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Effect of water quality on performance and health of dairy calves

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ABSTRACT

Water quality plays a crucial role in calf health and performance, mainly when it is used to dilute milk replacers, which can affect the incidence of diarrhea. This study assessed the effects of municipal tap water (TW) compared with purified water (PW) on calf performance and health. Thirty Holstein calves were randomly assigned to 2 treatment groups in a randomized block design. Calves received 6 L/d of milk replacer (14% solids) diluted with the respective water treatment until gradual weaning (49–53 d). A pelleted starter was offered from birth, and chopped hay was provided ad libitum from 49 to 70 d. Dry matter intake, water intake, and health scores were recorded daily. Average daily gain was measured weekly, whereas body measurements and blood samples were collected biweekly. The PW tended to improve feed efficiency and increased ADG in the third week of life, but the final BW did not differ between treatments. Calves that received PW consumed more hay and tended to have greater water intake. Moreover, PW reduced day with diarrhea and the number of antibiotic treatments. Blood metabolites varied primarily with age, except for higher albumin concentrations in TW calves at wk 10. In summary, the PW tended to enhance performance during preweaning, particularly after diarrhea episodes. These findings highlight the importance of water quality during early life, but further studies are needed to determine the effect on dairy calf health.
Key words: calf nutrition, preweaning, purified water, water intake

INTRODUCTION

Water is a vital nutrient involved in thermoregulation, nutrient transport, digestion, metabolism, and immune function in animals (Schütz, 2012). In dairy calves, adequate water intake supports growth, rumen development, and overall health (Gottardo et al., 2002). Although early-life nutrition strongly influences long-term productivity, water quality is often neglected in calf-rearing systems, where management typically prioritizes feed and housing (Hersom and Crawford, 2008). Evidence suggests that poor water quality can impair water and feed intake, gastrointestinal health, and growth performance in young ruminants (Willms et al., 2002; NASEM, 2021). Therefore, ensuring water quality from the earliest stages is essential to promote healthy growth and productive potential in dairy calves.

Calves obtain water from drinking, feed moisture, and metabolic processes (Jensen and Vestergaard, 2021). Water consumed voluntarily enters the rumen, stimulating microbial colonization and fermentation, which are essential for rumen maturation. In contrast, water from milk replacers bypasses the rumen via the esophageal groove and reaches the abomasum, supporting enzymatic digestion (Jones and Heinrichs, 2022). This physiological distinction highlights that water quality can differentially influence digestive development, particularly the establishment of ruminal function.

Water quality may be compromised by physical, chemical, and microbiological factors. High total dissolved solids, nitrates, sulfates, pathogenic microorganisms, and variations in pH may reduce water palatability and intake and increase health risks (Beede, 2006; Brew et al., 2009, 2011). Contaminated water is linked to diarrhea, impaired growth, and higher morbidity, especially in immunologically immature calves (Kamal et al., 2024). Moreover, water used to reconstitute milk replacers may act as a microbial vehicle, undermining passive immunity (Umar et al., 2014). Thus, poor water quality can jeopardize both health and performance, representing a critical yet often overlooked risk factor in calf-rearing systems.

Ensuring high-quality water is a low-cost yet effective strategy to improve calf health and performance. However, the effects of purified water on young calves remain poorly understood. We hypothesized that providing purified water, especially in milk replacer preparation, could improve health and thereby performance of preweaning Holstein dairy calves accordingly. Therefore, this study

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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.

aimed to evaluate the effects of purified water on water and feed intake, growth performance, and health indicators in preweaning dairy calves.

MATERIALS AND METHODS

All study procedures involving the animals in this study were approved by the "Luiz de Queiroz" College of Agriculture – University of São Paulo Institutional Animal Care and Use Committee (Protocol no. 4298170323). This study was conducted between January and July of 2022 at the calf facilities of the Animal Science Department of the "Luiz de Queiroz" College of Agriculture, Piracicaba, São Paulo, Brazil. During this period, the average temperature was 21°C (maximum of 36°C and minimum of 5°C), the mean relative humidity was 78%, and the average rainfall was 131.7 mm/mo.

Animals, Experimental Design, and Treatments

Thirty Holstein calves (24 males; and 6 females) from the university's dairy herd and a commercial farm near the campus were enrolled in this study. Calves were separated from their dam at birth, weighted, and colostrum-fed with 1.5 doses of colostrum replacer (Alta Genetics, Brazil, SCCL, Saskatoon, Canada; 150 g of IgG) within 3 h after birth, navel disinfection was done using 7% iodine solution. A blood sample was collected 48 h after colostrum feeding to assess the transfer of passive immunity by analyzing Brix with a handheld Brix refractometer (Deelen et al., 2014). There were no differences ($P = 0.18$) between groups for passive immune transfer, with an average and SD of 7.9% ± 0.28% Brix.

Calves were individually housed in suspended individual pens (1.13 × 140 cm) with rubber mats and sawdust bedding in an open shed with fans until they were 21 d. Then, they were housed in individual wooden hutches (135 cm × 100 cm × 145 cm), using chains with an average length of 2 m, allowing an area for walking but no physical contact between calves. The pens were cleaned daily, the bedding was replaced, and the hutches were moved to avoid the accumulation of feces and to keep the calves in a clean and dry area. The shelters had an east-west lateral orientation to ensure the animals had access to shade. Each hutch had a fixed stationary brush at the side, allowing calves to rub themselves.

Calves were blocked by herd, sex, and birth weight and randomly assigned into 2 treatments: (1) tap water (**TW**, control group; n = 15) and (2) purified water (**PW**; n = 15). Calves received their respective water treatment from birth, which was used to dilute their liquid diet and as drinking water, and were monitored for 70 d. The liquid diet consisted of a milk replacer (**MR**), diluted to 14% solids, using the respective water of each treatment.

Table 1. Nutritional values of liquid and solid diets, and hay fed to calves receiving different water treatments during pre- and postweaning periods

Item	Milk replacer[1]	Starter[2]	Tifton hay
DM, %	96.00	87.50	85.57
Chemical composition, % DM			
CP	21.00	21.00	17.00
Ether extract	18.00	3.30	0.93
Ash	7.00	10.00	7.30
Starch	—	25.00	1.40
NDF	—	26.00	71.30
ADF	0.10	13.00	35.43
Lignin	—	—	4.00
NFC	—	39.70	15.20

[1]Milk replacer Kaliber Lacto (De Heus Indústria e Comércio de Nutrição Animal Ltda., Rio Claro, SP, Brasil. Composition = 6 g/kg Ca; 6 g/kg P; 10 mg/kg Cu; 110 mg/kg Fe; 1.3 mg/kg I; 60 mg/kg Mn; 0.3 mg/kg Se; 5,500 mg/kg Na; 110 mg/kg Zn; 27,500 UI/kg vitamin A; 16 mg/kg vitamin B1; 9.5 mg/kg vitamin B2; 7 mg/kg vitamin B6; 80 µg/kg vitamin B12; 160 mg/kg vitamin C; 4,500 UI/kg vitamin D; 300 IU/kg vitamin E; 4.5 mg/kg vitamin K; 1 mg/kg folic acid; 22.5 mg/kg pantothenic acid; 120 µg/kg biotin; 40 mg/kg niacin; 3.2 × 109 *B. Licheniformis;* 3.2 × 109 *B. Subtilis*.

[2]Kaliber Initial, De Heus Indústria e Comércio de Nutrição Animal Ltda., Rio Claro, SP, Brasil, Premix: 7 g/kg Ca; 4.5 g/kg P; 0.9 mg/kg Co; 27 mg/kg Cu; 3 g/kg S; 1.3 mg/kg I; 2.5 g/kg Mg; 60 mg/kg Mn; 8,000 mg/kg K; 0.6 mg/kg Se; 4.5 g/kg Na; 100 mg/kg Zn; 5,400 UI/kg vitamin A; 1,300 UI/kg vitamin D; 40 IU/kg vitamin E; 45 mg/kg sodium monensin.

The MR was composed mainly of milk protein (skim milk powder) and vegetable protein (wheat gluten), with 21% CP and 18% ether extract. The calves were fed 6 L/d, divided into 2 meals (0700 and 1700 h) using individual teat-bucket (Milkbar). Liquid diet intake and the leftovers were measured daily.

From the first day of life, calves had ad libitum access to water and commercial concentrate (21% CP). The commercial pelleted concentrate (Kaliber Inicial, De Heus Indústria e Comércio de Nutrição Animal Ltda., Rio Claro, SP, Brazil; Table 1) was provided in the morning. The leftovers from the previous day were weighed daily on a digital scale (Toledo 9094, Dracena, Brazil) to calculate the daily starter and total DMI.

The drinking water provided was separated into 2 treatments, according to the water treatment method, namely tap water from the university's water supply system (TW) and purified water from a PW5660 water purifier (PW; PWTECH Indústria e Comércio de Purificadores Ltda., São Carlos, SP, Brazil). In both treatments, water was provided ad libitum, and consumption was recorded daily on a digital scale (Toledo 9094, Dracena, Brazil) by measuring the leftovers. Calves were gradually weaned with a 1 L/d reduction in the liquid diet, regardless of the solid diet intake, starting at 49 d until the end of 53 d. From 49 d of age, chopped hay (medium-quality Tifton hay) was offered ad libitum to the calves, and their consumption was recorded daily. Calves were followed until 70 d of age and caretakers were not blinded because buckets and feeding utensils were identified.

Once a week, each nipple was uncoupled from its respective teat-bucket, cleaned internally using a brush, and reattached to the same teat-bucket to prevent cross-contamination. Cleaning solutions were prepared by mixing hot water at 60°C, neutral detergent at a 1:10 mL ratio and an alkaline detergent containing sodium hypochlorite at a 1:20 ratio. Teat-bucket, water buckets, and feeding utensils were first rinsed with cold water to remove organic residues. Subsequently, all items were scrubbed with a sponge and a detergent solution, soaked for 10 min to allow the disinfectant action, and then rinsed with warm water at 32°C. After rinsing, a disinfectant solution of Dioxiplus (Dioxide Ind. Química Ltda.), 1 mL/L of water, was sprayed onto all surfaces. The cleaned and disinfected teat-bucket and utensils were stored in a cold room maintained at 4°C until their subsequent use. This protocol was all done using tap water.

Feed samples were collected monthly for chemical composition analysis. The DM and ash were determined according to AOAC International (2002; method 925.40; 942.05). Total nitrogen concentration was determined using the Leco TruMac N apparatus (Leco Corporation, St. Joseph, MI; AOAC International, 2002; method 968.06), and CP was estimated by converting total nitrogen using a factor of 6.25. Ether extract concentration was determined using petroleum ether (AOAC International, 2002; method 920.39). Total Starch Assay Kit AA/AMG–Megazyme (AOAC International, 2002; method 996.11). Sequential detergent fiber analyses were used to determine the concentration of NDF (Van Soest et al., 1991) and ADF (Goering and Van Soest, 1970) on an Ankon 2000 fiber analyzer (Ankom Tech. Corp., Fairport, NY). Heat stable α-amylase and sodium sulfite were included in the NDF analysis. The NFC content of the diets was estimated according to Mertens (1997). The chemical composition and ingredients of the liquid and solid diets are shown in Table 1.

Performance and Health

Calves were weighed at birth and then weekly before morning feeding on a mechanical scale (ISO-300, Coimma Ltda., Dracena, SP, Brazil). Body measurements were collected immediately after weighing at wk 0, 2, 4, 6, 8, and 10. The wither height and hip width were measured using a ruler with a scale in centimeters (Carci, São Paulo, SP, Brazil), and the heart girth was measured with measuring tape (Bovitec, São Paulo, SP, Brazil), also with a scale in centimeters.

Fecal scores were assessed daily to monitor the occurrence of diarrhea. Scores were given according to consistency: (0) normal; (1) pasty or semi-formed; (2) fluid; or (3) liquid-aqueous (Reis et al., 2021). Only scores ≥2 for at least 2 consecutive days were considered diarrhea. Oral rehydration protocol (2 L/d of warm water with 5 g of common salt, 4 g of sodium bicarbonate, 25 g of dextrose, and 1 g of potassium chloride for each liter) was offered between milk feedings for every calf that presented diarrhea until the fecal consistency was ≤1.

Rectal temperature was measured daily as part of a health scoring system adapted from the School of Veterinary Medicine at the University of Wisconsin–Madison (Madison, WI), aiming to monitor overall health status and support the identification and diagnosis of diseases.

The diagnosis of babesiosis and anaplasmosis, was made based on the evaluations of the clinical evaluation of the veterinarian. Antimicrobials were used only when the calves presented symptoms of systemic involvement, such as anorexia, refusal of liquid diet, loss of appetite, and fever, and were performed according to the veterinarian's instructions. All occurrences of liquid diet refusal, diarrhea, fever, respiratory diseases, nasal discharge, and cough were individually recorded.

Blood Parameters

Blood samples were collected at wk 2, 4, 6, 8, and 10, 2 h after the morning milk feeding via jugular vein puncture, using 3 different vacuolated tubes: one containing sodium fluoride as an antiglycolytic and potassium EDTA as an anticoagulant to obtain plasma and determine hematocrit, and another with a clot activator to obtain serum (VACUETTE do Brasil, Campinas, SP, Brazil).

Samples were centrifuged (Universal 320R, Hettich, Tuttlingen, Germany) at 2,000 × *g* for 20 min, at a temperature of 4°C, and were placed in plastic tubes and stored in a freezer for later determination of plasma glucose, total protein, albumin, and urea concentrations. Blood parameters were determined using an Automatic Biochemistry System Model SBA 200 (CELM, Barueri, SP, Brazil) using specific commercial kits for glucose, total proteins, albumin, and urea from LABTEST Diagnóstica S.A. (Lagoa Santa, MG, Brazil).

To determine hematocrit, capillaries without heparin were centrifuged for 15 min at 12,000 × *g* using a microhematocrit centrifuge Model SPIN 1000 (MICRO SPIN), with subsequent reading using a hematocrit ruler; the result was expressed as a percentage (%).

Water Analysis

The water quality of both treatments was monitored during the experimental period through chemical analysis of samples collected twice a week. One aliquot was used to measure the hydrogen potential (pH) values using a digital benchtop pH meter (Model Tec-5, Tecnal Ltda., Piracicaba, SP, Brazil) and electrical conductivity using a benchtop conductivity meter. The other 2 aliquots were stored in plastic tubes and a freezer at −20°C for later determination of nitrite and nitrate, 100 mL of water from each treatment was collected weekly for microbiological analysis of *Escherichia coli* and total coliforms using the Colipaper Microbiological Kit (Alfakit, Florianópolis, SC, Brazil). In total, 16 water samples were collected for each type of water treatment.

The nitrite and nitrate analyses were conducted at the Fish Nutrition Laboratory of the Department of Animal Science of the "Luiz de Queiroz" College of Agriculture. The concentration of nitrite ($N\text{-}NO_2$) and nitrate ($N\text{-}NO_3$) was measured using the Standard Methods for the Examination of Water and Wastewater (APHA, 2017) in a portable spectrophotometer (DR 2800, Hach, Loveland, CO).

Statistical Analysis

Data were assessed for normality using the PROC UNIVARIATE procedure and then analyzed using the MIXED procedure of the SAS statistical package (version 9.4, SAS Institute Inc., Cary, NC). The 30 calves were allocated into 15 randomized blocks according to calving herd, birth weight, and sex. Performance and blood parameters were analyzed as repeated measures over time, using the following statistical model: Yijk = μ + Ti+ Bj +Eij+ Ik + (Ti)Ik + Eijk. Where Yijk = response variable; μ = overall mean; Ti = fixed effect of treatment (different liquid diets and water quality); Bj = random effect of block; Eij = residual error (A); Ik = fixed effect of animal age (weeks of life); (Ti)Ik = fixed effect of the interaction between treatment and age; Eijk = residual error (B). The covariance matrices were tested and defined according to the lowest value obtained for Akaike's information criteria corrected.

The least squares method was used to compare means through the LSMEANS command. The model included the effects of treatments, age (week of life of the calves), and the interaction between treatment and age as fixed effects. All data were analyzed across the entire experimental period (1 to 70 d), without separating pre- and postweaning phases.

Health parameters were analyzed using a generalized linear mixed model (PROC GLIMMIX, SAS Institute Inc., Cary, NC), with a block included as a random effect. Calves were treated as a repeated measures design nested within blocks. The fixed effects of water treatment, herd, and sex were included in the model for each health-related outcome. Diarrhea was defined as a fecal score ≥2, and fever was characterized by a rectal temperature ≥39.5°C recorded. A binomial distribution with a logit link function was used for dichotomous outcomes. Odds ratios were obtained by exponentiating the fixed-effect estimates from the GLIMMIX model and were interpreted as the relative change in the odds of each health outcome occurring in the purified water group compared with the tap water group. The data were analyzed across the entire experimental period (1 to 70 d), without separating pre- and postweaning phases.

The number of days with health change and the number of days under antibiotic treatment were analyzed using PROC GLIMMIX. A model with Poisson distribution and log link function was initially fitted, including treatment as a fixed effect and block as random effect. The ratio of Pearson's chi-squared to the degrees of freedom was used for model evaluation and values greater than 1 indicated overdispersion, the negative binomial distribution was adopted to account for the extra variance present in the data. The number of calves with diarrhea was analyzed by nonparametric method. A binary variable was created (0 = absence, 1 = presence of diarrhea), and the frequency of calves with diarrhea was compared among treatments using contingency tables, using the chi-squared test. When all animals presented diarrhea, no statistical comparison was used, and only descriptive results were reported.

The value of $P \leq 0.05$ was adopted as a significant effect, and the value of $0.05 < P \leq 0.10$ as a trend for interpreting and discussing the results obtained.

RESULTS

Water Quality

Water quality differed between treatments (Table 2), and purified water had a higher pH ($P < 0.01$) and lower electrical conductivity ($P < 0.04$), indicating a lower total dissolved solids concentration. The experimental week influenced both pH ($P < 0.01$) and electrical conductivity ($P < 0.03$), suggesting variations in tap water quality throughout the study. There was a significant treatment × week interaction for nitrite concentration, with values fluctuating over time. However, no treatment effect was observed for nitrate concentration. Regarding microbiological quality, purified water did not show bacterial growth in any sample during the trial. In contrast, tap water showed variation, with some samples presenting no bacterial growth and others ranging from 80 to 7,200 cfu/100 mL.

Intake and Performance

Treatments did not affect MR, starter, and total DMI ($P > 0.05$). However, an age effect was observed for feed intake ($P < 0.01$; Figure 1A). Between wk 2 and 3, the MR intake decreased, followed by an increase until wk 7, when weaning began, ending at wk 8 (Figure 1A). Throughout

Table 2. Chemical and microbiological composition of tap water and purified water

	Water treatment[1]			P-value[2]		
Item	TW	PW	SEM	T	A	T × A
pH	6.71	7.00	0.035	0.01	0.01	0.01
Electrical conductivity, uS/cm	375.47	358.47	5.476	0.04	0.01	0.14
Nitrite, mg/L	0.129	0.145	0.0271	0.96	0.24	0.01
Nitrate, mg/L	1.672	0.970	0.2431	0.17	0.64	0.78
Total coliforms, cfu/mL	854	ND[3]	—	—	—	—

[1]TW = tap water; PW = purified water; 15 calves per treatment.

[2]T = treatment effect; A = age effect; T × A = interaction between treatment and age effect.

[3]ND = not detected.

the experiment, starter and total DMI increased with age ($P < 0.01$; Figure 1B and 1C), with no treatment or treatment × age interaction effects ($P > 0.05$).

An age effect was observed for starter and total DMI, with intakes increasing as calves age. Hay intake was greater in PW treatment (Table 3; Figure 1D) and increased as calves aged. The water intake tended to be greater at wk 10 for calves receiving PW ($P = 0.08$; Figure 2). Drinking water intake also increased with age, reaching its peak at wk 7 (Figure 2).

A significant treatment × age interaction was observed for ADG, calves with PW presented greater ADG in wk 3 ($P < 0.05$; Figure 3A). No differences were observed between treatments, age, or treatment × age interaction after weaning. A treatment × age interaction was observed for feed efficiency, calves given PW presented greater feed efficiency than TW at wk 3 (Figure 3B).

Body measurements (withers height, hip width, and heart girth) were not affected by treatments and interaction but increased as calves aged. ($P < 0.01$; Table 3).

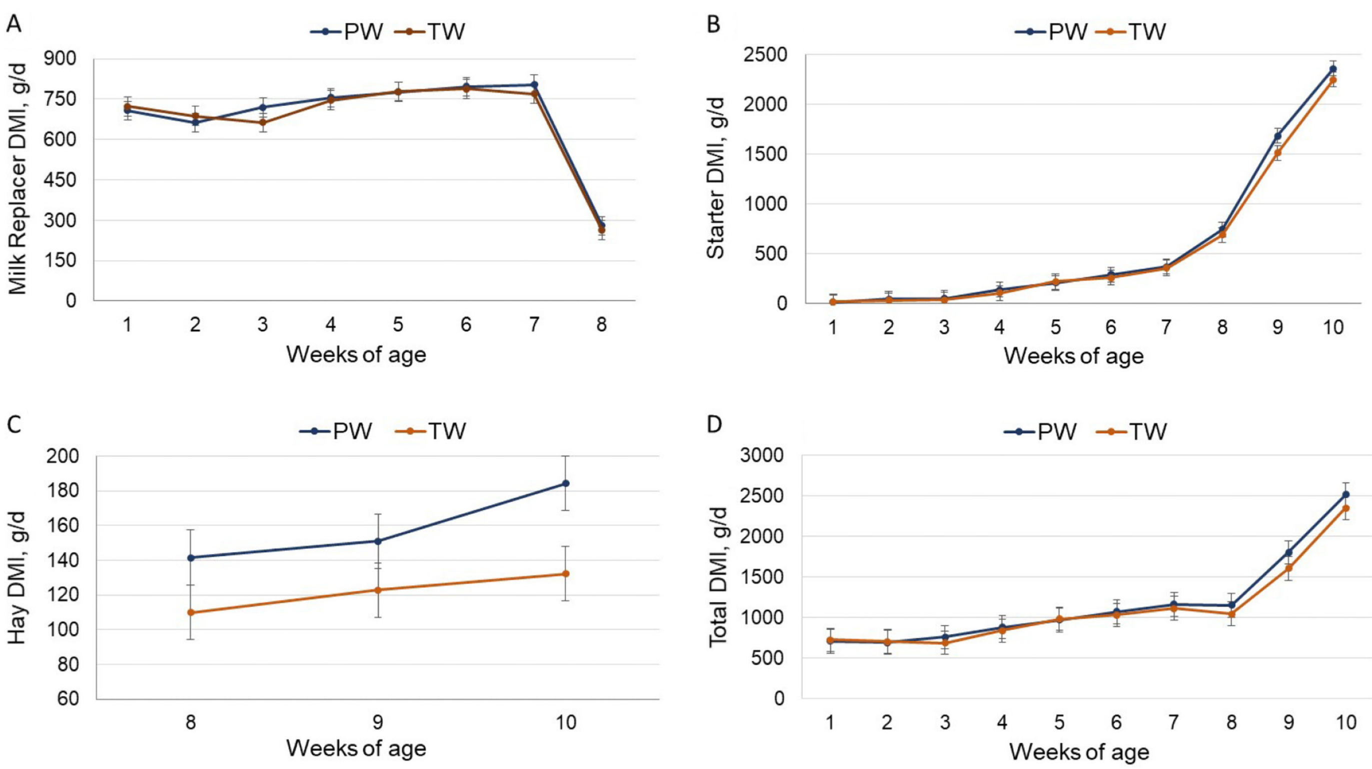

Figure 1. (A) Milk replacer DMI (g/d), (B) starter DMI (g/d), (C) hay DMI (g/d), and (D) total DMI (g/d) of calves fed with different water treatments during pre- and postweaning. TW = tap water, PW = purified water; 15 calves per treatment. Calves on the PW treatment had a greater intake of chopped Tifton hay ($P = 0.03$). Error bars indicate SEM.

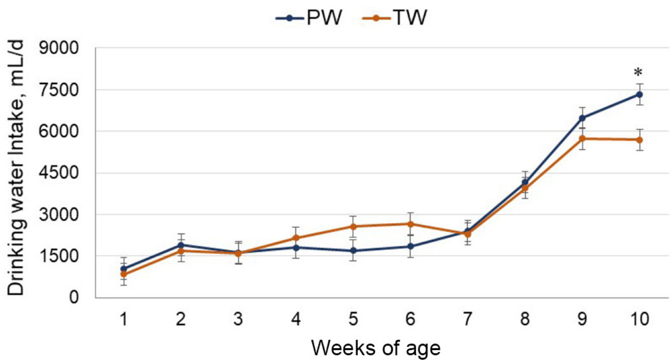

Figure 2. Water intake of calves fed different water treatments during pre- and postweaning (g/d). TW = tap water, PW = purified water; 15 calves per treatment. *Denotes difference between treatments within a week. Calves from PW treatment tended to have a greater water intake postweaning than calves from TW treatments (P = 0.08). Error bars indicate SEM.

Health Parameters

Water quality did not affect the incidence of diarrhea, as all calves experienced diarrhea at some point during the experimental period (1 to 70 d). Calves that received PW consistently showed less days with diarrhea compared with those that received TW (31.68 vs. 25.86; P = 0.01), indicating a reduction of about 18% in days with diarrhea for the PW treatment. No significant differences were found between treatments regarding days with fever, nasal discharge or health score ≥4. Calves that received TW showed less days with cough than calves from the PW treatment (7.88 vs. 11.75; P < 0.01). Calves that received PW showed a tendency to fewer days of antibiotic use compared with those that received TW (14.16 vs. 9.96; P = 0.08; Table 4).

Blood Metabolites

None of the selected blood metabolites were affected by treatment; however, all were affected by age (P < 0.01; Table 5). Glucose concentrations peaked in wk 6, followed by a decline at weaning at wk 8, and stabilization at wk 10. Urea concentrations decreased from wk 2 to wk 4, then increased from wk 6 onward. Although the hematocrit percentage did not differ between treatments, an age effect was observed (P < 0.01), with a reduction in hematocrit values at wk 4 and stabilization thereafter.

Except for albumin, there were also no effects for the interaction between water quality and age of the calves (P > 0.05). A treatment × age interaction was observed for albumin concentration (P = 0.04), and calves with TW presented greater albumin concentrations than PW at wk 10 (4.18 vs. 3.72; Figure 4).

DISCUSSION

In this study, the expected ADG was up to 600 g/d, based on the volume offered and the MR dilution (14% solids), which typically promotes greater nutrient intake (NASEM, 2021). However, this expectation was not achieved, as calves exhibited lower-than-expected weight gain. Compared with similar experiments that also evaluated milk-replacer-fed calves (Huang et al., 2015; Grice et al., 2020), our results suggest that additional factors, such as health status or environmental conditions, may

Table 3. Feed intake and performance of calves receiving different water treatments during pre- and postweaning periods

	Water treatment[1]			P-value[2]		
Item	TW	PW	SEM	T	A	T × A
DMI, g/d						
Milk replacer	676.2	707.1	14.80	0.60	<0.01	0.23
Starter	546.0	588.5	57.57	0.61	<0.01	0.85
Hay	121.3	159.5	12.27	0.03	0.07	0.61
Total	1,139	1,202	63.97	0.49	<0.01	0.78
Water intake, g/d	2,890	3,073	238.40	0.50	<0.01	0.08
BW, kg	36.79	37.39	1.907	0.71	<0.01	0.38
Initial	27.35	26.31	0.948	0.21	—	—
Weaning	46.29	49.12	3.726	0.35	—	—
Final	57.60	59.97	4.863	0.54	—	—
ADG, g/d	420.0	474.9	35.33	0.22	<0.01	0.02
Feed efficiency[3]	0.32	0.37	0.028	0.22	<0.01	0.01
Body measures, cm						
Withers height	83.0	83.1	1.06	0.90	<0.01	0.85
Hip width	23.0	22.4	0.37	0.25	<0.01	0.30
Heart girth	82.1	84.3	1.19	0.10	<0.01	0.65

[1]TW = tap water; PW = purified water; 15 calves per treatment.

[2]T = treatment effect; A = age effect; T × A = interaction between treatment and age effect.

[3]Gain/total DMI.

Table 4. Health parameters of calves receiving different water treatments during the total period

	Water treatment[1]			
Health parameter	TW	PW	SEM	*P*-value
Days with fever,[2] n	2.73	1.73	0.07	0.23
Calves with diarrhea,[3] %	100.00	100.00	—	1.00
Days with diarrhea, n	31.68	25.86	0.01	0.01
Days with cough, n	7.88	11.75	0.02	<0.01
Days nasal discharge, n	4.93	3.73	0.05	0.38
Days with health score ≥4,[4] n	4.94	3.10	0.01	0.19
Days of antibiotics,[5] n	14.16	9.96	0.03	0.08

[1]TW = tap water; PW = purified water; 15 calves per treatment.

[2]Fever was defined as a calf with a rectal temperature of ≥39.5°C.

[3]Diarrhea was defined as a calf with a score of ≥2 using a 4-point scale (0 to 3).

[4]Following the calf health scoring criteria from the School of Veterinary Medicine at the University of Wisconsin–Madison.

[5]Days each calf was treated with antibiotic.

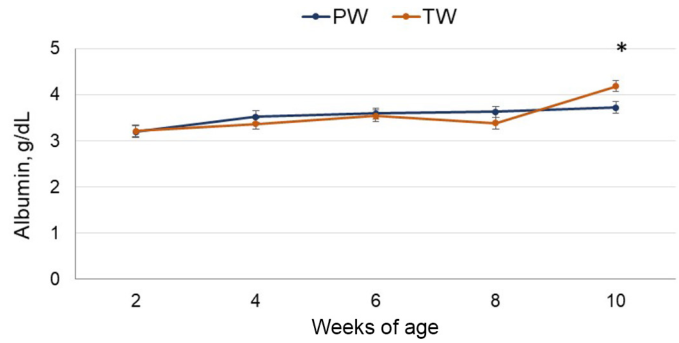

Figure 4. Albumin concentrations of calves fed different water treatments during pre- and postweaning (g/dL). TW = tap water, PW = purified water; 15 calves per treatment. *Denotes interaction of treatment and age. Calves that received purified water presented lower concentrations of albumin in wk 10 of life ($P = 0.04$). Error bars indicate SEM.

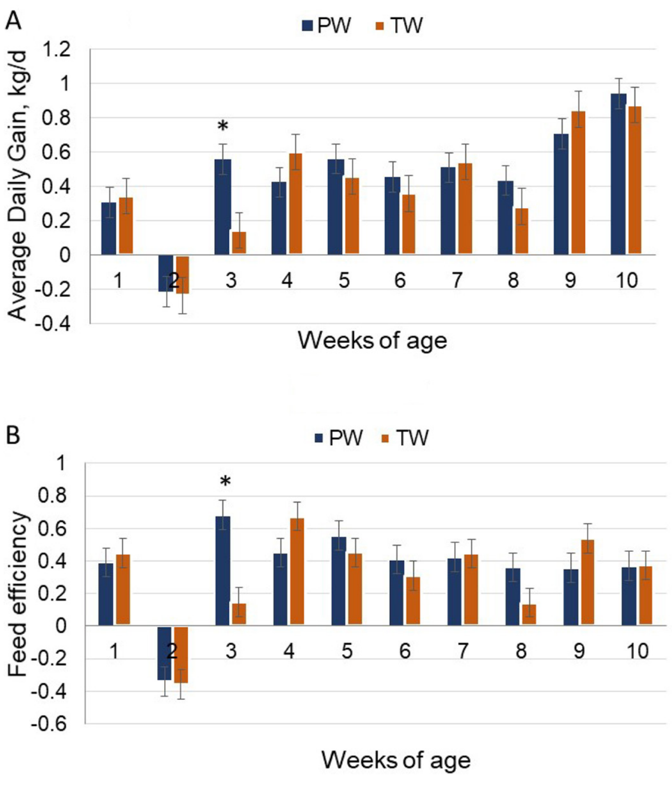

Figure 3. (A) Average daily gain of calves fed different water treatments during pre- and postweaning (g/d). TW = tap water, PW = purified water; 15 calves per treatment. *Denotes interaction of treatment and age. Calves that received purified water had a greater ADF than those receiving tap water on the third week of age ($P = 0.01$). (B) Feed efficiency of calves fed different water treatments during pre- and postweaning (%). *Denotes interaction effect of treatment and age. Calves with purified water had a greater feed efficiency in wk 3 of life ($P = 0.01$). Error bars indicate SEM.

have contributed to the reduced performance of these calves. Indeed, the health data of the present study reveals a great health challenge for the calves.

The literature recommendations for achieving puberty with an optimal age, the suggests for weight gain is between 800 to 900 g/d (Lammers et al., 1998). The medium quality of the MR used in this trial may also have contributed to this result, as it contained low protein and fat levels, likely leading to reduced energy and protein intake and, consequently, poorer performance.

Milk replacers with protein and fat levels below 20% can negatively affect calf performance. Even when containing highly digestible proteins and fats, studies have shown that calves fed milk replacers with low protein, but high fat exhibit lower weight gain and feed efficiency compared with those fed whole milk or MR with higher CP levels (Bascom et al., 2007; Swank et al., 2013). The study of de Paula et al. (2017) suggested that increasing liquid diet DM feeding does not overcome the restriction on ADG when MR presents medium protein levels. Literature also suggests that MR used in intensive calf feeding programs should contain between 24% and 28% protein and highly digestible nutrients to achieve the ideal weight gain of 800 g/d (NASEM, 2021). In this study, the milk replacer had fat levels of 18% and protein levels of 21%, with a composition including vegetable protein sources such as wheat gluten feed, which may have contributed to the lower weight gains and feed efficiency observed.

Throughout the experimental period, weather conditions were challenging, particularly regarding heat and humidity, leading to signs of heat stress in the calves. According to Shiasi Sardoabi et al. (2021), heat stress can reduce concentrate intake by half, negatively affecting growth and weight gain. It is worth noting that until the third week of age, calves were housed in suspended pens within a semi-open shed equipped with one column

Table 5. Blood metabolites of calves receiving different water treatments during pre- and postweaning periods

Item	Water treatment[1]		SEM	*P*-value[2]		
	TW	PW		T	A	T × A
Total protein, g/dL	6.2	5.9	0.19	0.19	<0.01	0.93
Albumin, g/dL	3.5	3.5	0.05	0.93	<0.01	0.04
Urea, mg/dL	22.1	20.7	1.95	0.55	<0.01	0.72
Glucose, mg/dL	93.7	91.2	2.24	0.44	0.01	0.25
Hematocrit, %	25.5	27.0	0.63	0.09	<0.01	0.47

[1]TW = tap water; PW = purified water; 15 per treatment.

[2]T = treatment effect; A = age effect; T × A = interaction between age and treatment.

Blood samples from Holstein dairy calves were collected within 2 hours after milk replacer feeding.

fan per row. Afterward, they were moved to tropical-type housing in an open experimental field, which has some natural shade, but with calves relying on hutches shade most of the time. Heat stress symptoms observed included elevated rectal temperature, increased heart and respiratory rates, prostration, excessive salivation, increased water intake, and refusal of liquid and solid diets. Panting was frequently noted, particularly during the hottest hours (midday to 4 p.m.). Heat stress likely influenced feed intake and overall performance, exacerbating health issues, particularly during weaning, when stress levels naturally increase. In these conditions, the availability and quality of drinking water is even more important for the calves.

Initial starter intake was low across all treatments, with significant consumption beginning around the fourth week of life (>100 g/d for 3 consecutive days). As expected, the intake increased during and after weaning, with calves progressing from an average of 350 g/d in the seventh week to over 1,450 g/d in the ninth week; this delay in starter intake may be linked to low intake during the second and third weeks when calves experience diarrhea. However, after recovering in the fourth week, a more substantial increase in stater intake was observed. Additionally, as calves aged, starter intake increased across all treatments, supporting the hypothesis that liquid diets fed in those levels are insufficient to meet the growing nutritional needs of calves. Late starter intake can affect rumen development, resulting in lower preweaning weight gain and impaired postweaning performance (Kertz et al., 2017; Grice et al., 2020).

In this study, the different water sources provided during preweaning did not affect starter or total DMI, aligning with findings from Senevirathne et al. (2021). However, unlike previous studies, hay DMI was higher in calves receiving purified water. The reason for this remains unclear, warranting further investigation into whether purified water influences hay consumption or if this was an isolated experimental effect. Despite differences in water quality, no effects or interactions were observed concerning performance, intake, or health, maybe because both water sources met the recommended physical-chemical quality range (NASEM, 2021; Beede, 2006).

Before weaning, the average drinking water intake was 2.1 kg/d ± 0.26 kg, higher than values reported by Broucek (2019), where calves fed 6 L of MR daily consumed an average of 1.2 kg of drinking water daily. In another study by Wickramasinghe et al. (2019), calves receiving drinking water from birth had an average intake of 0.82 kg/d until weaning at 42 d, which doubled during weaning (43–49 d) and further increased postweaning (50–70 d) to approximately 5 kg/d. In this study, postweaning water intake approximately tripled compared with preweaning, averaging 6.4 kg/d ± 0.49 kg. Calves in the PW treatment tended to consume more water than those in the TW treatment (6.9 kg/d vs. 5.7 kg/d), possibly due to differences in palatability, a feature difficult to measure. The higher drinking water intake observed in this study may indicate heat stress, as increased ambient temperatures lead to higher water consumption to regulate body temperature and compensate for losses through perspiration and respiration (Pereira et al., 2014; NASEM, 2021).

Although a few studies explore the relationship between water quality and calf performance, some references suggest its importance. Willms et al. (2002) found that beef heifers feed clean, treated water presented 23% more weight than those drinking untreated water. Lardner et al. (2005) observed that beef cattle given filtered and chlorinated water had higher diet and water intake, resulting in 9% to 10% greater weight gain than those drinking untreated water.

In this study, an interaction of age and treatment was observed during preweaning weeks for weight gain and feed efficiency. The calves fed PW showed superior performance, particularly in wk 3, with a weight gain of 0.558 kg/d compared with 0.144 kg/d for TW. Similarly, feed efficiency was higher in PW calves (0.682 vs. 0.146), suggesting that purified water may have aided recovery from diarrhea in the second week of life due to better microbiological quality.

Studies indicate that diarrhea increases water consumption by 25% to 50% (Jenny et al., 1978; McGuirk, 2008), which was observed in this study, with water intake peaking between the first and second weeks before stabilizing and increasing with age. Water analysis revealed an average bacterial count of 865 cfu/100 mL of fecal coliforms in tap water, exceeding the recommended 200 cfu/100 mL for dairy cattle (NASEM, 2021). These results suggest that municipal tap water may have contributed to recurrent intestinal infections, as water can serve as a transmission route for pathogens such as *Salmonella* spp., *Escherichia coli*, and *Cryptosporidium* spp. (Foster and Smith, 2009).

Calves that received purified water had fewer days of diarrhea, which likely contributed to their reduced need for antibiotic treatments compared with calves receiving regular water. As no differences were observed in other health indicators, such as days with fever, nasal discharge, or health scores ≥4, the lower incidence of diarrhea appears to be the main factor explaining this reduction. These findings suggest that improving water quality can positively influence gastrointestinal health and reduce the need for therapeutic interventions. Providing high-quality water, especially for MR dilution, is therefore crucial for calf health, as it directly enters the abomasum and can affect intestinal microbiota colonization and disease transmission (Jones and Heinrichs, 2022). Similarly, Senevirathne et al. (2018) reported improvements in calf health when water treated by reverse osmosis was provided instead of municipal city water.

Blood parameters, except for albumin, were affected only by age, which is consistent with the expected physiological and nutritional changes during calf development. Glucose levels observed were within the normal range for healthy calves (80–120 mg/dL), around 100 mg/dL, and showed a decline in the last week of preweaning. This decrease reflects the metabolic transition from using glucose from the liquid diet to VFA produced by ruminal fermentation of solid feeds, especially starter, as described by Khan et al. (2008).

Total protein, albumin, and urea concentrations were similar to values reported in literature, influenced by dietary protein intake and the gradual increase in solid feed intake. The increased plasma urea concentration after weaning indicates greater availability of rumen-degradable protein and increased fermentation activity, which is expected physiologically (Hayashi et al., 2006). The interaction between week and treatment for albumin at wk 10, with greater variation in TW compared with the PW group, suggests that other factors may be affecting this response. This variability in albumin levels in the TW group highlights the need for further studies to understand the underlying reasons.

The findings of this study underscore the need for further research on the effects of purified water on hay intake, health, and performance. However, it is evident that ensuring high-quality water in early life, particularly for liquid diets dilution, is essential due to the calves' developing digestive and immune systems. Water quality is often overlooked, yet it significantly affects health, disease incidence, intake, and feed efficiency, as demonstrated in this study.

CONCLUSIONS

Purified water is a promising strategy for feeding dairy calves, particularly during the first weeks of life when they are fed MR and are more susceptible to gastrointestinal diseases. Although both types of water exhibited relatively good quality, the results indicate that even minor changes in water quality, particularly microbiological quality, can improve the health and performance of dairy calves.

NOTES

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Nonstandard abbreviations used: A = age effect; MR = milk replacer; ND = not detected; PW = purified water; T = treatment effect; TW = tap water.

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