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Effects of reducing crude protein content in starter feed with different milk allowances on the performance and nitrogen balance in dairy calves

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ABSTRACT

Associate milk allowance and starter feed for dairy calves to achieve high performance and adequate nitrogen (N) balance can be challenging. Thus, this study aimed to evaluate the effects of reducing the CP content of the starter feed on the performance, mammary gland development, and N balance of dairy calves in the preweaning, weaning, and postweaning phases. The study was conducted according to a completely randomized design in a 3 × 2 factorial scheme, using 3 milk volumes (4, 6, or 8 L/d) and 2 strategies for starter feed supply (fixed or decreasing CP content). The first strategy consisted of feeding the animals a fixed starter feed with 18% CP (DM basis) throughout the preweaning phase (from 4 to 73 d), and in the second strategy, the animals were fed a starter feed with 24% CP (DM basis) from 4 to 24 d of age, 18% CP (DM basis) from 25 to 45 d of age, and 14% CP (DM basis) from 46 to 73 d of age. Therefore, 60 female crossbred calves were distributed through the 6 treatments formed: 4 L/d of milk and starter feed with fixed CP (FCP) content; 6 L/d of whole milk + FCP; 8 L/d of whole milk + FCP; 4 L/d whole milk and starter feed with decreasing CP (DCP) content; 6 L/d whole milk + DCP; 8 L/d milk + DCP. Calves receiving DCP had lower intake and fecal excretion on d 66. Moreover, younger calves (24 d old) had higher N utilization efficiency than calves at 45 and 66 d old, but when fed 8 L/d of milk, all calves had the similar N-use efficiency regardless of age. Additionally, calves receiving 8 L/d of milk had higher N intake and retention and lower N excretion. In the preweaning phase, animals fed FCP showed higher performance. In the weaning phase, higher ADG and feed efficiency were observed in animals fed 4 L/d whole milk compared with 8 L/d whole milk, and calves fed FCP had a higher BW. In the postweaning period, calves in the DCP group showed better feed efficiency, but animals fed FCP had higher ruminal ammonia-N and propionate concentrations. Moreover, calves on 4 and 6 L/d and FCP had higher blood urea N levels. Finally, for the mammary gland, age affected area, perimeter, circularity, and eccentricity, which increased in size during the 80 d of the trial. Female calves receiving 4 L/d of whole milk had a smaller area than the ones with 6 L/d, and, for perimeter, 4 L/d treatment promoted reduced parenchyma growth compared with 6 and 8 L/d treatment. To conclude, animals receiving 6 L/d of whole milk showed good performance, mammary parenchyma growth, greater N efficiency, and reduced N excretion. The FCP strategy improved performance, whereas the DCP strategy increased feed efficiency postweaning and tended to reduce fecal N excretion. However, it should be noted that this reduction was modest at the individual level, and the use of multiple starters on the farm should be carefully evaluated.
Key words: decreasing protein, Holstein × Gyr, mammary gland, nitrogen excretion

INTRODUCTION

Due to growing environmental concerns, livestock systems are under increasing pressure to improve efficiency (FAO, 2022). Dairy farms are sources of pollution because fecal and urinary nitrogen (N) losses contribute to atmospheric ammonia emissions, nitrate contamination of water, and economic losses due to inefficient N use (Tamminga, 1992; Appuhamy et al., 2011; Rodrigues et al., 2022). Dairy calves could become more efficient and environmentally sustainable if diets are adjusted to age. Efficiency improvements should include an increase in the performance of these animals in the preweaning, while reducing dietary CP intake in the preweaning phase could be a strategy to increase animal performance and reduce N excretion.

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

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

Item[1]	Starter feed (% CP)			Corn silage	Milk
	High (24%)	Medium (18%)	Low (14%)		
Ingredients (g/kg of DM)					
Corn meal	555	706	832	—	—
Soybean meal	401	249	123	—	—
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]	1.2	1.2	1.2	—	—
Chemical composition (g/kg DM)					
DM	841	872	868	310	125
OM	926	942	947	946	944
CP	242	165	126	70.0	222
EE	20.5	30.5	24.4	35.2	330
NDFap	135	119	109	493	—
Starch	463	555	632	272	—

[1]EE = ether extract; NDFap = NDF-corrected for ash and protein.

[2]Content (g/kg): zinc sulfate, 180; copper sulfate, 150; cobalt sulfate, 10; sodium selenite, 10; potassium iodate, 10.

Despite common use of a single starter formulation containing 18% to 22% CP (NASEM, 2021), several studies have reported greater starter intake and growth when higher-CP starters are provided in early life. Stamey Lanier et al. (2021) reported higher growth rates when calves were fed a 25% CP starter compared with 19% CP. Similarly, Kazemi-Bonchenari et al. (2022) observed improvements in intake and performance of calves receiving a 23% CP starter compared with 18% CP. These results likely reflect the elevated protein requirement of young animals, which is necessary for the accelerated synthesis of muscle tissue (Silva et al., 2017; Marcondes and Silva, 2021).

Milk allowance must also be considered when defining starter CP concentration, as it affects both nutrient supply and voluntary starter intake (Silva et al., 2015, 2019). Greater milk intake can meet requirements for high performance (Labussière et al., 2011) but suppresses starter feed intake (Rosenberger et al., 2017). Therefore, precision-feeding strategies should be designed to match the protein requirements of calves at each stage of development, balancing milk allowance with starter CP concentration (Stamey Lanier et al., 2021).

Considering the equations proposed by Silva et al. (2017, 2019), starter CP should gradually decrease with age rather than have a fixed CP content. For an animal receiving 4 L/d of milk, the starter CP should be ~24% from d 4 to 24, 18% from d 25 to 45, and 14% from d 46 to 66.

We hypothesized that decreasing the starter CP according to age (**DCP**) would improve performance and reduce N excretion compared with a fixed CP starter (**FCP**), dependent on milk allowance and persisting in the postweaning phase. The objective was to evaluate the effects of reducing starter CP on performance and N balance of dairy calves in the preweaning, weaning, and postweaning phases.

MATERIALS AND METHODS

The study was conducted at the José Henrique Bruschi Experimental Field of Embrapa Dairy Cattle (Minas Gerais, Brazil) and approved by the Institutional Ethics Committee for Animal Welfare and Use under the number 4422240120.

Experimental Design and Treatments

Calves were produced using a fixed-time artificial insemination protocol, which concentrated calvings within a 10-d period and allowed allocation of animals into a completely randomized design in a 3 × 2 factorial arrangement, with 3 whole milk allowances (4, 6, or 8 L/d) and 2 strategies for starter feed supply (fixed or decreasing CP content, on a DM basis). The first strategy consisted of feeding a single starter feed with 18% CP throughout the entire preweaning phase (from 4 to 73 d). The second strategy consisted of feeding starter feeds with 24% CP from d 4 to 24, 18% CP from d 25 to 45, and 14% CP from d 46 to 73 (Table 1).

Therefore, 6 treatments were formed: 4 L/d of milk and fixed CP starter (**4L_FCP**); 6 L/d of milk and fixed CP starter (**6L_FCP**); 8 L/d of milk and fixed CP starter (**8L_FCP**); 4 L/d of milk and decreasing CP starter (**4L_DCP**); 6 L/d milk and decreasing CP starter (**6L_DCP**); and 8 L/d milk and decreasing CP starter (**8L_DCP**; Figure 1). Ten animals were used per treatment, totaling 60

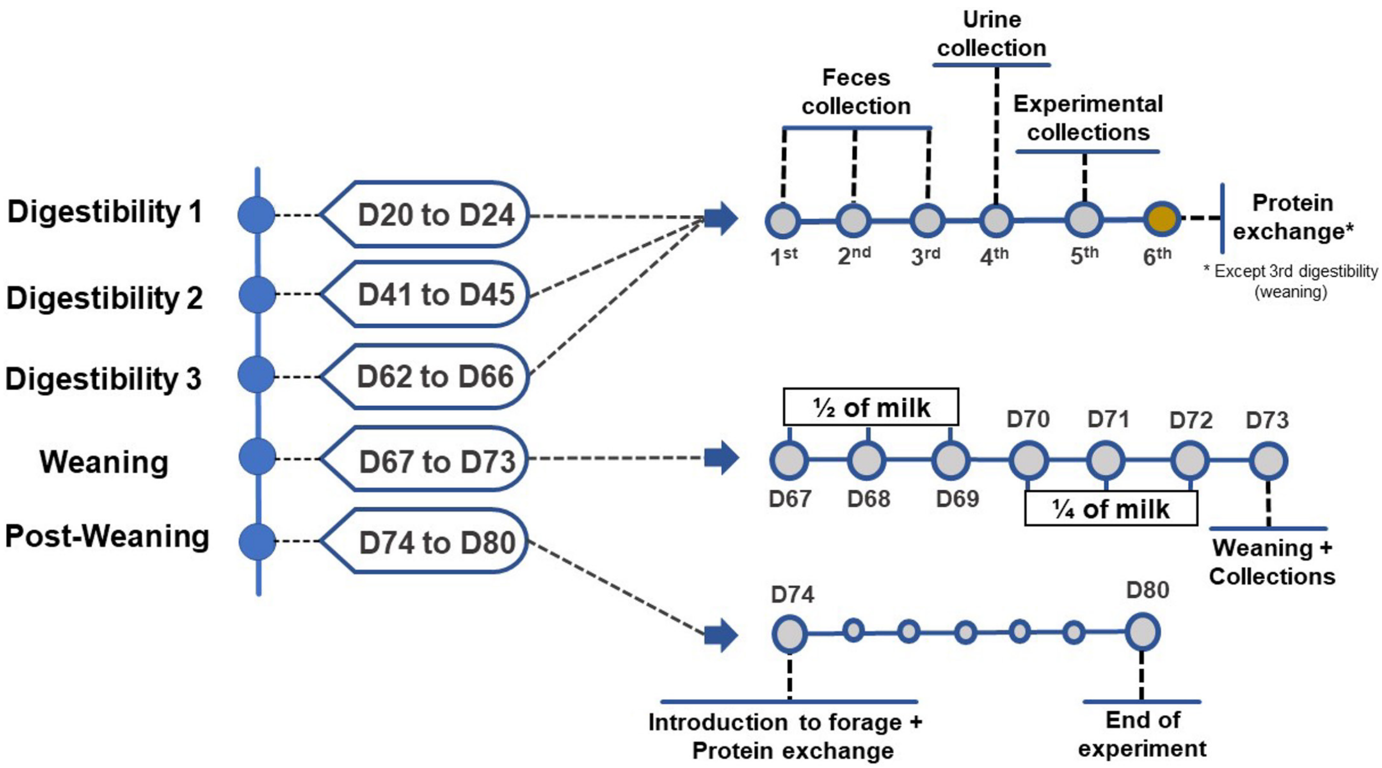

Figure 1. Summary of the experimental design and main collections carried out during the experiment.

female Holstein × Gyr crossbred calves. Sample size was determined by power analysis using ADG as the primary variable. The analysis assumed an α level of 0.05, power of 0.95, cv of 7% (SD = 46.5 g/d), and a detectable difference between treatments of 10% (Ryan, 2013). Power analysis indicated that 9.85 animals per treatment were required; therefore, 10 animals per treatment were used.

Animal Management

Immediately after birth, calves were dried, weighed, and fed colostrum (25% Brix at 10% BW). On d 2 and 3, calves received 4 L/d of transition milk. On d 2, blood samples were collected to evaluate passive immunity (de Souza et al., 2021). On d 4, calves were randomly assigned to 1 of the 6 treatments. Three hours after the morning feeding of the same day, blood was collected for baseline measurements of IGF-I, glucose, BUN, total protein, and albumin, which were used as a covariate for subsequent blood serum samples, as described below. On the same day, calves were weighed and measured for withers height (**WH**), rump height (**RH**), heart girth (**HG**), and body length (**BL**). Body weight and morphometric measures were recorded weekly thereafter.

Calves were housed individually in 5 m^2 pens with rubber bedding and ad libitum access to water. Saleable whole milk was offered 2 times daily (0700 and 1500 h), while starter feed and water were freely available to the animals. Milk intake was calculated at each meal as the difference between the allowance and orts, whereas starter feed and water intakes were measured daily as the difference between offered and refused amounts.

Rectal temperature and fecal score were monitored every morning. The feces were scored on a scale of 1 to 4 (Slanzon et al., 2022). Calves were disbudded on d 46 using a hot iron under local anesthesia (5 mL lidocaine, 5%), followed by analgesic administration.

Weaning began on d 67 using a step-down method. The weaning process consisted of reducing milk intake to 50% from d 67 to 70, to 25% from d 71 to 73, and to 0% on d 74. From d 74 to 80, calves from all treatments consumed the starter containing 18% CP and corn silage.

Digestibility Trials, Sampling, and Analysis

Digestibility trials were performed on d 20, 41, and 62. Each trial lasted 4 d, with feces collected for 3 d and urine for 1 d (Silva et al., 2015; Figure 1). Feces were collected immediately after defecation, and urine was collected using metabolic cages that directed flow through polyethylene tubing into ice-cooled tanks to prevent N losses (Knowlton et al., 2010). At the end

Table 2. Nutrient intake and apparent digestibility of dairy calves fed 4, 6, or 8 L/d of milk plus and a fixed or decreasing CP content in the starter feed

Item[3]	Milk (L/d)			SEM	SF[1]		SEM	Probability[2]			
	4	6	8		FCP	DCP		M	S	M × S	A
Intake (g/d)											
DM	730[c]	961[b]	1082[a]	27.4	952	897	22.4	<0.01	0.58	0.99	<0.01
DM starter	233	223	134	22.9	225	169	18.8	0.20	0.76	0.08	<0.01
OM	689[c]	908[b]	1022[a]	25.9	899	847	21.2	<0.01	0.57	0.99	<0.01
CP	148[c]	198[b]	232[a]	5.3	199	186	4.3	<0.01	0.23	0.89	<0.01
CP starter	37	34	21	3.7	37	25	3	0.09	0.28	0.24	<0.01
EE	169[c]	254[b]	320[a]	4	252	244	3.3	<0.01	0.07	0.82	0.01
NDFap	31	30	18	2.9	28	21	2.3	0.01	0.01	0.55	<0.01
DE (Mcal/d)	3.7[c]	5.2[b]	6.0[a]	0.1	5.1	4.8	0.09	<0.01	0.44	0.97	<0.01
Apparent digestibility (g/kg)											
DM	926[b]	939[ab]	945[a]	4.1	934	940	3.3	0.01	0.32	0.09	0.02
OM	940[b]	959[a]	963[a]	2.3	952	956	1.9	<0.01	0.12	0.35	0.01
CP	885[b]	920[a]	931[a]	4.9	908	915	4.1	<0.01	0.12	0.29	0.49
EE	962[b]	973[a]	976[a]	2.5	967	973	2.1	<0.01	0.05	0.15	0.14
NDFap	710	666	635	22.9	685	656	19	0.25	0.19	0.93	<0.01

[a–c]Mean values in the same row with different superscripts differ ($P < 0.05$) among milk levels by Tukey's test.

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

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

[3]EE = ether extract; NDFap = NDF-corrected for ash and protein; DE = digestible energy.

of each 24-h period, feces were weighed and homogenized, and a subsample of ~200 g was taken. Urine volume was measured, homogenized, filtered through gauze, and a 50-mL aliquot was stored at −20°C. At the end of each collection period, a fecal sample was prepared based on the 3 d of sampling. Whole milk was sampled and pooled weekly for analysis. Starter feed was sampled with each new mixture, and corn silage was sampled weekly at 10 points along the silo face, homogenized, and pooled for analysis.

Fecal and silage samples were partially dehydrated in a forced-air oven (TE-394/3, Tecnal) at 55°C (INCT-AS G-001/2), and milk samples were freeze-dried (INCT-AS G-002/2; Detmann et al., 2025). Subsequently, the samples of feces, milk, silage, and starter feed were ground to 1 mm in a knife mill. Feed and feces were analyzed for DM (method INCT-AS G-003/2), ash (method INCT-AS M-001/3), CP (method INCT-AS N-001/3), NDF-corrected for ash and protein (**NDFap**; methods INCT-AS N-004/3 and INCT-AS M-002/2), and ether extract (**EE**; method INCT-AS G-005/2). Urine N was determined using method N-001/3 (Detmann et al., 2025). The N balance was calculated as N intake minus fecal and urinary N excretion. Feed efficiency was defined as BW gain divided by total DMI. Nonfiber carbohydrates were calculated according to Detmann and Valadares Filho (2010) as follows:

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

where NDFap = NDF-corrected for ash and protein, and CA = crude ash. All values are given in grams per kilogram.

Digestible energy (**DE**) intake was calculated as the sum of digestible nutrients multiplied by their respective energy values (NRC, 2001):

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

where DE = digestible energy (Mcal/kg), dCP = digestible CP (kg/kg), dEE = digestible EE (kg/kg), dNFC = digestible NFC (kg/kg), and dNDFap = digestible NDFap (kg/kg).

Blood was collected from the jugular vein on d 3, 24, 45, 66, 73, and 80 into vacuum tubes with gel separator and coagulation activator. Samples were kept on ice and centrifuged at 3,000 × *g* for 20 min at 4°C. Serum was aliquoted into Eppendorf tubes and stored at −20°C for later analysis of IGF-I, glucose, BUN, total protein, and albumin. Glucose, BUN, total proteins, and albumin were determined using a Bioclin kit and a Mindray Automated Biochemistry Analyzer (model BS200E Shenzhen, Guangdong, China). The IGF-I was quantified by ELISA microplate reader using Beckman Coulter assay kits (intra-assay CV of 6.4% and interassay CV of 5.8%).

Ruminal fluid was sampled on d 24, 45, 66, 73, and 80 using an esophageal probe constructed from a 0.5-inch diameter silicone plastic hose. To minimize saliva contamination, the first portion of the obtained rumen

Table 3. Nitrogen balance of dairy calves receiving 4, 6, or 8 L/d of milk plus fixed or decreasing CP starter feed content

	Milk (L/d)				SF[1]			Probability[2]			
Item	4	6	8	SEM	FCP	DCP	SEM	M	S	M × S	A
N balance (g/d)											
Intake	23.6[c]	31.8[b]	36.3[a]	1.01	31.8	29.3	0.82	<0.01	0.36	0.99	<0.01
Urine excretion	2.6[a]	2.1[b]	2.4[ab]	0.13	2.4	2.3	0.10	0.03	0.81	0.06	0.02
Feces excretion	1.5[a]	1.2[b]	1.2[b]	0.09	1.4	1.2	0.07	0.04	0.08	0.61	<0.01
Retained	15.6[c]	25.4[b]	29.4[a]	1.08	24.7	22.2	0.88	<0.01	0.24	0.98	<0.01
Efficiency of N use (g/kg)	640[b]	788[a]	803[a]	13	752	736	10	<0.01	0.21	0.75	<0.01

[a–c]Mean values in the same row with different superscripts differ ($P < 0.05$) among milk levels by Tukey's test.

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

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

fluid was discarded before collecting the sample for analysis. Samples were filtered through sterile gauze and transported to the laboratory for pH measurement (portable pHmeter, T-1000, Tekna, Araucaria, Brazil). Two 10-mL aliquots were then stored: one for VFA (acetate, propionate, butyrate), preserved with metaphosphoric acid (200 g/L), and one for rumen ammonia-N (**RAN**), preserved with sulfuric acid (Leão et al., 2020). Samples for VFA analysis were filtered (0.22-μm) and analyzed for acetate, propionate, and butyrate by HPLC (Alliance e2695, PAD 2998 detector, Waters Corp., Milford, MA) using a C18 reversed-phase column (ODS 80A, 150 × 4.6 mm, 5 μm). Quantification was based on external calibration standards. The RAN was measured with a microplate spectrophotometer (Multiskan GO, Thermo Scientific, Waltham, MA).

Mammary Gland Image Analysis

Mammary gland ultrasound images were obtained on d 24, 45, 66, and 80 using a B-mode device with a 6-MHz microconvex transducer (resolution 640 × 480 pixels; DP2200, Mindray, China). Two images were collected per quarter in a standardized position, with the probe held at a 45° angle to the teat insertion in the caudocranial direction, following Nishimura et al. (2011) and Albino et al. (2017).

Image segmentation was performed using a U-Net model (Ronneberger et al., 2015) trained on 2,767 ultrasound images from 115 animals. Masks were manually annotated with Roboflow software (https://roboflow.com/). The network used ResNet34 as encoder (Matovinovic et al., 2019), pretrained on ImageNet, and was fine-tuned with supervised learning. Training was run for 370 epochs (batch size 128) using Dice Loss, stochastic gradient descent (initial learning rate 0.1), and cosine annealing scheduling (minimum learning rate 0.01; cycle length 10 epochs; Loshchilov and Hutter, 2016). Images were resized to 256 × 256 pixels and augmented with random vertical/horizontal flips (probability 0.5). The pipeline was implemented in PyTorch (Paszke et al., 2019) in Python.

Segmentation accuracy was assessed using the Intersection over Union (**IoU**) metric, defined as the ratio of overlap to total area between predicted and reference masks. Ground truth annotations were manually created with Roboflow. The IoU was calculated on 156 randomly selected images:

$$\text{IoU} = \frac{\left|reference \cap prediction\right|}{\left|reference \cup prediction\right|},$$

where *reference* refers to the ground truth (i.e., the manually annotated area representing the actual parenchymal region), and *prediction* refers to the segmented results generated by the U-Net model. An IoU score of 1.0 indicates perfect segmentation, whereas a score of 0.0 indicates no overlap between the predicted and actual regions.

Statistical Analysis

Data were subjected to ANOVA using the lm function of the basic package of R (R Core Team, 2025), testing the fixed effects of milk quantity, starter feed supply strategy, and their interaction according to the following model:

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

where 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, and ε_{ijk} = random error.

The effect of age (for digestibility trials, rumen fluid, and blood samples) was included as a repeated measure

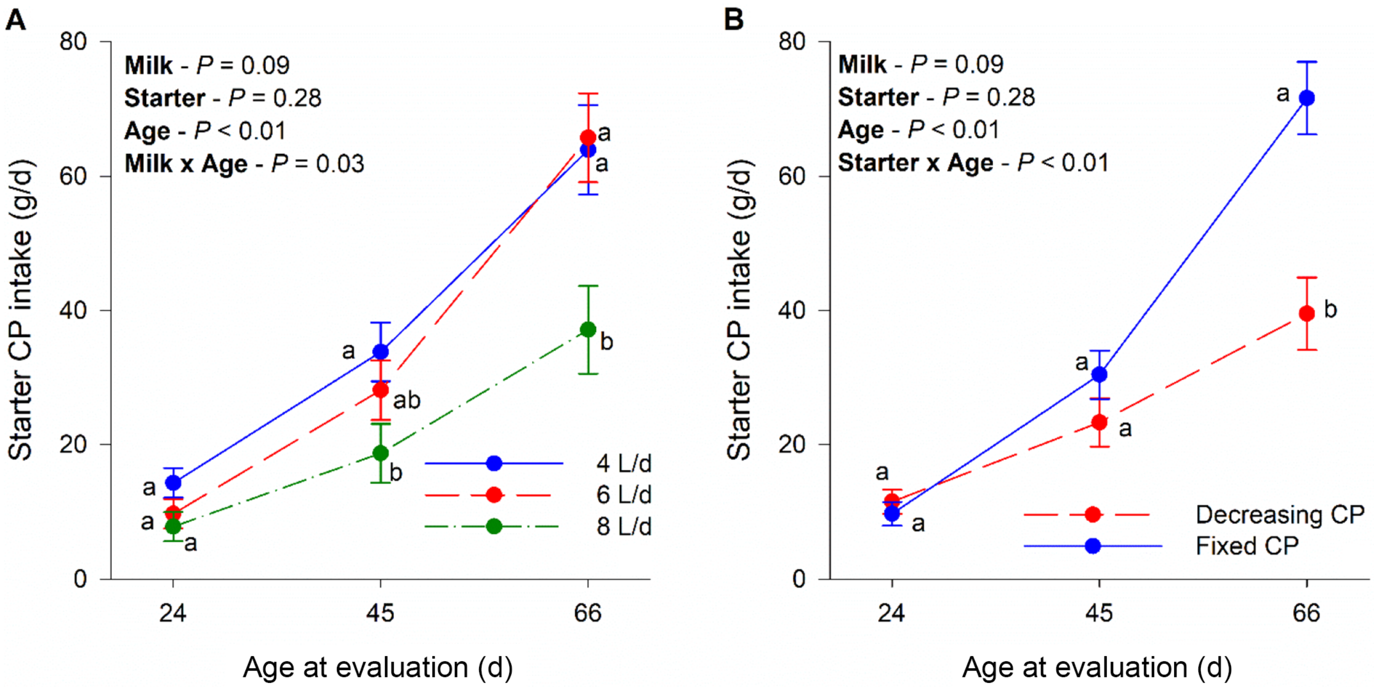

Figure 2. Effect (mean ± SEM) of milk allowance (A), starter feed strategy (B), and starter feed CP intake according to the age of calves. Different letters indicate differences ($P < 0.05$) within day of evaluation.

in the statistical model by using the gls function of the nlme package of R as follows:

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

where 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 ($M + S + M \times S$) is equal to the covariance between repeated measurements within animals, A_l = fixed effect of age, $(M \times A)_{il}$ = fixed effect of interaction between milk volume and age, $(S \times A)_{jl}$ = fixed effect of interaction between starter feed strategy and age, $(M \times S \times A)_{ijl}$ = fixed effect of interaction among milk volume, starter feed strategy, and age, and ε_{ijlkm} = random error.

The variance components, compound symmetry, heterogeneous compound symmetry, first-order autoregressive, and heterogeneous first-order autoregressive matrices of the (co)variance were tested. Matrix selection was based on the lowest value found for the corrected Akaike information criterion, depending on the variable analyzed. The initial BW was used as a covariate for performance analysis, while blood data from the first collection (d 4) was used as a covariate for the respective hormones/metabolites.

Observations with studentized internal residuals greater than |2.5| were considered "outliers" and removed from the model (Pell, 2000). When necessary, LSM were separated by Tukey's test, using $P < 0.05$ as the significance level for type I errors and $0.05 \leq P < 0.10$ as the trend.

RESULTS

Nutrient Intake, Digestibility, and Nitrogen Balance

The milk × age interaction (Figure 2A) showed that calves fed 8 L/d of milk generally had the lowest CP starter intake across ages, except on d 24. For the starter × age interaction (Figure 2B), CP starter intake decreased only on d 66 in the DCP treatment.

Nutrient intake and digestibility generally increased with age (Supplemental Table S1, see Notes), except for OM digestibility, which decreased from d 24 to 66. Milk allowance influenced both intake and digestibility ($P < 0.01$): higher allowances increased nutrient intake but reduced digestibility of OM, CP, and EE in calves fed 4 L/d compared with 6 or 8 L/d, and reduced DM digestibility compared with 8 L/d (Table 2).

A starter × age interaction affected N intake and fecal excretion ($P = 0.02$) and tended to affect retained N ($P =$

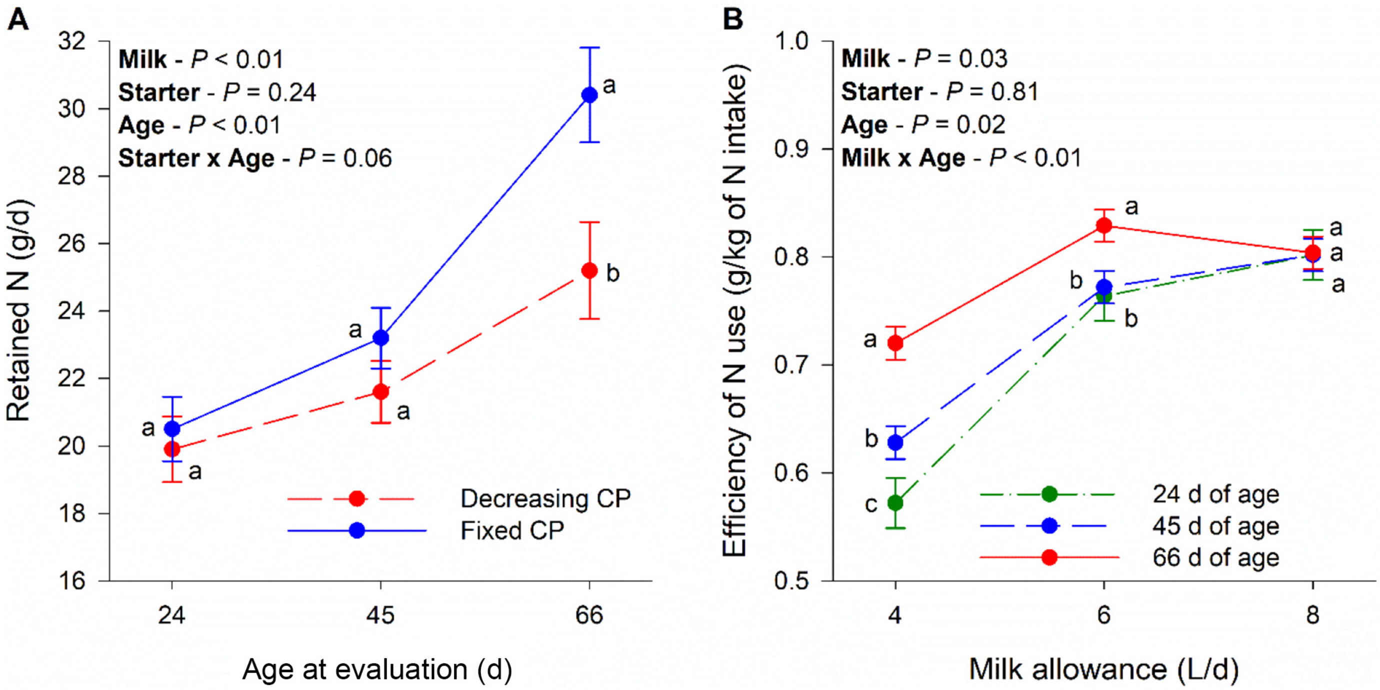

Figure 3. Effect (mean ± SEM) of starter feed strategy on retained N according to the age (A) and effect of age on the efficiency of N use according to the milk allowance (B). Different letters indicate differences ($P < 0.05$) within day of evaluation.

0.06; Figure 3A). Calves receiving DCP had lower intake and fecal excretion on d 66 but did not differ from FCP on d 24 and 45. The milk × age interaction influenced N-use efficiency ($P < 0.01$; Figure 3B): at 4 L/d, calves at d 24 had higher efficiency than those at d 45 and 66; at 6 L/d, efficiency was lower at d 45 and 66 compared with d 24; and at 8 L/d, efficiency did not differ with age. Overall, calves fed 8 L/d retained more N and excreted less N than those fed 4 L/d, with intermediate values at 6 L/d. The FCP treatment tended to increase fecal N excretion compared with DCP ($P = 0.08$; Table 3).

Performance, Feed Efficiency, and Body Measures

In the preweaning phase, BW at d 67 and ADG were influenced by milk allowance and starter feed, where calves receiving 8 L/d had the highest BW and ADG, followed by 6 and 4 L/d. Calves fed FCP also had greater performance than those fed DCP (Table 4). Feed efficiency was lower in calves fed 4 L/d than in those fed 6 or 8 L/d.

During weaning, calves receiving 8 L/d were heavier than those receiving 6 or 4 L/d. However, calves fed 4 L/d had greater ADG and feed efficiency (**FE**) than those fed 8 L/d. Calves fed FCP also had greater BW at weaning (d 73) compared with DCP. In the postweaning phase (d 80), calves fed 8 L/d had the highest BW compared with 6 and 4 L/d and tended to have higher ADG ($P = 0.09$). Feed efficiency during this period was influenced only by starter feed, with calves in the DCP group being more efficient than those in the FCP group.

Body measurements (WH, RH, HG, BL) were not affected by interactions between milk allowance and starter feed (Supplemental Table S4, see Notes). The only effect of starter feed was observed for HG during preweaning, when calves fed FCP had greater HG than those fed DCP ($P = 0.04$). A similar trend was observed postweaning.

Blood and Rumen Parameters

A starter × age interaction affected BUN, with calves on DCP showing decreased BUN at d 66 (Figure 4A). A milk × starter interaction also affected BUN, and calves receiving 4 or 6 L/d and FCP had higher BUN, whereas those receiving 8 L/d had similar BUN across starter treatments (Figure 4B). Milk allowance influenced IGF-I and BUN ($P \leq 0.03$; Table 5).

For rumen parameters, a starter × age interaction was observed for RAN ($P = 0.04$), with higher concentrations in FCP calves on d 66. Butyrate was lower in calves fed 4 L/d and DCP ($P = 0.02$). The milk × starter interaction tended to affect ruminal pH and acetate ($P = 0.08$ and 0.07, respectively). Age affected RAN and VFA profile ($P = 0.01$; Supplemental Table S2, see Notes). Calves

Table 4. Performance and feed efficiency of dairy calves fed 4, 6, or 8 L/d of milk and a starter feed with fixed or decreasing CP content during the preweaning, weaning, and postweaning periods

	Milk (L/d)				SF[1]			Probability[2]		
Item[3]	4	6	8	SEM	FCP	DCP	SEM	M	S	M × S
Preweaning										
Birth BW (kg)	32.1	32.1	32.3	1.14	32.9	31.5	0.95	0.97	0.26	0.19
BW at 67 d (kg)	63.9[c]	77.1[b]	85.1[a]	4.91	77.0	73.7	4.95	<0.01	0.03	0.55
ADG (g/d)	496[c]	712[b]	837[a]	7.8	708	655	7.7	<0.01	0.03	0.23
FE (g/kg)	630[b]	725[a]	762[a]	20.9	710	701	17.2	<0.01	0.75	0.81
Weaning										
BW at 73 d (kg)	69.9[c]	81.8[b]	87.8[a]	6.38	82.2	77.3	6.35	<0.01	0.01	0.30
ADG (g/d)	607[a]	545[ab]	293[b]	9.8	563	401	8.4	0.03	0.10	0.31
FE (g/kg)	514[a]	382[ab]	146[b]	9.95	402	293	7.6	0.02	0.36	0.27
Postweaning										
BW at 80 d (kg)	72.9[c]	85.6[b]	91.8[a]	5.48	84.6	82.3	5.40	<0.01	0.36	0.18
ADG (g/d)	717	899	1,095	11.9	824	984	9.8	0.09	0.25	0.85
FE (g/kg)	508	453	568	11.3	364	655	9.3	0.74	0.03	0.78

[a–c]Mean values in the same row with different superscripts differ ($P < 0.05$) among milk levels by Tukey's test.

[1]SF = starter feed (where FCP is the starter feed with fixed CP content and DCP is starter feed decreasing CP content).

[2]Probability of treatment effects: M = effect of milk volume, S = effect of starter feed type, and M × S = interaction effect between milk volume and starter feed type.

[3]FE = feed efficiency.

fed FCP had greater RAN and propionate concentrations ($P \leq 0.03$). The RAN concentration also tended to be greater in calves fed 4 and 8 L/d ($P = 0.09$; Table 5).

Mammary Gland

Age influenced parenchyma area, perimeter, circularity, and eccentricity, which increased over the 80-d trial ($P < 0.01$; Supplemental Table S3, see Notes). Milk allowance also affected area and perimeter: calves fed 4 L/d had smaller parenchyma area than those fed 6 L/d and smaller perimeter than those fed 6 or 8 L/d (Table 5).

DISCUSSION

The interaction effect of milk and age on intake has been widely reported. According to NASEM (2021), calves fed large volumes of milk (>900 g/d in large breeds) have lower starter DMI, likely because energy needs are met by milk (Kazemi-Bonchenari et al., 2022). Our results agree with these reports, as calves fed 8 L/d had the lowest starter intake and lower starter CP intake compared with calves on lower milk volumes. The increase in DM intake with age reflects higher maintenance and growth requirements. Silva et al. (2019) described an exponential rise in starter intake after 25 to 30 d of age. The lower NDFap intake in calves receiving 8 L/d of milk is explained by the reduced starter intake. For the starter × age interaction, the lower starter and CP starter intake observed in the DCP treatment, mainly at d 66, regardless of milk volume, is also consistent with previous findings. Stamey et al. (2012) and Kazemi-Bonchenari et al. (2022) reported that higher-CP starters increase intake and support higher ADG in the postweaning period. The higher EE and DE intake observed with advancing age and higher milk volumes reflects the greater contribution of milk in the diet.

Our results showed a higher digestibility of DM, OM, CP, and EE in calves consuming larger amounts of milk (8 and 6 L/d), which is to be expected because milk has a higher overall digestibility (about 93% digestibility; Silva et al., 2015), in addition to the partially substituted starter intake due to higher milk consumption. The NDF digestibility improves with age and time after weaning and may be reduced in animals fed high-milk or milk-replacer diets (NASEM, 2021). Thus, the lower NDFap digestibility observed with a higher amount of milk can be explained as described by NASEM (2021) and can also be associated with the lower starter intake observed in our results, which could cause a dilution effect (Silva et al., 2015).

Ruminants convert only 20% to 30% of dietary N into body protein, with the remainder excreted in urine and feces (Zhang et al., 2021). In our study, the starter × age interaction showed reduced N intake and fecal excretion in calves fed DCP at d 66, with a similar trend for lower N retention before weaning. This is likely reflected by the lower CP content (14%) of the starter offered in this period. Although DCP did not improve N retention, it reduced fecal N excretion, which has environmental benefits.

Milk allowance × starter interaction indicated greater urinary N excretion in calves fed FCP and 4 L/d of milk,

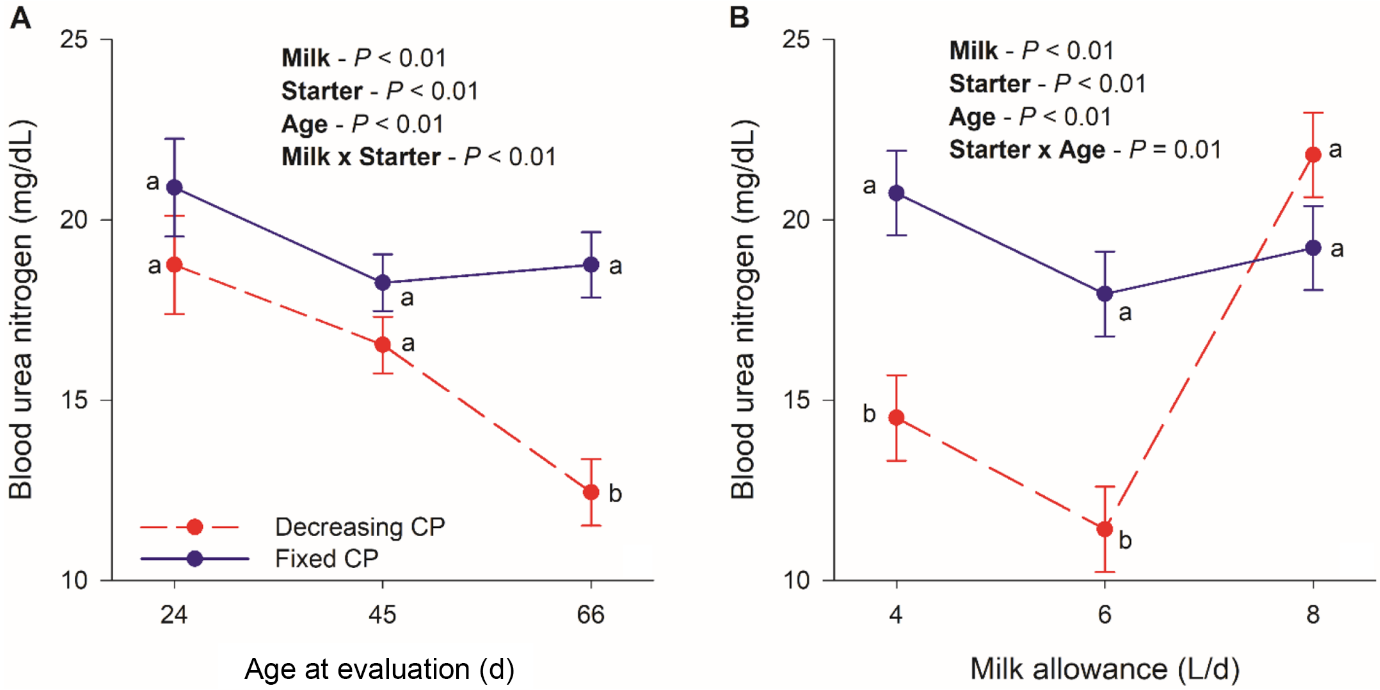

Figure 4. Effect (mean ± SEM) of starter feed strategy on blood urea N based on age (A) or milk allowance (B). Different letters indicate differences ($P < 0.05$) within day of evaluation (A) or milk allowance (B).

associated with lower N-use efficiency at d 24 and 45 (Figure 4). Previous work shows that urinary N excretion is correlated with starter intake rather than milk intake (Silva et al., 2015; Kazemi-Bonchenari et al., 2022). In our trial, calves on 4 and 6 L/d had higher starter intake early in life, but incomplete rumen development may have limited fermentation and N utilization, resulting in higher urinary N losses. The effect of age on N balance reflects increasing intake and performance as calves grow (Silva et al., 2015; Sharma et al., 2020). Higher N efficiency at 8 L/d was associated with greater N intake and retention (Zhang et al., 2021). Thus, calves with higher N intake achieved greater efficiency and higher N availability for tissue deposition.

Growth performance and FE were more influenced by milk allowance than by starter strategy. In the preweaning phase, calves fed 8 L/d and FCP had higher BW and ADG, likely due to greater DM intake, digestibility, DE intake, and N retention. During weaning, however, calves fed 8 L/d showed lower ADG and FE than those on 4 L/d despite having greater BW. Similar results have been reported (NASEM, 2021; Kazemi-Bonchenari et al., 2022) and are attributed to reduced starter intake and delayed rumen development in calves fed high milk allowances. Starter intake promotes epithelial development via carbohydrate fermentation and VFA production (Diao et al., 2019), which supports rumen adaptation during weaning and postweaning. The higher BW in FCP calves, along with the lower ADG and FE in 8 L/d calves during weaning, is consistent with this explanation.

The greater BW observed in 8 L/d calves in the postweaning phase likely reflects cumulative milk intake rather than improved growth after weaning. In contrast, higher FE in DCP calves postweaning supports the predicted reduction in protein requirements for this stage (Silva et al., 2017, 2019). Body measurements across all phases also showed positive effects of higher milk volumes, indicating not only greater weight but also skeletal and muscle growth. The effect of FCP on HG in the pre- and postweaning phases agrees with previous reports associating high milk allowances with larger body dimensions (Chapman et al., 2017; Stamey Lanier et al., 2021).

The only 3-way interaction (milk × starter × age) was a trend for serum IGF-I, which was higher at d 66 in calves fed 8 L/d plus FCP. Maresca et al. (2018) reported that IGF-I increases with age until ~90 d, and Haisan et al. (2018) showed that intake level regulates IGF-I production. In our study, greater N retention in FCP calves at d 66 was reflected in higher IGF-I, consistent with its role in protein metabolism. Thus, increased intake of DM, starter DM, and DE in FCP and high-milk calves likely promoted higher IGF-I and supported growth.

For BUN, Jafari et al. (2020) found values increase with age, and Khan et al. (2007) proposed BUN as an indicator of rumen development. In contrast, our results

Table 5. Blood and ruminal parameters and mammary gland parenchyma measurements of dairy calves receiving 4, 6, or 8 L/d of milk plus fixed or decreasing CP starter feed content

	Milk (L/d)				SF[1]			Probability[2]			
Item[3]	4	6	8	SEM	FCP	DCP	SEM	M	S	M × S	A
Blood parameters											
IGF-I (ng/mL)	79.7[b]	129.9[a]	146.6[a]	12.18	129.6	107.9	9.72	0.03	0.13	0.77	<0.01
Glucose (mg/dL)	107.8	116.2	115.3	3.67	110.1	116.2	3.03	0.22	0.16	0.79	0.70
BUN (mg/dL)	17.6[b]	14.7[c]	20.5[a]	0.82	19.3	15.9	0.68	<0.01	<0.01	<0.01	<0.01
Total protein (g/dL)	5.65	5.64	5.42	0.126	5.53	5.61	0.104	0.36	0.58	0.14	0.86
Albumin (g/dL)	2.92	2.96	2.88	0.062	2.87	2.97	0.051	0.69	0.18	0.39	<0.01
Ruminal parameters											
pH	5.13	5.10	5.23	0.084	5.17	5.14	0.069	0.52	0.76	0.08	0.84
RAN (mg/L)	112	88	114	9.34	118	92	7.62	0.09	0.02	0.79	0.01
Acetate (mmol/mL)	34.8	30.9	33.3	2.34	35.1	31.0	1.88	0.49	0.13	0.07	0.01
Propionate (mmol/mL)	18.6	17.3	17.9	1.93	20.8	15.3	1.58	0.89	0.03	0.23	0.01
Butyrate (mmol/mL)	7.5	6.1	8.2	1.01	7.6	6.9	0.82	0.34	0.52	0.02	0.21
Mammary gland measurements											
Area (pixels^2)	4,193[b]	5,070[a]	4,879[ab]	232	4,715	4,712	193	0.03	0.99	0.64	<0.01
Perimeter (pixel)	347[b]	388[a]	384[a]	9.0	371	375	7.5	0.01	0.68	0.56	<0.01
Circularity (ranging from 0 to 1)	0.385	0.371	0.370	0.0072	0.379	0.372	0.0058	0.25	0.45	0.82	<0.01
Eccentricity (ranging from 0 to 1)	0.951	0.949	0.953	0.0026	0.952	0950	0.0021	0.55	0.65	0.23	<0.01

[a–c]Mean values in the same row with different superscripts differ ($P < 0.05$) among milk levels by Tukey's test.

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

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

[3]RAN = rumen ammonia nitrogen.

suggest that FCP stabilized BUN in the preweaning phase. The FCP calves had higher BUN at 4 and 6 L/d, while FCP and DCP were similar at 8 L/d. These results suggest that DCP may limit rumen function due to an imbalanced supply of degradable and undegradable protein (Stamey et al., 2012), contributing to higher fecal N excretion and lower N retention at d 66. Albumin increased with age, consistent with previous reports (Feitosa et al., 2001; Elkhair, 2021). Lower concentrations after 24 d reflect the gradual rise during calf development.

Before starter intake begins, rumen pH is about 6.0 to 6.3 and decreases with starch fermentation, limited VFA absorption, and low saliva flow (NASEM, 2021). In our study, calves fed higher milk and FCP tended to have higher rumen pH, likely reflecting greater CP and MP supply from milk and starter. For RAN, higher dietary rumen-degradable protein is expected to increase ammonia (NASEM, 2021). Because soybean meal was the main CP source (~67% of RDP), calves fed FCP received more degradable protein in the final phase and had higher RAN at d 66, consistent with reports in lambs (Yang et al., 2016).

The VFA production stimulates rumen epithelial growth, with butyrate having the strongest effect, followed by propionate and acetate (Diao et al., 2019). In our study, VFA concentrations increased with age, reflecting normal rumen development, as its relative size rose from 38% of stomach weight at birth to 61% at 8 wk (Davis and Drackley, 1998; Diao et al., 2017). The observed interaction for butyrate, trend for acetate, and effect of starter on propionate indicate that higher-CP starter intake enhanced epithelial development and function. This combination of greater dietary energy and CP likely promoted rumen papillae growth and higher IGF-I availability, supporting calf performance.

Age affected all parenchymal traits, confirming a ductal growth pattern consistent with previous studies (Capuco and Ellis, 2013; Esselburn et al., 2015; Akers, 2017). Circularity and eccentricity indicated a shift toward a more elongated ductal structure, as also observed by Furini et al. (2018). In contrast to their report of greater circularity at 8 wk, our data showed decreasing circularity with age, supporting ductal elongation. Mammary development can be influenced by preweaning nutrition, with higher energy intake stimulating parenchyma growth (Soberon and Van Amburgh, 2017) and unbalanced protein:energy supply impairing it (Albino et al., 2015). Molenaar et al. (2020) found no difference in parenchyma area between 4 and 8 L/d but greater fat pad deposition at high-milk intake. Our results suggest that an intermediate milk allowance (6 L/d) promoted greater parenchyma area and perimeter in Holstein × Gyr calves, which may be linked to improved future milk production.

Finally, it is important to recognize that the reduction in fecal N excretion observed with the DCP strategy (~200 mg/calf per d) is modest at the individual level. On its own, this difference may not justify the management complexity of using multiple starters on a farm. However, when considered at the herd level and across production cycles, even small daily reductions could accumulate and contribute to lower overall N output. Therefore, while the environmental benefit is limited, our findings suggest that variable-CP starters could complement other precision-feeding strategies aimed at improving nutrient efficiency and sustainability in calf rearing.

CONCLUSIONS

Animals receiving 6 L/d of milk have good performance and mammary parenchyma growth, often not differing from animals that consume 8 L/d, and greater N efficiency and reduced excretion, indicating that it is an efficient feeding strategy that improves N usage efficiency and reduces N pollution. Regarding the starter feed, animals submitted to the FCP strategy had better performance compared with DCP animals. However, DCP calves showed improved FE postweaning and tended to have lower fecal N excretion, proving also to be an alternative to decrease N excretion. It should be noted, however, that this reduction was modest at the individual level and that the practical feasibility of using multiple starters on a farm requires careful evaluation. Therefore, animal performance could potentially be improved with this feeding strategy, but other combinations of age at which starter CP content is reduced and magnitude of CP reduction should be explored to optimize animal performance and N-use efficiency.

NOTES

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Nonstandard abbreviations used: 4L_DCP = 4 L/d of milk and decreasing CP starter; 6L_DCP = 6 L/d milk and decreasing CP starter; 8L_DCP = 8 L/d milk and decreasing CP starter; 4L_FCP = 4 L/d of milk and fixed CP starter; 6L_FCP = 6 L/d of milk and fixed CP starter; 8L_FCP = 8 L/d of milk and fixed CP starter; BL = body length; DCP = decreasing CP; DE = digestible energy; EE = ether extract; FCP = fixed CP; FE = feed efficiency; HG = heart girth; IoU = Intersection over Union; NDFap = NDF-corrected for ash and protein; NFC = nonfiber carbohydrates; RAN = rumen ammonia-N; RH = rump height; WH = withers height.

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