Tropical Animal Health and Production (2024) 56:41
https://doi.org/10.1007/s11250-024-03888-1

REGULAR ARTICLES

Effect of pregnancy and feeding level on voluntary intake, digestion, and microbial nitrogen synthesis in Zebu beef cows

Mateus Pies Gionbelli[1] · Marcio de Souza Duarte[2] · Sebastião de Campos Valadares Filho[3] · Tathyane Ramalho Santos Gionbelli[1] · German Dario Ramirez-Zamudio[1] · Luiz Henrique Pereira Silva[4] · Karolina Batista Nascimento[1] · Thais Correia Costa[1]

Received: 26 June 2023 / Accepted: 29 December 2023 / Published online: 12 January 2024

Abstract

The objective of this research was to evaluate how pregnancy and feeding regimens affect the feed intake, digestibility, and efficiency of microbial nitrogen (N) synthesis in beef cows. Forty-four multiparous Nellore cows, comprising 32 gestating and 12 non-gestating cows, with an average weight of 451 ± 10 kg, were assigned to either a HIGH (*ad libitum*) or LOW (limited feeding at 1.2 times maintenance based on the NRC) feeding regimen during the gestational period. The dry matter intake (DMI) in kg/d was significantly greater ($P < 0.01$) in HIGH-fed cows. The DMI reduced ($P < 0.05$) in proportion to the shrunk body weight (SBW) as days of pregnancy (DOP) increased. The interaction between feeding level and DOP was significant ($P < 0.05$) for the digestibility of dry matter (DM), organic matter (OM), N compounds, ether extract (EE), ash- and protein-free neutral detergent fiber (NDFap), gross energy (GE), and total digestible nutrients (TDN). Except for DM and TDN digestibility, there was a reduced nutrient digestibility as gestation progressed in HIGH-fed cows. In contrast, digestibility increased as a function of DOP in LOW-fed cows. Microbial N synthesis (g/day) was significantly higher in HIGH-fed cows ($P < 0.001$) compared to LOW-fed cows. The efficiency of microbial N production per g of N intake and kg of digestible OM intake was ($P = 0.021$) and tended ($P = 0.051$) to be greater in LOW-fed cows compared to HIGH-fed cows. In summary, HIGH-fed Nellore cows reduce feed intake and digestibility with advancing gestation, affecting feed utilization. In addition, LOW-fed cows, showed higher microbial protein synthesis efficiency, potentially making them more nutrient-efficient under challenging nutritional conditions.

Keywords Beef cattle · Nellore cows · Voluntary feed intake · Gestational period · Total digestible nutrients

Introduction

Homeorhetic regulation encompasses the coordinated control of metabolism essential for maintaining the physiological state of pregnancy (Daniel et al. 2018). During this stage, effective nutrient partitioning becomes critical to fulfill the requirements for fetal growth and the development of maternal tissues, including the placenta and mammary glands. Studies indicate that there is improvement in the cardiovascular system to ensure an adequate supply of nutrients and oxygen to maternal tissues, particularly the uteroplacental region (Moreira et al. 2021). As gestation advances, a variety of physical and physiological changes related to feed intake and digestion may manifest. It has been reported that feeding intake gradually decreases, with a more pronounced reduction observed during the last days of gestation, which can be attributed to the limited ruminal space caused by the expansion of the gravid uterus (Rotta et al. 2015). Additionally, physical limitations may be compensated for by an increase in passage rate (Linden et al. 2014; Moyo and Nsahlai 2018), negatively impacting nutrient digestibility in pregnant cows (Ribeiro et al. 2015). The synthesis of microbial protein

✉ Mateus Pies Gionbelli
mateus.pg@ufla.br

1 Department of Animal Science, Universidade Federal de Lavras, Lavras, MG 37203-202, Brazil

2 Department of Animal Biosciences, University of Guelph, Guelph, ON N1G 2W1, Canada

3 Department of Animal Science, Universidade Federal de Viçosa, Viçosa, MG 36570-900, Brazil

4 Department of Agriculture and Food Science, Western Kentucky University, Bowling Green, Kentucky 42101-1066, USA

relies on achieving a balance between the degradation rates of degradable protein and carbohydrate sources (Hackmann and Firkins 2015). In combination, both the stage of gestation and the nutritional plan can influence microbial protein production, consequently affecting maternal conditions.

In tropical regions, beef cows often face feed shortages during the mid-to late-gestation period, as these periods align with the dry season (Barcelos et al. 2022; Costa et al. 2021a; Carvalho et al. 2022; Nascimento et al. 2022). In this context, protein supplementation during crucial stages of gestation, has been employed as a strategy to improve nutrient intake and digestibility in beef cows raised on low-quality pastures (Marquez et al. 2017). Comparing maintenance to *ad libitum* feeding in crossbred cows (Holstein × Gyr), Rotta et al. (2015) showed greater dry matter apparent total-tract digestibility in maintenance-fed cows during mid-to-late gestation. Moreover, the mobilization of maternal reserves is minimized by the nutrient supply offered (Lopes et al. 2020; Rodrigues et al. 2021; Meneses et al. 2022). Addressing the elevated maternal requirements during gestation not only results in well-nourished dams but also yields healthier calves with enhanced growth potential (Barcelos et al. 2022). Additionally, well-nourished dams contribute to better meat quality at harvest, as reviewed by Costa et al. (2021b) and Santos et al. (2022).

However, given that pregnancy is a physiological state that triggers notable metabolic changes, additional interactions with feeding level and digestion may occur in ruminants. Additionally, it is crucial to emphasize that most research investigating the influence of pregnancy on feed intake and digestibility in cattle has been performed in temperate regions, with *Bos taurus* cattle serving as the primary model species. Research investigating these variables in Zebu cattle remains limited. Hence, it is crucial to address this knowledge gap to furnish pertinent information for Zebu animals, extensively utilized as a genetic resource in tropical regions. Consequently, this experiment aimed to assess the effects of pregnancy and feeding level on intake, digestibility, and microbial N production in the rumen of pregnant and non-pregnant Zebu cows.

Material and methods

This research was conducted at the Universidade Federal de Viçosa (Viçosa, MG, Brazil), adhering to established protocols for ethical animal care and handling (protocol number: 047/2012), following the guidelines of Universidade Federal de Viçosa (Brazil).

Animals

Forty-nine multiparous Nellore cows, exhibiting an average initial body weight (BW) of 451 ± 10 kg, an age of 5.6 ± 0.5 years, and a body condition score of 4.4 ± 0.2 on a scale of 1 to 9, were employed in this investigation. From the initial pool of 49 cows, a randomized selection process led to the separation of 32 cows, which were mated with Nellore bulls to form the pregnant group. These cows were randomly hand-mated with five Nellore bulls during a 50-day breeding period. Estrous synchronization was initiated through the administration of a gonadotropin-releasing hormone (GnRH) agonist injection, followed by a prostaglandin F2α (PGF2α) injection seven days later. The day of breeding served as the starting point for the pregnancy timeline, and 28 days post-breeding, an ultrasound was utilized to confirm and validate the pregnancy. Moreover, twelve additional cows were randomly selected and allocated to the non-pregnant group, with the remaining five cows specifically assigned to the baseline group.

Diet and management

Cows were housed in pens (5–6 cows), each spanning 48 m^2 and featuring a concrete floor and an additional covered area of 15 m^2. Furthermore, a continuous supply of potable water was ensured for the animals throughout the experimental period. Individual feed intake was quantified using an electronic headgate system (Kloppen Soluções Tecnológicas, Pirassununga, SP, Brazil). Cows in the gestational phase were at 47 ± 3 days of pregnancy (DOP) at the initiation of the feeding trial. The diet composition is presented in Table 1. Daily records were kept for both the provided feed and the orts. Samples of corn silage and orts were collected daily, and a composite sample was assembled weekly for subsequent analysis. Samples of the ground corn and soybean meal were taken each time the concentrate portion was mixed.

All cows received identical total mixed rations twice daily, at 0700 and 1500 h. Cows were categorized into two groups based on feeding levels: HIGH (*ad libitum*; $n = 16$ pregnant and 5 non-pregnant) or LOW [restricted feeding at 1.2 times maintenance according to the National Research Council (NRC 2000); $n = 16$ pregnant and 7 non-pregnant]. Restricted feeding was calculated to support pregnancy at 1.2 times the maintenance level, while the HIGH-fed regimen facilitated the accumulation of maternal tissue. The average dry matter intake (DMI) was 16.0 ± 2.0 and 10.8 ± 1.5 g/kg of shunk body weight (SBW)/d for cows fed HIGH and LOW levels of intake, respectively.

Table 1 Ingredients and chemical composition of the diet

Item	Silage	Concentrate	Diet
Ingredient, % of DM			
Corn Silage	100.0	-	84.3
Ground corn	-	54.6	8.5
Soybean meal	-	33.0	5.1
Urea	-	7.3	1.2
Sodium chloride	-	2.1	0.37
Ammonium sulfate	-	1.5	0.25
Dicalcium phosphate	-	1.3	0.23
Microminerals mixture[†]	-	0.17	0.028
Chemical composition, %			
DM	28.0	89.2	37.6
OM	94.7	92.8	94.4
CP	7.8	44.1	13.5
EE	2.9	2.5	2.8
NDF_{ap}	45.8	8.2	39.9
iNDF	20.8	0.65	17.6
NDIN	38.2	7.0	11.4
NFC	38.2	53.0	40.6
TDN	-	-	66.6
GE (Mcal/kg)	3.82	3.49	3.77

OM = organic matter; EE = ether extract; NDF_{ap} = neutral detergent fiber corrected to ash and protein, iNDF = indigestible neutral detergent fiber; NDIN = neutral detergent insoluble nitrogen; NFC = non-fiber carbohydrates; TDN = total digestible nutrients; GE = gross energy

[†] Zinc sulfate (56.3%), manganese sulfate (26.2%), copper sulfate (16.8%), potassium iodate (0.37%), cobalt sulfate (0.23%) and sodium selenite (0.10%)

The cows utilized in this study originated from a comparative slaughter experiment (Gionbelli et al. 2015), which is a methodology involving the collection of data by slaughtering animals at different developmental stages. This approach allows for a direct comparison between different animal groups, such as animals in different physiological stages, as evaluated in this study. As a result, the 32 pregnant cows were randomly distributed into four groups, each comprising eight cows (with four cows per feeding level). These cows were slaughtered at 136, 189, 239, and 269 days of pregnancy. To ensure a similar duration of participation in the study as the pregnant cows, the non-pregnant cows were also slaughtered at various time points throughout the experiment, ranging from 85 to 216 days of gestation of pregnant cows.

Digestibility and dry matter intake

The digestibility trials consisted of nine collection periods, each lasting for 5 consecutive days, with an interval varying from 21 to 28 days. Owing to variations in harvest periods, the intervals between collection periods were irregular, aiming to maximize the number of collections per cow. Fecal samples (approximately 60 g) were obtained directly from the rectum of each cow during all five consecutive days (at 1800, 1500, 1200, 0900, and 0600 h), pooled for each animal in each period, and stored at -20 ºC for further analyses. The *in vivo* apparent total tract digestibility was estimated by using the indigestible neutral detergent fiber (iNDF) as an internal marker.

The dry matter intake (DMI) was calculated every 7 days through the composite samples collected from feeds and leftovers. The DMI in proportion to BW (g/kg) for each cow was obtained at each period of fecal sampling when cows were weighed. The SBW was calculated in non-pregnant (SBWnp) and pregnant (SBWp) cows as a function of the BW according to Gionbelli et al. (2015). The equation used to estimate the SBW was $0.8084 \times BW^{1.0303}$.

Chemical analyses

All samples were lyophilized. Subsequently, the samples were ground using a Willey mill (TE-650, Tecnal, Piracicaba, SP, Brazil) to pass through a 2-mm screen for the determination of iNDF using the *in situ* incubation method for 10 days (Casali et al. 2008). The incubation process involved three rumen-cannulated Holstein × Nellore cows, which were provided *ad libitum* access to the same diet employed in the experiment. To quantify dry matter (DM), ash, crude protein (CP), ether extract (EE), and ash- and protein-free neutral detergent fiber (NDFap), the samples were ground using a knife mill equipped with a 1-mm sieve. The analysis methods employed were as follows: DM (method 934.01; AOAC 2000), ash (method 942.05; AOAC 2000), CP (method 920.87; AOAC 2000), EE (method 920.39; AOAC 2000), and NDFap (method described by Van Soest et al. 1991). Non-fiber carbohydrates (NFC) were calculated according to the approach proposed by Detmann and Valadares Filho (2010), where NFC = 100 – [(% CP – % CP derived from urea + %urea) + NDFap + EE + ash]. The determination of total digestive nutrients (TDN) concentration was calculated following the method outlined by Detmann et al. (2016). Gross energy was assessed using an adiabatic bomb calorimeter (C5001, IKA-Werke Co, Staufen, Germany) (Cadenas-Soberanis et al. 2021).

Microbial nitrogen synthesis

Spot urine samples were obtained by stimulating the area below the vulva to assess the excretion of urinary nitrogenous compounds. Two spot urine samples were gathered during each collection period, specifically on the second and fifth days of fecal sampling, at 1600 and 0800 h, respectively. The estimation of urine volume utilized creatinine concentration as a marker, and the calculation was performed using the following equation (Valadares Filho et al. 2016):

$$\text{UCE(mg/day)} = 37.88 \times \text{SBW}^{0.9316}$$

where: UCE = urinary creatinine excretion (mg/d); and SBW = shrunk body weight. Urinary concentrations of creatinine, allantoin, and uric acid were determined by employing a high-performance liquid chromatography method, as described by George et al. (2006).

Microbial nitrogen (N) synthesis was assessed utilizing the purine derivatives in the urine technique (Chen and Gomes 1992). The excretion of purine derivatives (PD) in urine was determined by the combined excretions of allantoin and uric acid, calculated by multiplying their concentrations in urine by the daily urinary volume. The calculation of absorbed purines (AP) from the excretion of PD followed the method described by Prates et al. (2012) as follows:

$$\text{AP(mmol/day)} = \text{PD} - \left(0.405 \times \text{BW}^{0.75}\right)/0.99$$

where: $0.405 \times \text{BW}^{0.75}$ value = endogenous excretion of purine derivates (mmol) in the urine per unit of metabolic body weight ($\text{BW}^{0.75}$); and 0.99 = recovered of absorbed purines as purine derivates in the urine (mmol/mmol).

The computation of ruminal synthesis of nitrogen compounds (Nmic) was determined based on the AP (Prates et al. 2012), as follows:

$$\text{Nmic(g/day)} = \text{AP} \times 70/(0.93 \times 0.11 \times 1000)$$

where: 70 = N content in purine derivates (mg/mmol); 0.93 = purine digestibility; and 0.11 = ratio of purine N: total N of microorganisms.

Statistical analysis

The MIXED procedure in SAS version 9.2 (SAS Inst. Inc., Cary, NC) was employed to assess the impact of feeding level and pregnancy on the variables under study. This analysis took into account the influence of collection periods, repeated measures within the same animal, and interactions among the mentioned factors. A previous evaluation of the estimated TDN of the diet based on the chemical composition (Detmann et al. 2016) revealed a significant variation between collection periods justifying the use of a period of measure as a random effect in the model.

The response variables were evaluated as follows:

$$Y_{ijk} = \mu + F_k + \beta_1\left(G_l\right) + \beta_2(F \times G)_{kl} + \delta_{kl} + \varepsilon_{ijkl}$$

where Y_{ijk} is the observed measurement; μ is the overall mean; β_1 and β_2 are the regression coefficients; F_k is the fixed effect of feeding level; G_l is the fixed effect of DOP of pregnant cows; $(F \times G)_{kl}$ is the interaction between feeding level and DOP of pregnant cows; δ_{kl} is the random error with mean 0 and variance σ^2_{δ}. The variance between cows (subjects) within feeding level and DOP of pregnant cows is equal to the covariance between repeated measurements within cows; and ε_{ijkl} is the random error with the mean 0 and variance σ^2, the variance between measurements within cows.

To eliminate probable misinterpretation effects and to certify the true relationship between the voluntary feed intake and DOP a new analysis was performed removing the LOW-fed cow's information from the database and the feeding level from the statistic model to check the quadratic and cubic effects on DM intake. Only the linear parameter was significant ($P = 0.012$, 0.522, and 0.739, respectively).

As the interval between collection periods exhibited variability, the response variables were regarded as irregularly measured. In such instances, a continuous-time model, as proposed by Moser (2004) to describe covariances among errors, was applied. The spatial data covariance structures available in PROC MIXED of SAS were utilized, with the spatial power serving as the chosen covariance function. The least-square means were estimated for feeding level. A significance level of 0.05 was chosen as the critical probability threshold to determine the occurrence of Type I errors.

Results

Body weight

Due to the applied feeding levels, the final SBW significantly differed ($P = 0.003$) between feeding groups, with an average of 564 ± 6 kg for HIGH-fed cows and 481 ± 5 kg for LOW-fed cows. The shrunk body gain (SBG, $P < 0.001$) among feeding level groups was 0.86 ± 0.04 kg/d for HIGH-fed cows and 0.26 ± 0.04 kg/d for LOW-fed cows (Gionbelli et al. 2015). Furthermore, within each evaluated gestational period, comparisons revealed that HIGH-fed cows consistently had greater ($P < 0.001$) SBG than LOW-fed cows.

Dry matter intake

Figure 1 presents a graphical representation of the voluntary dry matter intake for each pregnant cow in the HIGH-fed group. The interaction between feeding level and DOP on the DMI expressed as g/kg of SBWp and g/kg of SBWnp was significant ($P \leq 0.018$, Table 2). The DMI decreased as a proportion of SBW concerning DOP. Thus, the linear relationship between the voluntary intake and DOP in Nellore cows fed *ad libitum* can be described as follows (based on functions shown in Table 2):

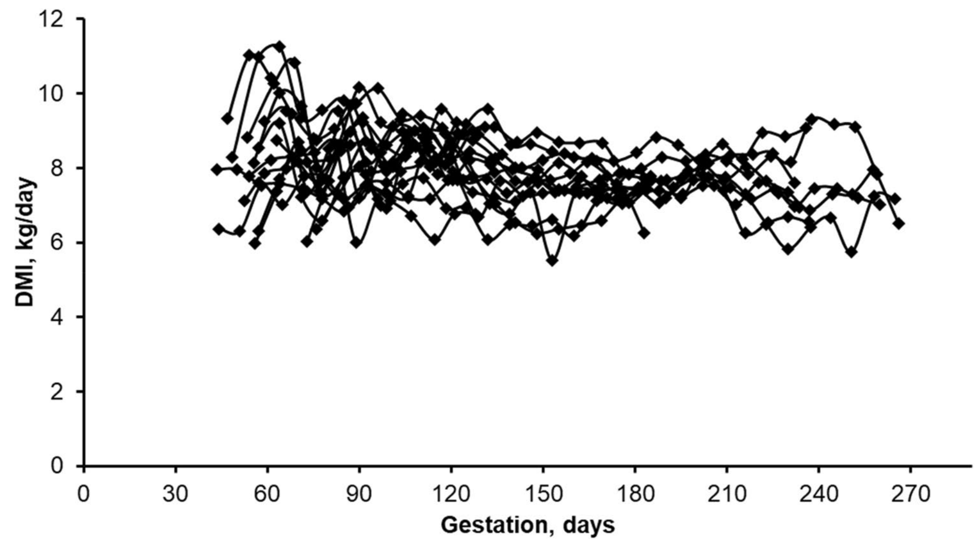

Fig. 1 Relationship between voluntary dry matter intake and days of pregnancy. Data of sixteen pregnant cows feeding *ad libitum*. The dots represent the weeks of evaluation

Table 2 Effects of feeding level and pregnancy on dry matter intake in Nellore cows

Variable	Functions		Feeding level[†]		SEM	*P*-value		
			Low	High		FL	DOP	FL × DOP
DMI, kg		-	4.60	7.62	0.16	< 0.001	0.779	0.286
DMI, g/kg of SBW_p	Low	$\hat{y} = 10.1 \pm 0.3$	10.1	15.0	0.3	< 0.001	0.113	0.002
	High	$\hat{y} = 16.0 \pm 0.6 - 0.0093 \pm 0.003 \times DOP$						
DMI, g/kg of SBW_{np}	Low	$\hat{y} = 10.2 \pm 0.4$	10.2	15.3	0.4	< 0.001	0.168	0.018
	High	$\hat{y} = 16.4 \pm 0.7 - 0.0109 \pm 0.004 \times DOP$						

DMI = dry matter intake; SBW_p = shrunk body weight to pregnant cows; SBW_{np} = shrunk body weight to non-pregnant cows; FL = feeding level, DOP = days of pregnancy and FL × DOP = interaction between feeding level and days of pregnancy

[†] Low is restricted feeding 1.2 times maintenance according to the NRC, 2000 and High is *ad libitum* feeding

$$\text{DMI}(\text{kg/day}) = (16 - 0.0093 \times \text{DOP})/1000 \times \text{SBWp}$$

$$\text{DMI}(\text{kg/day}) = (16.4 - 0.0093 \times \text{DOP})/1000 \times \text{SBWnp}$$

Digestibility

The interaction between feeding level and DOP was significant ($P < 0.05$) for all the components of the diet, except for the NFC (Table 3). Except for DM digestibility and TDN content, there was a decline in digestibility as gestation time increased in HIGH-fed cows, whereas digestibility in LOW-fed cows increased with the increase in DOP. The DM, OM, NDFap, and GE digestibility coefficients were higher in LOW-fed cows ($P < 0.05$) compared to HIGH-fed cows. The EE digestibility was higher in HIGH-fed cows than in LOW-fed cows ($P = 0.015$). Figure 2 illustrates the graphical representation of the association between DM digestibility and pregnancy time, incorporating the functions detailed in Table 3.

Microbial N production

There were no effects ($P \geq 0.180$) of DOP and interaction between feeding level and pregnancy ($P \geq 0.368$) on microbial N production and efficiency (Table 3). The microbial N production (g/d) and the efficiency of microbial N production by total digestible nutrients were higher in HIGH-fed cows than in LOW-fed cows ($P < 0.001$ and 0.049, respectively). However, the efficiency of microbial N production by g of N intake and kg of digestible organic matter intake was higher ($P = 0.021$) or tended to be higher ($P = 0.051$) in LOW-fed cows than in HIGH-fed cows, respectively.

Discussion

Regarding energy requirements, LOW-fed cows ingested 102%, 98%, and 67% of their estimated energy requirements according to the NRC (2000) at 0, 135, and 270 DOP, respectively. In contrast, HIGH-fed cows surpassed their energy requirements, consuming 168%, 162%, and 111%

Table 3 Effects of feeding level and pregnancy on total-tract apparent digestibility of diet components and microbial N production in Nellore cows

Variable	Functions		Feeding level[†]		SEM	*P*-value		
			Low	High		FL	DOP	FL × DOP
Digestibility, %								
DM	Low[‡]	$\hat{y} = 62.8 \pm 1.6$	62.8	59.9	1.6	0.047	0.112	0.002
	High	$\hat{y} = 60.9 \pm 1.6 - 0.0093 \pm 0.005 \times DOP$						
OM	Low[‡]	$\hat{y} = 65.5 \pm 1.6 + 0.0062 \pm 0.004 \times DOP$	66.2	62.9	1.4	0.038	0.042	0.003
	High	$\hat{y} = 64.0 \pm 1.6 - 0.0102 \pm 0.005 \times DOP$						
Nitrogenous compounds	Low	$\hat{y} = 66.3 \pm 1.6 + 0.0083 \pm 0.004 \times DOP$	67.1	65.7	1.6	0.217	0.722	0.011
	High	$\hat{y} = 66.3 \pm 1.6 - 0.0053 \pm 0.005 \times DOP$						
EE	Low	$\hat{y} = 80.8 \pm 1.2 + 0.0043 \pm 0.006 \times DOP$	81.2	82.1	0.9	0.015	0.506	0.044
	High	$\hat{y} = 83.2 \pm 1.2 - 0.0107 \pm 0.006 \times DOP$						
NDFap	Low	$\hat{y} = 51.0 \pm 1.7 + 0.0052 \pm 0.007 \times DOP$	51.5	44.6	1.4	0.045	< 0.001	0.019
	High	$\hat{y} = 46.3 \pm 1.7 - 0.0161 \pm 0.008 \times DOP$						
NFC		-	80.9	80.0	1.6	0.552	0.972	0.083
GE	Low	$\hat{y} = 63.1 \pm 1.6 + 0.0069 \pm 0.004 \times DOP$	63.8	60.3	1.5	0.035	0.032	0.002
	High	$\hat{y} = 61.5 \pm 1.6 - 0.0107 \pm 0.005 \times DOP$						
TDN	Low[‡]	$\hat{y} = 67.6 \pm 1.4$	67.6	64.9	1.4	0.031	0.160	0.002
	High	$\hat{y} = 66.0 \pm 1.6 - 0.0103 \pm 0.005 \times DOP$						
Microbial N production								
Nmic, g/day		-	49.6	74.4	3.2	< 0.001	0.744	0.617
Nmic, g/g of N		-	495	437	22	0.021	0.616	0.835
Nmic, g/kg of DOM		-	17.7	16.7	0.6	0.051	0.180	0.368
Nmic, g/kg of TDN		-	16.7	17.9	0.6	0.049	0.196	0.399

OM = organic matter; EE = ether extract; NDFap = neutral detergent fiber corrected to ash and protein; NFC = non-fiber carbohydrates; GE = gross energy; TDN = total digestible nutrients; Nmic = microbial nitrogen; DOM = digestible organic matter; FL = feeding level, DOP = days of pregnancy and FL × DOP = interaction between feeding level and days of pregnancy

[†] Low is restricted feeding 1.2 times maintenance according to the NRC, 2000 and High is *ad libitum* feeding

[‡] The slope for days of pregnancy was not significant ($P > 0.10$)

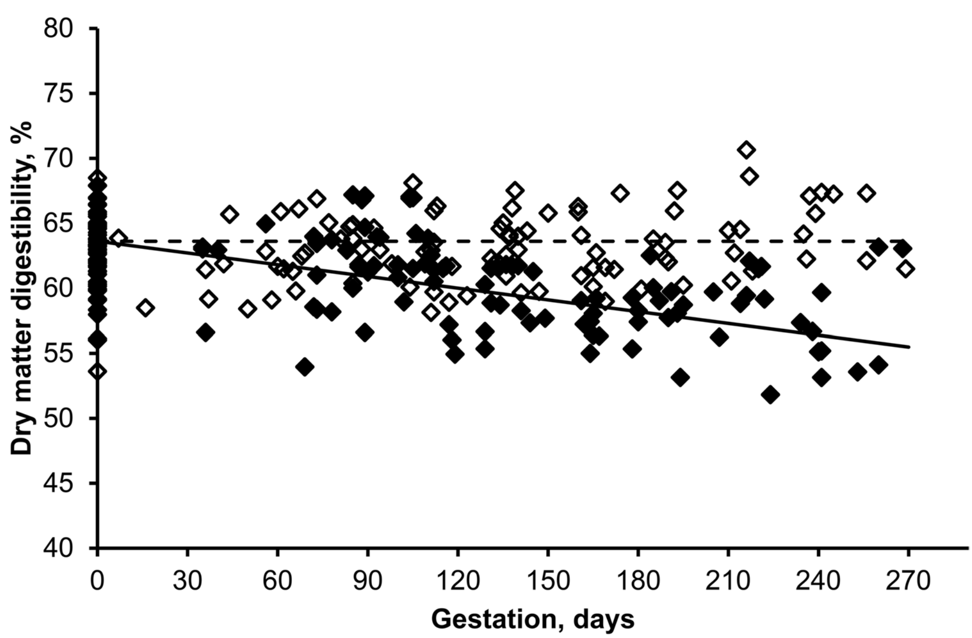

Fig. 2 Relationship between dry matter digestibility and days of pregnancy. Full dots and continuous lines represent HIGH-fed cows. Empty dots and dashed lines represent LOW-fed cows. The lines fitted to the data represent the functions shown in Table 2

during the corresponding gestational periods evaluated in the LOW group. This suggests that cows on the HIGH-fed treatment, which consistently met a larger proportion of their energy requirements throughout gestation, had a greater supply of substrates available for nutrient accretion. As a result, they were able to achieve weight gain throughout gestation and maintain maternal body reserves in comparison to LOW-fed cows, as previously described by Gionbelli et al. (2015). Consistently, findings from other studies (Lopes et al. 2020; Rodrigues et al. 2021; Barcelos et al. 2022; Meneses et al. 2022) demonstrated that increasing the proportion of nutritional requirements met has contributed to weight gain during gestation and preservation of maternal body condition.

The DM intake voluntarily decreases in pregnant cows as they progress through late gestation (Hummel et al. 2021). In the current study, was also observed a reduction in DM intake (g/kg of BW) as pregnancy advanced. This decline aligns with a reduction in both wet and dry matter content in the rumen during gestation, suggesting a diminished ruminal capacity in pregnant Zebu heifers, as reported by Moreira et al. (2023). The decrease in DMI is probably a result of limited ruminal space due to compression by the gravid uterus and visceral fat (Forbes 2007). Additionally, using the same females and experimental design, Gionbelli (2013) observed a reduction in the weight of the rumen-reticulum and omasum with advancing pregnancy. The author also noted that the 8.18% reduction in rumen weight between 136 and 239 days of pregnancy corresponds to approximately a 37% reduction in rumen volume (80.0 to 58.3 liters).

Additionally, it's worth noting that in ruminant animals, around 75% of fetal growth takes place during late gestation, which further contributes to the constraint on rumen capacity (LeMaster et al. 2017). In the present study, HIGH-fed cows produced heavier offspring compared to LOW-fed cows at 269 DOP (Gionbeli et al. 2013), which in turn, could magnify this effect. In addition to the physical control of DMI in pregnant cows, other factors could play a role, including physiological, metabolic, and behavioral aspects. Nevertheless, these intricacies, such as the impact of calf weight on the reduction of reticulum-rumen volume, hormonal regulation during pregnancy, or the homeorhetic process governing nutrient utilization, are challenging to simulate and represent the primary drivers of the variations in voluntary intake observed during this specific physiological stage in cattle.

Accompanied by the decrease in DMI, HIGH-fed cows also experienced a decrease in DM digestibility as pregnancy progressed. The magnitude of this reduction was one point percent at every 107 days (1/0.0093), corresponding to 2.7 percentile points of difference between a non-pregnant and a pregnant cow at parturition (approx. 290 days of gestation for Nellore cows) (Cavalcante et al. 2001; Rocha et al. 2005). The lower nutrient digestibility as pregnancy advances is likely due to changes in ruminal kinetics, as evidenced by downregulated markers associated with cell proliferation and apoptosis that contribute to decreased turnover of the rumen epithelium in pregnant cows (Moreira et al. 2021). The variations in the abundance of nutrient transporters and the surface area available for absorption may potentially lead to compromised absorption of volatile fatty acids in cows during late gestation (Moreira et al. 2021).

Given that an increased feed passage rate has been associated with reduced nutrient digestibility (Ribeiro et al. 2015), it is reasonable to infer that the decline in digestibility observed in HIGH-fed cows as DOP increase might be linked to an accelerated passage rate. This heightened passage rate could potentially be a compensatory response to the reduced ruminal volume, as passage rates tend to be higher in late gestation in cattle (Gionbelli et al. 2016).

In line with this, Moreira et al. (2023), in a comparison of canulated pregnant and non-pregnant cows, consistently observed that pregnancy led to an increased passage rate and a decreased degradation rate during late gestation. Consequently, this led to a shortened retention time of DM in the rumen. The greater reduction in NDFap digestibility compared to DM digestibility during pregnancy is in agreement with the previous study. The NDFap digestibility reduces by one point percent every 62 days (1/0.0161), which was almost twice that in DM digestibility. On the other hand, the enhanced digestibility observed in LOW-fed cows could be attributed to the improved efficiency of cows subjected to restricted feeding, particularly as their pregnancy advances and they continue to receive limited amounts of feed over an extended duration.

Changes in dry matter intake and nutrient digestibility during gestation as a function of the nutritional plan have the potential to affect the pattern of ruminal fermentation and the synthesis of microbial nitrogen (Hare et al. 2019). The process of microbial N production in the rumen is intricate and poses challenges for precise measurement (Dewhurst et al. 2000). It's recognized that the availability and coordination of energy and nitrogen in the rumen are crucial factors affecting microbial protein synthesis (Zhang et al. 2020). The efficiency in the synthesis of Nmic expressed as kg of digestible organic matter (DOM) increased with the DM intake (Broderick et al. 2010), and therefore, it would be expected the decrease in the efficiency as pregnancy advances.

In this study, there was no observed variation in this parameter as gestational days progressed. However, Moreira et al. (2023) reported that despite the decreasing DM intake during gestation, pregnant cows exhibited greater efficiency in microbial nitrogen production. In the current study, the variations in microbial N production during pregnancy were probably related to the effects of the feeding level. In research involving beef cows, prepartum non-supplementation was found to enhance the efficiency of Nmic synthesis (g/ kg of DOM), possibly due to more efficient nitrogen utilization (Ferreira et al. 2020). Similarly, LOW-fed cows demonstrated higher efficiency in Nmic synthesis when expressed in g/kg of DOM and g/ kg of N.

Moreover, the improved efficiency in microbial nitrogen observed in LOW-fed cows is likely attributed to their extended ruminal retention time (Bach et al. 2005). In conditions where the passage rate is high, a reduction in microbial maintenance costs is expected due to a decrease in ruminal retention time. The passage rate is influenced by the level of dry matter intake, but this assumption holds primarily when the passage rate changes in response to variations in feed quality. In other words, a positive correlation exists between the passage rate and the digestibility of the feed. In instances where the passage rate decreases, as observed in this study due to feed restriction, it is suggested that the prolonged ruminal retention time facilitates greater ruminal degradation of the feed, thereby leading to increased microbial N production per unit of feed.

In summary, this study illustrates the intricate relationship between nutrition, gestational progression, and rumen dynamics in pregnant cows. Our findings suggest that the nutritional plan during gestation directly influences the cows' ability to maintain their body condition and support fetal growth. In practical terms, these results show the importance of adopting nutritional plans for pregnant cows, especially during late gestation when fetal growth accelerates. Additionally, our findings reveal that the decrease in DM intake, linked to the pressure of the pregnant uterus and visceral fat during pregnancy in cows fed *ad libitum*, triggers a higher passage rate as a compensatory response to rumen compression. Nevertheless, this shorter feed retention time leads to reduced nutrient digestibility as pregnancy advances, impacting feed utilization. To provide comprehensive guidance for managing pregnant cows, future research should aim to integrate the complex interactions among feed quality and rumen dynamics. Such efforts will lead to more effective nutritional strategies benefiting both producers and the cattle industry as a whole.

Author contributions All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Mateus Pies Gionbelli, Marcio de Souza Duarte, Tathyane Ramalho Santos Gionbelli, and Luiz Henrique Pereira Silva. Sebastião de Campos Valadares Filho was responsible for managing the resources of this project and supervising it. The first draft of the manuscript was written by Mateus Pies Gionbelli and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, Brazil) and Instituto Nacional de Ciência e Tecnologia – Ciência Animal (INCT-CA, Brazil).

Data availability The datasets generated during and analyzed during the current study will be available upon reasonable request based on university standards.

Declarations

Ethics approval All procedures involving animal care and management were approved by the Animal Care and Use Committee of the Department of Animal Science of the Universidade Federal de Viçosa – Brazil (protocol number: 047/2012).

Competing interests No competing financial, personal, or professional interests have influenced the writing of this paper.

References

AOAC, 2000. Official Methods of Analysis of AOAC International, (AOAC, Arlington, VA)

Bach, A., Calsamiglia, S., and Stern, M.D., 2005. Nitrogen metabolism in the rumen. Journal of dairy science, 88, E9-E21. https://doi.org/10.3168/jds.S0022-0302(05)73133-7

Barcelos, S.S., Nascimento, K.B., Silva, T.E., Mezzomo, R., Alves, K.S., Duarte, M.S. and Gionbelli, M.P., 2022. The Effects of Prenatal Diet on Calf Performance and Perspectives for Fetal Programming Studies: A Meta-Analytical Investigation, Animals, 12,2145. https://doi.org/10.3390/ani12162145

Broderick, G.A., Huhtanen, P., Ahvenjärvi, S., Reynal, S.M. and Shingfield, K.J., 2010. Quantifying ruminal nitrogen metabolism using the omasal sampling technique in cattle—A meta-analysis, Journal of Dairy Science, 93, 3216–3230. https://doi.org/10.3168/jds.2009-2989

Cadenas-Soberanis, A., Jiménez-Ocampo, R., Arceo-Castillo, J.I., López-Zapata, C.P., Aguilar-Pérez, C.F., and Ku-Vera, J.C., 2021. Net energy requirement for maintenance of crossbred beef heifers (Bos taurus x Bos indicus) as measured with the washed-rumen technique and indirect calorimetry, Livestock Science, 251, 104612. https://doi.org/10.1016/j.livsci.2021.104612

Carvalho, E.B., Costa, T.C., Sanglard, L.P., Nascimento, K.B., Meneses, J.A., Galvão, M.C., Serão N.V.L., Duarte M.S., Gionbelli, M.P. 2022. Transcriptome profile in the skeletal muscle of cattle progeny as a function of maternal protein supplementation during mid-gestation. Livestock Science, 263, 104995. https://doi.org/10.1016/j.livsci.2022.104995

Casali, A.O., Detmann, E., Valadares Filho, S.C., Pereira, J.C., Henriques, L.T., Freitas, S.G. and Paulino, M.F., 2008. Influência do tempo de incubação e do tamanho de partículas sobre os teores de compostos indigestíveis em alimentos e fezes bovinas obtidos por procedimentos in situ, Revista Brasileira de Zootecnia, 37,335–342. https://doi.org/10.1590/S1516-35982008000200021

Cavalcante, F.A., Martins Filho, R., Campello, C.C., Lobo, R.N.B. and Martins, G.A., 2001. Período de gestação em rebanho Nelore na Amazônia Oriental, Revista Brasileira de Zootecnia, 30,1451–1455. https://doi.org/10.1590/S1516-35982001000600010

Chen, X.B. and Gomes, M.J., 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives -an overview of the technical details, (Rowett Research Institute, Bucksburn Aberdee, UK)

Costa, T.C., Du, M., Nascimento, K.B., Galvão, M.C., Meneses, J.A.M., Schultz, E.B., Gionbelli, M.P., Duarte, M.D.S., 2021a.

Skeletal muscle development in postnatal beef cattle resulting from maternal protein restriction during mid-gestation. Animals, 11, 860.https://doi.org/10.3390/ani11030860

Costa, T.C., Gionbelli, M.P. and Duarte, M.S., 2021b. Fetal programming in ruminant animals: understanding the skeletal muscle development to improve the quality of meat, Animal Frontiers, 12,440–447.https://doi.org/10.1093/af/vfab061

Daniel, J.B., Friggens, N.C., Van Laar, H., Ingvartsen, K.L., and Sauvant, D., 2018. Modeling homeorhetic trajectories of milk component yields, body composition and dry-matter intake in dairy cows: Influence of parity, milk production potential and breed, Animal, 12, 1182-1195. https://doi.org/10.1017/S1751731117002828

Detmann, E., Silva, T.E., Valadares Filho, S.C., Sampaio, C.B. and Palma, M.N.N., 2016. Prediction of the energy value of cattle diets based on the chemical composition of feeds In:, Nutrient requirements of zebu beef cattle BR-CORTE, 85–118.

Detmann, E. and Valadares Filho, S.C., 2010. On the estimation of non-fibrous carbohydrates in feeds and diets, Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 62,980–984. https://doi.org/10.1590/S0102-09352010000400030

Dewhurst, R.J., Davies, D.R. and Merry, R.J., 2000. Microbial protein supply from the rumen, Animal feed science and technology, 85,1–21. https://doi.org/10.1016/S0377-8401(00)00139-5

Ferreira, M.F.D.L., Rennó, L.N., Detmann, E., Paulino, M.F., Valadares Filho, S.C., Moreira, S.S., Martins, H.C., De Oliveira, B.I.C., Marquez, J.A. and De Paula Cidrine, I., 2020. Performance, metabolic and hormonal responses of grazing Nellore cows to an energy-protein supplementation during the pre-partum phase, BMC Veterinary Research, 16,1–13. https://doi.org/10.1186/s12917-020-02309-3

Forbes, J.M., 2007. Voluntary food intake and diet selection in farm animals, (CABI, Wallingford, UK)

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

Gionbeli, M.P., 2013. Nutrient Requirements and Quantitative Aspects of Growth, Development and Digestion of Pregnant and Non- Pregnant Nellore Cows, (PhD thesis, Universidade Federal de Viçosa)

Gionbelli, M.P., Duarte, M.S., Valadares Filho, S.C., Detmann, E., Chizzotti, M.L., Rodrigues, F.C., Zanetti, D., Gionbelli, T.R.S. and Machado, M.G., 2015. Achieving body weight adjustments for feeding status and pregnant or non-pregnant condition in beef cows, PLOS ONE, 10,e0112111. https://doi.org/10.1371/journal.pone.0112111

Gionbelli, M.P., Valadares Filho, S.C., and Duarte, M.S., 2016. Nutritional requirements for pregnant and non-pregnant beef cows. Nutrient Requirements of Zebu and Crossbred Cattle. (Eds SC Valadares Filho, LFC Costa e Silva, MP Gionbelli, PP Rotta, MI Marcondes, ML Chizzotti, LF Prados.) pp, 251–272.

Hackmann, T.J., and Firkins, J.L., 2015. Maximizing efficiency of rumen microbial protein production, Frontiers in microbiology, 6, 465. https://doi.org/10.3389/fmicb.2015.00465

Hare, K.S., Wood, K.M., Acton, K., Fitzsimmons, C. and Penner, G.B., 2019. Oversupplying metabolizable protein in late gestation for beef cattle: effects on prepartum BW, ruminal fermentation, nitrogen balance, and skeletal muscle catabolism, Journal of Animal Science, 97,407. https://doi.org/10.1093/JAS/SKY410

Hummel, G., Woodruff, K., Austin, K., Knuth, R., Lake, S., and Cunningham-Hollinger, H., 2021. Late gestation maternal feed restriction decreases microbial diversity of the placenta while mineral supplementation improves richness of the fetal gut microbiome in cattle. Animals, 11, 2219. https://doi.org/10.3390/ani11082219

LeMaster, C.T., Taylor, R.K., Ricks, R.E., and Long, N.M., 2017. The effects of late gestation maternal nutrient restriction with or without protein supplementation on endocrine regulation of newborn and postnatal beef calves. Theriogenology, 87, 64-71. https://doi.org/10.1016/j.theriogenology.2016.08.004

Linden, D.R., Titgemeyer, E.C., Olson, K.C. and Anderson, D.E., 2014. Effects of gestation and lactation on forage intake, digestion, and passage rates of primiparous beef heifers and multiparous beef cows, Journal of Animal Science, 92,2141–2151. https://doi.org/10.2527/jas.2013-6813

Lopes, R.C., Sampaio, C.B., Trece, A.S., Teixeira, P.D., Gionbelli, T.R.S., Santos, L.R., Costa, T.C., Duarte, M.S. and Gionbelli, M.P., 2020. Impacts of protein supplementation during late gestation of beef cows on maternal skeletal muscle and liver tissues metabolism, Animal, 14,1867–1875, https://doi.org/10.1017/S1751731120000336

Marquez, D.C., Paulino, M.F., Rennó, L.N., Villadiego, F.C., Ortega, R.M., Moreno, D.S., Martins, L.S., De Almeida, D.M., Gionbelli, M.P., Manso, M.R., Melo, L.P., Moura, F.H. and Duarte, M.S., 2017. Supplementation of grazing beef cows during gestation as a strategy to improve skeletal muscle development of the offspring, Animal, 11,2184–2192. https://doi.org/10.1017/S1751731117000982

Meneses, J.A.M., Nascimento, K.B., Galvão, M.C., Ramírez-Zamudio, G.D., Gionbelli, T.R.S., Ladeira, M.M., Duarte, M.S., Casagrande, D.R. and Gionbelli, M.P., 2022. Protein Supplementation during Mid-Gestation Alters the Amino Acid Patterns, Hepatic Metabolism, and Maternal Skeletal Muscle Turnover of Pregnant Zebu Beef Cows, Animals 2022, Vol. 12, Page 3567, 12,3567. https://doi.org/10.3390/ANI12243567

Moreira, G.M., Aguiar, G.L., Meneses, J.A.M., Luz, M.H., Monteiro, M.G.B.B., Lara, L., Ladeira, M.M., Souza, J.C. de, Duarte, M.S. and Gionbelli, M.P., 2021. The course of pregnancy changes general metabolism and affects ruminal epithelium activity pattern in Zebu beef heifers, Livestock Science, 248,104496. https://doi.org/10.1016/j.livsci.2021.104496

Moreira, G.M., Aguiar, G.L., Meneses, J.A.M., Nascimento, K.B., Ramirez-Zamudio, G.D., Costa T.C., Duarte M.S., and Gionbelli, M.P., 2023. Pregnancy affects maternal performance, feed intake, and digestion kinetics parameters in beef heifers. Journal of Animal Science.

Moser, E.B., 2004. Repeated measures modeling with PROC MIXED In Proceedings of the 29th SAS Users Group International Conference, Montreal, 2004.

Moyo, M., and Nsahlai, I., 2018. Rate of passage of digesta in ruminants; Are goats different? Goat Science; Kukovics, S., Ed.; IntechOpen: London, UK, 39–74.

Nascimento, K.B., Galvão, M.C., Meneses, J.A.M., Moreira, G.M., Ramírez-Zamudio, G.D., Souza, S.P.D., Prezotto L.D., Chalfun L.H.L., Duarte M.S., Casagrande D.R., and Gionbelli, M. P., 2022. Effects of Maternal Protein Supplementation at Mid-Gestation of Cows on Intake, Digestibility, and Feeding Behavior of the Offspring. Animals, 12, 2865. https://doi.org/10.3390/ani12202865

National Research Council and NRC, 2000, Nutrient Requirements of Beef Cattle (National Academy Press, Washington, DC).

Prates, L.L., Valadares, R.F.D., Valadares Filho, S.C., Detmann, E., Santos, S.A., Braga, J.M.S., Pellizzoni, S.G. and Barbosa, K.S., 2012. Endogenous fraction and urinary recovery of purine derivatives in Nellore and Holstein heifers with abomasal purine infusion, Livestock Science, 150,179–186. https://doi.org/10.1016/j.livsci.2012.08.018

Ribeiro, R.C.O., Villela, S.D.J., Filho, S.C.V., Santos, S.A., Ribeiro, K.G., Detmann, E., Zanetti, D. and Martins, P.G.M.A., 2015.

Effects of roughage sources produced in a tropical environment on forage intake, and ruminal and microbial parameters, Journal of Animal Science, 93,2363–2374. https://doi.org/10.2527/jas.2014-8719
Rocha, J., Tonhati, H., Alencar, M.M. and Lôbo, R.B., 2005. Genetic parameters estimates for gestation length in beef cattle, Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 57,784–791. https://doi.org/10.1590/S0102-09352005000600013
Rodrigues, L.M., Schoonmaker, J.P., Resende, F.D., Siqueira, G.R., Rodrigues MacHado Neto, O., Gionbelli, M.P., Ramalho Santos Gionbelli, T. and Ladeira, M.M.H., 2021. Effects of protein supplementation on Nellore cows' reproductive performance, growth, myogenesis, lipogenesis and intestine development of the progeny, Animal Production Science, 61,371–380.https://doi.org/10.1071/AN20498
Rotta, P.P., Filho, S.C.V., Gionbelli, T.R.S., Costa e Silva, L.F., Engle, T.E., Marcondes, M.I., Machado, F.S., Villadiego, F.A.C. and Silva, L.H.R., 2015. Effects of day of gestation and feeding regimen in Holstein × Gyr cows: I. Apparent total-tract digestibility, nitrogen balance, and fat deposition, Journal of Dairy Science, 98,3197–3210.https://doi.org/10.3168/JDS.2014-8280
Santos, M.M., Costa, T.C., Ramírez-Zamudio, G.D., Nascimento, K.B., Gionbelli, M.P. and Duarte, M.S, 2022. Prenatal origins of productivity and quality of beef, Revista Brasileira de Zootecnia, 51,e20220061. https://doi.org/10.37496/RBZ5120220061
Valadares Filho, S.C., Silva, L.F.C., Gionbelli, M.P., Rotta, P.P., Marcondes, M.I., Chizzotti, M.L. and Prados, L.F., 2016. Nutrient Requirements of Zebu and Crossbred Cattle - BR-CORTE, (Suprema Gráfica, Viçosa, MG)
Van Soest, P.J., Robertson, J.B. and Lewis, B.A., 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition, Journal of Dairy Science, 74,3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2
Zhang, J., Zheng, N., Shen, W., Zhao, S., and Wang, J., 2020. Synchrony degree of dietary energy and nitrogen release influences microbial community, fermentation, and protein synthesis in a rumen simulation system. Microorganisms, 8, 231. https://doi.org/10.3390/microorganisms8020231