



Supplementation during the mid-gestation period of Nellore cows: calf ingestive behavior

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ABSTRACT. The objective of this study was to evaluate the ingestive behavior of calves born to Nellore cows subjected to protein supplementation during the middle third of gestation. In phase I, 20 cows were used, and the study spanned the entire middle third of gestation. The cows were divided into two groups: supplemented and unsupplemented. The supplement contained 40% CP and 78% TDN, provided at 0.5 kg animal⁻¹ day⁻¹ to maintain a BCS between 5 and 6. Phase II began after calving and ended at 240 days postpartum. Ingestive behavior was evaluated for 24h on the 120th day of life, with observations made every five minutes. Data were statistically interpreted through analysis of variance and the F-test at a significance level of 0.05, in a completely randomized design, using the SAEG statistical software. Supplementation had no effect on the ingestive behavior of the calves. Discrete bouts and rumination parameters showed no differences; however, among the bite-related variables, biting rate and number of bites per day differed statistically. Protein supplementation during the middle third of gestation did not influence calf ingestive behavior, except for bite-related variables.

Keywords: bovine; ethology; fetal programming; offspring.

Received on October 8, 2024.
Accepted on September 29, 2025.

Introduction

Meat production plays a crucial role in the global economy, contributing significantly to trade at local, national, and international levels (Ghotbaldini et al., 2018). During the middle and final thirds of gestation, pregnant cows managed in extensive systems face nutritional challenges (Rodrigues et al., 2021), strongly associated with environmental seasonality. In this context, nutritional strategies for pregnant cows can be an effective strategy to mitigate the negative effects of seasonality on the productive performance of cows and their offspring (Costa et al., 2021).

Gestational supplementation indicates that several factors may act during this developmental stage, leading to long-term changes in growth, fetal development, organ systems, and metabolic functions. The timing and duration of these factors determine future outcomes, which vary according to the specific tissue or organ developing at that moment (Abuelot, 2019; Caton et al., 2019). This approach has been investigated worldwide as a means of improving intake, digestibility, performance, and carcass quality in offspring.

Ingestive behavior can be described as a set of activities that are key for animal assessment, and understanding it is highly valuable in ruminant production and its interaction with the rearing environment. In grazing cattle, the main daily activities include grazing, rumination, and resting, with the proportion of time expended on each depending on pasture characteristics, climate, nutritional needs, and management (Pereira et al., 2018; Santos et al., 2020; Dias-Silva & Filho, 2021).

Feeding patterns in cattle tend to persist, although they are strongly influenced by the environment. Variability among individuals may occur within the herd (Naeve et al., 2018), and assessing ingestive behavior provides information about the nutritional, social, and health status of the group.

Visual assessment of three of the most common actions in grazing situations—chewing, prehension, and combined chewing-prehension—is widely used to monitor ruminant feeding (Deniz et al., 2017). Feeding time is negatively associated with dietary neutral detergent fiber content and positively associated with dry matter intake (Custodio et al., 2017).

In selection and management, some indicators are more commonly used as quality markers, the most important being body weight (Fernandes et al., 2020). The search for additional techniques and parameters to evaluate animal efficiency has driven numerous scientific studies aimed at broadening selection methods and promoting optimal development from early life stages.

Thus, the objective was to evaluate the ingestive behavior of calves born to Nellore cows subjected to fetal nutrition during the middle third of gestation.

Material and methods

The Ethics Committee on the Use of Animals (CEUA) of the State University of Piauí (UESPI) evaluated and approved the project (approval no. 0033/2017).

The field phase was conducted at Fazenda Uberlândia, located in the municipality of Parnaguá, in the Cerrado region of the state of Piauí, Brazil. According to the Köppen classification, the climate is tropical seasonal sub-humid dry (Aw). The study area comprised 20 ha, separately cultivated with Massai grass (*Panicum maximum* cv. Massai) and Mandante grass (*Echinochloa polystachya*), subdivided into eight paddocks.

This study was divided into two phases: supplementation of the dams, and the calf pre-weaning period.

The cows were weighed, identified, treated for ecto- and endoparasites (Doramectin – 200 mcg kg⁻¹), and allocated into two groups of 10 animals each: cows receiving protein concentrate supplementation; cows without protein concentrate supplementation. Both groups had free access to mineral salt and water.

In phase I, 20 third-parity Nellore cows were subjected to fixed-time artificial insemination (FTAI), and pregnancy was confirmed by ultrasound 45 days after insemination. Phase I began at the end of the first third of gestation and continued through the middle third, with supplementation provided for 90 days.

The concentrate supplement was supplied daily at 10:00 in uncovered, double-access collective plastic troughs with a linear space of 70 cm animal⁻¹. The supplement (Table 1) was formulated according to nutritional requirements (Valadares Filho, 2016), containing 40% CP and 78% TDN, and provided at 0.5 kg per animal per day to maintain a BCS between 5 and 6 (scale of 1 to 9).

Table 1. Proportion of ingredients in the supplement and physicochemical composition of simulated grazing and supplement during the experimental period.

Proportion of ingredients in the supplement (g kg DM ⁻¹)		
Soybean meal		800
Ground corn		100
Mineral salt ^e		100
Physicochemical composition		
Variable	Simulated grazing	Concentrate
Dry matter (g kg as-fed ⁻¹)	431	892
Mineral matter (g kg DM ⁻¹)	121	144
Organic matter (g kg DM ⁻¹)	879	856
Crude protein (g kg DM ⁻¹)	86	488
NDFap ¹ (g kg DM ⁻¹)	759	321
Acid detergent fiber (g kg DM ⁻¹)	524	130
Lignin (g kg DM ⁻¹)	82	35
Ether extract (g kg DM ⁻¹)	34	34
Non-fibrous carbohydrates (g kg DM ⁻¹)	241	679
Total digestible nutrients (g kg DM ⁻¹)	520	759

*Calcium 190-165 g kg⁻¹; Phosphorus 60 g kg⁻¹; Sodium 107 g kg⁻¹; Sulfur 12 g kg⁻¹; Magnesium 5000 mg kg⁻¹; Cobalt 107 mg kg⁻¹; Copper 1300 mg kg⁻¹; Iodine 70 mg kg⁻¹; Manganese 1000 mg kg⁻¹; Selenium 18 mg kg⁻¹; Zinc 4000 mg kg⁻¹; Fluorine 600 mg kg⁻¹; ¹Neutral detergent fiber corrected for ash and protein.

The second phase corresponded to the calf pre-weaning stage and involved the dams and their offspring. This phase began at birth and ended 240 days later. The two treatments from phase I were maintained, using 10 replicates: calves from cows receiving protein concentrate supplementation; and calves from cows without protein concentrate supplementation. Both treatments had free access to mineral salt and water *ad libitum*.

After birth, the umbilical cord was cut, and a 10% iodine solution was applied to the calves. The newborns were weighed and identified with ear tags for subsequent evaluations.

Pasture was assessed in both phases of the experiment on the first day and every 30 days thereafter. Dry matter availability was estimated according to total extraction method. To minimise the influence of biomass variation between paddocks, cows were kept in each paddock and randomly transferred to another every eight days, following the experimental design.

Pasture samples were weighed, and a composite sample of forage from grazed and ungrazed paddocks was obtained. The samples were manually separated into leaf blade, stem, and dead material fractions, which were weighed to determine their proportions and then stored in labelled plastic bags at $-10\text{ }^{\circ}\text{C}$ for later chemical analysis (Table 1).

To determine biomass accumulation in paddocks kept closed for 30 days as exclusion areas, the triple-pairing technique was used. Dry matter accumulation during each experimental period was calculated by multiplying the daily accumulation rate (DAR) by the number of days in the period.

The daily dry matter accumulation rate (DAR) was estimated using the equation proposed by Campbell (1966): $\text{DAR} = (\text{Gi} - \text{Fi-1}) / n$, where DAR = daily dry matter accumulation rate in period j , in $\text{kg DM ha}^{-1} \text{ day}^{-1}$; Gi = average final dry matter of the four empty paddocks at time i , in kg DM ha^{-1} ; Fi-1 = average initial dry matter in the empty paddocks at time $i-1$, in kg DM ha^{-1} ; and n = number of days in period j .

Potentially digestible dry matter (pdDM) of the pasture was estimated by the equation: $\text{pdDM} = 0.98 (100 - \% \text{NDF}) + (\% \text{NDF} - \% \text{iNDF})$, where 0.98 is the true digestibility coefficient of cell contents, NDF = neutral detergent fiber, and iNDF = indigestible NDF. The availability of potentially digestible DM (pdDMA) was calculated as $\text{pdDMA} = \text{TDMA} \times \text{pdDM}$, where pdDMA = availability of potentially digestible DM, in kg ha^{-1} ; TDMA = total DM availability, in kg ha^{-1} ; and pdDM = potentially digestible DM, in percentage.

Forage allowance (FA) was calculated using the equation of Prohmann et al. (2004): $\text{FA} = \{(\text{DRB} \times \text{area} + \text{DAR} \times \text{area}) / \text{BWtotal}\} \times 100$, where FA = forage allowance, in $\text{kg DM per 100 kg BW day}^{-1}$; DRB = daily total residual biomass, in $\text{kg DM ha}^{-1} \text{ day}^{-1}$; DAR = daily accumulation rate, in $\text{kg DM ha}^{-1} \text{ day}^{-1}$; and BWtotal = total body weight of animals, in kg ha^{-1} .

The stocking rate (SR) was calculated considering the animal unit (AU) as 450 kg of body weight (BW), using $\text{SR} = \text{AUt} / \text{Area}$, where SR = stocking rate, in AU ha^{-1} ; AUt = total animal units; and Area = total experimental area, in hectares.

For forage collection by simulated grazing, the animals in each treatment were observed during grazing to determine the height of the grazed stratum. A forage sample was then collected, aiming to reproduce the characteristics of the forage consumed by the animals, according to Johnson (1978).

Laboratory analyses were conducted at the Animal Nutrition Laboratory of the State University of Piauí (UESPI), Corrente/PI campus. Analyses of dry matter (DM), ash (MM), crude protein (CP), ether extract (EE), and acid detergent fiber (ADF) in concentrate and forage samples were performed according to Detmann et al. (2012).

Neutral detergent fiber corrected for ash and protein (NDFap) was estimated following Licitra et al. (1996). Non-fibrous carbohydrates (NFC) were calculated as proposed by Hall (2003): $100 - [\% \text{CP} - \% \text{CP derived from urea} + \% \text{urea} + \% \text{NDFap} + \% \text{EE} + \% \text{ash}]$.

Total digestible nutrients (TDN) were calculated according to Sampaio et al. (2025), using NDF and NFC corrected for ash and protein, by the formula: $\text{TDN} (\%) = \text{DCP} + \text{DNDFap} + \text{DNFCap} + 2.25\text{DEE}$, where DCP = digestible CP; DNDFap = digestible NDFap; DNFCap = digestible NFCap; and DEE = digestible EE.

Observations of ingestive behavior were conducted over 24 h on the 120th day of life. Animals were visually assessed every five minutes (Gary et al., 1970) by trained observers using digital stopwatches to record the time expended on each activity on an ethogram. Time expended grazing, ruminating, suckling, and engaging in other activities was recorded. Feeding time (grazing + feed consumption) and rumination time were calculated based on DM and NDF intake ($\text{min. kg}^{-1} \text{ DM}$ or NDF).

Grazing time was defined as the time expended selecting and prehending forage, including short intervals of movement associated with forage selection (Hancock, 1953). Rumination time included the processes of regurgitation, remastication, reinsalivation, and reswallowing. Suckling time was calculated by summing the observed periods of activity over 24h, while time allocated to other activities (rest, water intake, interactions, etc.) included all activities not described above.

The discretization of the time series was performed directly in the data collection spreadsheets by counting discrete bouts of feeding, rumination, and other activities. The average duration of each discrete bout was obtained by dividing the daily duration of each activity by the number of discrete bouts, according to Silva et al. (2006).

Total feeding time (TFT) and total chewing time (TCT) were obtained using the equations: $\text{TFT} = \text{GRA} + \text{TRO}$, where GRA = grazing time (min.) and TRO = feeding time at the trough (min.); and $\text{TCT} = \text{GRA} + \text{RUM} + \text{TRO}$, where GRA = grazing time (min.), RUM (min.) = rumination time, and TRO = feeding time at the trough (min.).

The number of rumination chews and the time expended ruminating each ruminal cud were recorded for each animal using a digital stopwatch. To obtain the average chewing and rumination times, three

observations of ruminal cuds were taken between 09:00 and 12:00 and between 16:00 and 19:00, following Burger et al. (2000). The number of daily cuds was estimated by dividing the total rumination time by the average time expended ruminating each cud.

The biting rate of the animals in each group was estimated as the time required for an animal to take 20 bites (Hodgson, 1982). Bite and swallowing observations were recorded on six occasions during the day, as described by Baggio et al. (2009), with three evaluations in the morning and three in the afternoon. These data were also used to determine the number of bites per day, calculated as the product of biting rate and grazing time.

The number of cuds ruminated per day (CRD, n), cud chewing time (CCT, s), and number of chews per ruminated cud (CRC, n) were calculated using the following equations: $CRD = RUM / CCT$, where RUM = rumination time (s/day) and CCT = cud chewing time (s); and $CRC = CRD \times CRC$. Feeding and rumination efficiency, expressed in $kg\ h^{-1}$ for DM, NDF, NFC, and CP, was calculated by dividing the intake of each nutrient by total feeding time (feeding efficiency) or by rumination time (rumination efficiency).

The data were statistically analyzed using analysis of variance and the F-test at a 0.05 significance level in a completely randomized design, with the aid of the statistical software SAEG (System for Statistical Analysis, version 9.0).

Results and discussion

There was no effect ($p > 0.05$) of concentrate supplementation during the middle third of gestation on the ingestive behavior of the calves (Table 2). Grazing time and rumination time did not differ ($p > 0.05$), which is expected since both treatments had access to the same forage conditions. The high NDF content of the pasture is a limiting factor for intake, and both the quantity and quality of NDF can influence grazing and rumination times. NDF is a variable that directly affects ingestive behavior parameters (Van Soest, 1994; Mendonça et al., 2004). Age also affects grazing and rumination times; “[...] the intensity and constancy of the rumination process are defined two to three weeks after birth in animals with access to fibrous feed” (Rosenberger, 1993, p. 169). Given that the calves were four to five months old at the time of data collection, the observed values for grazing and rumination were not influenced by age.

Table 2. Ingestive behavior (min day^{-1}) of offspring from dams subjected to protein-mineral supplementation in the middle third of gestation.

Variable	Supplementation		P-value	CV%
	Unsupplemented	Supplemented		
Grazing time	378.125	392.143	0.5999	13.10
Rumination	275.625	308.571	0.1712	15.10
Suckling time	41.875	47.857	0.5572	43.00
Time on other activities	744.375	691.429	0.0569	6.80
Total chewing time	653.750	700.714	0.1200	8.10

The values obtained for grazing and rumination may also reflect the time expended suckling, since at this stage calves still do not obtain their full nutritional requirements from grazing. Although the animals were already considered functional ruminants, the presence of milk in the diet may still influence ingestive behavior.

Suckling time did not differ ($p > 0.05$), with an average of 3.11% of the total time. This variable is influenced by factors such as nutritional requirements, dam size, and milk production. As no differences were observed for these factors between treatments, suckling time remained similar. Lopes et al. (2017), studying Nelore calves raised at foot and evaluated at approximately 120 days of age, reported an average suckling time of 3% of the total time.

The time expended on other activities was 744.375 and 691.429 min. day^{-1} , respectively, with no difference ($p > 0.05$). The pasture available during the evaluation period had characteristics of low-quality forage, due to high fiber and low crude protein content. Such conditions may reduce forage intake and consequently increase time expended on other activities.

Aldrighi et al. (2019) reported values of 746 and 735 min. day^{-1} for time expended on other activities when evaluating Nelore bulls and heifers, respectively, after weaning. The high values observed here may be attributed to the developmental stage of the animals, as during the pre-weaning period, socialization and learning dynamics still exert strong influence on daily activities.

Total chewing time also showed no effect ($p>0.05$). This result is expected because it is the sum of grazing and rumination times, which also showed no effect ($p>0.05$), as well as the absence of differences in NDF content in the animals' diet, a factor known to affect the number and duration of chews.

Another factor that may influence the ingestive behavior of the evaluated animals is the development of their ingestive behavior itself. In young animals, much of this behavior is shaped by social models, such as the dam and other members of the group (Naeve et al., 2018).

The discrete bouts of behavioral variables showed no significant variation ($p>0.05$) (Table 3). This result may be associated with the fact that all animals evaluated belonged to the same category and, therefore, likely had similar body dimensions, ruminal capacity, and nutritional requirements. Protein supplementation of cows during the middle third of gestation was not sufficient to modify the number or duration of behavioral activity cycles in the offspring, likely due to similar dry matter intake and milk consumption. These findings support the hypothesis of an absence of anatomical and physiological differences in the gastrointestinal tract of the offspring.

Table 3. Discrete bouts of biting and rumination behaviors of calves from dams receiving protein mineral supplementation in the middle third of gestation.

Variable	Supplementation		P-value	CV%
	Unsupplemented	Supplemented		
Discrete bouts				
NGB ¹	26.250	29.000	0.2692	16.70
NRB ²	23.750	27.714	0.0796	15.70
NSB ³	5.875	6.000	0.8925	29.90
NOB ⁴	45.250	46.714	0.5326	9.60
TGB ⁵	14.683	13.796	0.5371	18.90
TRB ⁶	11.587	11.256	0.5993	10.40
TSB ⁷	7.008	7.551	0.5391	22.90
TOB ⁸	16.535	14.948	0.0963	10.80
Bite-related variables				
BR ⁹	0.355	0.257	0.0236	23.90
BM ¹⁰	0.312	0.366	0.0346	17.82
BBS ¹¹	11.108	10.561	0.8485	49.60
TBS ¹²	31.150	43.185	0.221	49.20
NBD ¹³	7923.029	6041.500	0.0271	20.70
Rumination-related variables				
CRC ¹⁴	45.139	40.216	0.3658	23.70
TRC ¹⁵	44.197	42.188	0.5488	14.60
CS ¹⁶	1.007	0.954	0.3923	11.90
CCT ¹⁷	1.008	1.054	0.4547	11.30
CCD ¹⁸	16626.850	17793.930	0.5215	19.90
CRD ¹⁹	384.960	447.044	0.2422	23.60

¹Number of grazing bouts; ²Number of rumination bouts; ³Number of suckling bouts; ⁴Number of bouts of other activities; ⁵Time per grazing bout; ⁶Time per rumination bout; ⁷Time per suckling bout; ⁸Time per bout of other activities; ⁹Biting rate; ¹⁰Bite mass; ¹¹Number of bites between swallows; ¹²Time between swallows; ¹³Number of bites per day; ¹⁴Chews per ruminated cud; ¹⁵Time per ruminated cud; ¹⁶Chewing speed; ¹⁷Cud chewing time; ¹⁸Cud chews per day; ¹⁹Cuds ruminated per day.

According to Santana Júnior et al. (2013, p. 1346), "[...] time per bout corresponds to the quotient between the time expended on the activity and the number of bouts of the same activity". Therefore, because no differences ($p>0.05$) were observed in either time expended or number of bouts (Table 3), no differences in time per bout were expected. Silva et al. (2015) reported a relationship between grazing and rumination times and NDF and ADF intake, attributed to changes in forage passage rate, with NDF acting as either a limiting or stimulating factor. Under conditions of high dietary NDF concentration, a greater number of meals per animal is typically observed, directly affecting grazing and rumination times due to the prehension of smaller forage particles.

Among bite-related variables, biting rate, bite mass, and number of bites per day were affected ($p<0.05$) (Table 3). Offspring of supplemented cows exhibited a lower pasture intake rate (biting rate) and greater bite mass, which could indicate improved selectivity; however, this effect was not seen in the present study. During the data collection period, forage quality had already declined, and the observed differences may be associated with the animals' ability to more effectively select ingested material during grazing. Additionally, anatomical differences in mouth size may influence biting rate and number of bites per day, as animals with wider mouths can capture more forage per bite and may reach ruminal fill limits more rapidly.

Rumination-related variables were not influenced ($p > 0.05$) (Table 3). One of the primary functions of rumination is to reduce fibrous particle size, and because no differences were observed in the calves' diets, variation among rumination parameters was unlikely. Silva et al. (2015) also reported a direct relationship between rumination variables and neutral detergent fiber content in the diet.

Conclusion

Protein concentrate supplementation during the middle third of gestation did not influence the ingestive behavior of the offspring, except for bite-related variables.

Data availability

The data in this article are part of a doctoral thesis entitled "Fetal Nutrition in Beef Calf Production" which is available at <https://repositorio.ufpi.br/xmlui/handle/123456789/3155>

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