J. Dairy Sci. 107:6852–6865
https://doi.org/10.3168/jds.2024-24674
© 2024, The Authors. Published by Elsevier Inc. on behalf of the American Dairy Science Association®.
This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Effects of bulk tank milk, waste milk, and pasteurized waste milk on the nutrient utilization, gastrointestinal tract development, and antimicrobial resistance to *Escherichia coli* in preweaning dairy calves

H. C. Diniz Neto,[1] S. G. Coelho,[1] J. P. Campolina,[1] S. F. Vieira,[1] M. C. Lombardi,[2] B. P. Pereira,[3] B. S. F. Albuquerque,[3] S. F. Costa,[4] A. S. Guimarães,[3,4] M. A. V. P. Brito,[3] C. S. Silva,[3] F. S. Machado,[3] T. R. Tomich,[3] and M. M. Campos[3]*

[1]Departamento de Zootecnia, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, 30161-970
[2]Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, 30161-970
[3]Empresa Brasileira de Pesquisa Agropecuária–Embrapa Gado de Leite, Juiz de Fora, Minas Gerais, Brazil, 36038-330
[4]Departamento de Medicina Veterinária, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil, 30161-970

ABSTRACT

This study aimed to assess the effect of bulk tank milk (BTM), waste milk (WM), and pasteurized waste milk (PWM) on nutrient digestibility, ruminal and cecal fermentation, gastrointestinal tract (GIT) development, and antimicrobial resistance of fecal *Escherichia coli* from dairy calves at 2 periods (30 and 60 d of age). Calves were grouped according to BW, serum protein levels, and breed composition. Three treatments were included: BTM (n = 21), WM from cows under antibiotic treatment (n = 21), and PWM (WM submitted to HTST pasteurization; n = 21). A total of 63 calves were used, of which 18 animals (n = 6 per treatment) were evaluated in the period of 4 to 30 d, and 45 (n = 15 per treatment) from 4 to 60 d. During the experimental period, a daily intake of 6 L of milk was divided into 2 equal meals, with ad libitum access to water and starter. Milk and feed intakes were recorded daily. Apparent total-tract digestibility and nitrogen balance were conducted from 25 to 29 d of age (n = 6) and from 53 to 57 d of age (n = 15). Animals were slaughtered at 30 ± 1 and 60 ± 1 d of age for the assessment of ruminal and cecal fermentation and GIT development. Antimicrobial susceptibility testing was conducted at 1, 30, and 60 d of age (n = 15/treatment). Statistical analysis used a linear mixed-effects model for continuous outcomes and generalized linear models for single measurements (R software). Treatments WM and PWM had lower rumen pH, higher ruminal acetate concentration, larger reticulorumen and liver, and a higher prevalence of fecal-resistant *E. coli* compared with BTM at both 30 and 60 d. Up to 60 d, both BTM and WM treatments exhibited higher digestibility of ether extract and gross energy compared with the PWM, whereas WM and PWM treatments showed increased nitrogen intake and retention compared with the BTM treatment. These findings suggest that pasteurization of WM negatively affects nutrient digestibility and calf performance, while also affecting rumen development. Additionally, the use of milk containing antibiotic residue leads to the selection of resistant *E. coli* in the GIT over time.

Key words: calf feeding strategies, nutrient partitioning, rational use of antimicrobials, rumen development, volatile fatty acids

INTRODUCTION

Waste milk (**WM**) encompasses colostrum and milk derived from cows undergoing treatment for several ailments, including clinical mastitis, foot and reproductive diseases, and other related conditions (Ma et al., 2022). Additionally, this category further encompasses milk with an elevated SCC, rendering it unsuitable for commercial use (Aust et al., 2013). Worldwide, the use of WM in suckling programs is considered a common practice (Brunton et al., 2012). For example, in US dairy operations, the WM was the main dietary used in the liquid diet of calves (40.1%; Urie et al., 2018). The predominant use of WM in rearing programs has been perceived by producers as an economical feed alternative, effectively replacing calf nutrition derived from bulk tank milk (**BTM**) or milk replacer. From their standpoint, this approach mitigates the necessity for specific waste treatment systems, which would otherwise be required to manage microbial contamination and dispose of drug residues safely in the environment, given that the WM will be ingested by the calf (Brunton et al., 2012). However, despite its utilization as a feed, concerns persist

Received January 12, 2024.
Accepted April 11, 2024.
*Corresponding author: mariana.campos@embrapa.br

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

regarding antimicrobial resistance, which may prevail after internal metabolism, as well as soil contamination by resistant bacteria and antimicrobial shedding into the manure (Zhang et al., 2019a).

The indiscriminate use of antimicrobials and deviations from prescribed treatment protocols represent important contributors to the emergence of resistant bacteria in animal production (Wegener, 2003). Thus, feeding WM to preweaning calves introduces an additional risk factor for the development of microbial resistance (Pereira et al., 2014; Zhang et al., 2022). To minimize the risks related to the presence of microorganisms, pasteurization (fast or slow) has been used to improve WM quality due to the ability to inactivate pathogenic microorganisms and reduce the total microbial load (Macdonald et al., 2011). Nevertheless, pasteurization is not able to reduce antimicrobial residues (Garzon et al., 2020).

In addition to these considerations, it has been acknowledged that the quality of WM exhibits significant variability (Moore et al., 2009) and influences the composition and stability of the intestinal microbiota (**IM**) in calves (Maynou et al., 2019; Penati et al., 2021). Dennis et al. (2019) were pioneers in investigating the nutritional responses of calves fed WM, observing lower digestibility of DM, OM, ADF, and NDF in calves receiving WM containing antibiotics. Antibiotic residues have also been implicated in altered rumen microbiota and rumen fermentation profile due to variable quantities of WM reaching the rumen during feeding (Li et al., 2019; Zhang et al., 2019). Metagenomic analysis of rumen contents from calves fed WM not only revealed the effect of antibiotic residues on nutrient digestion of the solid diet but also highlighted the susceptibility of bacterial phyla to these residues (Naas et al., 2014; Zhang et al., 2019). These alterations are substantial enough to affect the development of the gastrointestinal tract (**GIT**), particularly the rumen (Li et al., 2019; Zhang et al., 2019). Despite these observations, scientific studies evaluating such effects remain limited.

The objective of this study was to assess the impact of utilizing BTM, WM, and pasteurized waste milk (**PWM**) on nutrient digestibility, ruminal and cecal fermentation, organ development, and antimicrobial resistance of fecal *Escherichia coli* in dairy calves. The research was structured around the following hypotheses: (1) the incorporation of WM and PWM for calf feeding influences nutrient digestibility through alterations in the fermentation pattern; (2) WM and PWM exert detrimental effects on organ development, particularly the rumen; and (3) calves fed with WM and PWM exhibit a higher prevalence of fecal *E. coli* resistant to various antimicrobial drugs.

MATERIALS AND METHODS

The experimental procedures employed in this study were approved by the Ethics Committee on Animal Use of Embrapa Dairy Cattle (CEUA number: 9849040419). The research was conducted over a period of 101 d on the Embrapa Dairy Cattle Experimental Farm (herd size: 400 animals) located in Coronel Pacheco, Minas Gerais, Brazil.

Animals, Treatments, and Management

A total of 63 male Holstein × Gyr crossbred dairy calves were used in the study. Immediately following birth, newborn animals were separated from their dams and had their umbilical cord immersed in a 10% iodine solution for 3 consecutive days. Within the first 6 h of life, the calves were weighed and provided with colostrum equivalent to 10% of their BW, with a Brix concentration of 25%. Colostrum with a result Brix <25% was enriched using a colostrum replacer (Saskatoon Colostrum Company, Saskatoon, Canada) until reaching a Brix value of 25%.

During the initial 3 d, calves were accommodated in individual suspended cages (1.50 m × 0.80 m; Intergado Ltd., Contagem, Brazil) with hay bedding, and fed with 6 L/d of transition milk from their dams (0800 and 1600 h), offered via commercial milk feeders (Milkbar, Waipu, New Zealand). Water was provided ad libitum from the first day. Blood samples were collected 48 h after birth to evaluate passive immune transfer through total serum protein. Subsequently, samples were centrifuged (1,800 × g for 10 min) at room temperature (22–25°C), and total serum protein levels were quantified using an electronic refractometer (Serum protein REF-301; Biocotek, Beilun, Ningbo, China). No differences in passive immune transfer ($P = 0.97$) and initial weight ($P = 0.98$) were observed between treatments using t-test evaluation. The total serum protein concentrations were 6.63, 6.63, and 6.58 g/dL and initial weight 37.36, 37.03, and 37.08 kg for calves in the BTM, WM, and PWM groups, respectively.

The experimental farm operated within a calf birth season, resulting in numerous calves being born simultaneously. Consequently, during the first 4 d of life, calves with comparable initial BW, serum protein levels, and genetic composition (3/4 or 5/8 Holstein × Gyr breed composition) were selected for random assignment to 1 of 3 treatments, in accordance with the principle of random distribution. These attributes were evaluated to confirm that any discrepancies observed among treatment groups were not attributable to initial variations in these factors. Minimal modifications were implemented

to guarantee that the calves conformed to random assignment to their respective experimental treatment: (1) BTM sold by the dairy, sourced solely from 60 healthy cows without any antimicrobial residue (n = 21), (2) WM from cows subjected to antimicrobial treatments (n = 21), (3) PWM processed for 15 s at 72 to 75°C (West, Juiz de Fora, Brazil; n = 21). The WM in the study was sourced from cows that had undergone antimicrobial treatments, including those treated for clinical mastitis, placental retention, metritis, or foot infections, across 3 experimental herds within the same research center. Each cow receiving antimicrobial treatment was individually identified and milked separately. Following milking, the milk from those cows was promptly transported to the calf experimental center for utilization. The WM was divided into 2 streams: one intended to be fed directly to animals in the WM treatment group, and the other to undergo pasteurization before being fed to the animals in the PWM treatment group. Following the pasteurization process, a milk sample was assessed for pasteurization efficiency by investigating peroxidase and phosphatase enzymes (CapLab, Ipiranga, São Paulo, Brazil), and it was only used after confirming the absence of phosphatase and the presence of peroxidase. Calves in the PWM treatment had their first meal immediately after pasteurization (milk temperature of 38°C). The remaining milk was refrigerated for 6 h (4°C) until the second meal. Milk samples from all 3 treatments were collected immediately before meal feeding to assess SCC and total bacterial count (Table 1). No significant bacterial differences were observed between preweaning milk fed in the first and second meal.

The study included 2 periods: period 1, comprising 18 animals (n = 6/treatment), assessed over 4 to 30 d; and period 2, consisting of 45 animals (n = 15/treatment), underwent evaluation for an extended duration of 4 to 60 d. During the experimental period, animals were housed in individual sand-bedded pens (1.25 × 1.75 m, tethered with 1.2 m long chains). The pens were separated by masonry plates to prevent cross-contamination between animals in different treatments, and each treatment had its dedicated utensils.

In all experimental periods, calves received 6 L/d of milk, divided into 2 meals (BTM at 0900 and 1500 h; WM at 1000 and 1600 h; and PWM at 1100 and 1700 h) in calf milk feeders (Milkbar, Waipu, New Zealand). The mealtimes differed between treatment groups because BTM was obtained at the beginning of the herd milking process, whereas WM was obtained at the end. Furthermore, a portion of the WM had to undergo pasteurization, which required additional time before it could be fed to the calves. To maintain consistency in the liquid diet of the calves throughout the experimental periods, meal times were precisely defined and adhered to. A solid diet was offered ad libitum from the fourth day of age. The diet consisted of ground corn, soybean meal, and mineral and vitamin supplements (Table 1).

Intake and Performance

Feed intake (milk and starter) was measured daily by calculating the difference between the amount of DM in the provided milk and solid feed and the amount refused. The total DMI was determined by summing the DMI

Table 1. Composition, SCC, and total bacterial count (TBC) of bulk tank milk (BTM), waste milk (WM), and pasteurized waste milk (PWM) and starter during the experimental period (means ± SD)

	Treatment			
Item	BTM	WM	PWM	Starter[1]
Milk composition, %				
DM	13.04 ± 0.58[a]	12.82 ± 0.64[b]	12.48 ± 0.53[c]	94.53
Fat	4.24 ± 0.54[a]	4.10 ± 0.58[a]	3.76 ± 0.43[b]	3.14
CP	3.30 ± 0.27[b]	3.46 ± 0.44[a]	3.49 ± 0.29	19.06
Casein	2.71 ± 0.19	2.64 ± 0.36	2.53 ± 0.44	—
NPN	0.10 ± 0.01[b]	0.13 ± 0.02[a]	0.12 ± 0.02[a]	—
Lactose	4.46 ± 0.12[a]	4.33 ± 0.20[b]	4.33 ± 0.17[b]	—
Ash	0.69 ± 0.03[b]	0.72 ± 0.04[a]	0.73 ± 0.03[a]	8.81
NDF	—	—	—	12.70
ADF	—	—	—	5.60
GE,[2] kcal/kg	—	—	—	4,168.63
Milk quality				
SCC, × 10^3 cells/mL	366.81 ± 175.13[c]	1,740.15 ± 1,638.03[a]	1,424.67 ± 784.44[b]	—
TBC, × 10^3 cfu/mL	19.79 ± 15.14[c]	548.37 ± 695.11[a]	295.41 ± 353.25	—

[a–c]Different superscripts within a row indicate significant differences between treatments ($P < 0.05$; Tukey test).

[1]Basic composition: soybean meal, ground corn, and minerals (Prima/DSM, São Paulo, Brazil).

[2]GE = gross energy.

of milk and starter. Body weight was measured on the fourth and seventh days of age and then weekly thereafter. Feeding efficiency was calculated by dividing the mean of ADG by the total DMI.

Digestibility

The apparent total-tract digestibility assay was conducted during 2 periods: from 25 to 29 d of age (n = 6/treatment) and from 53 to 57 d of age (n = 15/treatment). For 4 consecutive days (25–28 and 53–56 of age), the animals were housed in individual pens equipped with a rubber mat (WingFlex, Kraiburg TPE GmbH & Co., Waldkraiburg, Germany) for feces collection. The total feces samples from each animal were collected daily, weighed, and frozen at −20°C. On the last day (29 and 57 d of age), the animals were transferred to metabolic cages (1.50 m × 0.80 m, Intergado Ltd., Contagem, Brazil) for total urine and fecal collection. A tray placed below the cage drained all urine into 5-L containers, which were stored in coolers with ice. The total urine volume, weight, and density were measured, and a 50-mL sample was collected and stored at −20°C. Samples of the supplied starter and feed refusals were collected daily, and the equivalent amounts from each total daily sample were combined to create one sample per animal.

Nutrient Composition Analysis

Samples of milk supplied were collected daily for analysis of TS, CP, lactose, and fat (Table 1) using infrared spectroscopy (Bentley model 2000, Bentley Instruments Inc., Chaska, MN).

The starter samples from the entire experimental period and fecal samples from the digestibility assay were dried at 55°C for 72 h and ground through a 1-mm sieve using a Wiley mill (model 3, Arthur H. Thomas Co., Philadelphia, PA). After drying and grinding, 25 g of each sample were used to create a composite sample, representative of the digestibility test. The starter and fecal samples were analyzed for DM (method 934.01), CP (method 988.05), ether extract (**EE**, method 988.05), and ash (method 942.05), according to AOAC International (2012). Neutral detergent fiber and ADF contents were determined according to the method described by Van Soest et al. (1991). The gross energy was measured by an adiabatic bomb calorimeter (IKA-C5000, IKA Works, Staufen, Germany).

Urinary nitrogen and energy were analyzed using the Kjeldahl method (AOAC International, 2012) and adiabatic bomb calorimeter (IKA-C5000, IKA Works, Staufen, Germany), respectively.

Nutrient Digestibility

The DMI of each nutrient was calculated by summing the intake of each supplied component (milk and starter) and their respective DM and nutrient content, while subtracting the amounts of DM and nutrients obtained from refusals.

The apparent total-tract digestibility of nutrients (g/kg of DM) was determined using the amount consumed and the amount of each nutritional component recovered in the feces. Nitrogen balance was calculated as the difference between dietary nitrogen intake and nitrogen excreted in feces and urine. Gross energy (**GE**) intake was calculated by the difference between the GE content of each of the components supplied in the diet (milk and concentrate). The GE content in milk was calculated according to the equation proposed by Drackley (2008):

$$\text{GE (Mcal/kg of milk)} = (0.911 \times \%\ \text{fat}) + (0.0586 \times \%\ \text{protein}) + (0.0395 \times \%\ \text{lactose}).$$

Euthanasia, GIT, Internal Organs, and Viscera Weight Comparative Slaughter

The animals were randomly selected to be slaughtered at 30 ± 1 d (n = 6/treatment) and 60 ± 1 d (n = 15/treatment) for organ weighing and collection of samples for histological development evaluation. The animals were slaughtered in the morning, before morning feeding, following the protocols of the Brazilian Federal Council of Veterinary Medicine (CFMV, 2013). The slaughter procedure was conducted with sedation (0.05 mg/kg of intramuscular xylazine), followed by intravenous anesthesia (0.1 mg/kg ketamine) and the application of 20 mL of lidocaine via the foramen magnum. Confirmation of death was established through cardiopulmonary auscultation and absence of the corneal reflex. The abdominal cavity was opened and each organ of the GIT (reticulorumen, omasum, abomasum, small and large intestine), was isolated by lashing to preserve the contents, removed, and weighed. The GIT organs were then emptied, washed, and weighed again. Additional organs, such as the tongue, heart, lung, pancreas, liver, bladder, spleen, kidneys, and perirenal, omental, and mesenteric fat were also weighed. The organ weights were computed relative to the empty animal weight, defined as the total weight of the animal before slaughter minus the sum of the weight of visceral content and the weight of organs. The weight of the visceral content was determined by subtracting the weight of the empty GIT from the weight of the full GIT.

Rumen and Cecum pH, NH_3, and VFA

The rumen and cecum were promptly isolated upon opening the abdominal cavity. After isolating the organs, a sample of the contents of the rumen and cecum were collected (50 mL). After collection, the sample was filtered through gauze, and the pH was measured immediately (Mettler Toledo, Columbus, OH).

For determination of N-NH_3 and VFA concentrations, 5 and 10 mL of the filtered content samples from the rumen and cecum were acidified with 1 mL of sulfuric acid (50%) and 1 mL of metaphosphoric acid (20%). The quantification of N-NH_3 was performed according to the colorimetric distillation method proposed by Chaney and Marbach (1962). Absorbance was measured at 630 nm (Thermo Fisher Scientific, Madison, WI) after the Kjeldahl test with magnesium oxide and calcium chloride, following method 920.03 (AOAC, 1980). For VFA analysis the samples were centrifuged (1,800 × *g* 10 min) at room temperature (22–25°C), and subsequently analyzed by HPLC (Waters Alliance e2695 Chromatograph, Waters Technologies do Brazil LTDA, Barueri, SP, Brazil).

Histology

Immediately after slaughter, samples were collected for histological comparison from various sections of the dorsal sac and ventral sac of the rumen, omasum, abomasum, and portions of the small intestine including the duodenum, jejunum, and ileum. These samples were then fixed in formalin and kept in an ethanol solution. Afterward, they were embedded in paraffin blocks and sectioned into 5-µm-thick sections (microtome Olympus, Tokyo, Japan), and affixed to a glass slide for microscopy, stained with hematoxylin eosin. Microscopic images were captured using a microscope equipped with a camera (Olympus CX31 and Olympus OSIS SC30, Tokyo, Japan).

Morphometric analyses were carried out using AxioVision Software 4.8.2–06/2010 (Images Carl Zeiss Systems, Jena, Germany) to evaluate papillae height (ventral and dorsal sac of the rumen, and omasum), villus height (duodenum and ileum), papillae area (ventral and dorsal sac of the rumen, and omasum), villi area (duodenum and ileum), depth of the crypt (abomasum), gland depth (abomasum, duodenum, ileum, and colon), and cell proliferation (abomasum, duodenum, ileum, and colon). To determine the rate of cell division known as the mitotic index, 2,000 cells from the basal layer of the epithelium of the rumen ventral and dorsal sac and omasum were counted, including those with a nucleus in mitosis. The mitotic index was calculated as the ratio between the number of dividing nuclei, and the total number of nuclei (Costa et al., 2008), indicating possible adaptations of the rumen mucosa dietary changes.

Antimicrobial Susceptibility Testing

Fecal samples were directly collected from the rectal ampulla from 45 calves (15 per treatment) at 1, 30, and 60 d of age. The collected samples were stored in sterile 70-mL bottles (Prolab, São Paulo, Brazil) and frozen at −80°C. Stool samples were cultured on MacConkey Agar and incubated for 18 to 24 h at 37°C. After the incubation period, 3 potential *E. coli* isolates were selected from each sample based on morphological characteristics and cultured on Columbia Agar for 18 to 24 h. Molecular identification was performed using PCR (Juck et al., 1996).

The antimicrobials selected for evaluation were based on the antimicrobials used in the treatment protocols for cows that had their milk discarded during the experimental period. These included ampicillin (**AMP**), amoxicillin (**AMO**), ceftiofur (**CEF**), florfenicol (**FLO**), enrofloxacin (**ENR**), streptomycin (**STR**), and tetracycline (**TET**). The susceptibility test of *E. coli* isolates was conducted by the disk diffusion test. Each bacterium isolated from the pure culture was suspended in 2 mL of sterile NaCl solution (0.9%) and adjusted spectrophotometrically to an absorbance of 0.08 to 0.10 at 625 nm. The *E. coli* samples were inoculated onto plates containing Mueller-Hinton Agar (Oxoid), covering the entire surface. The antimicrobials AMP (10 µg), AMO (10 µg), CEF (30 µg), FLO (30 µg), ENR (5 µg), STR (10 µg), and TET (10 µg) were tested. The plates were then incubated at 16 to 18 h to 37°C for subsequent reading. The inhibition halo around each disc was measured in millimeters and classified into one of the categories (resistant, sensitive, or intermediate; Table 2).

Table 2. Zone diameters (mm) and interpretative criteria of tested antimicrobial agents used to categorize antimicrobial susceptibility of fecal *Escherichia coli* isolates in calves (n = 45) fed bulk tank milk, waste milk, and pasteurized waste milk from 4 to 60 d of age

Antimicrobial agent tested[1]	Resistant	Intermediate	Susceptible
Amoxicillin-clavulanic acid	≤13	14–17	≥18
Ampicillin	≤13	14–16	≥17
Ceftiofur	≤17	18–20	≥21
Florfenicol	≤14	15–18	≥19
Enrofloxacin	≤16	17–20	≥21
Streptomycin	≤11	12–14	≥15
Tetracycline	≤11	12–14	≥15

[1]Interpretation of results based on the Clinical and Laboratory Standards Institute (CLSI, 2008, 2013, 2014).

Statistical Analysis

Data were analyzed using R software (R Core Team, 2019; version 4.1.2). Power analysis was conducted to determine the appropriate sample size of 3 treatment groups, using a power of 80% and a significance level of 0.05 using PWR package. Using the parameter with the highest variation (VFA concentration in the rumen and cecum contents), with an SE of the mean of 3.67, it was found a Cohen's f of 2.410989. Therefore, the expected difference to detect was 5.809687 (i.e., 6 calves per group). The data were segmented into 2 periods: 4 to 30 d of age (n = 6/treatment) and 4 to 60 d of age (n = 15/treatment).

The data collected were summarized by period (period 1: calves from 4–30 d; and period 2: calves from 4–60 d). A randomized complete experimental design was implemented to test the hypothesis of the effect of milk type in each outcome. Continuous outcomes such as nutrient intake, performance, antimicrobial resistance in *E. coli* isolates were analyzed independently using a linear mixed model (nlme package). Each independent outcome was modeled as a function of the following fixed effects: treatment, breed composition, day, and their interaction were included as factors for fixed effects, with animals as random effects. There were 3 experimental groups and 2 levels for breed composition, 3/4 and 5/8 Holstein × Gyr. Birth weight and total serum protein were tested as covariates and included in the model only if found to be significant ($P < 0.05$). For variables such as digestibility, nitrogen balance, ruminal and cecum characteristics (pH, NH_3, and VFA), empty BW, the weight of organs and viscera, intestines length, and GIT development were analyzed using a generalized linear model.

All models underwent graphical verification for normality and homoscedasticity of residuals and were tested using the Shapiro-Wilk and Bartlett tests, respectively. A 95% CI was employed to assess the null hypothesis, and *P*-values were determined using the Tukey test. A *P*-value ≤0.05 was considered statistically significant for all analyses.

RESULTS

Nutrient Intake and Performance

Up to 30 d of age (period 1), the daily milk intake in the PWM treatment was 5% lower compared with the WM and BTM treatments (774.57 g DM/d; $P < 0.05$; Table 3). However, no differences were observed across treatments for starter intake, total DMI, and ADG with final BW at 30 d (Tables 3 and 4).

Similar trends were observed for calves evaluated up to 60 d of age (period 2). There were no significant effects of treatments on starter intake and total DMI. Nevertheless, milk intake by PWM-fed calves was significantly lower with around 4% less compared with those receiving WM and BTM ($P < 0.05$). Additionally, WM-fed calves exhibited a similar ADG to those fed BTM, but 14% higher ADG than calves receiving PWM ($P < 0.05$; Table 3). However, the final BW at 60 d of age did not exhibit any significant differences across treatments (Table 4).

Apparent Nutrient Digestibility

For period 1, no differences in nutrient digestibility were observed among BTM, WM, and PWM treatments for digestibility of DM, OM, CP, EE, and GE, respectively (Table 4).

Similar results were observed in period 2. There were no differences between BTM, WM, and PWM treatments for digestibility of DM, OM, and CP ($P > 0.05$; Table 4). However, the BTM and WM treatments exhibit higher digestibility of EE (96.29% and 95.55%, respectively;

Table 3. Nutrient intake and performance of dairy calves fed bulk tank milk (BTM), waste milk (WM), and pasteurized waste milk (PWM) in 2 different periods

	Calves (30 d)[1]					Calves (60 d)[2]				
Item[3]	BTM	WM	PWM	SEM	*P*-value	BTM	WM	PWM	SEM	*P*-value
DMI										
Milk, g/d	774.6[a]	765.2[a]	743.4[b]	2.6	<0.01	777.0[a]	769.4[a]	746.7[b]	2.6	<0.01
Starter, g/d	39.4	56.6	38.0	16.1	0.66	129.6	164.6	181.3	21.1	0.22
Total, g/d	808.9	818.2	775.5	14.5	0.12	895.1	910.7	900.2	22.0	0.87
Performance										
ADG, g/d	545.0	533.3	531.7	0.04	0.97	670.0[ab]	707.3[a]	618.0[b]	0.02	0.04
ADG/TDMI	0.67	0.64	0.68	0.05	0.41	0.76	0.78	0.69	0.01	0.38

[a,b]Different superscripts within a row indicate significant differences between treatments ($P < 0.05$; Tukey test).

[1]Calves (30 d): calves raised from 4–30 d (n = 6/treatment).

[2]Calves (60 d): calves raised from 4–60 d (n = 15/treatment).

[3]ADG/TDMI: calculated by dividing ADG (g) by total DMI.

Table 4. Digestibility of nutrients of dairy calves fed bulk tank milk (BTM), waste milk (WM), and pasteurized waste milk (PWM) in 2 different periods

	Calves (30 d)[1]					Calves (60 d)[2]				
Item[3]	BTM	WM	PWM	SEM	*P*-value	BTM	WM	PWM	SEM	*P*-value
BW, kg	50.74	50.91	49.26	2.69	0.89	76.03	77.43	74.09	0.82	0.77
DMI, kg/d	854.25	870.45	824.44	29.5	0.55	1,070.00	1,167.00	1,170.01	23.01	0.28
Digestibility, %										
DM	85.24	85.09	89.97	20.38	0.19	88.65	88.10	87.08	26.70	0.61
OM	93.92	94.38	94.84	0.81	0.76	95.22	94.94	93.78	3.73	0.06
CP	88.87	87.66	89.01	20.02	0.87	91.70	92.59	91.30	11.00	0.44
EE	95.52	96.50	95.768	5.55	0.30	96.29[a]	95.55[a]	93.28[b]	7.53	<0.01
GE	93.48	94.53	94.37	8.77	0.66	94.39[a]	94.87[a]	93.53[b]	3.51	0.01

[a,b]Different superscripts within a row indicate significant differences between treatments ($P < 0.05$; Tukey test).

[1]Calves (30 d): male calves raised from 4–30 d (n = 6/treatment).

[2]Calves (60 d): male calves raised from 4–60 d (n = 15/treatment).

[3]EE = ether extract; GE = gross energy.

$P < 0.01$) and GE (94.96% and 94.87%, respectively; $P = 0.01$) compared with the PWM (93.28 and 93.35%, respectively).

Nitrogen Balance

Calves from period 1 did not exhibit any differences in nitrogen balance across treatments ($P > 0.05$; Table 5). However, calves in period 2 fed WM and PWM exhibited 13.5% and 15.6% higher nitrogen intake ($P = 0.02$) and 14.6% and 12.0% higher nitrogen retention, respectively ($P = 0.04$) compared with those fed BTM. No differences in fecal nitrogen content and urine nitrogen were observed among the BTM, WM, and PWM treatments ($P > 0.05$; Table 5).

Ruminal and Cecum Parameters

Dietary treatment did not have a significant effect ($P > 0.05$) on the rumen and cecum fermentation parameters of calves from period 1 (Table 6). Nevertheless, in period 2, calves receiving PWM had lower rumen pH (5.03) compared with those receiving the BTM (5.58, $P < 0.01$). The same pattern was observed in ruminal acetate concentration, where PWM-fed animals had higher rumen acetate concentration than calves fed the BTM ($P < 0.01$; Table 6).

GIT Development

In period 1, no discernible differences were observed between treatments for empty BW, weight of internal organs and viscera, and intestine length (Table 7). Similar results were observed in period 2, with exceptions noted for reticulorumen, liver, omental, and perirenal fat weight. The WM- and PWM-treated calves exhibited 16% and 15% higher weights for reticulorumen ($P = 0.01$) and 6% and 8% larger livers ($P < 0.01$), respectively, compared with calves in the BTM treatment (Table 7). Furthermore, the BTM and WM treatments presented 25% and 13% greater weight of omental fat and 42% and 21% higher perirenal fat, respectively, in comparison to PWM ($P < 0.01$; Table 7).

Dietary treatment did not exert a significant influence on various aspects of GIT histology development, including papilla height (ventral and dorsal rumen sac, and omasum), villus height (duodenum and ileum),

Table 5. Nitrogen balance ($g/d/BW^{0.75}$) of dairy calves fed bulk tank milk (BTM), waste milk (WM), and pasteurized waste milk (PWM) in 2 different periods

	Calves (30 d)[1]					Calves (60 d)[2]				
Item	BTM	WM	PWM	SEM	*P*-value	BTM	WM	PWM	SEM	*P*-value
Nitrogen balance, $g/BW^{0.75}/d$										
Nitrogen intake	1.29	1.36	1.33	0.06	0.80	1.63[b]	1.82[a]	1.91[a]	0.05	<0.01
Feces nitrogen	0.15	0.16	0.15	0.02	0.75	0.12	0.14	0.17	0.01	0.31
Urine nitrogen	0.16	0.13	0.24	0.03	0.09	0.28	0.31	0.39	0.02	0.07
Retained nitrogen	0.98	1.07	0.94	0.07	0.51	1.20[b]	1.36[a]	1.35[a]	0.04	0.03

[a,b]Different superscripts within a row indicate significant differences between treatments ($P < 0.05$; Tukey test).

[1]Calves (30 d): calves raised from 4–30 d (n = 6/treatment).

[2]Calves (60 d): calves raised from 4–60 d (n = 15/treatment).

Table 6. Ruminal and cecum pH, ammonia nitrogen (NH_3), and VFA of dairy calves fed bulk tank milk (BTM), waste milk (WM), and pasteurized waste milk (PWM) in 2 different periods

Item	Calves (30 d)[1]			SEM	*P*-value	Calves (60 d)[2]			SEM	*P*-value
	BHM	WM	PWM			BHM	WM	PWM		
Rumen										
pH	5.70	5.71	5.66	0.26	0.99	5.58[a]	5.48[ab]	5.03[b]	0.13	0.01
Ammonia-N, mg/dL	19.88	18.55	27.16	4.97	0.46	26.88	25.20	27.02	4.62	0.95
VFA, µmol/mL										
Acetic (C2)	24.13	39.76	30.63	6.97	0.31	40.62[b]	44.26[ab]	55.03[a]	3.53	0.01
Propionic (C3)	12.61	23.58	20.48	4.55	0.25	32.56	33.03	37.82	3.67	0.54
Butyric (C4)	3.18	5.53	4.24	1.12	0.36	7.21	6.82	9.11	0.86	0.15
C2:C3	2.04	1.66	1.55	0.18	0.29	1.41	1.33	1.28	0.07	0.47
Cecum										
pH	6.42	5.72	5.85	0.23	0.20	6.41	6.55	6.35	0.27	0.46
Ammonia-N, mg/dL	21.87	19.82	30.38	4.65	0.28	17.35	19.65	20.41	4.82	0.51
VFA, µmol/mL										
Acetic (C2)	30.06	30.44	42.09	5.50	0.28	35.21	35.22	38.15	1.84	0.79
Propionic (C3)	11.34	13.87	23.27	4.03	0.15	14.66	16.44	17.72	2.22	0.58
Butyric (C4)	5.10	5.41	8.26	0.86	0.06	5.01	4.91	5.11	1.63	0.96
C2:C3	2.79	2.57	1.97	0.40	0.37	2.47	2.19	2.41	0.36	0.48

[a,b]Different superscripts within a row indicate significant differences between treatments ($P < 0.05$; Tukey test).

[1]Calves (30 d): calves raised from 4–30 d (n = 6/treatment).

[2]Calves (60 d): calves raised from 4–60 d (n = 15/treatment).

papillae area (ventral and dorsal sac of the rumen, and omasum), villi area (duodenum and ileum), crypt depth (abomasum), glandular epithelium depth (abomasum, duodenum, ileum, and colon), and cell proliferation (abomasum, duodenum, ileum, and colon). Therefore, there were no notable effects on the GIT development of calves up to 30 or 60 d of age (Table 8).

Antimicrobial Resistance of E. coli Isolates

No fecal *E. coli* isolate showed resistance to STR (Table 9). No treatment effects or treatment × week interactions were observed for FLO resistance. Additionally, no isolated effects of treatments or treatment × week interactions were noted on the prevalence on the resistant

Table 7. Empty BW (kg), weight of internal organs and viscera, and intestine length of dairy calves fed bulk tank milk (BTM), waste milk (WM), and pasteurized waste milk (PWM) in 2 different periods

Item	Calves (30 d)[1]			SEM	*P*-value	Calves (60 d)[2]			SEM	*P*-value
	BTM	WM	PWM			BTM	WM	PWM		
Empty BW, kg	51.81	51.77	49.46	2.58	0.72	73.15	73.66	70.17	4.81	0.08
Organ weight, % of empty BW										
Reticulo-rumen	0.65	0.73	0.71	0.036	0.28	1.06[b]	1.26[a]	1.24[a]	0.181	0.01
Omasum	0.17	0.17	0.18	0.012	0.99	0.20	0.21	0.22	0.001	0.38
Abomasum	0.52	0.53	0.60	0.028	0.11	0.50	0.48	0.49	0.002	0.33
Small intestine	3.63	3.66	3.28	0.148	0.18	2.94	2.69	3.02	0.071	0.06
Large intestine	1.06	1.8	1.03	0.042	0.07	1.21	1.17	1.19	0.011	0.79
Bladder	0.07	0.08	0.08	0.005	0.38	0.06	0.07	0.07	0.001	0.07
Liver	2.55	2.57	2.58	0.089	0.97	2.15[b]	2.29[a]	2.34[a]	0.012	<0.01
Spleen	0.38	0.47	0.56	0.053	0.11	0.52	0.51	0.57	0.021	0.19
Kidneys	0.49	0.50	0.56	0.034	0.40	0.47	0.51	0.52	0.003	0.11
Pancreas	0.06	0.07	0.07	0.003	0.14	0.08	0.08	0.08	0.001	0.38
Heart	0.68	0.71	0.72	0.026	0.60	0.63	0.63	0.66	0.003	0.09
Omental fat	0.41	0.40	0.39	0.034	0.97	0.61[a]	0.53[a]	0.46[b]	0.009	<0.01
Perirenal fat	0.63	0.62	0.50	0.064	0.22	0.82[a]	0.61[a]	0.48[b]	0.011	<0.01
Mesenteric fat	0.51	0.55	0.47	0.025	0.11	0.57	0.51	0.51	0.006	0.27
Tongue	0.43	0.44	0.46	0.014	0.26	0.47	0.45	0.47	0.001	0.46
Length, m										
Small intestine	23.07	22.84	21.97	0.842	0.62	22.96	23.02	23.46	2.461	0.97
Large intestine	2.58	2.68	2.62	0.121	0.83	3.09	3.15	3.24	0.131	0.78

[a,b]Different superscripts within a row indicate significant differences between treatments ($P < 0.05$; Tukey test).

[1]Calves (30 d): calves raised from 4–30 d (n = 6/treatment).

[2]Calves (60 d): calves raised from 4–60 d (n = 15/treatment).

Table 8. Gastrointestinal tract development of dairy calves fed bulk tank milk (BTM), waste milk (WM), and pasteurized waste milk (PWM) in 2 different periods

	Calves (30 d)[1]					Calves (60 d)[2]				
Item[3]	BTM	WM	PWM	SEM	*P*-value	BTM	WM	PWM	SEM	*P*-value
Height, µm										
Ruminal papillae DS	199.52	170.95	222.42	40.54	0.62	235.97	209.69	258.32	52.9	0.37
Ruminal papillae VS	140.74	191.46	128.61	29.18	0.27	219.86	263.13	233.41	61.9	0.23
Omasum papillae	138.48	179.04	190.81	23.98	0.32	205.41	219.65	213.52	56.7	0.89
Duodenum villi	151.88	126.51	140.15	11.65	0.31	136.87	160.88	144.05	8.86	0.09
Ileum villi	139.89	114.01	119.27	9.12	0.14	134.94	144.23	138.75	8.16	0.61
Area, µm^2										
Ruminal papillae DS	30.79	26.02	43.98	10.58	0.44	50.14	40.49	65.34	4.62	0.71
Ruminal papillae VS	15.90	33.17	20.93	6.47	0.18	34.98	43.12	33.09	4.11	0.56
Omasum papillae	12.19	19.66	21.86	3.67	0.20	26.41	32.91	26.34	2.03	0.41
Duodenum villi	7.83	5.65	5.81	0.74	0.15	5.29	7.48	6.71	0.42	0.14
Ileum villi	6.31	4.77	5.58	0.73	0.35	6.07	6.66	7.05	3.72	0.41
Crypt depth, µm										
Abomasum	114.79	94.18	111.17	9.76	0.29	99.27	110.64	108.77	5.6	0.54
Depth gland, µm										
Abomasum	96.03	75.61	80.62	9.98	0.35	62.54	63.59	70.54	13.21	0.38
Duodenum	93.03	96.10	80.75	7.07	0.29	102.79	92.11	108.67	7.66	0.32
Ileum	85.38	78.05	92.44	7.89	0.45	84.32	86.09	96.72	3.41	0.17
Colon	389.95	392.63	352.79	19.57	0.30	363.03	351.56	330.52	24.9	0.24
Cell proliferation, no.										
Abomasum	1.01	0.60	0.16	0.47	0.48	1.9	0.91	1.54	0.24	0.39
Duodenum	22.66	22.83	17.66	3.19	0.45	18.43	20.08	25.47	1.26	0.24
Ileum	15.00	11.83	12.33	2.26	0.58	14.21	14.14	16.53	0.72	0.61
Colon	8.83	3.75	7.16	1.50	0.11	4.54	6.69	5.69	0.17	0.41
Mitotic index, %										
Rumen DS	0.59	0.54	0.46	0.16	0.84	0.46	0.47	0.49	0.07	0.96
Rumen VS	0.67	0.45	0.44	0.13	0.53	0.69	0.65	0.67	0.09	0.93
Omasum	0.99	0.86	1.01	0.17	0.81	0.85	0.91	0.92	0.11	0.82

[1]Calves (30 d): calves raised from 4 to 30 d (n = 6/treatment).

[2]Calves (60 d): calves raised from 4 to 30 d (n = 15/treatment).

[3]DS = dorsal rumen sac; VS = ventral rumen sac.

fecal *E. coli* across AMO, AMP, CEF, ENR, and TET at 3 d of age. At 30 d of age, the WM and PWM treatments exhibited a higher prevalence of resistant fecal *E. coli* compared with BTM for AMO, AMP, CEF, ENR, and TET. Similar patterns were observed at 60 d of age, in which the WM and PWM treatments showed a higher prevalence of resistant fecal *E. coli* compared with BTM for AMO, AMP, CEF, and ENR.

DISCUSSION

This study aimed to explore the association between different types of milk (BTM, WM, and PWM) in the liquid diet of preweaning calves and their effect on calf development and performance. This research is unique in its simultaneous evaluation of these milk types over 2 distinct periods (30 and 60 d), assessing apparent nutrient digestibility, ruminal and cecal fermentation, GIT development, and antimicrobial-resistant fecal *E. coli* of dairy calves. Our key findings include (1) PWM-fed calves consumed less milk solids than those on BTM and WM, and presented lower EE digestibility at 60 d of age; (2) milk type influenced ruminal pH and acetate concentration; (3) BTM-fed calves exhibited lighter reticulum-rumen and livers, whereas PWM-fed calves had reduced omental and perirenal fat; and (4) calves fed WM and PWM demonstrated a higher prevalence of resistant fecal *E. coli* at both 30 and 60 d. Specifically, WM-fed calves showed resistance to 5 out of 7 tested antimicrobials, whereas PWM-fed calves demonstrated resistance to 4 out of 7 antimicrobials.

In our study, we hypothesized that the provision of WM and PWM may alter nutrient digestibility. Our observations partially support this hypothesis, as calves fed PWM (but not WM) exhibited lower EE and GE digestibility values. The lower digestibility of EE and GE observed in the PWM treatment in period 2 (4–60 d) can be attributed to the effects of thermal processing on milk fat content and fatty acid composition. In PWM, fat content tends to decrease due to the fat adherence to the container surface after processing (Pestana et al., 2015). Additionally, thermal processing may lead to oxidative losses of unsaturated lipids such as linoleic (C18:2 n-6) and arachidonic acid (C20:4 n-6), as well as certain SFA, such as butyric acid (C4:0), caproic acid (C6:0), and caprylic acid (C8:0; Fidler et al., 2001; Pestana et

Table 9. Prevalence of resistant fecal *Escherichia coli* isolates of dairy calves fed bulk tank milk (BTM, n = 15), waste milk (WM, n = 15), and pasteurized waste milk (PWM, n = 15) at 3, 30, and 60 d of age[1]

	BTM			WM			PWM				*P*-value[2]		
Item[3]	Day 3, %	Day 30, %	Day 60, %	Day 3, %	Day 30, %	Day 60, %	Day 3, %	Day 30, %	Day 60, %	SEM	T	D	T × D
AMO	24.92^{a,A}	11.11^{b,B}	16.66^{b,B}	44.44^{b,A}	67.74^{a,A}	55.88^{a,A}	37.51^{b,A}	72.72^{a,A}	55.00^{a,A}	3.27	0.05	0.03	<0.01
AMP	29.26^{a,A}	11.11^{b,B}	14.28^{ab,B}	41.66^{b,A}	70.96^{a,A}	55.88^{ab,A}	25.00^{b,A}	72.72^{a,A}	50.00^{a,A}	3.98	0.04	0.04	<0.01
CEF	0.00^{A}	2.77^{B}	0.00^{B}	5.55^{A}	48.38^{A}	32.35^{A}	7.50^{A}	57.57^{A}	30.00^{A}	5.76	0.03	0.54	<0.01
FLO	0.00	2.77	2.38	0.00	3.22	5.88	0.00	18.18	10.00	3.84	0.99	0.21	0.14
ENR	2.43^{a,A}	0.00^{a,B}	2.38^{a,C}	5.55^{b,A}	58.06^{a,A}	58.82^{a,A}	2.50^{c,A}	75.75^{a,A}	40.00^{b,B}	4.49	0.04	0.62	<0.01
STR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	—	—	—	—
TET	53.65^{a,A}	41.66^{a,B}	42.85^{a,B}	66.66^{b,A}	96.77^{a,A}	70.58^{b,A}	45.00^{b,A}	96.96^{a,A}	75.00^{a,A}	6.39	0.02	0.57	<0.01

[a–c]Different lowercase superscripts within a row indicate significant differences between time (days).

[A–C]Different uppercase superscripts within a row indicate significant differences between treatments ($P < 0.05$; Tukey test).

[1]Percentage of resistant *E. coli* based on CLSI (2008, 2013, 2014).

[2]T = treatment effect; D = day effect; and T × D = treatment by day interactions.

[3]AMO = amoxicillin-clavulanic acid; AMP = ampicillin; CEF = ceftiofur; FLO = florfenicol; ENR = enrofloxacin; STR = streptomycin; TET = tetracycline.

al., 2015). In calves, triglycerides present in the liquid diet undergo enzymatic hydrolysis in the abomasum, forming free fatty acids and 2-monoglyceride, both of which require long-chain fatty acids for chain formation. These molecules are then incorporated into the micelles for absorption in the small intestine. The stability of fat micelles, which are crucial for absorption, depends on the presence of 2-monoglyceride molecules and bile salts. However, the reduction of long-chain UFA in the diet may compromise the total apparent digestibility in the GIT due to insufficient 2-monoglyceride formation for micelle incorporation (Spanski et al., 1997).

Sixty-day-old calves fed WM and PWM consumed, on average, 6 g/BW$^{0.75}$ per day more nitrogen than calves receiving BTM, likely attributable to the differences in CP intake previously reported by Vieira et al. (2021). They have demonstrated that calves fed WM and PWM had higher CP intake (57.35 g/d and 59.22 g/d, respectively) between 53 and 60 d of age compared with those in the BTM treatment (50.75 g/d). Furthermore, the higher nitrogen intake and lack of differences in CP digestibility by WM- and PWM-fed calves may elucidate the greater nitrogen retention observed in animals receiving these treatments.

During suckling, a relatively small quantity of milk enters the rumen (Berends et al., 2012; Ellingsen et al., 2016). Antibiotics present in WM reaching the rumen can exert detrimental effects on rumen fermentation by directly affecting microorganisms (Owens and Basalan, 2016). However, in the first weeks of life, calves still possess a rudimentary microbiota and rumen epithelium (Diao et al., 2019), which likely contributed to the similar fermentation pattern observed for animals evaluated at the 30-d mark in our study. In contrast, older calves exhibit a more-developed epithelium and microbiota, making modifications of the rumen fermentation pattern more easily detected. Accordingly, 60-d-old calves subjected to the PWM treatment had higher concentrations of acetic acid and lower pH values compared with the BTM treatment. This difference may be attributed to penicillin and streptomycin present in the WM, which has been shown to increase acetate concentrations under anaerobic conditions (Wasserman et al., 1952; Xiong et al., 2017). Our results align with Li et al. (2019), who evaluated the effects of several antibiotics in the milk replacer on the rumen fermentation pattern of preweaning calves, observing increased acetic acid concentration in the rumen. Thus, the effect of the antibiotics in the rumen is believed to influence specific microbial abundance groups and, consequently, result in different ruminal fermentation patterns and ruminal pH levels (Zhang et al., 2019b).

The absorption of VFA through the ruminal epithelium provides the chemical stimuli required for epithelium

proliferation (Diao et al., 2019). In the present study, the higher reticulorumen weight observed for calves fed WM and PWM at 60 d may be partially attributed to differences in starter consumption, known to influence VFA synthesis and overall rumen development. However, this observation does not fully account for the greater reticulorumen development in calves fed WM and PWM. The stimulatory effects of VFA on papillae and proliferation are specific, with butyrate being a potent stimulator, followed by propionate (Tamate et al., 1962). In our work, we did not observe significant treatment effects on butyrate and propionate concentrations. Although these specific VFA are recognized for their crucial roles in stimulating papillae and the proliferation of the ruminal epithelium, it is essential to consider that factors beyond VFA levels may contribute to the observed differences in reticulorumen development among calves fed WM and PWM within 60 d of age. Considering that Li et al. (2019) suggest that the development of ruminal papillae may be more closely associated with blood insulin levels, a deeper exploration into research would be interesting to examine the effects of insulin on ruminal development in calves, particularly under conditions resembling those in our study.

Additionally, Nishihara et al. (2019) uncovered molecular insights into ruminal development in young calves by linking the growth of rumen papillae to the expression of genes associated with insulin-like growth factors synthesis in the ruminal epithelium. Previous investigations (Li et al., 2019; Zhang et al., 2019b) have associated distinctions in GIT development among preweaning calves fed BTM, WM, and milk replacer with starter intake, often without significant alterations in VFA production. Specifically, Zhang et al. (2019)) explored the GIT development of calves fed BTM, WM, and milk replacer, finding that calves fed BTM and milk replacer exhibited higher pre-stomach weight and rumen weight compared with the those fed WM due to increased starter consumption.

Li et al. (2019) evaluated the rumen development of calves receiving milk replacer without antibiotics or milk replacer containing antibiotics and found no significant histological differences in papillae length and papillae width in the dorsal sac. However, a higher papillae length was observed in the ventral sac of the group treated with milk replacer containing antibiotics. The authors suggested that the difference in the ventral sac histology could be elucidated by the ruminal concentration of VFA, despite no discernible difference in total VFA concentrations between treatments.

In our investigation, greater liver weight was only observed in calves from the WM and PWM treatments at the 60-d mark. The liver serves as a primary site for the metabolism of antibiotics, where these antimicrobial undergo transformation into active or inactive metabolites through enzymatic processes, contingent upon the specific drug administered (Linhares et al., 1998). We posit that the daily antibiotic consumption by the animals may have imposed an additional load on liver function, potentially contributing to the higher organ weight values observed in WM and PWM-fed calves in our study. Moreover, the greater deposition of omental and perirenal fat observed in the BTM and WM treatments was likely linked to higher fat intake over the experimental period (Vieira et al., 2021) and greater deposition at these specific anatomical sites.

The presence of antimicrobial residues in WM raises significant concerns within the scientific community. Despite the potential fluctuations in antimicrobial levels in WM, its use poses a risk by exerting selective pressure, fostering the emergence and spread of resistant bacteria in the IM of calves (Maynou et al., 2017a; Firth et al., 2021; Zhang et al., 2022). In our study, both WM and PWM treatments exhibited a higher prevalence of fecal *E. coli* resistant to amoxicillin-clavulanic acid, AMP, CEF, ENR, and TET. Notably, the majority of treatments administered in the cow herd, including those for mastitis, retained placenta, and metritis, involved β-lactams and TET. Maynou et al. (2017b) reported an increased prevalence of *E. coli* resistant to CEF and FLO in the group of animals receiving PWM, as these 2 antimicrobials were among the most frequently administered for treating diseases within the herd. Similarly, Foutz et al. (2018) observed a higher prevalence of fecal *E. coli* resistant to AMP, CEF, ENR, FLO, gentamicin, oxytetracycline, sulfadimethoxine, and trimethoprim in animals that received PWM and milk replacer containing antibiotics. These findings underscore the potential role of milk types in contributing to the dissemination of antimicrobial resistance.

In our study, the emergence of fecal *E. coli* resistant to FLO was unexpected, as this drug was not used in animal treatment protocols. Nevertheless, all treatments exhibited FLO-resistant fecal *E. coli* at 30 and 60 d of age. Previous studies have suggested that resistance to FLO in *E. coli* isolates has been mediated by the *floR* gene (White et al., 2000; Doublet, 2002). When evaluated in conjunction with mobile genetic elements, the *floR* gene has been found alongside other resistance genes, heightening the risk of co-selection and horizontal dissemination of resistant *E. coli* (Meunier et al., 2010). Consistent with our findings, Maynou et al. (2017b) also observed FLO-resistant fecal *E. coli* in the PWM treatment, despite the absence of antibiotic use for treating conditions in the herd. This highlights the possibility of resistance dissemination, even in scenarios where the antibiotic is not directly used in specific treatments.

Exposure to antibiotics typically found in the liquid diet diminishes following weaning, thereby reducing

the selection pressure, and consequently, the emergence of resistant bacteria in the GIT (Foutz et al., 2018). However, in our study, all experimental animals were slaughtered at 60 d for assessments of organ and viscera development. As a result, we advocate for future research that tracks animals in transition from liquid to solid diets beyond weaning. Understanding the effect of antibiotic residues is crucial for developing new feeding and residue-disposal strategies within calf-rearing systems.

It is important to acknowledge certain limitations of the study that may benefit from future investigation. We anticipated variations in milk composition among treatments, which could influence the animal's microbiota and development. Adjusting the milk replacer to stabilize its nutritional values could potentially provide insights into this effect. However, the primary aim of our study was to compare different types of liquid diets as they are typically provided on farms. Therefore, altering the nutritional content would have deviated from the original study objective. It is important to note that the WM used in this trial was derived specifically from cows with mastitis. Thus, this type of milk is known for its lower fat and TS content. Additionally, due to experimental logistics, the milk of the WM and PWM treatments comprised only the morning milking, unlike the BTM treatment, which included both morning and evening milk. Consequently, due to dilution factors, the levels of fat and TS are lower in the morning milk compared with the evening milk. Furthermore, the presence of bacterial lipase in the milk of the PWM treatment could have contributed to increased lipolysis during the storage period, consequently reducing fat and energy content in this treatment. Therefore, nutritional differences between treatments could have obscured the benefits of the pasteurization process. From an antimicrobial perspective, WM and PWM could have also exhibited differences in residue composition. Because calves were born sequentially over a 2-mo period and enrolled at different times into the trial, the antimicrobial treatments administered to the herd may have varied over time. Consequently, the spectrum of antimicrobials contained in WM and PWM treatments might have fluctuated throughout the observation period.

CONCLUSIONS

Pasteurizing WM has adverse effects on nutrient digestibility of calves, potentially compromising their performance. Nevertheless, the changes in ruminal fermentation patterns hint at a potential effect on rumen development. The use of WM for calf feeding may contribute to the gradual selection of resistant fecal *E. coli* in the GIT over time, posing a potential threat to the effectiveness of disease treatments within the herd. The identification of florfenicol-resistant *E. coli* suggests that other factors beyond direct antibiotic exposure may be involved, such as the horizontal bacterial transfer from the environment or direct contact with other animals.

NOTES

Gratitude is extended to Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, Belo Horizonte, Brazil), for funding this study. Additional appreciation goes to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brasília, Brazil), Universidade Federal de Minas Gerais (UFMG, Minas Gerais, Brazil), Universidade Federal de Lavras (UFLA, Minas Gerais, Brazil), Instituto de Laticínios Cândido Tostes (EPAMIG, Minas Gerais, Brazil), and Embrapa Gado de Leite (Minas Gerais, Brazil) for providing the necessary infrastructure to carry out this project. The experimental procedures employed in this study were approved by the Ethics Committee on Animal Use of Embrapa Dairy Cattle (CEUA number: 9849040419). The authors have not stated any conflicts of interest.

Nonstandard abbreviations used: AMO = amoxicillin; AMP = ampicillin; BTM = bulk tank milk; CEF = ceftiofur; EE = ether extract; ENR = enrofloxacin; FLO = florfenicol; GIT = gastrointestinal tract; GE = gross energy; IM = intestinal microbiota; PWM = pasteurized waste milk; STR = streptomycin; TBC = total bacterial count; TDMI = total DMI; TET = tetracycline; WM = waste milk.

REFERENCES

AOAC (Association of Official Analytical Chemists). 1980. Official Methods of Analysis. 13th ed. AOAC, Washington, DC.

AOAC International. 2012. Official Methods of Analysis. 19th ed. AOAC International, Gaithersburg, MD.

Aust, V., K. Knappstein, H.-J. Kunz, H. Kaspar, J. Wallmann, and M. Kaske. 2013. Feeding untreated and pasteurized waste milk and bulk milk to calves: Effects on calf performance, health status and antibiotic resistance of faecal bacteria. J. Anim. Physiol. Anim. Nutr. (Berl.) 97:1091–1103. https://doi.org/10.1111/jpn.12019.

Berends, H., C. G. van Reenen, N. Stockhofe-Zurwieden, and W. J. J. Gerrits. 2012. Effects of early rumen development and solid feed composition on growth performance and abomasal health in veal calves. J. Dairy Sci. 95:3190–3199. https://doi.org/10.3168/jds.2011-4643.

Brunton, L. A., D. Duncan, N. G. Coldham, L. C. Snow, and J. R. Jones. 2012. A survey of antimicrobial usage on dairy farms and waste milk feeding practices in England and Wales. Vet. Rec. 171:296. https://doi.org/10.1136/vr.100924.

CFMV (Conselho Federal de Medicina Veterinária). 2013. Guia Brasileiro de Boas Práticas Para Eutanásia Em Animais—Conceitos e Procedimentos. 1st ed. CFMV, Brasília.

Chaney, A. L., and E. P. Marbach. 1962. Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130–132.

CLSI (Clinical and Laboratory Standards Institute). 2008. Performance Standards for Antimicrobial Susceptibility Testing. 18th ed. Clinical and Laboratory Standards Institute, Wayne, PA.

CLSI (Clinical and Laboratory Standards Institute). 2013. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests

for Bacteria Isolated from Animals; Approved Standard. VET01–A4. 4th ed. Clinical and Laboratory Standards Institute, Wayne, PA.

CLSI (Clinical and Laboratory Standards Institute). 2014. Performance Standards for Antimicrobial Susceptibility Testing: 24th Informational Supplement. 24th ed. Clinical and Laboratory Standards Institute, Wayne, PA.

Costa, S. F., M. N. Pereira, L. Q. Melo, J. C. Resende Júnior, and M. L. Chaves. 2008. Alterações morfológicas induzidas por butirato, propionato e lactato sobre a mucosa ruminal e a epiderme de bezerros: I Aspectos histológicos. Arq. Bras. Med. Vet. Zootec. 60:1–9. https://doi.org/10.1590/S0102-09352008000100001.

Dennis, T. S., F. X. Suarez-Mena, W. Hu, T. M. Hill, J. D. Quigley, and R. L. Schlotterbeck. 2019. Effects of milk replacer feeding rate and long-term antibiotic inclusion in milk replacer on performance and nutrient digestibility of Holstein dairy calves up to 4 months of age. J. Dairy Sci. 102:2094–2102. https://doi.org/10.3168/jds.2018-15652.

Diao, Q., R. Zhang, and T. Fu. 2019. Review of strategies to promote rumen development in calves. Animals (Basel) 9:490. https://doi.org/10.3390/ani9080490.

Doublet, B. 2002. Molecular analysis of chromosomally florfenicol-resistant Escherichia coli isolates from France and Germany. J. Antimicrob. Chemother. 49:49–54. https://doi.org/10.1093/jac/49.1.49.

Drackley, J. K. 2008. Calf nutrition from birth to breeding. Vet. Clin. North Am. Food Anim. Pract. 24:55–86. https://doi.org/10.1016/j.cvfa.2008.01.001.

Ellingsen, K., C. M. Mejdell, N. Ottesen, S. Larsen, and A. M. Grøndahl. 2016. The effect of large milk meals on digestive physiology and behaviour in dairy calves. Physiol. Behav. 154:169–174. https://doi.org/10.1016/j.physbeh.2015.11.025.

Fidler, N., T. U. Sauerwald, H. Demmelmair, and B. Koletzko. 2001. Fat content and fatty acid composition of fresh, pasteurized, or sterilized human milk. Adv. Exp. Med. Biol. 501:485–495.

Firth, C. L., K. Kremer, T. Werner, and A. Käsbohrer. 2021. The effects of feeding waste milk containing antimicrobial residues on dairy calf health. Pathogens 10:112. https://doi.org/10.3390/pathogens10020112.

Foutz, C. A., S. M. Godden, J. B. Bender, F. Diez-Gonzalez, M. Akhtar, and A. Vatulin. 2018. Exposure to antimicrobials through the milk diet or systemic therapy is associated with a transient increase in antimicrobial resistance in fecal *Escherichia coli* of dairy calves. J. Dairy Sci. 101:10126–10141. https://doi.org/10.3168/jds.2018-14598.

Garzon, A., P. Pandey, L. Tell, S. Aly, R. Poppenga, and R. Pereira. 2020. Evaluation of heat and pH treatments on degradation of ceftiofur in whole milk. Front. Vet. Sci. 7:288. https://doi.org/10.3389/fvets.2020.00288.

Juck, D., J. Ingram, M. Prévost, J. Coallier, and C. Greer. 1996. Nested PCR protocol for the rapid detection of *Escherichia coli* in potable water. Can. J. Microbiol. 42:862–866. https://doi.org/10.1139/m96-110.

Li, J. H., M. H. Yousif, Z. Q. Li, Z. H. Wu, S. L. Li, H. J. Yang, Y. J. Wang, and Z. J. Cao. 2019. Effects of antibiotic residues in milk on growth, ruminal fermentation, and microbial community of preweaning dairy calves. J. Dairy Sci. 102:2298–2307. https://doi.org/10.3168/jds.2018-15506.

Linhares, M. C., L. F. Harran, T. J. Strelevitz, J. W. Gauthier, M. J. Cole, R. L. Hassfurther, and J. E. Risk. 1998. Disposition and metabolism of the novel macrolide antibiotic CP-163505 in cattle. J. Vet. Pharmacol. Ther. 21:99–106. https://doi.org/10.1046/j.1365-2885.1998.00125.x.

Ma, Y., M. Z. Khan, J. Xiao, G. M. Alugongo, X. Chen, S. Li, Y. Wang, and Z. Cao. 2022. An overview of waste milk feeding effect on growth performance, metabolism, antioxidant status and immunity of dairy calves. Front. Vet. Sci. 9:898295. https://doi.org/10.3389/fvets.2022.898295.

Macdonald, L. E., J. Brett, D. Kelton, S. E. Majowicz, K. Snedeker, and J. M. Sargeant. 2011. A systematic review and meta-analysis of the effects of pasteurization on milk vitamins, and evidence for raw milk consumption and other health-related outcomes. J. Food Prot. 74:1814–1832. https://doi.org/10.4315/0362-028X.JFP-10-269.

Maynou, G., A. Bach, and M. Terré. 2017a. Feeding of waste milk to Holstein calves affects antimicrobial resistance of *Escherichia coli* and *Pasteurella multocida* isolated from fecal and nasal swabs. J. Dairy Sci. 100:2682–2694. https://doi.org/10.3168/jds.2016-11891.

Maynou, G., H. Chester-Jones, A. Bach, and M. Terré. 2019. Feeding pasteurized waste milk to preweaned dairy calves changes fecal and upper respiratory tract microbiota. Front. Vet. Sci. 6:159. https://doi.org/10.3389/fvets.2019.00159.

Maynou, G., L. Migura-Garcia, H. Chester-Jones, D. Ziegler, A. Bach, and M. Terré. 2017b. Effects of feeding pasteurized waste milk to dairy calves on phenotypes and genotypes of antimicrobial resistance in fecal *Escherichia coli* isolates before and after weaning. J. Dairy Sci. 100:7967–7979. https://doi.org/10.3168/jds.2017-13040.

Meunier, D., E. Jouy, C. Lazizzera, B. Doublet, M. Kobisch, A. Cloeckaert, and J.-Y. Madec. 2010. Plasmid-borne florfenicol and ceftiofur resistance encoded by the *floR* and $bla_{\text{CMY-2}}$ genes in *Escherichia coli* isolates from diseased cattle in France. J. Med. Microbiol. 59:467–471. https://doi.org/10.1099/jmm.0.016162-0.

Moore, D. A., J. Taylor, M. L. Hartman, and W. M. Sischo. 2009. Quality assessments of waste milk at a calf ranch. J. Dairy Sci. 92:3503–3509. https://doi.org/10.3168/jds.2008-1623.

Naas, A. E., A. K. Mackenzie, J. Mravec, J. Schückel, W. G. T. Willats, V. G. H. Eijsink, and P. B. Pope. 2014. Do rumen *Bacteroidetes* utilize an alternative mechanism for cellulose degradation? MBio 5:e01401-14. https://doi.org/10.1128/mBio.01401-14.

Nishihara, K., Y. Suzuki, D. Kim, and S. Roh. 2019. Growth of rumen papillae in weaned calves is associated with lower expression of insulin-like growth factor-binding proteins 2, 3, and 6. Anim. Sci. J. 90:1287–1292. https://doi.org/10.1111/asj.13270.

Owens, F. N., and M. Basalan. 2016. Pages 63–102 in Ruminal Fermentation. Springer International Publishing.

Penati, M., G. Sala, F. Biscarini, A. Boccardo, V. Bronzo, B. Castiglioni, P. Cremonesi, P. Moroni, D. Pravettoni, and M. F. Addis. 2021. Feeding pre-weaned calves with waste milk containing antibiotic residues is related to a higher incidence of diarrhea and alterations in the fecal microbiota. Front. Vet. Sci. 8:650150. https://doi.org/10.3389/fvets.2021.650150.

Pereira, R. V. V., J. D. Siler, R. C. Bicalho, and L. D. Warnick. 2014. In vivo selection of resistant *E. coli* after ingestion of milk with added drug residues. PLoS One 9:e115223. https://doi.org/10.1371/journal.pone.0115223.

Pestana, J. M., A. Gennari, B. W. Monteiro, D. N. Lehn, and C. F. V. Souza. 2015. Effects of pasteurization and ultra-high temperature processes on proximate composition and fatty acid profile in bovine milk. Am. J. Food Technol. 10:265–272. https://doi.org/10.3923/ajft.2015.265.272.

R Core Team. 2019. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.

Spanski, N. A., J. K. Drackley, C. L. Davis, and E. H. Jaster. 1997. Utilization of supplemental triglycerides or free fatty acids by calves from 4 to 10 weeks of age. J. Dairy Sci. 80:573–585. https://doi.org/10.3168/jds.S0022-0302(97)75973-3.

Tamate, H., A. D. McGilliard, N. L. Jacobson, and R. Getty. 1962. Effect of various dietaries on the anatomical development of the stomach in the calf. J. Dairy Sci. 45:408–420. https://doi.org/10.3168/jds.S0022-0302(62)89406-5.

Urie, N. J., J. E. Lombard, C. B. Shivley, C. A. Kopral, A. E. Adams, T. J. Earleywine, J. D. Olson, and F. B. Garry. 2018. Preweaned heifer management on US dairy operations: Part I. Descriptive characteristics of preweaned heifer raising practices. J. Dairy Sci. 101:9168–9184. https://doi.org/10.3168/jds.2017-14010.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.

Vieira, S. de F., S. G. Coelho, H. C. Diniz Neto, H. C. M. Sá, B. P. Pereira, B. S. F. Albuquerque, F. S. Machado, L. G. R. Pereira, T. R. Tomich, I. R. T. Renhe, and M. M. Campos. 2021. Effects of bulk tank milk, waste milk, and pasteurized waste milk on the intake, ruminal parameters, blood parameters, health, and performance

of dairy calves. Animals (Basel) 11:3552. https://doi.org/10.3390/ani11123552.

Wasserman, R. H., C. W. Duncan, E. S. Churchill, and C. F. Huffman. 1952. The effect of antibiotics on in vitro cellulose digestion by rumen microorganisms. J. Dairy Sci. 35:571–580. https://doi.org/10.3168/jds.S0022-0302(52)93739-9.

Wegener, H. C. 2003. Antibiotics in animal feed and their role in resistance development. Curr. Opin. Microbiol. 6:439–445. https://doi.org/10.1016/j.mib.2003.09.009.

White, D. G., C. Hudson, J. J. Maurer, S. Ayers, S. Zhao, M. D. Lee, L. Bolton, T. Foley, and J. Sherwood. 2000. Characterization of chloramphenicol and florfenicol resistance in *Escherichia coli* associated with bovine diarrhea. J. Clin. Microbiol. 38:4593–4598. https://doi.org/10.1128/JCM.38.12.4593-4598.2000.

Xiong, Y., M. Harb, and P.-Y. Hong. 2017. Performance and microbial community variations of anaerobic digesters under increasing tetracycline concentrations. Appl. Microbiol. Biotechnol. 101:5505–5517. https://doi.org/10.1007/s00253-017-8253-1.

Zhang, R., W. Zhang, Y. Bi, Y. Tu, Y. Beckers, H. Du, and Q. Diao. 2019. Early feeding regime of waste milk, milk, and milk replacer for calves has different effects on rumen fermentation and the bacterial community. Animals (Basel) 9:443. https://doi.org/10.3390/ani9070443.

Zhang, X., X. Yi, H. Zhuang, Z. Deng, and C. Ma. 2022. Invited Review: Antimicrobial use and antimicrobial resistance in pathogens associated with diarrhea and pneumonia in dairy calves. Animals (Basel) 12:771. https://doi.org/10.3390/ani12060771.