ANA CAROLINE RAMOS TELES DA SILVA

EFFECT OF REDUCING STARTER FEED CRUDE PROTEIN CONTENT WITH DIFFERENT VOLUMES OF MILK ON THE PERFORMANCE OF CALVES

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

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ABSTRACT

SILVA, Ana Caroline Ramos Teles, M.Sc., Universidade Federal de Viçosa, March, 2023. **Effect of reducing starter feed crude protein content with different volumes of milk on the performance of calves.** Adviser: Alex Lopes da Silva. Co-advisers: Marcos Inácio Marcondes, Mariana Magalhães Campos, and Polyana Pizzi Rotta.

The objective was to evaluate the influence of decreasing crude protein (CP) of the starter feed content according to increasing age on the development of dairy calves during the pre-weaning, weaning and post-weaning phases. Sixty crossbred heifers were randomly assigned at 4 days of age to one of six treatments: 4 L/d and starter feed with fixed CP (**4L_FCP**), 4 L/d and starter feed with decreasing CP (**4L_DCP**), 6 L/d and starter feed with fixed CP (**6L_FCP**), 6 L/d and starter feed with decreasing CP (**6L_DCP**), 8 L/d and starter feed with fixed CP (**8L_FCP**) and 8 L/d and starter feed with decreasing CP (**8L_DCP**). Animals submitted to the FCP treatment received 18% PB in the starter feed throughout the experiment, while those included in the DCP treatment received 25% PB in the starter feed from day 4 to day 24, 18% from day 25 to day 45 and 14% from day 46 to day 66. The animals had free access to water and starter feed and their consumption was measured daily, in addition, they were submitted to 3 digestibility tests, in which samples of feces, urine, blood and ruminal fluid were collected. At 67 days, gradual weaning began and at 73 days the animals were weaned and submitted to blood and ruminal fluid collection. At 74 days, the animals began to have access to corn silage and remained in the experiment until 80 days of age, when blood and ruminal fluid were collected again. In addition, body measurements and body weight were measured weekly since the beginning of the experiment and at 66 and 80 d the animals were submitted to ultrasonography of the mammary gland, to evaluate the influence of the treatments. In general, animals that received 8 and 6L/d had higher performance, digestibility, feed efficiency and N utilization than 4L/d, but at weaning, 4L/d animals had a higher ADG (607 g/d for 4 L, 545 g/d for 6 L and 293 g/day for 8 L). Body weight was higher in animals that consumed starter feed with CP fixed in the starter at 66 d (73.1 kg for FCP and 68.4 kg for DCP) and at weaning (77.4 kg for FCP and 71.2 kg for DCP). Post-weaning feed efficiency was higher in DCP animals (655 g/kg) compared to FCP animals (364 g/kg), in addition to the intake and excretion of N in feces, at 66 d it was higher for animals that consumed FCP (37.8 g/d for ingestion and 1.76 g/d for N in feces) when compared to those that consumed DCP (31.7 g/d for N intake and 1.31 g /d for N stools). The efficiency of N use and the serum concentration of IGF -I and glucose were lower for 4 L/d animals. For blood urea nitrogen (NUS), at 80 d we observed higher concentrations of NUS for all milk volumes; compared to starter feed at 24 (20.8 mg/dL), 66 (18 mg/dL), 73 (22.8 mg/dL) and 80 d (27.7 mg/dL) FCP animals had higher concentrations of NUS than DCP (17.6 mg /dL at 24 d, 12.5 mg/dL at 66 d, 11.8 mg/dL at 73 d, 21.1 mg/dL at 80 d). Acetic, propionic and butyric acids increased in relation to days, respectively. In addition, the concentrations of butyric and acetic acid in animals that received 4L/d were higher when associated with FCP (9.33 µmol/mL for butyric acid and 43.9 µmol/mL for acetic acid) than with DCP (5.73 µmol/mL for butyric acid and 30.8 µmol/mL/mL for acetic acid). In the mammary gland, the animals had better parenchyma deposition at 80 d (67.4 pixels/mm^2) compared to 66 d (71.7 pixels/mm^2). Regarding milk volume, 4 L/d (64.9 pixels/mm^2) was better than 8 L/d (75.1 pixels/mm^2) and 6 L/d (69.3 pixels/mm^2) did not differ from 4 or 8 L. Animals of FCP treatments (67 .2 pixels/mm^2) had better results compared to DCP animals (72.1 pixels/mm^2). It can be concluded that animals that consume 6 L/d have shown a good performance, in addition to reducing costs and bringing less harm to the animals at the time of weaning. In relation to the starter diet, the animals submitted to the FCP strategy presented higher consumption and better performance in relation to the DCP animals. However, DCP animals had lower fecal N excretion at 66 d, and better EF post-weaning, proving to be an effective alternative to decrease N excretion, and that there is a potential for improvement in animal performance with the use of this strategy that should be better explored.

Keywords: Nutrition. Development. Dairy calves.

RESUMO

SILVA, Ana Caroline Ramos Teles, M.Sc., Universidade Federal de Viçosa, março de 2023. **Efeito da redução do teor de proteína bruta do concentrado associado a diferentes volumes de leite sobre o desempenho de bezerras.** Orientador: Alex Lopes da Silva. Coorientadores: Marcos Inácio Marcondes, Mariana Magalhães Campos e Polyana Pizzi Rotta.

O objetivo foi avaliar a influência da diminuição do teor de proteína bruta (**PB**) do concentrado de acordo com o aumento da idade no desenvolvimento de bezerras leiteiras durante as fases de pré-desmame, desmame e pós-desmame. Sessenta bezerras mestiças foram distribuídas aleatoriamente, aos 4 dias de vida, em um dos seis tratamentos: 4 L/d e concentrado com PB fixa (**4L_PBF**), 4 L/d e concentrado com PB decrescente (**4L_PBD**), 6 L/d e concentrado com PB convencional (**6L_PBF**), 6 L/d e concentrado com PB decrescente (**6L_PBD**), 8 L/d e concentrado com PB convencional (**8L_PBF**) e 8 L/d e concentrado com PB decrescente (**8L_PBF**). Animais submetidos ao tratamento PBF recebiam 18% de PB no concentrado durante todo o experimento, já os inclusos no tratamento PBD recebiam 25% PB no concentrado do dia 4 ao dia 24, 18% do dia 25 ao dia 45 e 14% do dia 46 ao dia 66. Os animais tiveram livre acesso a água e concentrado e seu consumo foi medido diariamente, além disso, foram submetidos a 3 ensaios de digestibilidade, nos quais foram amostrados fezes, urina, sangue e líquido ruminal. Aos 67 dias iniciou-se o desmame gradual e aos 73 dias os animais foram desmamados e submetidos à coleta de sangue e líquido ruminal. Aos 74 dias, os animais passaram a ter acesso à silagem de milho e permaneceram no experimento até os 80 dias de idade, quando o sangue e o líquido ruminal foram novamente coletados. Além disso, medidas corporais e peso foram aferidos semanalmente desde o início do experimento e aos 66 e 80 d os animais foram submetidos à ultrassonografia da glândula mamária, a fim de avaliar a influência dos tratamentos. De modo geral, animais que receberam 8 e 6L/d tiveram desempenho, digestibilidade, eficiência alimentar e utilização do N superior aos 4L/d, porém no desmame os animais 4L/d tiveram um maior GMD (607 g/d para 4 L, 545 g/d para 6 L e 293 g/dia para 8 L). O peso corporal foi maior nos animais que consumiam concentrado com PB fixa no concentrado aos 66 d (73,1 kg para PBF e 68,4 kg para PBD) e no desmame (77,4 kg para PBF e 71.2 kg para PBD). A eficiência alimentar no pós-desmame foi maior nos animais PBD (655 g/kg) em relação aos animais PBF (364 g/kg), além disso a ingestão e a excreção de N nas fezes, aos 66 d foi maior para os animais que consumiram PBF (37,8 g/d para ingestão e 1,76 g/d para N nas fezes) quando comparados aos que consumiram PBD (31,7 g/d para ingestão de N e 1,31 g/d para N fezes). A eficiência de uso de N e a concentração sérica de IGF -I e glicose foram menores para animais de 4 L/d. Para nitrogênio uréico sanguíneo (NUS), aos 80 d observamos maiores concentrações de NUS para todos os volumes de leite; com relação ao concentrado aos 24 (20,8 mg/dL), 66 (18 mg/dL), 73 (22,8 mg/dL) e 80 d (27,7 mg/dL) animais de PBF apresentaram maior concentração de NUS do que o PBD (17,6 mg /dL aos 24 d, 12,5 mg/dL aos 66 d, 11,8 mg/dL aos 73 d, 21,1 mg/dL aos 80 d). Os ácidos acético, propiônico e butírico aumentaram em relação aos dias, respectivamente. Além disso, as concentrações de ácido butírico e acético nos animais que receberam 4L/d foram maiores quando associado a PBF (9,33 µmol/mL para butírico e 43,9 µmol/mL para acético) do que com PBD (5,73 µmol/mL para butírico e 30,8 µmol/mL/ mL para acético). Na glândula mamária os animais tiveram maior deposição de parênquima aos 80 d (67,4 pixels/mm²) em comparação aos 66 d (71,7 pixels/mm²), animais que consumiram, 4 L/d (64,9 pixels/mm²) foram melhores que 8 L/d (75,1 pixels/mm²) e 6 L/d (69,3 pixels/mm²) não diferiram de 4 ou 8 L, já no concentrado animais PBF (67,2 pixels/mm²) tiveram melhor resultado comparados a animais PBD (72,1 pixels/mm²). Pode-se concluir que, animais que consomem 6 L/d demostraram um bom desempenho, além de diminuir custos e trazer menos prejuízos aos animais no momento do desmame. Em relação à ração inicial, os animais submetidos à estratégia PBF apresentaram maior consumo e melhor desempenho em relação aos animais PBD. No entanto, os animais PBD tiveram menor excreção fecal de N aos 66 d, e melhor FE no pós-desmame, mostrando-se uma alternativa eficaz para diminuir a excreção de N, e que existe um potencial de melhora no desempenho animal com o uso dessa estratégia que deve ser melhor explorado.

Palavras-chave: Nutrição. Desenvolvimento. Bezerras leiteiras.

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SUMMARY

1. INTRODUCTION

Because of increased environmental concerns, animal production systems are increasingly pressured to become more efficient (FAO, 2022). Dairy farms are recognized as critical sources of environmental pollution, mainly due to methane emission and N excretion (Rodrigues et al., 2022; Richardson et al., 2021). Fecal and urinary N excretion contributes to increasing the atmospheric levels of ammonia and the contamination of watercourses by nitrates, in addition to representing an economic loss due to feeding costs (Tamminga, 1992; Appuhamy et al., 2011).

In this context, dairy calves could be more efficient and environmentally friendly with adequate nutrition adjustments (Niekert et al., 2021). Efficiency improvements should involve an increase in the performance of these animals during the pre-weaned phase, contributing to higher rates of ADG also during the entire rearing phase (Silva et al., 2015). One of the tools that can be used to increase animals' performance, as well as mitigate N excretion in the pre-weaned phase is the chemical composition of the starter feed, especially its CP content.

Although we know that requeriment for calves are rapidly changing, we feed those animals with just one stater, ignoring that the proportion of milk in the diet is decreasing when the animals starts to eat starter feed. Most of the studies and farms use starter feed with CP content ranging from 18 to 22% (NASEM, 2021). Conversely, Stamey et al. (2021) demonstrated positive effects on calf's performance when fed a starter feed with 25% of CP from 3rd d of life until post-weaning compared to the starter feed with 19% of CP. Additionally, Kazemi-Bonchenari et al. (2022) observed that animals that consumed starter feed with 23% CP during the rearing phase had higher starter feed intake and a tendency of higher ADG compared to starter feed with 18% of CP. These results are probably associated with the high protein requirement of young animals, which demand high muscle tissue synthesis (Silva et al., 2017; Marcondes and Silva, 2021).

Additionally, milk intake must be considered as a source of variation to determine the starter feed CP composition, once that has an influence on the achievement of nutrients requirements of calves and in the starter feed DMI (Silva et al., 2015, 2018; Kertz and Loften, 2013). For instance, the supply of higher amounts of milk is associated with the achievement of nutrients requirements for high performances (Labussiere et al. 2011), as well as limitation starter feed intake (Rosenberger et al., 2017). In view of this, precision nutrition should be adopted (Stamey et al., 2021), which meets the protein needs of these animals according to their age, balancing milk intake and starter feed intake, through the association between amounts of milk offered and the drop in the starter feed CP content.

In swine nutrition, commercial feeding programs can be divided into nursery, growth and finishing categories, for each category, the total dietary supply of crude protein (CP) must be sufficient and to meet the need for essential amino acids, and with that reduce N and P excretion to the environment (Rocha et al., 2022). In this context, calves have low starter feed intake in the first weeks of life, following an exponentially increasing pattern according to age, with considerable growth from 25-30 d of life. This low starter feed intake allied to the high protein requirements could cause a protein deficit for these animals, justifying the higher performance when a starter with higher protein content is provided (Silva et al., 2015, 2018).

Observing the equations for estimating starter feed intake proposed by Silva et al. (2018) and the protein requirement recommended by Silva et al. (2017), it is observed that the starter feed should not have a fixed content of 18-19% of CP throughout the pre-weaned phase but should present a gradual decrease according to age. Considering an animal receiving 4 L/d of milk, the starter feed CP content should be around 24% of CP from 4 to 24 d of age; around 18% of CP from 25 to 45 d of life; around 14% of CP from 46 to 66 d of life.

Thus, we hypothesized that the decrease in the starter feed CP content (**DCP**), according to the increase in the calves' age, results in greater animal performance and lower N excretion when compared to the starter feed with fixed CP content (**FCP**) supply strategy, and these effects are dependent of the milk allowance, and they are propagated to the post-weaning phase. The objective was to evaluate the influence of the reduction in the crude protein content of the concentrate on the performance of dairy calves, during the lactation, weaning and post-weaning phases.

2. MATERIAL AND METHODS

The study was carried out at the Experimental Field José Henrique Bruschi of Embrapa Dairy Cattle, State of Minas Gerais, Brazil. The study was approved by the ethics committee of animal care and use of Embrapa Dairy Cattle under number 4422240120.

2.1 Experimental design and treatments

The study was carried out following a completely randomized design in a 3×2 factorial scheme; where were used 3 volumes of milk (4, 6, or 8 L/d) and 2 starter feed supply strategies (fixed or decreasing CP content). The first strategy consisted of supplying fixed starter feed with 18% of CP throughout the pre-weaning phase (from 4 to 73 d of age). In the second strategy, animals received a starter feed with 24% CP from 4 to 24 d of age; 18% CP from 25 to 45 d of the experiment; and 14% CP from the 46 to the 73 d of age (Table 1). Nevertheless, the average CP content achieved for each starter feed was slightly lower than the planned treatments, as shown in Table 1.

Therefore, 6 treatments were formed: 4 L/d of milk and start feed with fixed CP content (**4L_FCP**); 6 L/d of milk and start feed with fixed CP content (**6L_FCP**); 8 L/d of milk and start feed with fixed CP content (**8L_FCP**); 4 L/d of milk and starter feed with decreased CP content (**4L_DCP**); 6 L/d of and starter feed with decreased CP content (**6L_DCP**); 8 L/d of milk and starter feed with decreased CP content (**8L_DS**). Ten animals were used per treatment, totaling 60 female crossbred calves, with blood degree ranging from 5/8 to 3/4 Holstein × Gyr.

2.2 Animal management

Immediately after birth, animals were dried, and their navels were treated with iodine 10% to prevent infections. Navel cure continued twice a day until the navel fell off. Additionally, animals were weighed and received 10% of BW in colostrum, standardized at 25% of Brix. At days 2 and three of life, they received 4L/d of transition milk from their own dams. Forty-eight hours after colostrum feeding, the animals were subjected to blood collections to evaluate the transfer passive immunity efficiency. Only animals with serum Brix greater than 8.1% entered the study (Lombard et al., 2020).

On the fourth d of life, animals were randomly assigned to one of the 6 treatments. Three hours after morning feeding of the same d, a blood collection was performed and the serum was later analyzed for contents of IGF-1, glucose, blood urea nitrogen (**BUN**), total protein, and albumin. These measures were used as a covariate for the other blood serum collections, as described below. Additionally, in the same d, animals were weighed and measured for withers height (**WH**), rump height (**RH**), Heart girth (**HG**), and body length (**BL**). Weighing and body measurements were repeated weekly to evaluate the performance of the animals during the experiment.

Throughout the experiment, animals were housed in individual stalls of 5 m^2, provided with feeders, drinkers, and rubber bedding. Milk was provided twice a day, at 0700 and 1500, while starter feed and water were kept freely accessible to the animals. Milk intake was computed at each meal, while starter and water intakes were measured every morning, through the difference between offered and leftovers from the previous day.

Stalls were cleaned every morning with water and later sprayed with Dioxiplus® (4% sodium hypochlorite) to control *Cryptosporidium*. After each use, the buckets were cleaned with soap and water and submerged in a chlorine solution to control microorganisms' growth before the next use they were removed from the solution and rinsed. The water buckets were washed every day, after weighing the leftovers, with soap, and water, while the buckets of starter feed were cleaned when necessary.

Health score was evaluated every morning, where rectal temperature and feces score were checked. Feces score was analyzed on a scale from 1 to 4, where 1 represented normal feces, and 4 represented liquid feces (Slanzon et al., 2022). When necessary, animals were analyzed by a veterinarian and medicated. In the 46 d of age, animals were disbudded using hot iron. The animals were previously anesthetized locally and subsequently received anti-inflammatory to control pain (AVMA, 2014).

In the 67 d of age, the weaning process began by a step-down method. Amounts of milk supplied in each treatment dropped by half with 67 d and again at 70 d; on d 73, weaning was carried out. During weaning, the starter feed supply strategies were maintained, the animals allocated in the fixed starter feed strategy continued with 18% of CP in the starter feed, whereas those animals with decreasing CP starter feed maintained the CP content of 14%.

In the 74 d of age, the weaned animals began to have access to corn silage. Intake was computed daily through the difference between offered and leftovers. During this period, all animals received starter feed with 18% of CP and in 80 d the experiment ended.

2.3 Digestibility trials, sampling, and analysis

At 20, 41, and 62 d of age, animals were submitted to digestibility trials with total collection of feces and urine, lasting 4 days (Figure 1). Feces collections lasted 3 d of and urine collection 1 d, as described by Silva et al. (2015). Feces were collected directly from the floor, immediately after defecation. The urine was collected through a metabolic cage, where the animals were suspended and the urine was directed through polyethylene hoses to tanks containing ice, to avoid the loss of urinary N (Knowlton et al., 2010). At the end of each 24 hours of collection, feces were weighed, homogenized, and sampled in an approximate amount of 200 g. Likewise, the total amount of urine produced was measured, homogenized, filtered through gauze, and a 50 mL aliquot was taken and stored at –20ºC. At the end of each collection period, a sample composed of feces was prepared based on the 3 days of sampling.

Milk samples were collected daily and later a composite sample was made weekly to be analyzed. Starter feed samples were collected at each new mixture and corn silage samples were collected weekly directly from the silo profile at ten different points, homogenized and finally the sample was taken for analysis.

Feces and silage samples were partially dehydrated in an oven with forced ventilation (55°C), according to the method INCT-CA G-001/2, while the milk samples were partially dehydrated by lyophilization according to the method INCT-CA G-002/2, described in Detmann et al. (2021). Subsequently, samples of feces, milk, silage, and starter feed were ground to 1 mm in a knife mill. Feeds and feces samples were evaluated for DM content (method INCT–CA G-003/2), mineral matter (**MM**; method INCT–CA M-001/2), CP (method INCT–CA N-001/2), NDF (method INCT–CA F-002/2), NDF corrections for protein and ash (**NDFap**; methods INCT–CA N-004/2 and INCT –CA M-002/2) and ether extract (**EE**; method G-005/2). Urine samples were used to determine total N urinary excretion (**NTU**), according to the INCT-CA N-001/2 method, described in Detmann et al. (2021). Nitrogen balance was calculated by the difference between the amount of N consumed and excreted in feces and urine. Feed efficiency was obtained by the difference between final weight and initial weight, divided by total DMI. Nonfibrous carbohydrates (**NFC**) were calculated according to Detmann and Valadares Filho (2010) as:

$$NFC = 1{,}000 - (CP + NDFap + EE + CA),$$

where: CP = crude protein, NDFap = neutral detergent fiber corrected for ash and protein, EE = ether extract, and CA = crude ash. All values are in grams per kilogram.

The intake of digestible energy (**DE**) of the diet was calculated by multiplying the digestible fraction of each caloric component by its respective energy value (NRC, 2001) as:

$$DE = (5.6 \times dCP) + (9.4 \times dEE) + (4.2 \times dNFC) + (4.2 \times dNDFap),$$

where: DE is in megacalories per kilogram; dCP = digestible CP concentration (kg/kg); dEE = digestible EE concentration (kg/kg); dNFC = digestible NFC concentration (kg/kg); and dNFDap = digestible NDFap concentration (kg/kg).

Blood collection was performed by puncturing the jugular vein on d 3, 24, 45, 66, 73, and 80. Blood was collected in vacuum tubes containing separator gel and a coagulation activator. The tubes were placed in a polystyrene box with ice and then transferred to the laboratory, where they were centrifuged at 3000*g* for 20 minutes. Two serum samples were taken from each animal, which were placed in eppendorf tubes and stored at -20ºC for further analysis of IGF-1, glucose, BUN, total protein, and albumin. BUN, glucose, total proteins, and albumin was determined using a Bioclin® kit and a Mindray automatic biochemistry device (model BS200E). The quantification of IGF-I levels was carried out in an ELISA (Enzyme-linked immunosorbent assay) microplate reader, using determination kits from Beckman Coulter.

Ruminal fluid samples were collected on d 24, 45, 66, 73 and 80 through an esophageal probe. After collection, samples were filtered through sterile gauze and transported to the laboratory for pH measurement using a portable Phmeter (Phmetro T-1000, Tekna, Araucaria, Brazil). Two 10 mL aliquots of ruminal fluid were separated for subsequent analysis of VFA's and rumen ammonia N (**RAN**). Samples destined for analysis of VFA's (acetate, propionate, and butyrate) were preserved with metaphosphoric acid, and those destined for RAN were preserved with sulfuric acid as described by Leão et al. (2020).

The samples for VFA’s analysis were filtered and had their acetic, propionic and butyric acid levels measured using the Waters alliance e2695 equipment with Detector PAD 2998 (photodiode array detector), separation system consisting of a C18 reverse phase column ODS 80A (150 ×x 4.6 mm ×x 5 μm) and quantification was obtained by calibration curve, using standards. The samples destined for rumen ammonia N were analyzed using a microplate spectrophotometer (Thermo Scientific Multiskan GO).

Mammary glands ultrasound images were taken on d 66 and 80 using ultrasound equipment (B-mode) equipped with a micro-convex transducer working at a frequency of 6 MHz (DP2200, Mindray, China). Two images were taken of each breast quarter, in a standardized position of the probe with an inclination of 45° in relation to the insertion of the teat, always in the caudocranial direction, according to the technique described by Nishimura et al. (2011) and Albino et al. (2017).

The mammary gland ultrasound images were saved in BPM format and subsequently processed using ImageJ® software (NIH, USA). The most hypoechoic region (black) of the image was identified as breast parenchyma and the most hyperechoic region (white) was identified as the fat pad (**FP**), as described by Esselburn et al. (2015). Within each parenchyma area (**PAR**), three squares were randomly superimposed (each square measuring 0.15 cm²) and located close to the growth of the mammary duct, then, the mean value of pixels within each square was evaluated in 8-bit images and represented numerically in a scale of 256 shades of gray (0=black, 255=white) according to their brightness intensity as suggested by Nishimura et al. (2011).

2.4 Statistical analysis

The data were submitted to analysis of variance, using the lm function of base package of R (RStudio Team, 2022), where the fixed effects of milk volume, starter feed supply strategy, and their interaction were tested according to the following model:

$$Y_{ijk} = \mu + M_i + S_j + (M \times S)_{ij} + \varepsilon_{ijk}$$

Y_{ijk} = dependent variable, μ = overall mean, M_i = fixed effect of milk volume, S_j = fixed effect of starter feed strategy, $M{\times}S_{ij}$ = fixed effect of interaction between milk volume and starter feed strategy, ε_{ijk} = random error.

The effect of age (for digestibility trials, rumen fluid, and blood samples) was included as a repeated measure in the statistical model, using the function gls of nlme package of R as follows:

$$Y_{ijlkm} = \mu + M_i + S_j + (M \times S)_{ij} + \delta_{ijk} + A_l + (M \times A)_{il} + (S \times A)_{jl} + (M \times S \times A)_{ijl} + \varepsilon_{ijlkm}$$

Y_{ijlkm} = dependent variable, μ = overall mean, M_i = fixed effect of milk volume, S_j = fixed effect of starter feed strategy, $M{\times}S_{ij}$ = fixed effect of interaction between milk volume and starter feed strategy, δ_{ijk} = random error where the variance between animals within treatments (M + S + M × S) is equal to the covariance between repeated measurements within animals; $M{\times}A_{il}$ = fixed effect of interaction between milk volume and age, $S{\times}A_{jl}$ = fixed effect of interaction between starter feed strategy and age, $M{\times}S{\times}Ai_{jl}$ = fixed effect of interaction among milk volume, starter feed strategy, and age, ε_{ijklm} = random error.

The variance components (VC), compound symmetry (CS), heterogeneous compound symmetry (CSH), first-order autoregressive (AR1), and heterogeneous first-order autoregressive (ARH1) matrices of (co)variance were tested. The matrix selection was based on the lowest value found for the corrected Akaike information criterion, according to each variable analyzed. The initial BW was used as a covariate for performance analysis, while blood records from the first collection (d 4) were used as a covariate for each respective hormone/metabolite.

Observations with studentized internal residuals greater than |2.5| were considered “outliers” and removed from the model. When necessary, lest square means were separated by the Tukey’s test, using $P < 0.05$ as a significance level for type I error and $0.05 \leq P < 0.10$ as trend.

3. RESULTS

3.1 Nutrient intake, digestibility, and nitrogen balance

No two or three-way interaction effects among milk, starter feed, and age were observed on total DM, OM, and EE intakes (*P* > 0.05; Tables 2 and S1). Additionally, we did not observe effect of starter feed strategy and interaction between milk and starter feed on any of the variables tested (*P* > 0.05; Table 2). We observed an increase in DM, OM, CP, DE, and EE intakes (*P* < 0.01) according to increasing milk allowance (Table 2). Effect of age was also observed in DM, OM, DE, and EE intakes (*P* < 0.01), where intake increased as animal's age increase.

For total CP intake (*P* < 0.05; Figure 2A) and CP intake from starter feed (*P* < 0.01; Figure 2B), we observed that at 66 d of age, animals which received starter feed with decreased CP content had lower intakes (202 g/d for CP and 71 g/d for CP for starter) compared to those receiving fixed starter feed (232 g/d for CP and 39 g/d for CP for starter). A similar pattern (trend) was observed for total DMI (*P* = 0.097; Figure 2C) e DMI from starter feed (*P* = 0.079; Figure 2D), with averages of 1.171 g/d for FCP and 1.062 g/d for DCP in DMI and 435 g/d for FCP and 321 g/d for DCP in DMI from starter. Furthermore, DMI from starter feed presented interaction between milk and age (*P* = 0.008), where, at 24 (69 g/d), 45 (201 g/d) and 66 d (428 g/d), animals that consumed 4 L/d of milk had higher intake when compared to those that consumed 8 L/d (37 g/d for 24 d, 112 g/d for 45 d, and 252 g/d for 66 d). Additionally, animals that received 6 L/d had similar intake of 8 L/d animals at 24 days (50 g/d), while at 45 (164 g/d) and 66 d (453 g/d), the intake was similar of the 4 L/d animals (Figure 3A).

The OM intake from starter feed presented an interaction between milk and age, where at 66 d animals that consumed 6 L/d of milk had higher intake (483 g/d) when compared to those that consumed 8 L/d of milk (250 g/d), while 4 L/d animals showed intermediate results (404 g/d) (Figure 3B). The CP and EE intakes from starter feed also presented interaction between milk and age. Concerning CP intake (*P* < 0.01; Figure 3C), at 45 (33 g/d) and 66 d (63 g/d) animals that received 4 L/d had higher intake when compared to animals that consumed 8 L/d (18 g/d for 45 d and 37 g/d for 66 d). At 45 d, animals that received 6 L/d showed equal results (28 g/d) to the 4 and 8 L/d, and at 66 d their intake was equal to the 4 L/d animals (65 g/d). For EE intake, on d 66 animals that consumed 8 L/d of milk had lower (*P* = 0.023; 7.02 g/d) intake when compared to the animals receiving 6 (16.49 g/d) or 4 L/d (12.93 g/d).

Regarding NDFap intake, it was observed a three-way interaction among milk, starter, and age (*P* = 0.027). At 66 d of age a significant difference was observed, animals consuming fixed starter feed and 4 L/d of milk had higher NDFap intake (69.44 g/d) when compared to 8 L/d animals (35.27 g/d), while 6L/d animals showed intermediate results, not differing of the treatments of 4 and 8 L/d (50.97 g/d). Whereas, in the group that consumed starter feed with decreased CP content, at 66 d, the 6 L/d animals had a higher NDFap intake (49.44 g/d) when compared to the 8 L/d animals (25.18 g/d), while the 4L/d animals had intermediate results, not differing of the treatments of 6 and 8 L/d (33.11 g/d).

We did not observe interaction effect between starter feed and milk on CP, OM, and NDFap digestibilities ($P > 0.05$). Additionally, it was not observed effect of starter feed on DM, CP, OM, and NDFap digestibilities ($P > 0.05$; Table 2). The EE digestibility presented a three-way interaction between milk, starter, and age ($P = 0.040$; Table S1). Summarizing, at 24 d of age it was not observed differences in EE digestibility among treatments. However, at 45 d the EE digestibility of 4L_FCP treatment was 955 g/kg, which was lower than other treatments, which averaged 979 g/kg. At 66 d of age, a similar pattern was observed, while 4L_FCP EE digestibility was 946 g/kg, other treatments averaged 974 g/kg.

Animals that consumed 8 L/d of milk had higher CP, OM, and DM digestibilities when compared to animals that consumed 4 L/d ($P < 0.05$). For CP and OM, the 6 L/d animals were statistically equal to those of 8 L/d, and for DM the animals that intake 6 L/d did not differ from those that intake 4 and 8 L/d. The DM and OM digestibilities were affected by age. The DM ($P = 0.016$) presented lowest digestibility at 24 d (929 g/kg), compared to 45 (941 g/kg) and 66 d (941 g/kg; Table 2). The OM ($P = 0.01$) presented the greater digestibility at 24 d (958 g/kg), intermediate at 45 (956 g/kg) and lowest at 66 d (949 g/kg; Table 2).

For DM digestibility, a trend of interaction between milk and starter feed was observed ($P = 0.089$), where animals that consumed 4 L/d of milk and decreasing starter feed CP content (936 g/kg) had greater digestibility when compared to those that received the same volume of milk and fixed starter cp content (917 g/kg; Figure 4A). For NDFap digestibility, only on d 24, the digestibility was higher in animals that consumed 4 L/d (0.732 g) of milk when compared to those that consumed 6 (574 g/kg) and 8 L/d ((485 g/kg) $P < 0.01$; Figure 4B)).

It was no observed three-way interactions among milk, starter feed, and age or two-way interactions between milk and starter feed for any of the nitrogen (**N**) balance variables ($P > 0.05$; Table 5). Milk volume affected N intake ($P < 0.001$), urine N excretion ($P = 0.03$), feces N excretion (P = 0.039), retained N ($P < 0.001$), and N efficiency ($P < 0.001$; Table 5). For N intake and retained N, animals that consumed 8L/d had higher values when compared to those that consumed 6 L/d, and 6L/d animals presented greater values than 4 L/d animals. For feces N excretion 6 and 8 L/d were equal and higher than 4 L/d, and for urine N excretion 6L/d was greater than 4 L/d and 8 L/d was intermediate. Concerning age, it was observed a difference in retained N, at 66 d the average was statistically higher than those on d 45, and on d 45 it was higher than on d 24 (Table 5).

The N intake (Figure 5A) and feces N (Figure 5B) excretion presented interaction between starter feed and age (Table S4). At 66 d animals that consumed fixed starter had higher values (37.8 g/d for intake and 1.76 g/d for N feces) when compared to those with decreasing starter feed CP content (31.7 for N intake and 1.31 for N feces). An interaction between milk and age (Table S4) was observed for N efficiency, where at 24 (572 g/kg), 45 (628 g/kg), and 66 d (720 g/kg), 4 L/d animals showed lower efficiency compared to animals with 6 (764 g/kg for 24, 772 g/kg for 45 and 829 g/kg for 66 d) and 8 L/d (802 g/kg for 24, 802 g/kg for 45 and 804 g/kg for 66 d; Figure 5C).

3.2 Performance, and feed efficiency

There was no interaction effect between milk and starter feed for any of the performance variables analyzed, nor any of them did differ on the first d of the experiment ($P > 0.05$, Tables 3 and 4).

The BW showed interaction between milk and pre-weaning time ($P < 0.001$) where on 24 d of the experiment the animals that consumed 4 L/d had a lower BW compared to those 6 and 8 L/d, at 45 and 65 d 8L/d had greater weight compared to 6 L/d and 6L/d had higher BW compared to 4 L/d. At 45 and 66 d, animals consuming 8 L/d were higher than those consuming 6 L/d and these were higher than those consuming 4 L/d. Even in pre-weaning there was an interaction between starter feed and time ($P = 0.020$), where at 66 d the animals that consumed starter feed with fixed CP had higher body weight when compared to those that consumed starter feed with decreasing CP. At weaning ($P < 0.001$) and post-weaning ($P < 0.001$) the animals that consumed 8 and 6 L/d had higher BW compared to those that consumed 4 L/d, even at post-weaning there was a starter feed effect ($P = 0.033$), where the animals that consumed the starter feed with fixed CP had higher BW when compared to the animals that consumed starter feed with decreasing CP.

At pre-weaning, there was an effect of milk volume on ADG ($P < 0.01$; Table 3), animals that consumed 8 liters of milk had greater gains compared to those that consumed 6 L/d, which had better results compared to those that consumed 4L/d. Even in pre-weaning, a tendency ($P = 0.096$) was observed between starter feed and time, where on day 63, animals that consumed starter feed with fixed CP had higher ADG when compared to animals that consumed starter feed with decreasing CP. At weaning ($P = 0.026$), animals that consumed 8 L/d had lower ADG when compared to those that consumed 6 L/d, whereas those that consumed 4L/d did not differ from those that consumed higher volumes. In post-weaning (P = 0.039), the animals that consumed 6 L/d during the experiment had a higher ADG when compared to those that consumed 4L/d, whereas those that consumed 8L/d showed statistically equal results to those of 4 and 6L/d.

The WH showed interaction between milk and time (P < 0.001) in pre-weaning, where at 42 and 63 days the animals that consumed 6 and 8L/d had statistically equal HW, and higher than the animals that consumed 4 L/d, the same behavior was observed in relation to the volume of milk in the weaning period (P < 0.001) and post-weaning (P = 0.002). Pre-weaning rump height showed interaction between milk and time (P = 0.016) at 42 and 63 days, animals that consumed 8 L/d had higher AG when compared to animals that consumed 4 L/d, whereas animals consuming 6 L/d did not differ from those 4 and 8 L/d, the same behavior was observed in the weaning period for milk volume (P = 0.007). At post-weaning (P = 0.004) animals that consumed 6 and 8 L/d did not differ among themselves and had higher AG when compared to those that consumed 4 L/d.

The Heart girth of the pre-weaning animals showed interaction between milk and time (P <0.001), at 21, 42 and 63 days the animals that consumed 6 and 8 L/d had statistically the same perimeter, being greater than the PT of the animals that consumed 4L/d. At weaning (P <0.001) and post weaning (P <0.001) the animals showed the same behavior, in addition, at post weaning there was an effect of starter feed (P = 0.023), animals that consumed starter feed with fixed CP had greater perimeters when compared to animals that consumed starter with decreasing CP. The body length of the pre-weaning animals showed interaction between milk and time (P = 0.002) where at 42 days the animals that consumed 8 liters had a greater body length than the 4 liter animals, the 6 L/d animals did not differ from the 4 and 8 L/d animals, and at 63 days the 6 and 8 L/d animals had the same perimeter and these were smaller than the 4L/d animals. At weaning (P <0.001) and post weaning (P <0.001) the animals had the same behavior as at 42 days.

Feed efficiency was influenced by pre-weaning milk volume (P <0.001), animals consuming 6 and 8L/d had greater feed efficiency compared to those consuming 4 L/d, at weaning (P = 0.018) efficiency was reversed, animals consuming 4 L/d had greater efficiency compared to those consuming 8 L/d, whereas animals with 6 L/d did not differ from those with the highest and lowest milk volumes. At post-weaning, there was an effect of starter feed (P = 0.029), animals that consumed FCP had lower feed efficiency when compared to animals that consumed DCP.

3.3 Blood and rumen parameters

No three-way interaction among milk, starter feed, and age was observed in blood parameters. The IGF-1, glucose, total protein, and albumin were not affected by starter feed, and interaction between milk and starter feed was not observed ($P > 0.05$; Table 6). For IGF-1 there was a significant difference between milk volumes ($P = 0.001$) and between ages ($P < 0.001$; Table 6). The concentration was lower for animals that consumed 4 L/d of milk when compared to 6 and 8 L/d animals. Concerning age, on d 24, animals had lower serum IGF-1 when compared to d 45, 66, and 73, and the greatest serum IGF-1concentration was observed on d 66.

Glucose concentration presented an interaction between milk and age ($P = 0.021$). At 66 d, the 6 L/d animals had a higher glucose concentration (122.2 mg/dL) than 4 L/d (104.7 mg/dL), and 8 L/d did not differ (116.6 mg/dL) from 4 and 6 L/d. At 73 d, the 8 L/d animals presented higher concentration (104.8 mg/dL) than 4 L/d (93.3 mg/dL), and 6 L/d did not differ (100.2 mg/dL) from 4 and 6 L/d (Figure 6A). Albumin presented an interaction between starter feed and age (Table S5), where animals that consumed fixed starter feed CP content (2.85 g/dL) and decreasing starter feed CP content (2.89 g/dL) had lower serum concentrations on d 24 (Figure 6B).

The BUN concentration presented two-way interactions between milk and starter feed, between milk and age, and between starter feed and age (Table S5). In the interaction between milk and starter feed ($P = 0.001$), decreasing starter feed CP content animals, that consumed 8 L/d of milk, had the highest concentration of BUN (20.4 mg/dL for 8L/d, 12.9 mg/dL for 6 L/d and 14.2 mg/dL for 4 L/d). For the interaction between milk and age ($P = 0.023$), animals from the 4 (23.5 mg/dL), 6 (21.7 mg/dL) and 8 L/d (27.9 mg/dL) of milk treatments had a higher N concentration at 80 d (Figure 6C). Finally, in the interaction between starter feed and age ($P < 0.001$), at 24 (20.8 mg/dL), 66 (18 mg/dL), 73 (22.8 mg/dL), and 80 d (27.7 mg/dL), the animals that consumed fixed starter feed CP content presented higher values of BUN when compared to the animals that consumed starter with decreasing CP content (17.6 mg/dL for 24 d, 12.5 mg/dL for 66 d, 11.8 mg/dL for 73 d, and 21.1 mg/dL for 80 d; Figure 6D).

No three-way interaction among milk, starter feed, and age was observed ($P > 0.05$; Table S5) for rumen variables. The pH was not different between treatments and their interactions ($P > 0.05$). In a general way, acetic, propionic, and butyric acids increased in relation to days 24, 45, 66, and 73 ($P < 0.001$), respectively; with means of 27.6, 31.5, 40.1, and 42.0 µMol/mL for acetic, 14.6, 17.6, 21.5, and 21.5 µMol/mL for propionic and 6.2, 6.9, 7.5, and 10.2 µMol/mL for butyric (Table 6).

The RAN concentration presented interaction between starter feed and age, being higher for animals that consumed fixed starter feed CP content (117.5, 87.5 and 80.9 mg/L for 66, 73, and 80 d, respectively) when compared to those that consumed decreasing starter CP content at d 66 (70.9 mg/L), 73 (39.9 mg/L), and 80 (50.5 mg/L; Figure 7A). Butyric, propionic, and acetic acids showed an interaction between milk and starter feed. For butyric (Figure 7B) and acetic acids (Figure 7C) animals of 4L_FCP treatment (9.33 µmol/mL for butyric and 43.9 µmol/mL for acetic) had higher concentrations when compared to the 4L_DCP (5.73 µmol/mL for butyric and 30.8 µmol/mL for acetic). For propionic acid (Figure 7D) the animals of 4L_FCP (25.0 µmol/mL) and 6L_FCP (13.9 µmol/mL) treatments had higher concentrations when compared 4L_DCP (22.5 µmol/mL) and 6L_DCP (14.5 µmol/mL; Table S5).

3.4 Mammary Gland

No two or three-way interaction effects among milk, starter feed, and age were observed in the mammary gland development ($P > 0.05$; Figure 8). Among the milk volumes ($P = 0.01$), animals receiving 4L/d (64.9 pixels/mm²) were statistically better than those with 8 L/d (75.1 pixels/mm²), whereas those with 6L/d (69.3 pixels/mm²) did not differ from 4 and 8 L/d. Concerning age ($P < 0.01$), at 80 d (67.9 pixels/mm²) the animals were better than at 66 d (71.7 pixels/mm²). For starter feed, it was observed a trend ($P = 0.052$) where animals from the CS treatment (67.2 pixels/mm²) had a higher value compared to DS (72.4 pixels/mm²).

4. DISCUSSION

The observed effect of age on DM and nutrient intakes of all animals is directly linked to an increase in starter feed intake. Silva et al. (2018) found that an exponential increase in starter feed intake is observed with increasing age, which is more pronounced after 25-30 d of life. At 24 d, when we observed the lowest intakes, the gastrointestinal tract of animals is still primitive, due to its slow development, which becomes more accelerated from the eighth week onwards (Hulsen, 2019; König and Liebich, 2016; Furlan et al., 2011). Diao et al. (2019) state that rumen development can directly affect the digestibility of nutrients, so the lower ruminal development could also reflect lower DM digestibility, as we observed at 24 d (Table 2). Regarding N balance, we observed greater urinary N excretion and N retention as animals got older. These results are closely linked to N intake and, probably, to the increase in their performance (Silva et al., 2015; Sharma et al., 2020). The protein requirement equations proposed by Marcondes and Silva (2021), suggest that as the ADG increase, the requirement for metabolizable protein for gain increase as well. In our study the animals had a progressive increase in BW according to age, with a consequent increase in requirements of protein, reflecting in the greater retention of N. The greater retention of N in the animals at 66 d is also reflected in the increase in circulating IGF-I in these animals, since this hormone is directly linked to protein metabolism (Maresca et al., 2018).

Maresca et al. (2018) evaluated serum IGF-I of calves monthly, from birth to weaning (which occurred at 180 d). They observed that serum IGF-I was lower at birth and increased until d 90. In this context, Breier and Sauerwein (1994), state that the endocrine production of IGF-I seems to be regulated by the ingestion of feed in calves; therefore, with the increase of the DMI according to the age, the circulating levels of IGF-I got higher. We observed similar results, where at 66 d animals had the highest serum concentrations of IGF-I; however, at 73 d the concentrations observed were lower than at 66 d (Table 6). From 64 to 70 d of life, the animals were gradually weaned, which impact their starter feed intake, mainly for treatments of higher amounts of milk (8 L/d). This pattern may be responsible to reduce the synthesis of IGF-I, and can be linked to the lower ADG that was observed in this phase, mainly for high milk-fed animals (Table 3). Similar results were observed by Haisan et al. (2018) when comparing a high-nutrition plan with a low-nutrition plan. The authors observed that animals fed a high-nutrition plan during the weaning period had a decrease in ADG, reflecting a drop in IGF-I levels of these animals. Belli et al. (2018) observed a 62% decrease in the plasma concentration of IGF-I from d 0 to d 3 of weaning, which was like our report. By observing ruminal VFA's, we noticed that there was a gradual increase according to the animals' age increased, achieving the maximum at 66 d. This result is due to the increase in the rumen volume, which moves from 38% of the total stomach weight at birth to 61% at eight weeks of age (Davis and Drackley, 1998; Diao et al., 2017). This gradual rumen development is linked to an increase in the starter feed intake capacity, which resulted in greater VFA production as obtained in this study (Tables 2 and 6). Concerning the mammary gland development, it is considered that as the ultrasonic waves are more reflected by tissue structures (hyperechoic), the brighter the structure appears on the ultrasound image and thus shows a higher average pixel value. Fat tissue is hyperechoic and thus appears brighter (white); hence, the pixel values are higher and represent worse results (Albino et al., 2015; Seibt et al., 2023). At 80 d had a higher parenchyma deposition compared to 66 d, which coincides with the idea that breast growth becomes allometric (faster in relation to body growth) from weaning until puberty (Nishimura et al., 2011), thus this result was expected.

As expected, and observed by several other studies, milk volume affected BW, ADG, body measurements, N retention, and FE (Tables 3, 4, and 5). In general, animals that consumed higher amounts of milk performed better, independently of age (Rosenberg et al., 2017; Ivemeyer et al., 2022; Ahmadi et al., 2022). Nevertheless, it was observed an inversion in ADG at weaning, where animals that received 4 L/d had higher ADG and FE (Table 3). A similar result was observed by Hill et al. (2010), when they observed that calves fed 1.09 kg of milk replacer DM had lower ADG (950 g/d) when compared to treatments of 0.66 kg of milk replacer DM (1.060 g/d) at post-weaning. Suggesting that digestion is compromised for at least a few weeks after weaning, as ruminal development would be impaired (Schaff et al., 2018). Additionally, this result reflects the lower starter feed intake by animals that receive higher volumes of milk (Rosenberg et al., 2017) which results in a worse performance of these animals at weaning. Hill et al. (2016) suggest that weaning must be done gradually over 3 weeks to reduce this performance damage, which would allow for increased DMI at weaning, helping rumen development, and optimizing post-weaning digestion. In our study, the weaning was carried out over one week, despite that, animals of 6 L/d treatments showed a similar ADG to 4 L/d animals over weaning and the best ADG among all treatments in the post-weaning, proving to be an interesting feeding strategy (Caixeta & Carmo, 2020). Additionally, 6 L/d animals presented similar BW to 8 L/d animals at 24 d of age, where starter feed intake is still very low (Table 3).

The observed performance results are probably linked to the greater intake and digestibility of DM, OM, DE, and CP according to the increase in milk supply. Milk is a highly digestible food, with about 93% of digestibility (Silva et al., 2015); therefore, when milk intake is higher, the diet digestibility is increased. Silva et al. (2015) also observed that N intake increases linearly with milk supply, results that corroborate to the present study. We observed that N intake, retained N, and N efficiency increased according to the increase in milk intake. Regarding serum IGF-I, animals that consumed more milk had a higher amount of IGF-I (Table 6). Similar results were found by Smith et al. (2002) and Schäff et al. (2016), who attributed the high concentration of plasma IGF-I to the higher intake of nutrients.

In the mammary gland, animals that consumed 8L/d had greater fat deposition when compared to animals that consumed 4L/d, a result like that observed by Geiger et al. (2016). These authors observed that the concentration of fat in the mammary gland of calves subjected to intensified treatment with a milk replacer (1.13 kg/d of milk replacer containing 28% CP and 25% fat) was higher (667g) compared to calves submitted to restricted treatment (0.45 kg/d of milk replacer, 20% CP and 20% fat; 112 g). This fat accumulation may be directly related to the high ADG that, in turn, is supported by the level of energy consumed by the animal, which was higher in animals that consumed 8L/d.

The intake of DM, CP, EE, and OM from starter feed presented interaction between milk and age (Table S1). Overall, we observed an increase in intake according to age, which was less pronounced for 8 L/d animals (Figure 3). These results corroborate with several studies that evaluated the effect of milk supply on starter feed intake and observed that animals that consumed small volumes of milk had higher starter feed intake and stated that calves fed less milk probably try to compensate for the lack of nutrients by consuming more starter feed (Rosenberg et al., 2017; Fischer et al., 2019; Borderas et al., 2009). Demonstrating that the effect of providing larger amounts of milk on starter feed intake is associated with meeting nutrients requirements and high circulating glucose levels (Labussiere et al. 2011; Mirzaei et al., 2018). This relationship between glucose and milk intake was observed in the present study, where animals had higher levels of glucose in the pre-weaning period and a drop in these levels in the weaning and post-weaning periods (Figure 6A).

The NDFap digestibility was higher in 4 L/d animals on d 24, although the DMI from starter feed was similar among milk volumes at this age (Figure 3A). This result is probably related to the leakage of milk from the esophageal groove into the rumen, which could increase feed rumen scape. Martín-Alonso et al. (2019) show that the esophageal groove can transport 75-90% of the milk ingested to the abomasum, that is, 10-25% of the total milk ingested escapes to the rumen. Therefore, animals that consume larger amounts of milk would have a greater leakage of milk into the rumen, which would lead these animals to have a higher passage rate and, consequently, a lower fiber digestibility.

The better N efficiency of 6 and 8 L/d animals, when compared to the 4 L/d animals, is directly linked to the N intake, which drives the N retention (Zhang et al., 2021). Hence, animals that have a higher N intake tend to have better N efficiency, because when the intake is a high level, the animals have more N available for deposition (Silva et al., 2015). Animals with 6 L/d showed a N efficiency similar to 8 L/d animals, probably because they balanced the milk and starter feed intakes. Glucose concentration was lower in animals consuming 4 L/d on 66 and 73 d (Figure 6A), which was probably because the glucose concentration is driven by milk intake. Other studies have also reported higher blood glucose levels in calves fed high milk amounts (MacPherson et al., 2016; Mirzaei et al., 2018).

The BUN concentration was higher at 80 d for all milk volumes. Jafari et al. (2020) when evaluating the BUN concentration in calves fed a high supply of milk and textured starter feed at different ages, observed that the animals had an increase in BUN with increasing age (13.7 mg/dL on d 35, 22.9 mg/dL on d56, and 27 mg/dL on d70). Additionally, Khan et al. (2007) state that BUN is considered an indicator of rumen development in calves, which is why it is present in greater quantity at 80 d, when rumen development is more advanced. In addition, the higher protein intake due to the higher intake of solid feed and its ruminal degradation evolved in higher concentrations of ruminal ammonia and BUN.

At 66 d, animals that consumed fixed PB in starter feed had greater BW (73.1 kg) compared to treatments decreasing CP in starter feed (68.4 kg), which may be related to the lower amount of PB ingested by animals in the DCP treatment at 66 d. At post-weaning, the same behavior was observed for BW and HG, where FCP animals (82.7 kg for BW, and 103 cm for HG) had higher means compared to DCP animals (77.2 kg for BW, and 101 cm for HG). However, the EF of animals that consumed starter feed with decreasing CP was higher (655 g/kg) compared to animals that consumed starter feed with fixed CP (354 g/kg), indicating that the lower performances of DCP animals are related to a lower intake of starter feed in this group of animals.

Animals that consumed the fixed starter feed showed higher N intake and feces N excretion when compared to those that consumed the starter feed decreasing CP content, at 66 d. A similar result was found by Chapman et al. (2017), when feeding calves with milk replacer with different CP contents, they observed a higher N excretion in animals that fed MR with higher CP content. In opposite, post-weaning FE was higher for animals that received starter feed decreasing CP content (Table 3). This may be associated with a compensatory gain in this group of animals in the post-weaning, since at weaning the FE and ADG of these animals were numerically lower (293 g/kg for FE and 419 g/d for ADG, respectively) than those of the animals in the fixed starter feed (402 g/kg for FE, 558 g/d for ADG), the same pattern was observed by Taylor et al. (2020).

The albumin concentration was lower at 24 d, independently of the starter feed strategy. Albumin acts in the transport of nutrients and lipophilic hormones, as well as in the transport and distribution of lipids, including fatty acids (Scanes, 2015). Feitosa et al. (2001) quantified the serum proteins of calves up to one year of age and observed that the concentration of albumin has a gradual increase, which explains the lower amount of this protein in the plasma at 24 days. In addition, Elkhair (2021) observed lower serum albumin in younger animals, when observing concentrations in animals from birth to 28 d of age. The concentration of BUN was higher in all ages (except 45 d) for animals that consumed fixed starter feed CP content, with a more pronounced pattern in the advanced ages (Figure 6D). This can be explained by the higher CP content of the starter feed compared to the starter feed with CP decreasing from d 46. Stamey et al. (2021) observed similar results, when offering milk replacer with a higher CP content to Holstein calves they found an increase in BUN. At 24 d, it was expected that the result would be the opposite, since the animals receiving starter feed with decreasing CP content had access to a starter with greater CP. However, when observing the DMI from starter feed on d 24 we observed that the intake was lower for the animals with decreasing starter CP content (47.7 g/d) when compared to fixed starter feed CP content (57.6 g/d), which resulted in a higher BUN in the fixed starter feed animals at 24 d.

The ruminal degradation of proteins results in nitrogenous compounds, mainly ammonia (NASEM, 2021). Thus, if dietary rumen degradable protein increase, an increase in RAN is expected. The animals receiving fixed starter feed CP content were fed a higher protein diet in the final phase of the study when compared to treatments decreasing starter CP content. As soybean meal was the main CP source of the starter feed and considering that about 67% of the CP is rumen degradable protein (NASEM, 2021), consequently fixed starter feed animals had a higher amount of ruminal ammonia on d 66, 73, and 80. The animals in the FCP treatment had a lower pixel count when compared to the treatment with DS treatments, indicating a lower fat position. At 66 d, DCP animals had lower DMI compared to FCP, as mammary gland development is responsive to nutrient supply (Piantoni et al., 2012; Albino et al., 2015), DCP animals had worse results.

The DMI from starter feed in the treatment 4L_DS was numerically lower at 24 d (72.5g FCP and 67.3g DCP) and 45 d (239g FCP and 164g DCP), and statistically lower at 66 d (544g FCP and 313g DCP) when compared to 4L_FCP. The starter feed intake drives the concentration of ruminal VFA's (NASEM, 2021). Therefore, animals of 4L_FCP had higher concentrations of acetate, butyrate, and propionate. The BUN of the animals that consumed starter feed with decreasing CP content was higher for those that consumed 8L/d of milk when compared to other volumes of milk from the same starter feed. The same result was observed by Alimirzaei et al. (2020) who observed a higher concentration of BUN in animals fed high milk (6.20 mmol/mL) compared to low milk (5.42 mmol/mL) at 28 d, which may be associated with a higher intake of protein from milk in the animals of 8 L/d.

Animals receiving starter feed with decreased CP content had higher EE digestibility when compared to those that consumed fixed starter feed CP content. This may have happened because DMI was numerically lower at 45d (913 g/d FCP and 860 g/d DCP) and at 66d (1171 g/d FCP and 1062 g/d FCP) for animals on treatments with fixed starter feed, causing a lower rate of passage in the food, which caused a greater digestibility of EE (Bosa et al., 2012).

5. CONCLUSION

It can be concluded that animals receiving 6 L/d of milk have a good performance, often not differing from animals that consume 8L/d, indicating that it is an efficient feeding strategy that improves the efficiency of animals compared to smaller volumes and brings less damage to the animals at the time of weaning compared to larger volumes. Regarding the starter feed, animals submitted to the FCP strategy, higher consumption and better performance compared to animals DCP. However, DCP animals had lower fecal N excretion at 66 d, and a better FE in post-weaning compared FCP, proving to be an alternative to decrease N excretion and that there is a potential for improvement in animal performance with the use of this strategy that should be better explored.

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Table 1. Dietary ingredients used for starter feed and chemical composition of starter feed, silage, and milk used for calves.

Items[1]	Starter feed (% of CP)			Silage	Milk
	24%	18%	14%		
Ingredients, g/kg of DM					
Corn meal	555.3	706.5	832.5	-	-
Soybean meal	400.6	249.4	123.4	-	-
Wheat bran	26.4	26.4	26.4	-	-
Dicalcium Phosphate	2.9	2.9	2.9	-	-
Limestone	11.9	11.9	11.9	-	-
Sodium Chloride	2.7	2.7	2.7	-	-
Mineral Premix[2]	0.2	0.2	0.2	-	-
Chemical composition, g/kg of DM					
DM	841.5	872.4	867.7	310	125.5
OM	925.6	942.2	947.1	946	944.4
CP	242	165.4	126.3	70	222.3
EE	20.5	30.5	24.4	35.2	330.5
NDFap	134.6	119	109	493	-

[1] DM = dry matter, OM = organic matter, CP = crude protein, EE = ethereal extract, and NDFap = neutral detergent fiber corrected for ash and protein.

Table 2. Nutrient intake and apparent digestibility of dairy calves receiving 4, 6, or 8 L/d of milk plus fixed or decreasing CP starter feed at different ages.

	Milk (L/d)			SEM	SF[2]		SEM	Age (d)			SEM	*P*- value[3]			
Item[1]	4	6	8		FCP	DCP		24	45	66		M	S	A	M × S
Intake (g/d)															
DM	730c	961b	1082a	27.4	952	897	22.4	771c	886b	1116a	12.9	<0.001	0.585	<0.001	0.994
DM starter	233	223	134	22.9	225	196	18.8	53	159	378	6.2	0.201	0.766	<0.001	0.105
OM	689c	908b	1022a	25.9	899	847	21.2	728c	838b	1054a	12.1	<0.001	0.575	<0.001	0.992
OM starter	228	231	131	25.9	234	159	21.3	56	155	380	9.6	0.043	0.203	<0.001	0.515
CP	148c	198b	232a	5.3	199	186	4.3	172	189	217	3.3	<0.001	0.234	<0.001	0.898
CP starter	37	34	21	3.7	37	25	3	11	27	56	1.26	0.093	0.288	<0.001	0.243
EE	169c	254b	320a	4	252	244	3.3	243b	245ab	256a	3.4	<0.001	0.073	0.004	0.820
NDFap	30	27	17	2.9	28	21	2.3	9	21	44	1.1	0.99	0.462	<0.001	0.633
DE, Mcal/d	3.7	5.2	6.0	0.1	5.1	4.8	0.09	4.4	4.8	5.7	0.06	<0.001	0.446	<0.001	0.978
Digestibility (g/kg)															
DM	926b	939ab	945a	4.1	934	940	3.3	929b	941a	941a	3.4	0.015	0.318	0.016	0.089
CP	885b	920a	931a	4.9	908	915	4.1	908	915	912	3.4	<0.001	0.124	0.497	0.294
OM	940b	959a	963a	2.3	952	956	1.9	958a	956ab	949b	2.2	<0.001	0.124	0.01	0.348
EE	963	976	976	1.6	969	975	1.3	972	975	969	1.4	<0.001	<0.001	0.011	0.007
NDFap	710	666	635	22.9	685	656	19	597	685	730	19	0.255	0.19	<0.001	0.926

[1] DM = dry matter, DMs = dry matter from starter feed, OM = organic matter, OMs = organic matter from starter feed, CP = crude protein, CPs = crude protein from starter feed, EE = ethereal extract, and NDFap = neutral detergent fiber corrected for ash and protein, DE = digestible energy.

[2] SF = starter feed (where FCP is fixed starter feed and DCP is starter feed decreasing crude protein content).

[3] M = effect of milk volume, S = effect of starter feed type, A = effect of age, and M×S = interaction effect between milk volume and starter feed type.

Table 3. Performance, body measurements and feed efficiency of dairy calves receiving 4, 6, or 8 L/d of milk plus fixed or decreasing CP starter feed in pre-weaning.

	Milk (L/d)			SEM	SF[2]		SEM	Age (d)				SEM	*P*- value[3]			
Item[1]	4	6	8		FCP	DCP		4	24	45	66		M	S	A	M × S
BW (kg)	44.7	52.4	56.1	1.24	52.4	49.7	1.01	33.2	44.1	56.2	70.7	0.78	<0.001	0.056	<0.001	0.433
ADG (kg)	0.43c	0.63b	0.76a	0.02	0.63	0.58	0.01	-	0.54	0.58	0.69	0.02	<0.001	0.046	<0.001	0.489
HW (cm)	80.1	82.8	82.8	0.72	82.5	81.2	0.58	74	79.5	84.7	89.3	0.45	0.0209	0.140	<0.001	0.573
RH (cm)	84.9	86.8	87.1	0.68	86.9	85.6	0.57	78.3	83.7	89.2	93.9	0.44	0.0613	0.108	<0.001	0.244
HG (cm)	84.1	87.6	89.1	0.74	87.7	86.2	0.61	75.7	83.5	90.4	98.2	0.49	< 0.001	0.072	<0.001	0.174
BL (cm)	69.1	70.9	72.8	0.75	71.4	70.5	0.61	61.2	68	74.3	80.3	0.55	0.005	0.286	<0.001	0.748
FE (g/kg)	625b	697a	749a	1.91	692	688	1.64	-	-	-	-	-	<0.001	0.943	-	0.521

[1] BW = body weight, ADG = average daily gain, WH = withers height, RH = rump height, HG = Heart girth, BL = body length, and FE = feed efficiency.

[2] SF = starter feed (where FCP is fixed starter feed and DCP is starter feed decreasing crude protein content).

[3] M = effect of milk volume, S = effect of starter feed type, A = effect of age, and M×S = interaction effect between milk volume and starter feed type.

Table 4. Performance, body measurements and feed efficiency of dairy calves receiving 4, 6, or 8 L/d of milk plus fixed or decreasing CP starter feed in weaning and post-weaning.

	Milk (L/d)			SEM	SF[2]		SEM	*P*- value[3]		
Item[1]	4	6	8		FCP	DCP		M	S	M × S
Weaning										
BW (kg)	63.3b	76.7a	82.6a	1.94	77.4	71.2	1.64	<0.001	0.012	0.945
ADG (kg)	0.56ab	0.57a	0.32b	0.07	0.56	0.42	0.06	0.026	0.111	0.296
HW (cm)	88.8b	92.8a	92.6a	0.72	91.7	90.9	0.59	<0.001	0.335	0.696
RH (cm)	93b	95.6ab	97a	0.84	95.6	94.8	0.69	0.007	0.276	0.622
HG (cm)	95.6b	101a	102.7a	0.92	100.7	98.9	0.76	<0.001	0.098	0.835
BL (cm)	78.6b	83.8a	85.1a	1.07	83.1	81.9	0.87	<0.001	0.355	0.973
FE (g/kg)	514a	382ab	146b	8.83	402	293	7.63	0.018	0.36	0.275
Post-Weaning										
BW (kg)	68.4b	83.6a	88a	2.15	82.7	77.2	1.75	<0.001	0.033	0.931
ADG (kg)	0.68b	0.984a	0.75ab	0.083	0.836	0.776	0.069	0.039	0.53	0.877
HW (cm)	90.1b	93.9a	93.6a	0.73	93.3	91.8	0.6	0.002	0.088	0.615
RH (cm)	94.2b	97.4a	97.9a	0.76	97.1	95.9	0.62	0.004	0.192	0.557
HG (cm)	97.4b	103.8a	104.8a	0.91	103	101	0.74	<0.001	0.023	0.717
BL (cm)	80.1b	85.1a	87a	1.05	84.7	83.3	0.85	<0.001	0.271	0.944
FE (g/kg)	508	453	568	1.1	364b	655a	9.31	0.741	0.029	0.78

[1] BW = body weight, ADG = average daily gain, WH = withers height, RH = rump height, HG = Heart girth, BL = body length, and FE = feed efficiency.

[2] SF = starter feed (where FCP is fixed starter feed and DCP is starter feed decreasing crude protein content).

[3] M = effect of milk volume, S = effect of starter feed type, A = effect of age, and M×S = interaction effect between milk volume and starter feed type.

Table 5. Nitrogen balance of dairy calves receiving 4, 6, or 8 L/d of milk plus fixed or decreasing CP starter feed at different ages.

	Milk (L/d)			SEM	SF[1]		SEM	Age (d)			SEM	*P*- value[2]			
Item	4	6	8		FCP	DCP		24	45	66		M	S	A	M × S
N balance (g/d)															
Intake	23.6c	31.8b	36.3a	1.01	31.8	29.3	0.82	27.3	29.7	34.8	0.60	<0.001	0.362	<0.001	0.990
Urine excretion	2.6a	2.1b	2.4ab	0.13	2.4	2.3	0.10	2.1b	2.4ab	2.5a	0.10	0.030	0.818	0.025	0.061
Feces excretion	1.5a	1.2b	1.2b	0.09	1.4	1.2	0.07	1.1	1.2	1.5	0.07	0.039	0.088	<0.001	0.614
Retained	15.6c	25.4b	29.4a	1.08	24.7	22.2	0.88	20.2c	22.4b	27.8a	0.64	<0.001	0.243	<0.001	0.982
Efficiency (g/kg)	640b	788a	803a	13	752	736	10	713b	734b	784a	10	<0.001	0.214	<0.001	0.756

[1] SF = starter feed (where FCP is fixed starter feed and DCP is starter feed decreasing crude protein content).

[2] M = effect of milk volume, S = effect of starter feed type, A = effect of age, and M×S = interaction effect between milk volume and starter feed type.

Table 6. Blood and Ruminal parameters of dairy calves receiving 4, 6, or 8 L/d of milk plus fixed or decreasing CP starter feed at different ages.

	Milk (L/d)			SEM	SF[1]		SEM	Age (d)					SEM	*P*- value[2]			
Item	4	6	8		FCP	DCP		24	45	66	73	80		M	S	A	M × S
Blood Parameters																	
IGF-1 (ng/mL)	82b	124a	147a	10.1	128	108	8.2	88a	114b	154c	116b	-	7.4	0.001	0.055	<0.001	0.36
Glucose (mg/dL)	102	109	110	2.4	106	108	2.1	111	115	114	99	93	1.4	0.049	0.635	<0.001	0.867
BUN (mg/dL)	18	16	21	0.7	22	16	0.6	19	18	15	17	24	0.5	<0.001	<0.001	<0.001	0.001
Total protein (g/dL)	5.7	5.7	5.7	0.73	5.7	5.7	0.06	5.7	5.6	5.6	5.8	5.9	0.06	0.962	0.905	<0.001	0.4
Albumin (g/dL)	2.9	3	3	0.39	2.9	3	0.03	2.9	3	3	3	3.1	0.03	0.506	0.177	<0.001	0.793
Ruminal Parameters																	
pH	5.1	5.1	5.2	0.07	5.1	5.1	0.06	5.2	5.2	5.1	5.1	5.2	0.05	0.497	0.706	0.859	0.303
RAN (mg/L)	96a	73b	98a	7.9	105	73	6.5	116	106	94	64	66	5.6	0.009	<0.001	<0.001	0.998
Acetate (µMol/mL)	37.4	34.3	34.2	2.06	38.2	32.3	1.68	27.6b	31.5b	40.1a	42a	-	2.08	0.351	0.018	<0.001	0.04
Propionate(µMol/mL)	19.5	18.5	18.4	1.55	21.7	15.9	1.26	14.6b	17.6ab	21.5a	21.5a	-	1.35	0.796	0.002	<0.001	0.015
Butyrate (µMol/mL)	7.5	6.8	8.7	0.78	8.0	7.3	0.64	6.2b	6.9b	7.5ab	10.2a	-	0.79	0.243	0.502	0.001	0.036

[1] SF = starter feed (where FCP is fixed starter feed and DCP is starter feed decreasing crude protein content).

[2] M = effect of milk volume, S = effect of starter feed type, A = effect of age, and M×S = interaction effect between milk volume and starter feed type.

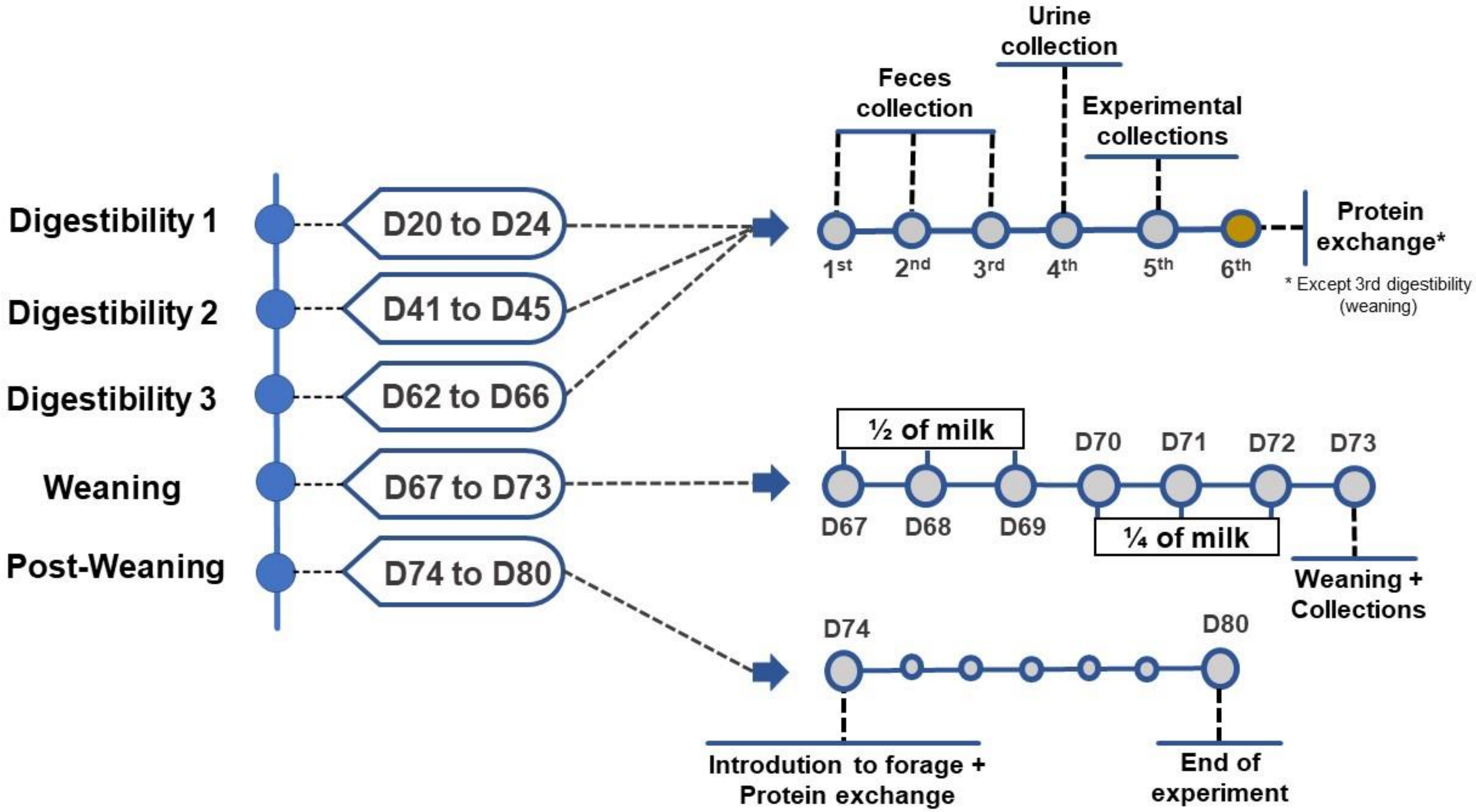

Figure 1. Summary of the experimental design and main collections carried out during the experiment.

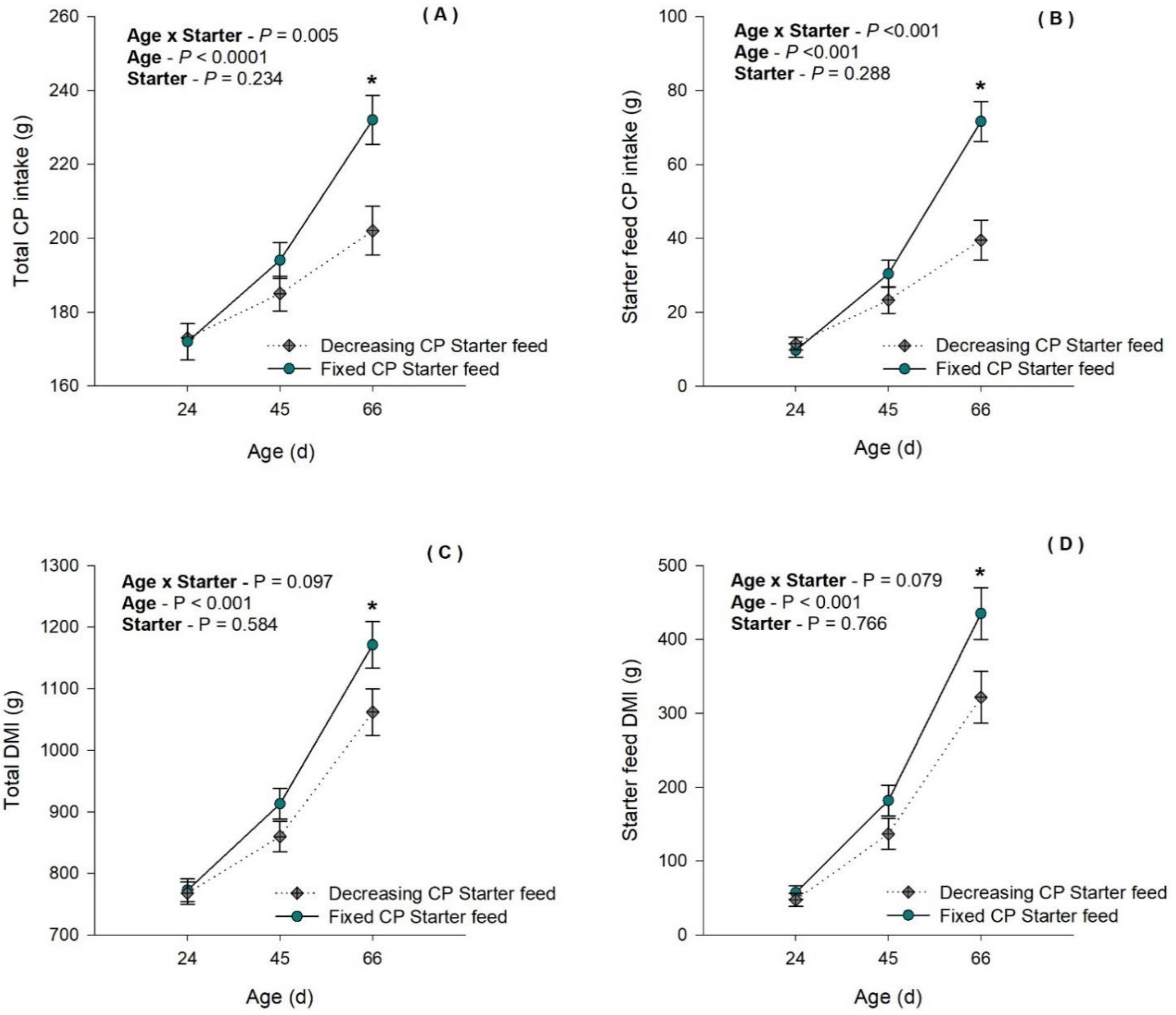

Figure 2. Interaction effect between age and starter feed strategy on total DMI, DMI from starter feed, CP intake, and CP intake from starter feed. The asterisk indicates differences between starter feed strategy within d of evaluation.

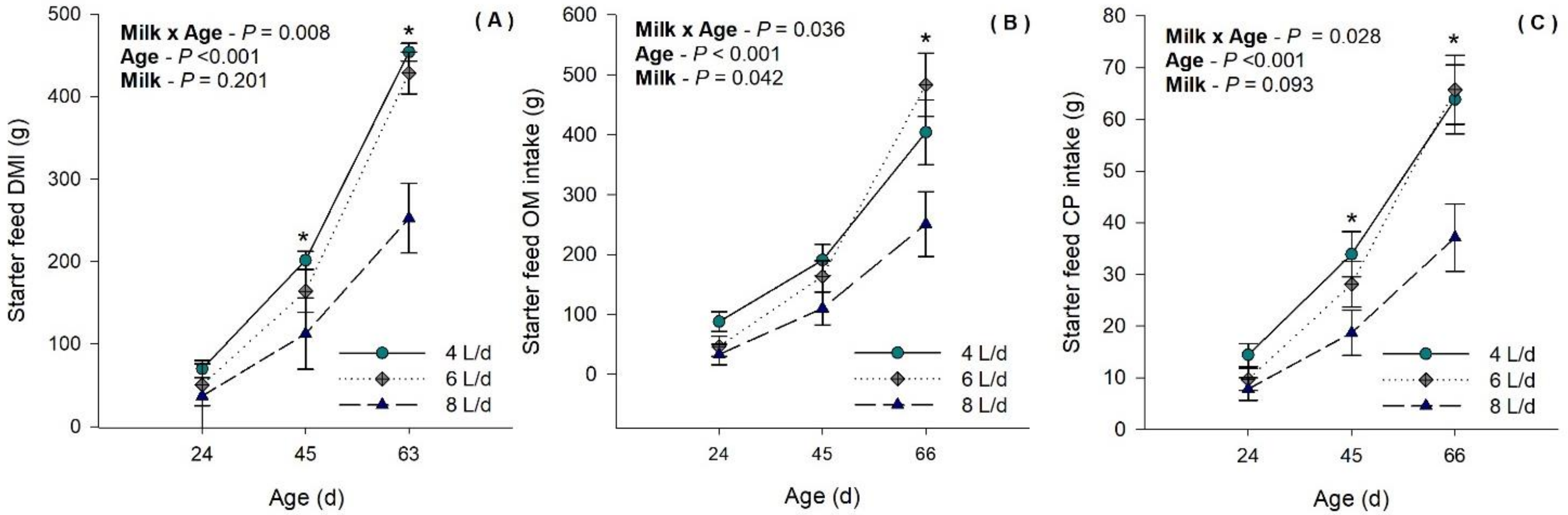

Figure 3. Interaction effect between age and milk on DMI from starter feed, OM intake from starter feed, and CP from starter feed. The asterisk indicates differences between milk strategy within d of evaluation.

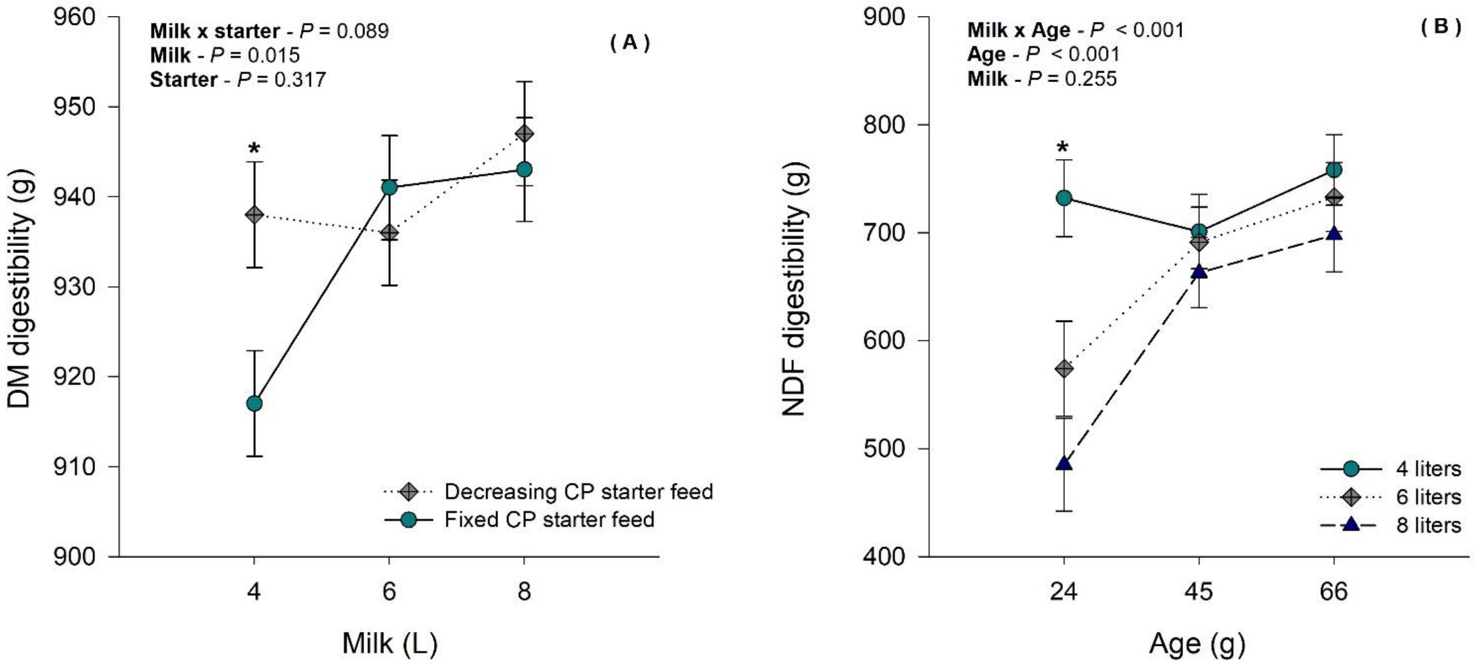

Figure 4. Interaction effect between age and milk and between age and starter feed on DM and NDF digestibility. The asterisk indicates differences between milk strategy within starter feed strategy (A) and between milk strategy within d of evaluation (B).

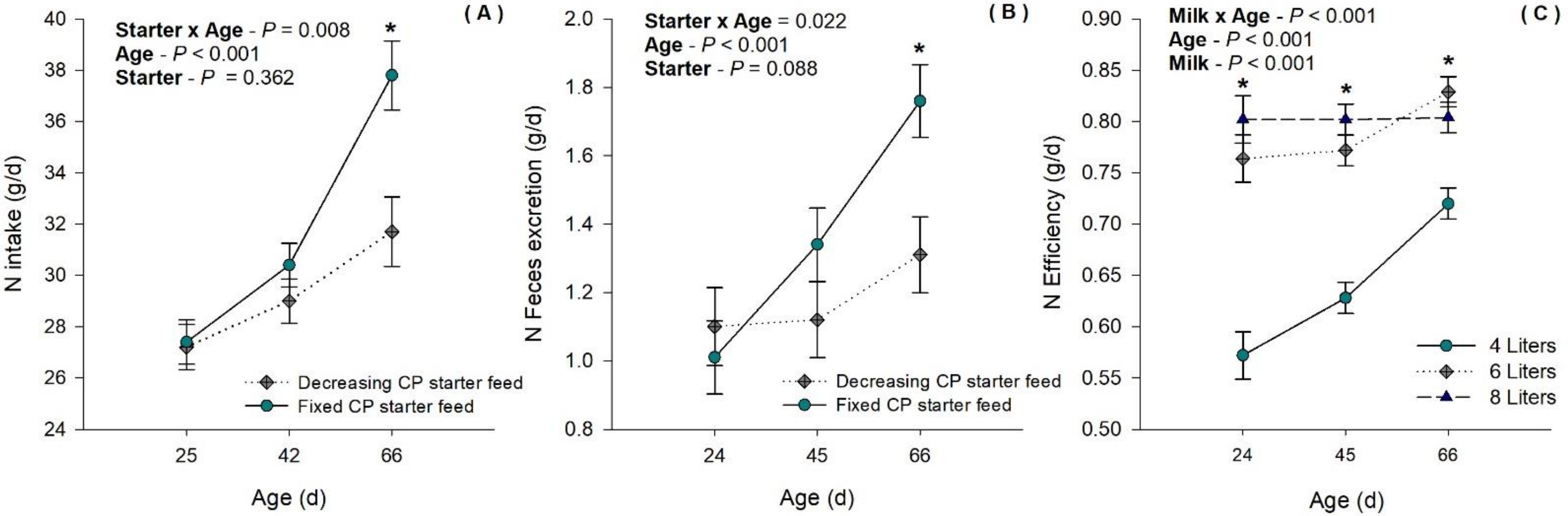

Figure 5. Interaction effect between age and starter feed and between age and milk on N intake, N feces excretion and N efficiency. The asterisk indicates differences between starter feed strategy within d of evaluation (A and B) and between milk strategy within d of evaluation (C).

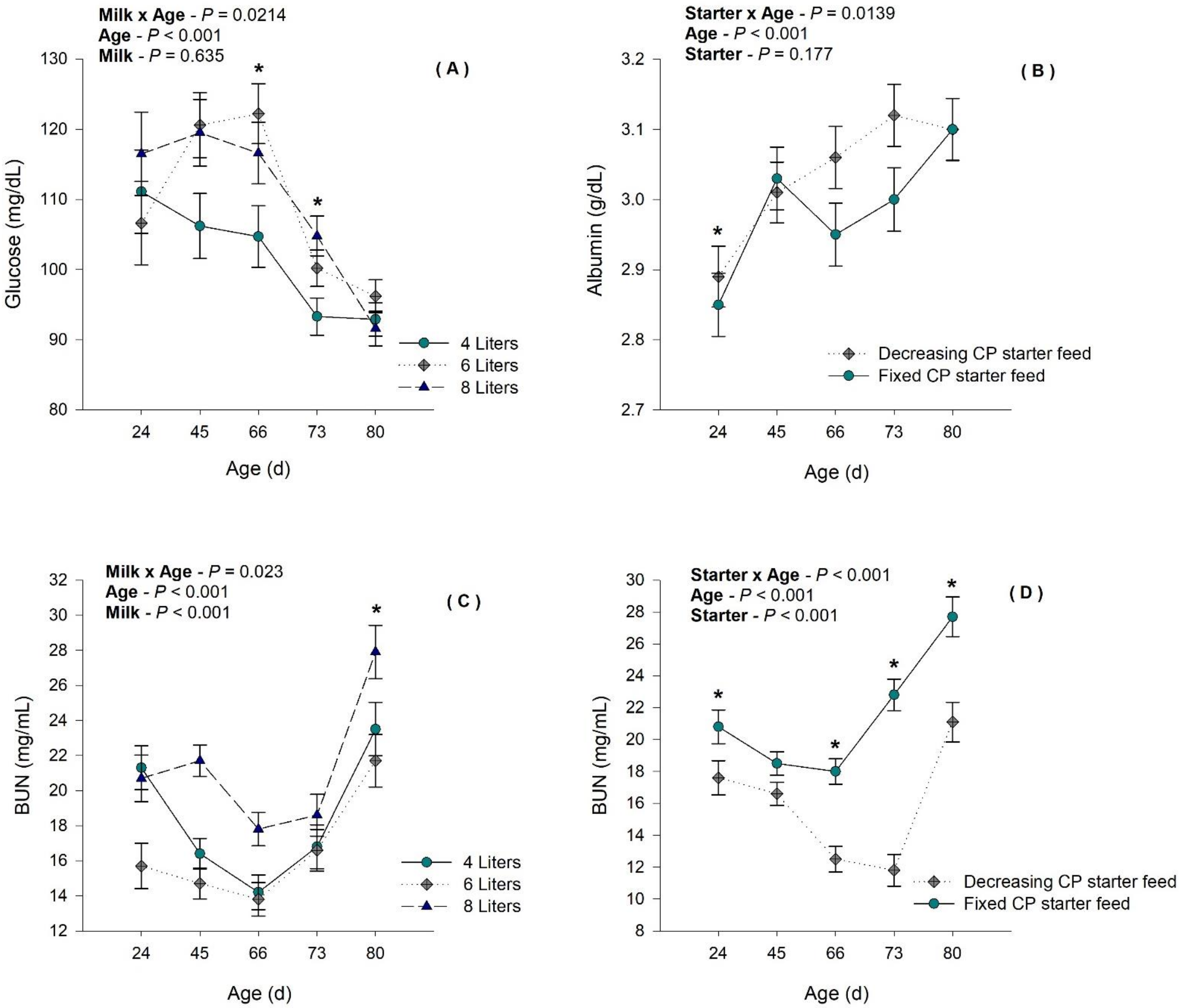

Figure 6. Interaction effect between age and starter feed and between age and milk on serum glucose, albumin, and BUN concentrations. The asterisk indicates differences between milk volumes within d of evaluation (A and C) and between starter feed strategy within d of evaluation (B and D).

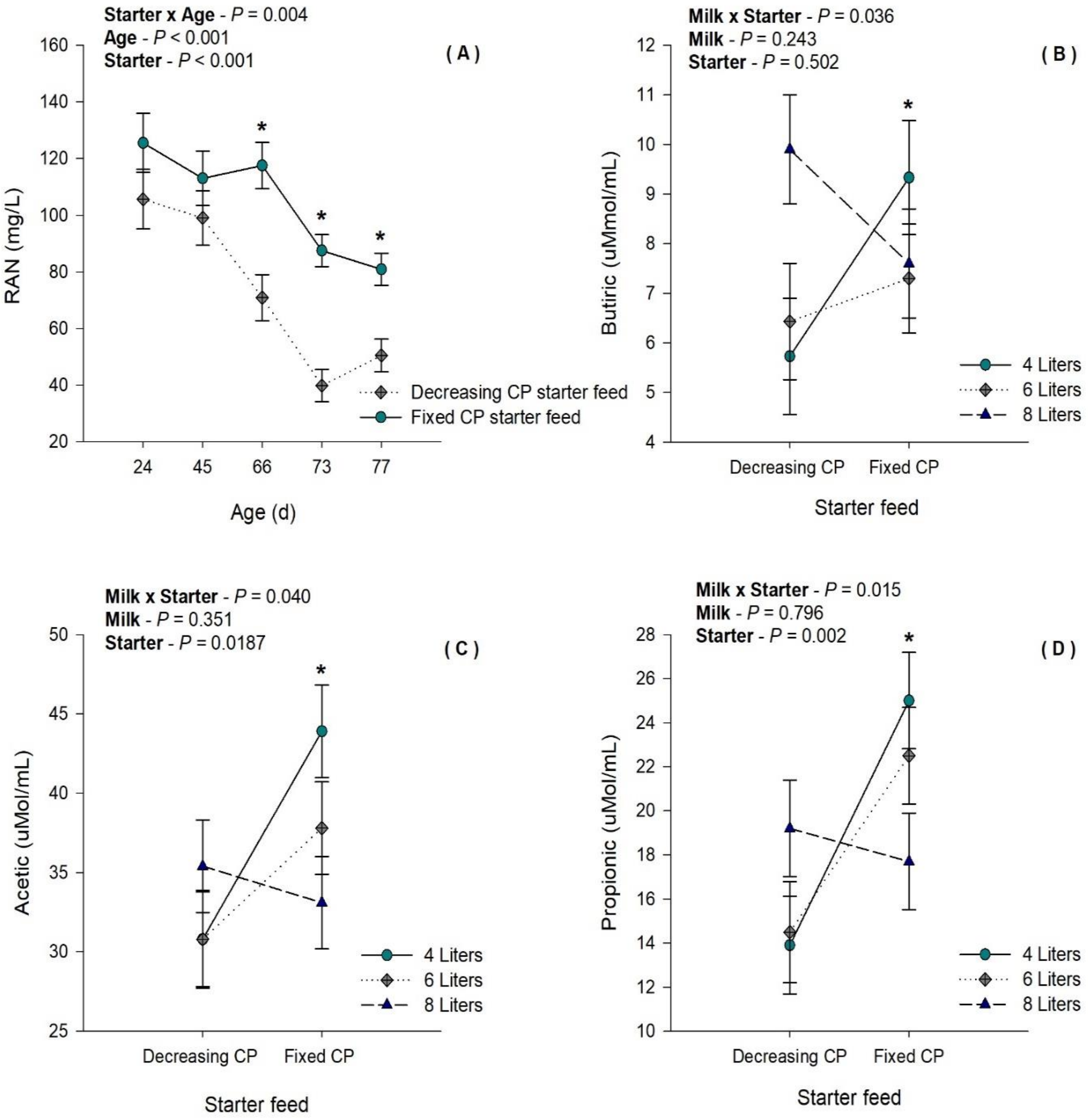

Figure 7. Interaction effect between age and starter feed and between milk and starter feed on RAN and acetic, butyric, and propionic acids. The asterisk indicates differences between starter feed strategy within d of evaluation (A) and between milk volumes within starter feed strategy (B, C, and D).

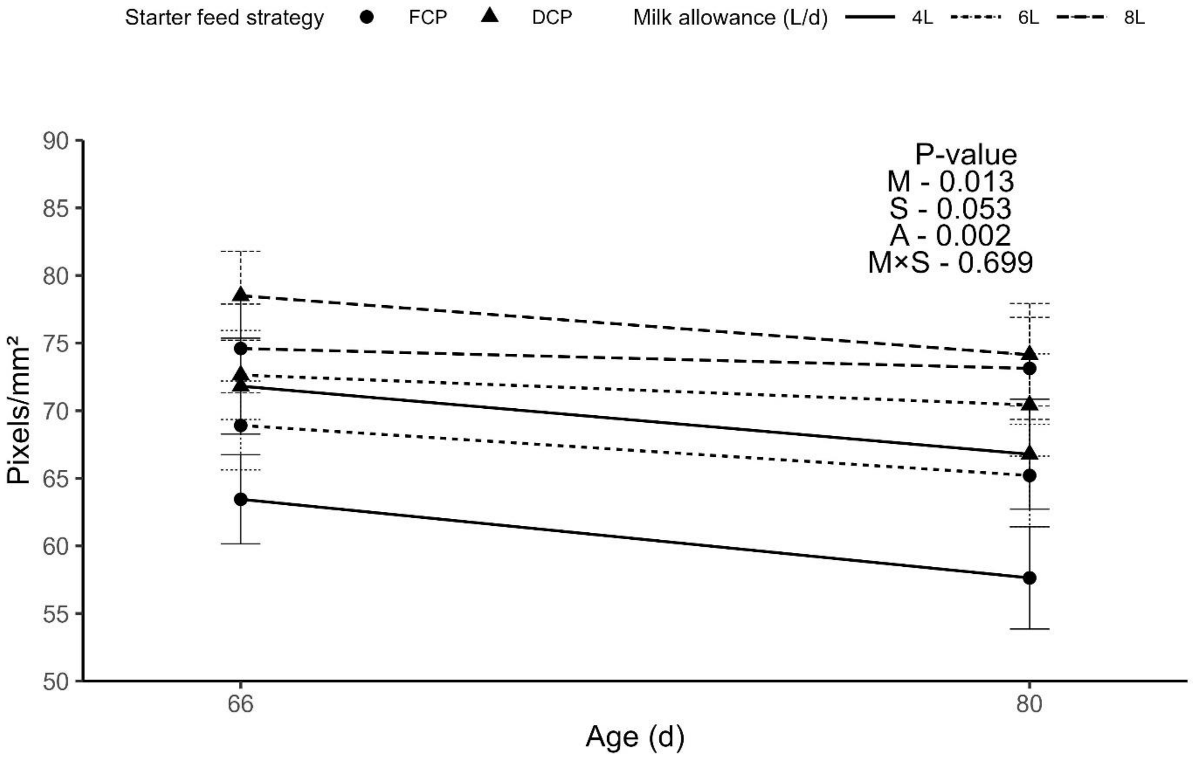

Figure 8. Effect milk allowance, starter feed strategy and age on the pixels concentration in the parenchyma of the mammary gland.

Supplementary Table S1. Treatments means of nutrient intake and apparent digestibility of dairy calves receiving 4, 6, or 8 L/d of milk plus fixed or decreasing CP starter feed.

	Treatment[2]						*P*- value[3]						
Item[1]	4L_FCP	6L_FCP	8L_FCP	4L_DCP	6L_DCP	8L_DCP	M	S	A	M × S	M × A	S × A	M × S × A
Intake (g/d)													
DM	779	967	1111	681	956	1054	<0.001	0.585	<0.001	0.994	0.094	0.097	0.189
DMs	285	230	159	185	216	109	0.201	0.766	<0.001	0.105	0.008	0.079	0.157
OM	735	913	1049	643	903	996	<0.001	0.575	<0.001	0.992	0.092	0.111	0.191
OMs	284	258	161	204	172	102	0.042	0.203	<0.001	0.514	0.036	0.089	0.751
CP	156	202	239	140	194	226	<0.001	0.234	<0.001	0.898	0.391	0.005	0.309
CPs	47.2	38.3	26.2	30.7	27.5	16.2	0.093	0.288	<0.001	0.243	0.028	<0.001	0.122
EE	172	258	326	167	251	315	<0.001	0.073	0.004	0.82	0.896	0.531	0.189
NDFap	37	27	21	23	26	14	0.990	0.462	<0.001	0.633	0.002	0.008	0.027
DE, Mcal/d	3.9	5.2	6.1	3.5	5.1	5.9	<0.001	0.446	<0.001	0.978	0.419	0.221	0.121
Digestibility (g/kg)													
DM	917	941	943	936	936	947	0.015	0.318	0.016	0.089	0.142	0.403	0.794
CP	876	922	927	893	917	936	<0.001	0.1241	0.497	0.294	0.516	0.689	0.914
OM	936	960	962	945	959	965	<0.001	0.124	0.01	0.348	0.112	0.254	0.712
EE	956	974	975	970	978	977	<0.001	<0.001	0.011	0.007	0.196	0.018	0.04
NDFap	722	691	642	699	641	628	0.255	0.19	<0.001	0.926	<0.001	0.544	0.812

[1] DM = dry matter, OM = organic matter, CP = crude protein, EE = ethereal extract, and NDFap = neutral detergent fiber corrected for ash and protein.

[2] 4L_FCP = 4 liters of milk and fixed starter feed crude protein content, 6L_FCP = 6 liters of milk and fixed starter feed crude protein content, 8L_FCP = 8 liters of milk and fixed starter feed crude protein content, 4L_DCP = 4 liters of milk and decreasing starter feed crude protein content, 6L_DCP = 6 liters of milk and decreasing starter feed crude protein content, and 8L_DCP = 8 liters of milk and decreasing starter feed crude protein content.

[3] M = effect of milk volume, S = effect of starter feed type, A = effect of age, and M×S = interaction effect between milk volume and starter feed type, M×A = interaction effect between milk volume and age, S×A = interaction effect between starter feed type and age, and M×S×A = interaction effect between milk volume, starter feed type, and age.

Supplementary Table S2. Treatments means of performance and feed efficiency of dairy calves receiving 4, 6, or 8 L/d of milk plus conventional or decreasing CP starter feed.

	Treatments[2]						*P*- value[3]						
Item[1]	4L_FCP	6L_FCP	8L_FCP	4L_DCP	6L_DCP	8L_DCP	M	S	A	M × S	M × A	S × A	M × S × A
BW (kg)	45.0	54.9	57.3	44.5	49.8	54.9	<0.001	0.056	<0.001	0.433	<0.001	0.020	0.253
ADG (kg)	0.477	0.633	0.783	0.389	0.627	0.748	<0.001	0.046	<0.001	0.489	0.747	0.096	0.292
HW (cm)	80.5	84.1	83.0	79.6	81.5	82.6	0.0209	0.140	<0.001	0.573	<0.001	0.717	0.891
RH (cm)	84.9	88.4	87.4	84.9	85.2	86.7	0.0613	0.108	<0.001	0.244	0.016	0.704	0.267
HG (cm)	83.8	89.4	89.9	84.3	85.9	88.3	< 0.001	0.072	<0.001	0.174	<0.001	0.294	0.829
BL (cm)	69.1	71.7	73.4	69.1	70.7	72.2	0.005	0.286	<0.001	0.748	0.002	0.147	0.421

[1] BW = body weight, ADG = average daily gain, WH = withers height, RH = rump height, HG = Heart girth, BL = body length, and FE = feed efficiency.

[2] 4L_CS = 4 liters of milk and conventional starter feed, 6L_CS = 6 liters of milk and conventional starter feed, 8L_CS = 8 liters of milk and conventional starter feed, 4L_DS = 4 liters of milk and decreasing starter feed crude protein content, 6L_DS = 6 liters of milk and decreasing starter feed crude protein content, 8L_DS = 8 liters of milk and decreasing starter feed crude protein content.

[3] M = effect of milk volume, S = effect of starter feed type, A = effect of age, and M×S = interaction effect between milk volume and starter feed type, M×A = interaction effect between milk volume and age, S×A = interaction effect between starter feed type and age, M×S×A = interaction effect between milk volume, starter feed type and age.

Supplementary Table S3. Treatments means of biometric measures of dairy calves receiving 4, 6, or 8 L/d of milk plus conventional or decreasing CP starter feed.

	Treatments[2]					
Item[1]	4L_FCP	6L_FCP	8L_FCP	4L_DCP	6L_DCP	8L_DCP
Weaning						
BW (kg)	66.9	79.4	85.8	59.6	74.0	80.0
ADG (kg)	0.727	0.576	0.370	0.411	0.569	0.278
HW (cm)	89.2	93.6	92.3	88.4	91.9	92.3
RH (cm)	93.3	97.1	97.1	92.8	94.7	96.8
HG (cm)	96.3	102.4	103.4	95.0	99.7	102.0
BL (cm)	79.3	84.3	85.6	83.2	77.8	84.7
Post-Weaning						
BW (kg)	71.8	85.9	90.5	65.0	81.2	85.5
ADG (kg)	0.689	0.931	0.706	0.678	1.037	0.793
HW (cm)	90.4	95.2	94.2	89.9	92.6	92.9
RH (cm)	94.5	98.7	98.0	93.8	96.2	97.8
HG (cm)	98.0	105.5	106.1	96.7	102.0	103.5
BL (cm)	80.9	85.5	87.8	79.2	84.7	86.1

[1] BW = body weight, ADG = average daily gain, WH = withers height, RH = rump height, HG = Heart girth, BL = body length, and FE = feed efficiency.

[2] 4L_CS = 4 liters of milk and conventional starter feed, 6L_CS = 6 liters of milk and conventional starter feed, 8L_CS = 8 liters of milk and conventional starter feed, 4L_DS = 4 liters of milk and decreasing starter feed crude protein content, 6L_DS = 6 liters of milk and decreasing starter feed crude protein content, 8L_DS = 8 liters of milk and decreasing starter feed crude protein content.

Supplementary Table S4. Treatments means of nitrogen balance of dairy calves receiving 4, 6, or 8 L/d of milk plus fixed or decreasing CP starter feed.

	Treatment[1]						*P*- value[2]						
Item[1]	4L_FCP	6L_FCP	8L_FCP	4L_DCP	6L_DCP	8L_DCP	M	S	A	M×S	M × A	S × A	M × S × A
N balance (g/d)													
Intake	25.1	33.2	37.2	22.1	30.5	35.4	<0.001	0.362	<0.001	0.990	0.289	0.008	0.75
Urine excretion	2.9	1.9	2.4	2.3	2.3	2.4	0.030	0.818	0.025	0.061	0.595	0.133	0.435
Feces excretion	1.6	1.2	1.3	1.3	1.1	1.1	0.039	0.088	<0.001	0.614	0.101	0.022	0.388
Retained	16.9	27	30.1	14.2	23.8	28.7	<0.001	0.243	<0.001	0.982	0.161	0.064	0.235
Efficiency (g/kg)	653	803	799	627	773	806	<0.001	0.214	<0.001	0.756	<0.001	0.777	0.329

[1] 4L_FCP = 4 liters of milk and fixed starter feed crude protein content, 6L_FCP = 6 liters of milk and fixed starter feed crude protein content, 8L_FCP = 8 liters of milk and fixed starter feed crude protein content, 4L_DCP = 4 liters of milk and decreasing starter feed crude protein content, 6L_DCP = 6 liters of milk and decreasing starter feed crude protein content, and 8L_DCP = 8 liters of milk and decreasing starter feed crude protein content.

[2] M = effect of milk volume, S = effect of starter feed type, A = effect of age, and M×S = interaction effect between milk volume and starter feed type, M×A = interaction effect between milk volume and age, S×A = interaction effect between starter feed type and age, M×S×A = interaction effect between milk volume, starter feed type and age.

Supplementary Table S5. Treatments means of blood and Ruminal parameters of dairy calves receiving 4, 6, or 8 L/d of milk plus cfixed or decreasing CP starter feed.

	Treatment[1]						*P*- value[2]						
Item	4L_FCP	6L_FCP	8L_FCP	4L_DCP	6L_DCP	8L_DCP	M	S	A	M × S	M × A	S × A	M × S × A
Blood Parameters													
IGF-1 (ng/mL)	98	138	148.5	66.7	109.9	146.7	0.001	0.055	<0.001	0.36	0.378	0.627	0.073
Glucose (mg/dL)	99	108	109	104	110	110	0.049	0.635	<0.001	0.867	0.021	0.317	0.506
BUN (mg/dL)	22.7	20	22	14.2	12.9	20.7	<0.001	<0.001	<0.001	0.001	0.023	<0.001	0.107
Total protein (g/dL)	5.76	5.83	5.65	5.66	5.65	5.76	0.962	0.905	<0.001	0.4	0.135	0.22	0.321
Albumin (g/dL)	2.96	2.98	3.01	2.97	3.07	3.07	0.506	0.177	<0.001	0.793	0.595	0.013	0.61
Ruminal Parameters													
PH	4.97	5.2	5.25	5.18	5.2	5.16	0.497	0.706	0.859	0.303	0.894	0.6	0.161
NH3 (mg/L)	113.7	85	115.9	78.8	60.3	80.5	0.009	<0.001	<0.001	0.998	0.759	0.004	0.881
Acetic (µMol/mL)	43.9	37.8	33.1	30.8	30.8	35.4	0.351	0.018	<0.001	0.04	0.14	0.483	0.409
Propionic (µMol/mL)	25	22.5	17.7	13.9	14.5	19.2	0.796	0.002	<0.001	0.015	0.359	0.606	0.16
Butiric (µMol/mL)	9.33	7.3	7.6	5.73	6.43	9.9	0.243	0.502	0.001	0.036	0.8	0.497	0.722

[1] 4L_FCP = 4 liters of milk and fixed starter feed crude protein content, 6L_FCP = 6 liters of milk and fixed starter feed crude protein content, 8L_FCP = 8 liters of milk and fixed starter feed crude protein content, 4L_DCP = 4 liters of milk and decreasing starter feed crude protein content, 6L_DCP = 6 liters of milk and decreasing starter feed crude protein content, and 8L_DCP = 8 liters of milk and decreasing starter feed crude protein content.

[2] M = effect of milk volume, S = effect of starter feed type, A = effect of age, and M×S = interaction effect between milk volume and starter feed type, M×A = interaction effect between milk volume and age, S×A = interaction effect between starter feed type and age, M×S×A = interaction effect between milk volume, starter feed type and age.