradable nitrogen, minimizing losses arising from asynchronous substrate availability. In this study, the decline in MPS at higher concentrate levels was associated with ruminal conditions typically observed under rapid fermentation, including reductions in pH, inhibition of fibrolytic species, and increased microbial competition for limiting substrates (Russell & Dombrowski, 1980; Carvalho *et al.*, 2011). These mechanisms are consistent with the reduction in NDF digestibility reported in this experiment and align with the findings of Bach *et al.* (2005), who demonstrated that under lower ruminal pH conditions, ATP from carbohydrate fermentation is redirected toward cellular maintenance rather than protein synthesis, reducing microbial growth efficiency.

However, the responses observed in this study contrast with those of Pucetti *et al.* (2024), who reported a linear increase in MPS and MPSE using sorghum silage diets containing 0% to 80% concentrate. The lower fermentability and slower starch degradation of sorghum may promote a more stable ruminal environment, improving nitrogen utilization. Similarly, Granja-Salcedo *et al.* (2015) reported a linear increase in MPS with greater concentrate inclusion (30% to 80%) in corn silage-based diets, though without effects on MPSE. In their study, crude protein content increased concurrently with concentrate level (13.9% to 17.1%), likely enhancing nitrogen availability and mitigating the negative effects of high concentrate inclusion on ruminal environment.

Rapid fermentation of NFC increases the production of VFA and lactic acid, potentially exceeding ruminal absorptive capacity and reducing pH (Hungate, 1966; Nagaraja & Titgemeyer, 2007). Ruminal acidification compromises fibrolytic bacteria, which are particularly sensitive to low pH, resulting in reduced enzymatic activity, impaired fiber degradation, and less efficient microbial protein synthesis (Russell & Wilson, 1996; Hiltner & Dehority, 1983; Roger *et al.*, 1990; Bach *et al.*, 2005). This was evidenced in the present study by the association between RDapNDF and ruminal pH. Although pH did not affect MPS or MPSE when expressed relative to rumen-degraded DM, OM, or starch, a clear negative relationship emerged when MPSE was expressed relative to rumen-degraded NDF, reinforcing the high sensitivity of fibrolytic microorganisms to acidotic conditions.

Furthermore, Hackmann & Firkins (2015) describe that excess fermentable carbohydrates in isonitrogenous diets can lead to “energy spilling,” where surplus ATP is diverted toward futile metabolic cycles instead of microbial biomass synthesis, decreasing MPS efficiency. This emphasizes that optimal microbial protein synthesis depends not only on substrate availability but also on the equilibrium between fermentable carbohydrate supply, adequate nitrogen availability, and ruminal stability. As Dewhurst *et al*. (2000) noted, efficient ruminal systems ensure both adequate supply and temporal alignment of fermentable carbohydrates and nitrogen, optimizing ammonia capture and minimizing nitrogen losses. Therefore, nutritional strategies that promote ruminal stability and synchronize substrate release, such as gradual adaptation protocols and the balanced formulation of TDN and RDP, as recommended by Pinto *et al*. (2020), are essential to enhance microbial protein synthesis in ruminant feeding systems. Taken together, the quadratic responses of MPS and MPSE, the decline in fiber digestibility, and the reduction in nitrogen utilization at higher concentrate levels closely align with the broader literature. They demonstrate that although increasing concentrate enhances fermentable energy supply, this strategy has physiological limits. Beyond the threshold of approximately 48% concentrate in the present study, ruminal acidification, microbial imbalance, and substrate asynchrony become dominant constraints that impair fermentation efficiency and reduce microbial protein yield.

3.6 CONCLUSION

Increasing dietary concentrate levels shifts ruminal degradation dynamics by enhancing the utilization of rapidly fermentable nutrients while progressively depressing fibrolytic activity. This study demonstrates that the accuracy of the *in situ* methodology is highly dependent on the dietary matrix, as evidenced by the wide variation in equivalence times required to match *in vivo* data. These findings challenge the use of standardized, fixed incubation periods for diverse diets. While the *in situ* model proved more precise for true protein sources, its ability to represent fiber digestion is severely compromised in high-starch systems. Furthermore, the quadratic responses of microbial protein synthesis and efficiency identify a biological threshold at approximately 48% concentrate inclusion, beyond which ruminal acidification and substrate asynchrony impair fermentation efficiency. Overall, these findings underscore that while moderate concentrate levels optimize nitrogen utilization and microbial yield, excessive levels exceed the rumen's adaptive capacity, requiring dynamic modeling and balanced starch-to-fiber ratios to sustain high-performance beef cattle production.

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