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Animal Health and Well Being

Effects of isoenergetic supplementation as water use mitigation strategy on water footprint and health of nursing bull calves

Arturo Macias Franco,[*] Aghata Elins Moreira da Silva,[*] Felipe Henrique de Moura,[*] Aaron B. Norris,[†,*] Serena Breanne Roloson,[*] David E. Gerrard,[‡] Amilton de Mello,[*] and Mozart A. Fonseca[*,1]

[*]Department of Agriculture, Veterinary, and Rangeland Sciences, University of Nevada, Reno, Reno, Nevada 89557, USA
[†]Department of Natural Resources Management, Texas Tech University, Texas 79409USA
[‡]Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, Virginia 24061, USA
[1]Corresponding author: mfonseca@unr.edu

Abstract

Sustainable livestock systems focus on mitigating natural resource use such as water. Dietary management strategies can significantly reduce the water footprint of livestock animals; however, animal health is of concern when animals reduce water intake due to subacute dehydration. To evaluate potential consequences of this nutritional management intervention, a total of 23, 60 ± 3 days old nursing Holstein bull calves, weighing 94.7 ± 12.07 kg, were distributed in a completely randomized design and received one of three diets. Control was a basal diet composed of a non-medicated milk replacer (milk replacer; n = 7), and the additional two diets, were composed of the same non-medicated milk replacer in addition to either lipid [n = 8; milk replacer + menhaden fish oil (3 %)] or soluble carbohydrate [n = 8; milk replacer + corn starch (7%) isoenergetic to fat group] supplements. Animals were offered ad libitum mineral mix and water, as well as 120 g/day of a composite mix of dried microbrewery's spent grains. Data were analyzed as linear and generalized linear mixed models with diet as a fixed effect and animal as random utilizing R studio (R Core Team, 2021, Vienna, Austria; SAS Inst., Cary, NC). Within supplementation groups, lipid supplemented calves had the highest lymphocyte (63.24 vs 57.69 counts/100 lymphocytes; $P < 0.033$), and lowest neutrophil counts (29.3 vs 35.3 counts/100 lymphocytes; $P < 0.047$). Supplementation significantly increased total serum protein ($P = 0.001$) and skin moisture ($P < 0.011$), with carbohydrate group having the highest skin moisture (5.30 vs 3.99; $P < 0.047$). Supplementation also decreased fecal fluidity scores ($P < 0.001$) with no significant change in serum electrolytes ($P > 0.256$). No significant differences were found amongst treatments for the ingestive behavior ($P > 0.338$). The carbohydrate-supplemented calves significantly decreased all daily water footprints compared to the control and fat-supplemented groups: blue a 47.55 L decrease, ($P < 0.001$), green a 265.62 L decrease ($P = 0.005$), and gray a 55.87 L decrease ($P = 0.009$) water footprint, as well as total water footprint (369.04 L, $P = 0.004$). Our results indicate the potential to maintain animal performance while increasing water use efficiency through diet supplementation tailored to mitigate water use, without adverse effects on animal health.

Lay Summary

This study investigates the development of a novel line of supplements for nursing cattle tailored to make production more sustainable. The fat and sugar-based supplement lines explored decreased water intake of the animals compared to the non-supplemented group. Further, when evaluating the water footprint from this decrease, significant decreases were found on water footprint of the supplemented animals. These decreases were possible without adversely affecting the health, hydration, and behavior of the animals. Our work highlights how water requirements of cattle can be adjusted through metabolic water production for nursing calves. Further, we demonstrate that livestock environmental impact, particularly water, can be modified through dietary supplementations without any adverse effects.

Keywords: calf health, calf hydration, metabolic water, nursing calf, water footprint

Introduction

Until recently, the belief that nursing calves' water requirements were met through milk or milk replacer alone was a commonplace. Free access to ad libitum water was shown to positively benefit performance, health, and digestibility of nursing calves (Wickramasinghe et al., 2019). However, growing concerns regarding the amount of water used by livestock operations may influence water use and distribution across the agricultural sector. Our previous work with nursing calves has demonstrated that voluntary water intake reductions and feed efficiency increases are achievable through dietary supplementation of soluble carbohydrates and fats without adversely affecting animal performance, digestibility, and carcass traits (Macias Franco et al., 2021). The mechanisms for water use mitigation are rather complex and still not fully understood. Decreasing voluntary water intake as an attempt to reduce water footprint could have potential adverse effects on animal health, and hence, need to be part of a strategic coordinated plan in sustainable husbandry practices that also improve animal welfare.

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One of the major mortality causes in nursing calves, is calf diarrhea, or scours, which is often paired with dehydration of the animals that are undergoing a negative water balance. Previous research has investigated the inclusion of soluble carbohydrates and n-3 fatty acids for increased immune response (Ballou and DePeters, 2008; McDonell et al., 2019). These findings and our previous work highlight the potential implementation of energetic supplements to increase immune response, performance, and feed efficiency at a lower water intake. We hypothesized that supplementation plans aimed at decreasing water use by nursing calves would compromise their immune system because of potential dehydration, and that ingestive behavior could be used to detect a departure from normal behavior of animals with a depleted immune system in individual cattle management systems. The goals herein involved the quantification of reductions in water use and water footprint due to isoenergetic supplementation. Additionally, we aimed at using modifications in animal normal behavior and skin hydration as an indication of health for early identification of disease.

Materials and Methods

All experimental and animal husbandry procedures were approved by the Institutional Animal Care and Use Committee, protocol #00750, of the University of Nevada, Reno, Nevada, USA.

Animals, water, diets, and facilities

Twenty-three nursing Holstein bull calves were acquired from a commercial farm located in Northern NV and were raised in a closed and controlled environment until harvesting. All calves were weighed at birth, monitored for expected behavior including their ability to stand and nurse within the first two hours, and had their umbilicus treated with an iodine solution (10% w/v). The animals were moved to the experimental barn at the Nevada Agricultural Experimental Station and upon arrival [4 ± 3 days; body weight 43.5 ± 3 kg], a clinical veterinarian evaluated the health of the animals confirming their adequacy for the trial. Animals were randomly assigned to individual 3-m^2 galvanized steel pens (Seneca Dairy Systems, LLC; Seneca Falls, NY). The barn was equipped with heaters, fans, and a swamp cooler for temperature and relative humidity control. The animals were adapted to the barn facilities for 67 days and were maintained on the experimental diets for 68 days (135 days total). Pens were originally bedded with wood shavings and replaced by rubber stall mats on day 45. Rubber mats were sanitized twice daily after the feeding times.

Animals were randomly distributed to one of three experimental diets: control (CON; n = 7), consisting of a commercial non-medicated milk replacer (milk replacer; 200 g/kg crude protein, 200 g/kg crude fat, 15 g/kg crude fiber, 95.8% DM); and two additional diets based on the supplementation of milk replacer with either lipid [n = 8; 3% inclusion of menhaden fish oil (5.2 g/L of milk replacer)], or soluble carbohydrate sourced isoenergetically matching the gross energy content of the fat-supplemented treatment [n = 8; 7% inclusion of corn starch (11.8 g/L of milk replacer)]. All experimental groups were provided 120 g of microbreweries spent grains daily. Water composition and quality analysis, as well as additional dietary information are described in detail by Macias Franco et al. (2021).

Individual water intakes were collected twice daily before feeding. Individual water troughs with float regulators were installed inside all individual pens. The individual troughs were previously described by Macias Franco et al. (2021), and are pictured in Supplementary Figure S1.

Temperature humidity index was computed according to Berman et al. (2016) and evaluated twice daily to ensure animals remained within their thermoneutral zone throughout the experimental period to avoid confounding factors. Temperature, relative humidity, wind speed, wind direction, and solar radiation were recorded daily using a H-21 HOBO data logging station (Onset Computer Corp, Boston, MA) equipped with a Davis Wind Speed and Direction Smart Sensor (P# S-WCF-M003), a Solar Radiation Shield (P# RS3-B), a temperature and relative humidity Sensor (12-bit; P# S-THB-M002), and a light sensor level (P# M-LLA) all collecting data points every 30 s.

Water losses

Spot urine samples were collected 4 h before and after feeding during spontaneous urination on days 67, 100, and 135 of the experiment. Urine-specific gravity was measured with a refractometer (MASTER-SUR/Nα, Atago Co Ltd, Bellevue, WA, USA) to determine its osmolarity compared to water. Samples from each collection were proportionally composited and filtered through cheesecloth layers, 10 mL aliquots were diluted into 40 mL of H_2SO_4 (0.036 N), and an additional sample was stored as concentrate. All urine samples were frozen at –20 °C for further analysis.

Creatinine was analyzed through high-performance liquid chromatography with an Agilent 1100 liquid chromatograph with addition of a guard column with Spherisorb ODS2 guard cartridges, 80Å, 5 µm, 4.6 mm × 30 mm following recommendations by Shingfield and Offer (1999). The creatinine excretion was estimated according to empirical equations presented by Chizzotti et al. (2008). In brief, the ratio of the expected creatinine daily excretion, and the measured creatinine in urine were utilized to estimate urinary volume. Water loss from urine was estimated based on urine production by means of creatinine concentration as a biological marker according to Chizzotti et al. (2008).

Fecal samples were collected on days 95 through 100 and 127 through 132. Samples were collected and weighed hourly and kept in air-tight sealed containers; composites were re-weighed daily prior to drying for 72 h at 65 °C. Representative composites of the total fecal collection were homogeneously mixed and stored at –20 °C until time of analysis. Water loss from feces was calculated as the weight difference in total fecal collection before and after drying (accounting for dry matter and the moisture from the pre-drying after collection). Sensible water loss was then calculated as the sum of water loss from urine and feces, insensible water loss was computed according to Church (1976); briefly, water loss from urine and feces were subtracted from the total water intake (including metabolic water).

Water footprint

For analysis of water footprint, water was divided into blue, green, and gray water footprint according to Mekonnen and Hoekstra (2010). The green, blue, and gray water footprint values utilized accounted for the water directly consumed by the animals, as well as the water utilized on the feedstuffs the animals consumed. The green water footprint refers to

water from precipitation (excluding run-off), gray water footprint refers to the freshwater required to assimilate the load of pollutants from our system, and lastly blue water footprint refers to surface and groundwater resources. The sum of these water footprints constitutes the total water footprint. The water footprint contribution of corn starch for the carbohydrate-supplemented group was accounted for based on the values reported by Mekonnen and Hoekstra (2011), as green water footprint = 1,295, blue water footprint = 111, gray water footprint = 265 m^3/t, and total water footprint = 1,671 m^3/t. The fish oil contribution to the water footprint calculations for the fat-supplemented group was computed according to Pahlow et al., (2015), the weighted average for fish was estimated to be green water footprint = 1,629, blue water footprint = 179, gray water footprint = 166, and total water footprint = 1974 m^3/t respectively. Values from Mekonnen and Hoekstra (2010) were utilized for water footprint quantification of the milk replacer (green water footprint = 2,065, blue water footprint = 282, gray water footprint = 464 m^3/t). Values for water footprint of the microbreweries spent grains, were obtained from Mekonnen and Hoekstra, (2011). Our microbreweries spent grains (mixed from several microbreweries) was composed of 86% barley, 10% rye, and 4% wheat. The summed contributions of the respective grains accounted for a total green water footprint = 3,909, blue water footprint = 446, and gray water footprint = 437 m^3/t of water per t of microbreweries spent grains produced. In addition, blue water footprint also accounted for the water intake of the animals.

Ingestive behavior

The analysis of animal ingestive behavior was performed to ensure early detection of abnormal activities that would indicate the onset of illnesses related to dietary treatments. The animals were monitored using 16 Channel 5MP Extreme HD Night Owl XHD502-88P-B Video Security DVR & Wired Infrared Cameras with 2 TB HDD system with full area recording for all animal images. Videos were processed on the VLC Media Player (Version 3.0.1, VideoLan, France). A total of 6 d (days 30-32, 62-64) of recording were gathered throughout the experimental period to assess treatment effects on behavioral patterns. The parameters evaluated included the time spent laying, the time spent standing, the time spent ruminating/chewing, the time spent eating a mineral mix or brewer's grains, and the time spent drinking water. Video analysis involved training of three laboratory technicians blinded to the treatments who evaluated the behaviors, and the aggregate of their observations was combined to determine animal ingestive behavior and the time spent performing each activity. The four days of recordings were analyzed in 5-min intervals as reported by Martin and Bateson (1993), and Jensen and Larsen (2014), with continuous animal observations.

Health assessment

Health parameters and their relevance are briefly described in Table 1. Animals were monitored for health assessment by multiple trained animal caretakers blinded to experimental treatments twice daily before feeding. Animals were assigned a fecal fluidity score using a scale from 0 to 3 as described by Larson et al. (1977). Respiratory score was assessed by observation of nasal discharge, cough, or pulmonary auscultation; and lethargy scores performed as described by Schaefer et al. (2004) and Cortese et al. (1998). In the event of abnormal recordings for fecal fluidity, respiratory, or lethargy scores (values greater than 1), and during weekly collections, enophthalmia score was additionally measured by a trained technician. Briefly, the degree of suppression in the eye was scored from 0 to 3 with 3 being a sunken gap of 0.5 to 1.0 cm, laser corneal/lens temperature, and rectal temperature were also collected using a laser temperature gun and a digital thermometer, respectively.

Daily hydration was assessed through skin pliability score and skin moisture. Skin pliability, was accounted as the time taken for the skin to return to its initial state upon pinching 2.5 cm of skin for one second at the middle portion of the neck while rotating it at 90°, holding for 1s, and then releasing it (Constable et al., 1998). Skin moisture was first examined dorsally, 5 cm from the nasal cavity, and at the cross-point between the horns and the eyes using a skin moisture meter (MoistureMeterSC-2, Delfin Technologies Ltd, Kuopio, Finland; Supplementary Figure S2). In brief, 2 d before skin moisture assessment, calves were shaved in the area and site was thoroughly cleaned. On the day of the collection, after checking for dryness and cleanliness with a dry paper towel, skin moisture was collected by holding the moisture meter perpendicular to the skin with constant pressure for three seconds until the reading was done. The skin moisture meter measures epidermal capacitance which is interpreted as the percent of water content in superficial skin (Palma et al., 2012).

A basal blood sample was collected prior to treatment administration, and weekly thereafter for the duration of the experiment. Animals were manually restrained, and 5 mL of blood was collected into ethylenediaminetetraacetic acid (EDTA) and red serum tube containers (BD Vacutainer, Franklin Lakes, NJ). Immediately after collection, containers were placed in an ice chest and transported to the laboratory for immediate processing. All tubes were centrifuged at a speed of 2,000 × *g* for 10 min and stored at –80 °C until time of analysis. Red-serum tubes were allowed to clot for 30 min prior to centrifugation. Blood smear slides were prepared with a rapid 3-step staining kit (Hemacolor Rapid staining of a blood smear, Sigma-Aldrich, St. Louis, MO).

Blood samples were analyzed for white blood cell differential counts (values reported as total count of 100 total lymphocytes) and the neutrophil to lymphocyte ratio (NLR). Three technicians blindsided to treatments were trained by the clinical veterinarian before counting of basophils, neutrophils, lymphocytes, eosinophils, and monocytes. Counts were averaged and the average of the values was submitted to statistical analysis. Additionally, blood was analyzed for packed cell volume through the microhematocrit technique, where micro-capillary tubes were filled with blood, sealed on one end with clay, and centrifuged in a microhematocrit centrifuge (LW Scientific Inc., Lawrenceville GA) at 12,000 rpm for 5 min and assessed utilizing a caliper to read the liquid portion and the serum after centrifugation. Serum concentrations of Na, K, Cl, CO_2, Ca, P, and Mg, collected at the beginning (67 d), middle (100 d), and end (134 d) of the experiment, were shipped frozen for same-day analysis to the University of Georgia Tifton Veterinary Diagnostic and Investigational Laboratory. The analyses were performed on a Roche Hitachi 912, and an Advia 1200 fully automated chemistry analyzer (Roche Diagnostics, Indianapolis, IN; Siemens Medical Solutions Diagnostics, Tarrytown, NY, respectively).

Table 1. Descriptive assessment of clinical scored used throughout the experimental period

Parameters[1]	Significance
Fecal fluidity	0 = normal fluidity, 1 = feces spread slightly, pasty soft manure, 2 = moderate spread of feces, semi-liquid diarrhea), 3 = watery, pure liquid feces (severe scours)
Respiratory	0 = no symptoms, 1 = clear nasal discharge or slight cough, 2 = mucopurulent discharge or severe cough with subcreptatnt lung sound, 3 = severe pneumonia
Lethargy	0 = normal, 1 = mild depression, non-vigorous suckling, 2 = moderate depression, calf stands, weak suckling/disorganized, 3 = severe depression, calf unable to stand or suckle
Skin pliability	2 s = any values over 2 seconds reported
Enophthalmia	0 = normal, 1 = slightly sunken eye with no separation between globe and orbit, 2 = separation of globe and orbit, 3 = severe 0.5-1.0 cm gap between eye and orbit
Skin moisture	Skin capacitance as a measure of hydration, measured on forehead
White blood cell differentials count	
Neutrophil	Values greater than 6.0×10^9/L represent 5-fold increase in mortality (von Konigslow et al., 2020)
Lymphocyte	Values between 4.6 to 5.8×10^9/L reduced hazard of mortality, > 7.0×10^9/L 54% to 63% decrease in hazard of morbidity (von Konigslow et al., 2020)
Monocyte	Not sensible indicator for specific disease, but monocytosis can happen under acute stress, and during healing of chronic infections (Jones and Allison, 2007)
Eosinophil	Below reference at birth, increase with age, no specific thresholds defined (Jones and Allison, 2007)
Basophil	Intervals may include zero, but basophilia can be associated with allergies, ulceration, parasitic infections, and hyperlipidemia (Roland et al., 2014)
Packed cell volume, %	Increased values, paired with BW loss, represent dehydration of calves (Marcato et al., 2018)
Total protein, g/dl	Cut points below or above 5.2 g/dL (Buczinski et al., 2018; Roland et al., 2014; von Konigslow et al., 2020)
Urine specific gravity, w/v	Values > 1.025 indicate dehydration, normal values range between 1.004 and 1.015 in dairy cows (Peek and Divers, 2018)
Serum mineral panel	
Na, mEq/L	Electrolyte balance increase signals dehydration, low levels can signal dehydration
K, mEq/L	Low values signal dehydration
Cl, mEq/L	Electrolyte balance increase signals dehydration, low levels can signal dehydration
CO_2, mmol/L	Blood buffering, and O2 transport, high levels are bad
Ca, mg/dl	Muscle function, bone cycling, low values relate to metabolic disease, too high common in calves (high calcium milk)
P, mg/dl	6-8 mg per 100 mL of blood, less may reduce appetite and lead to lower gain (Noller et al., 1977)
Mg, mg/dl	Electrolyte balance increase signals dehydration, low levels can signal dehydration
Temperature recordings, °C	
Corneal laser eye	Evaluation of validity to establish thresholds
Rectal	Report temperatures above 103.5°F (39.7°C) or below 100°C F (37.8°C) to the project manager

[1]FFS = fecal fluidity score, RS = respiratory score, Le = lethargy score, SP = skin pliability, En = enophthalmia score, Mo = skin capacitance/moisture level measure, LTE = corneal laser temperature, RTE = rectal temperature.

Slaughter

Animals were slaughtered at Wolf Pack Meats, a USDA-inspected facility, at the University of Nevada, Reno on day 135. Briefly, animals were desensitized with use of a penetrating captive bolt and exsanguinated through incision of the jugular vein and carotid arteries. The water footprint was corrected for cold carcass weight.

Statistical methods

Statistical analyses were performed utilizing R Statistical Software v.4.0.1 and the GLIMMIX procedure of SAS 9.4 (R Core Team, 2021, Vienna, Austria; SAS Inst., Cary, NC). Data were analyzed as linear mixed models under a completely randomized design with the diet as fixed, and individual animals as random effects according to $Y_{ij} = \mu_{ij} + T_i + A_j + \epsilon_{ij}$; where Y_{ij} represents the response variables, μ_{ij} represents the respective means, T_i represents the diet effect, A_j represents the random effect of animal, and ε_{ij} represents the random error. Mean comparisons were performed using the LSMEANS statement and separated with *F*-protected *t*-tests. The PROC MIXED with COVTEST and a REPEATED command were utilized to evaluate treatment effects through time. Studentized residuals were plotted against predicted values with Cook's distance for influence on results; values outside of the 2.5 Studentized *t* distribution were considered outliers and removed from the analysis (Kutner et al., 2004). Significance was established at $P < 0.05$, with trends considered at $0.05 < P < 0.10$.

Results

Daily evaluation of temperature humidity index resulted on no moderate or severe stress days for the animals in our experiment.

Table 2. Sensible and insensible water loss and water footprint of nursing Holstein bull calves fed non-medicated milk replacer (MR) only (CON; $n = 7$), MR supplemented with 3% fish oil (FAT; $n = 8$), or MR isoenergetically supplemented (gross energy) with cornstarch (CHO; $n = 8$)

Item[1]	Treatments			SEM	*P*-value[2]
	CON	CHO	FAT		
Water loss, L/d					
WLF	0.59	0.61	0.53	0.047	0.410
WLU	7.52	8.29	8.00	0.697	0.881
SWL	8.03	8.90	8.47	0.710	0.839
IsWL	4.77	5.32	5.11	2.651	0.895
Water footprint daily values, L/d					
BWF	889.50[a]	842.08[b]	889.77[a]	9.033	<0.001
GWF	6,445.66[a]	6,193.95[b]	6,473.48[a]	93.598	0.005
GrWF	1,433.85[a]	1,372.51[bA]	1,422.91[a]	14.729	0.009
TWF	8,769.01[a]	8,408.55[b]	A	89.943	0.004
Water footprint, L/kg cold carcass weight					
BWF	238.86	224.84	222.82	10.404	0.535
GWF	1730.52	1653.72	1621.37	76.263	0.616
GrWF	384.95	366.44	356.30	16.935	0.518
TWF	2,354.33	2,244.99	2,200.49	103.600	0.593

[1]WLF = water loss from feces, WLU = water loss from urine, SWL = sensible water loss, IsWL = insensible water loss, BWF = blue water footprint, GWF = green water footprint, GrWF = gray water footprint, TWF = total water footprint.
[2]*P*-values: significance < 0.05, trend: <0.1.
[ab]Means within row without common superscript differ ($P < 0.05$).

Water loss

There were no significant differences in the amount of sensible water loss or insensible water loss ($P > 0.1$; Table 2). Similarly, no treatment differences were detected for water loss from feces, water loss from urine, and total water loss ($P > 0.1$; Table 2).

Water footprint

Significant differences were detected for green, blue, gray, and total water footprint ($P < 0.005$, $P < 0.001$, $P = 0.009$, $P = 0.004$, respectively) where carbohydrate-supplemented calves had the lowest footprint when compared to control and fat-supplemented calves. Similar results were observed between green, blue, gray, and total water footprint with the carbohydrate-supplemented group always having lower thresholds ($P < 0.0001$, $P = 0.0049$, 0.0087, and 0.0041 respectively; Table 2). For the water directly used by the animal, the blue water footprint, a significant reduction was observed for the soluble carbohydrate supplemented group with values of 842.08 ± 9.03 L, whereas control and fat-supplemented groups achieved 889.50 ± 9.66 and 889.77 ± 9.03 L/d during this experiment, respectively ($P < 0.0001$; Table 2). When corrected per unit of cold carcass weight (Control = 113.33 kg, carbohydrate = 116.01 kg, lipid = 121.39 kg cold carcass weight respectively, Macias Franco, et al., 2021), no significant differences amongst treatments were found for L of water required to produce a kg of cold carcass weight ($P > 0.5$; Table 2). Though not statistically different, the values were numerically different lower for supplemented groups compared to the control for green, blue gray, and total water footprints.

Behavior

No statistical differences amongst treatments were observed for any of the ingestive behavioral traits analyzed ($P < 0.338$; Table 3).

Table 3. Behavior analysis of nursing Holstein bull calves fed non-medicated milk replacer (MR) only (CON; $n = 7$), MR supplemented with 3% fish oil (FAT; $n = 8$), or MR isoenergetically supplemented (gross energy) with cornstarch (CHO; $n = 8$)

Item[1]	Treatments			SEM	*P*-value[2]
	CON	CHO	FAT		Treatment
Observations, min/d					
TSD	2.25	2.25	2.16	0.362	0.978
TSE	4.54	5.00	3.97	0.697	0.338
TSL	133.25	131.81	139.75	8.142	0.766
TSR	28.07	27.88	26.28	5.586	0.942
TSS	53.75	54.91	49.75	5.296	0.598

[1]TSD = time spent drinking water, TSE = time spent eating microbreweries spent grains or mineral mix, TSL = time spent laying, TSR = time spent ruminating/chewing, TSS = time spent standing, CON = control, CHO = carbohydrate, FAT = lipid.
[2]*P*-values: significance < 0.05, trend: < 0.1.

Health and hydration

White blood cell differential counts displayed significantly lower values for fat-supplemented compared to the carbohydrate-supplemented ($P < 0.05$). Neutrophil count was the lowest for fat-supplemented (29.30; $P < 0.047$; Table 4), and no difference was observed for control (34.04) and carbohydrate supplemented (35.30). Further, a time effect was detected for neutrophil count, most notably for the carbohydrate-supplemented group peaking at week 3 of the experiment (Figure 1). The mean lymphocyte count values were greater for the fat-supplemented group compared to carbohydrate-supplemented and control 62.24, 59.41, 57.69, respectively; $P < 0.033$). Further, the same critical mark was observed at week 3 with carbohydrate-supplemented

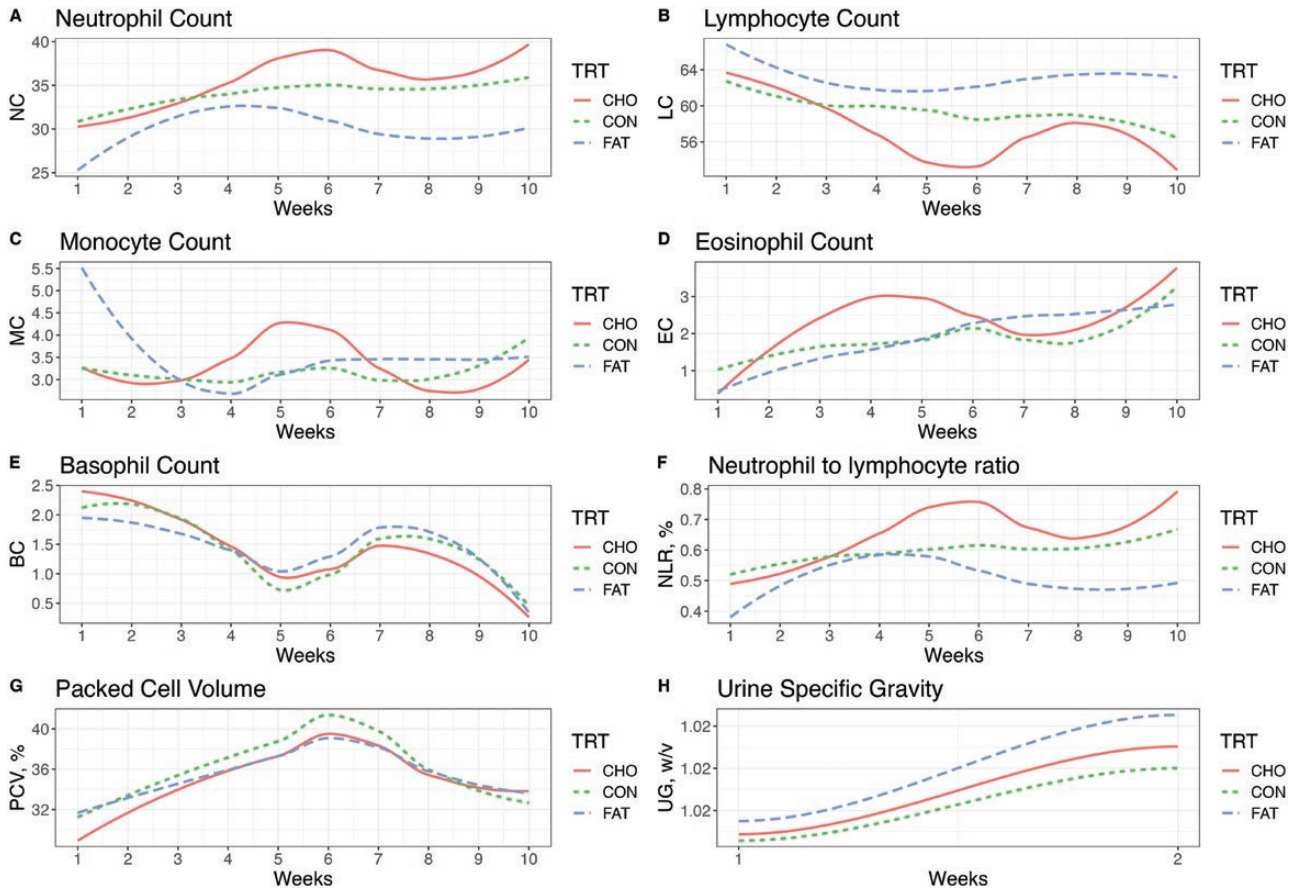

Figure 1. Treatment and time interactions for, urine specific gravity (UG), packed cell volume (PCV), neutrophil count (NC), lymphocyte count (LC), neutrophil to lymphocyte ratio (NLR), Eosinophil count (EC), basophil count (BC), monocyte count (MC) for 23 nursing Holstein bull calves fed with a non-medicated commercial milk replacer (Control [CON]; $n = 7$), supplemented isoenergetically (gross energy) with cornstarch (Carbohydrate [CHO]; $n = 8$), or with fish oil (fat [FAT]; $n = 8$).

having a more remarkable decrease (Figure 1). The neutrophil:lymphocyte of the fat-supplemented group was significantly lower, even among supplementation groups ($P \leq 0.003$; Table 4).

No differences were detected for monocyte, eosinophil, and basophil counts, nor for packed cell volume among treatments ($P > 0.10$; Table 4). Supplementation groups had significantly higher values of total serum protein ($P > 0.011$; Table 4). Urine specific gravity was not significantly affected by experimental diets but increased overtime ($P < 0.0001$; Table 4).

No statistically significant differences were detectable for laser-corneal and rectal temperatures, or for respiratory score ($P > 0.182$; Table 5). Supplementation decreased fecal fluidity score ($P < 0.001$; Table 5). Skin moisture was significantly affected by supplementation ($P < 0.011$; Table 5), with highest for carbohydrate-supplemented ($P < 0.013$; Table 5) while not differing between control and fat-supplemented groups. A time effect was also observed for skin moisture ($P < 0.05$; Table 5). Individual correlation between skin moisture and packed cell volume was statistically significant (–0.383, $P = 0.02$).

Electrolyte serum concentrations were not different amongst treatments; however, there was a slight decrease over time for serum K (Figure 2) and CO_2 (Figure 3) ($P < 0.0368$ and $P < 0.0001$, respectively; Table 6).

Discussion

Lipid and carbohydrate isoenergetic supplementation can reduce voluntary water intake of nursing bull calves while also increasing feed/gain ratios (Macias Franco et al., 2021); however, no studies have investigated how these supplementation strategies may be used as water mitigation tools to reduce water footprint, and consequently, how this may affect animal health.

Relevant to water balance, urinary excretion ranged from 6.02 to 8.61 L/d which is higher than values reported in the literature (Abe et al., 1999; Hill et al., 2008; Yousefinejad et al., 2021). This is expected since our animals were nursing (liquid-fed). Though urine excretion was higher, there were no detectable differences for sensible and insensible water loss which reinforces the results from our previous work (Macias Franco et al., 2021) showing supplemented animals were more water efficient and produced more metabolic water (lower water requirements) which could explain the higher rates of urinary excretion.

Regarding the water footprint, since the carbohydrate-supplemented group was more water and feed efficient (Macias Franco et al., 2021), its lower water footprint comes as no surprise. A potential mechanism for this reduction, which would be true for all animal species, would be the hepatic oxidation theory where the observed decrease in feed and water intakes is partially explained through membrane polarization

Table 4. Blood parameters repeated measures analysis of nursing Holstein bull calves fed non-medicated milk replacer (MR) only (CON; $n = 7$), MR supplemented with 3% fish oil (FAT; $n = 8$), or MR isoenergetically supplemented (gross energy) with cornstarch (CHO; $n = 8$)

Item[1]	Treatment			SEM	*P*-value[2]			
	CON	CHO	FAT		CON vs. SUP	CHO vs. FAT	Time	Trt × Time
Differential counts								
NC	34.04[a]	35.30[a]	29.30[b]	1.796	0.541	0.047	0.002	0.151
LC	59.41[b]	57.69[b]	63.24[a]	1.717	0.638	0.033	<0.0001	0.066
MC	3.19	3.25	3.52	0.215	0.479	0.387	<0.0001	0.216
EC	1.86	2.31	1.88	0.325	0.571	0.359	<0.0001	0.173
BC	1.47	1.44	1.46	0.145	0.909	0.904	<0.0001	0.939
NLR	0.61[a]	0.64[a]	0.50[b]	0.048	0.527	0.054	0.053	0.278
PCV, %	35.70	34.67	35.20	1.221	0.630	0.764	<0.0001	0.392
TP, g/dL	5.57[b]	5.91[a]	5.88[a]	0.091	0.011	0.804	0.393	0.957
UG, w/v	1.018	1.019	1.020	0.00	0.4276	0.5228	<0.0001	0.677

[1]NC = neutrophil count, LC = lymphocyte count, MC = monocyte count, EC = eosinophil count, BC = basophil count, NLR = neutrophil to lymphocyte ratio, PCV = packed cell volume, TP = total protein, UG = urine specific gravity, CON = control, CHO = carbohydrate supplement, FAT = lipid supplement, Trt = diet offered, CON vs. SUP = control treatment comparisons against supplemented groups CHO and FAT.
[2]*P*-value, <0.1 = trend; <0.05 = significant.
[ab]Means within row without common superscript differ ($P < 0.05$).

Table 5. Health assessment of nursing Holstein bull calves fed non-medicated milk replacer (MR) only (CON; $n = 7$), MR supplemented with 3% menhaden fish oil (FAT; $n = 8$), or MR isoenergetically supplemented (gross energy) with 7% with cornstarch (CHO; $n = 8$)

Item[1]	Treatment			SEM	*P*-value[2]			
	CON	CHO	FAT		CON vs. SUP	CHO vs. FAT	Time	Trt × Time
Mo	3.76[b]	5.30[a]	3.99[b]	0.380	0.011	0.013	<0.001	0.001
LTE, °C	38.29	38.31	38.25	0.044	0.528	0.423	0.501	0.537
RTE, °F	38.43	38.50	38.52	0.066	0.182	0.388	<0.001	0.278
Observations measured on 1 to 5 scale								
RS	0.043	0.013	0.025	0.018	0.510	0.983	0.999	0.999
FFS	0.40[a]	0.20[b]	0.10[b]	0.050	0.001	0.048	0.841	0.999

[1]Mo = skin moisture evaluated with moisture meter, PTE = pen temperature at time of collection, LTE = corneal eye laser temperature, RTE = rectal temperature, RS = respiratory score, FFS = fecal fluidity score, CON = control, CHO = carbohydrate supplement, FAT = lipid supplement, Trt = diet offered, CON vs. SUP = control treatment comparisons against supplemented groups CHO and FAT.
[2]*P*-values: significance < 0.05, trend: <0.1.
[ab]Means within row without common superscript differ ($P < 0.05$).

of hepatocytes that could trigger a satiety signaling cascade in the animals fed these diets (Allen et al., 2009). Additionally, given that the water intake composes a small portion of the water pool for growing animals, the increased cellular energy source coming from the diets would also result in higher levels of metabolic water production which could play a significant role in the reduction of the water requirements of nursing bull calves (Macias Franco et al., 2021). The water footprint values reported for this experiment are important in understanding both water requirements and water utilization of the livestock production sector for nursing calves. However, improper understanding of the modeling behind these values can be misleading. For instance, the values reported are unrealistically high. From a water use perspective, one could argue that the high water footprint values observed for the microbreweries spent grains should not be accounted here given that this was a co-product re-utilized for the feeding of animals and hence not providing a fair assessment of actual water use. Removal of the footprint of the microbreweries spent grains, would yield individual animal total water footprint values that were lower by more than 200 L for all treatments. The only direct water source the animals are consuming is through water intake which represents a small percentage of the total water footprint (mostly carryover from the feedstuff); water footprint estimates should remove the "double dipping" of co-products in feedstuffs to generate more accurate water footprint estimates that are biologically focused on the animals. Nearly 10% of the estimated water footprint came from the microbreweries spent grains; however, feeding rates of starter feeds are usually a lot higher and consequently carry higher water footprint. For instance, Wickramasinghe et al. (2019) reported lower water intake values than the ones we observed while using younger animals; however, the starter intake content was significantly higher than ours; therefore, the water footprint calculation in response to the high starter intake would result in higher water footprint for the solid intake in their animals. When adjusted for cold carcass weight, the lack of statistical differences could be attributed to the increased efficiencies in feed and water metabolism (such as residual feed intake, residual water intake, and average daily gain per unit of feed and water intake) for fat-supplemented calves. While the

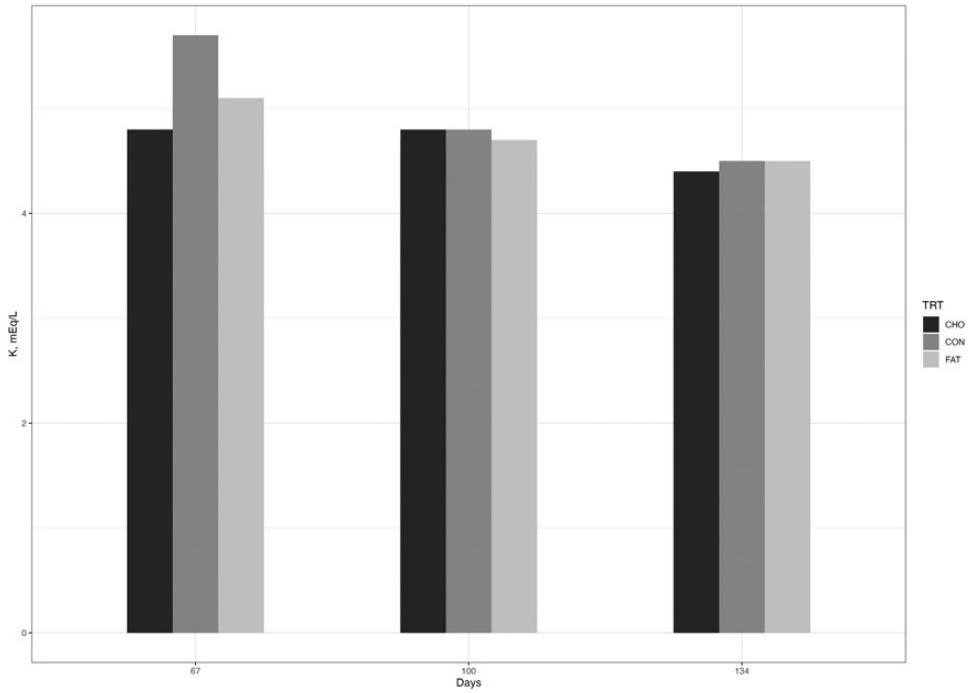

Figure 2. Treatment and time interactions for Serum potassium concentrations for 23 nursing Holstein bull calves fed with a non-medicated commercial milk replacer (Control [CON]; *n* = 7), supplemented isoenergetically (gross energy) with cornstarch (Carbohydrate [CHO]; *n* = 8), or with fish oil (fat [FAT]; *n* = 8).

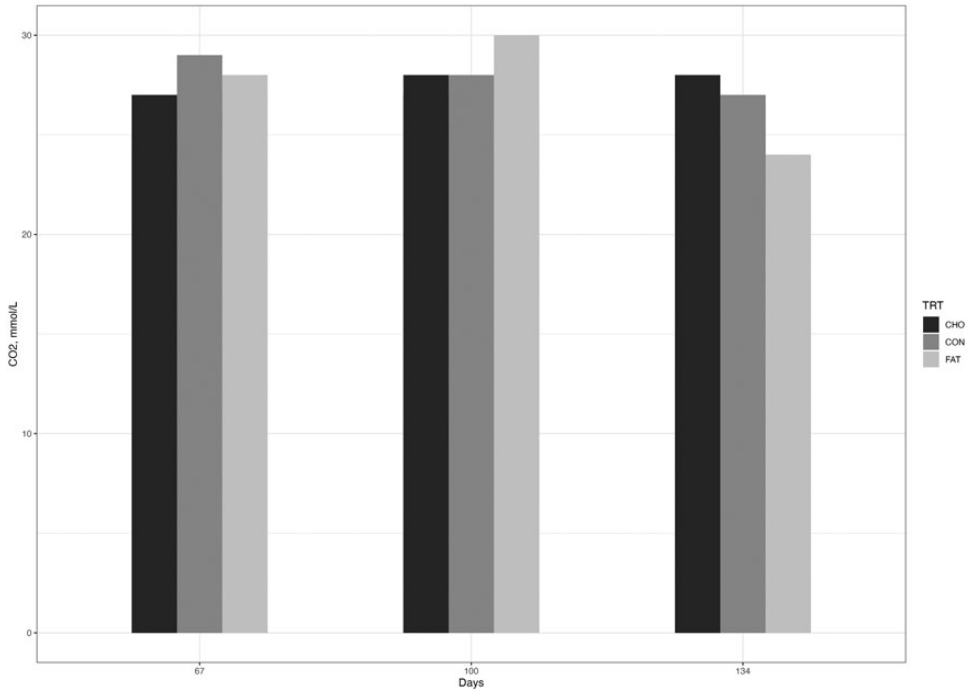

Figure 3. Treatment and time interactions for carbon dioxide serum concentration for 23 nursing Holstein bull calves fed with a non-medicated commercial milk replacer (Control [CON]; *n* = 7), supplemented isoenergetically (gross energy) with cornstarch (Carbohydrate [CHO]; *n* = 8), or with fish oil (fat [FAT]; *n* = 8).

animals were more feed and water-efficient, their numerically higher carcass weight resulted in statistically non-significant differences in feed intake per unit of body weight (Macias Franco et al., 2021), and therefore, when corrected, it could explain the results we observed. Our results highlight the potential decrease in water footprint for nursing bull calves by replacing starter feeds with energy supplements offered in milk replacer, thus, helping address the freshwater crisis and the role livestock production may play in it.

The implications of reducing water footprint and voluntary water intake potentially modify animal behavior. Dehydrated and heat-stressed animals shift intake patterns and behavior

Table 6. Serum electrolytes concentrations of nursing Holstein bull calves fed non-medicated milk replacer (MR) only (CON; $n = 7$), MR supplemented with 3% fish oil (FAT; $n = 8$), or MR supplemented (gross energy) with cornstarch (CHO; $n = 8$)

Item[1]	Treatment			SEM	*P*-value[2]			
	CON	CHO	FAT		CON vs. SUP	CHO vs. FAT	Time	Trt × Time
Na, mEq/L	129.52	130.67	127.67	1.431	0.256	0.386	0.723	0.876
K, mEq/L	4.38	4.23	4.22	0.074	0.443	0.155	<0.0001	0.869
Cl, mEq/L	91.71	90.75	91.91	1.203	0.773	0.502	0.315	0.756
CO_2, mmol/L	24.57	24.75	23.95	0.441	0.386	0.354	0.036	0.148
Ca, mg/dL	10.77	10.76	10.31	0.235	0.462	0.201	0.749	0.842
P, mg/dL	8.01	8.27	7.75	0.219	0.165	0.411	0.700	0.819
Mg, mg/dL	1.84	1.85	1.76	0.086	0.650	0.507	0.819	0.913

[1]CON = control, CHO = carbohydrate supplement, FAT = lipid supplement, Trt = diet offered, CON vs. SUP = control treatment comparisons against supplemented groups CHO and FAT.
[2]*P*-value, < 0.1 = trend; < 0.05 = significant.

in response to disease (Gaughan et al., 2019). The lack of differences in the ingestive behaviors amongst the treatments indicates our animals remained within healthy baselines, which further reinforces the idea that regulatory feed and water efficiency mechanisms may be interconnected and that animals consuming less water can do so without adverse effects on health and hydration.

Dehydration results in a loss of electrolytes and water in the feces, urine, respiration, and sweat (Hill et al., 2012). The lack of differences in serum electrolyte concentrations signal that our animals did not experience dehydration that could shift serum electrolytes. The kidney can increase filtration of water by selectively removing ions such as Na and Cl from plasma out through urine, increased urinary excretion may be related to an increase of Na and Cl excretion, decreasing its levels in the blood (Sherman and Eisinger, 1983). Concentrations of Na and Cl were below recommendation for all the treatments, whereas concentration of Ca was slightly higher for the indexes reported for hydrated and healthy animals (Goff, 2000; Dillane et al., 2018). Low serum concentrations of Na and Cl have been reported on scouring calves (Roy et al., 1959; Maach et al., 1992). The liquid nature of our diets could explain why these values were lower (similar loose stools as in scours). However, no other hydration parameters evaluated appear to signal that our animals were dehydrated whatsoever, suggesting an alternative mechanism for the decreased levels of serum Na and Cl exist such as water loss through respiration and sweat (insensible). Increased serum K has previously been associated with dehydration in hyperkalemic calves (Sweeney, 1999). The higher serum K levels observed in our animals could further reinforce the idea of a healthy hydration for our calves. The time effect observed on the CO2 concentrations (Figure 4) has previously been reported in mice fed ketogenic diets (Arsyad et al., 2020). The temporal decrease observed in the CO_2 represents observable changes during growth and are not necessarily alarming for hydration of the animals in this trial. In fact, for CO_2, the decreased levels through time observed for the fat-supplemented group could represent a potential higher affinity to serum O_2 which could be beneficial for overall animal health and in overall blood buffering ability (Hill, et al., 2012). Regarding Ca, a potential explanation for the high levels observed may be associated with the fact that calves are usually born hypercalcemic (Keaton et al., 1978). The observed levels of serum Ca concentrations of the animals may still be high due to the young age and partially because of the high Ca content in the milk replacer (Macias Franco et al., 2021).

Serum electrolytes are often troublesome to acquire, and therefore, alternative techniques and indices are necessary to evaluate animal hydration. Palma et al. (2015) showed that in humans, altered hydration levels could be detected with the use of the skin moisture meter utilized in this experiment; they further show that in humans, it is possible to detect hydration fluctuations in the skin in response to dietary changes. Such work highlights the potential of using skin moisture as a metric of indirect hydration/water loss capabilities. Kells et al. (2020) highlighted the significance of dehydration detection techniques for welfare and health assessment in nursing bull calves. Though indexes are not available for livestock species yet, dermal studies can serve as additional health and hydration biomarkers as assessed by epidermal transpiration in livestock (Scharf et al., 2010; Palma et al., 2012). Results of the present trial indicate that animals fed the carbohydrate-supplemented diet had significantly higher skin moisture than the other treatments. Higher water absorption rates could serve as representation of an enhanced skin hydration pattern and water tissue pool budgeting. The observed skin moisture differences could also be explained through animal subcutaneous fat cover. Though not examined in this study, provided animals would have similar hair coat, if the fat cover were less in the carbohydrate-supplemented animals, then water would be eliminated at higher rates due to less insulation. However, this alternative is unlikely given that higher evapotranspiration rates would result in increased water intake of the animals, and the opposite was observed in this experiment (Macias-Franco et al., 2021). A potential explanation could be that the carbohydrate-supplemented group had higher glucose levels, which would be expected for animals supplemented with soluble carbohydrates, this could also signal a potential more rapid rehydration mechanism. Higher glucose serum levels lead to hypertonic fluids within the animals' body, which paired with higher rates of glucose absorption, would yield a necessary water absorption (Smith, 2009) within the tissue of interest. Individual correlation investigation of the skin moisture with other health and hydration parameters (Figure 5) resulted in a significant correlation with packed cell volume and with the serum electrolytes examined (Figure 5). Given that packed cell volume and serum electrolytes are often utilized to assess hydration and

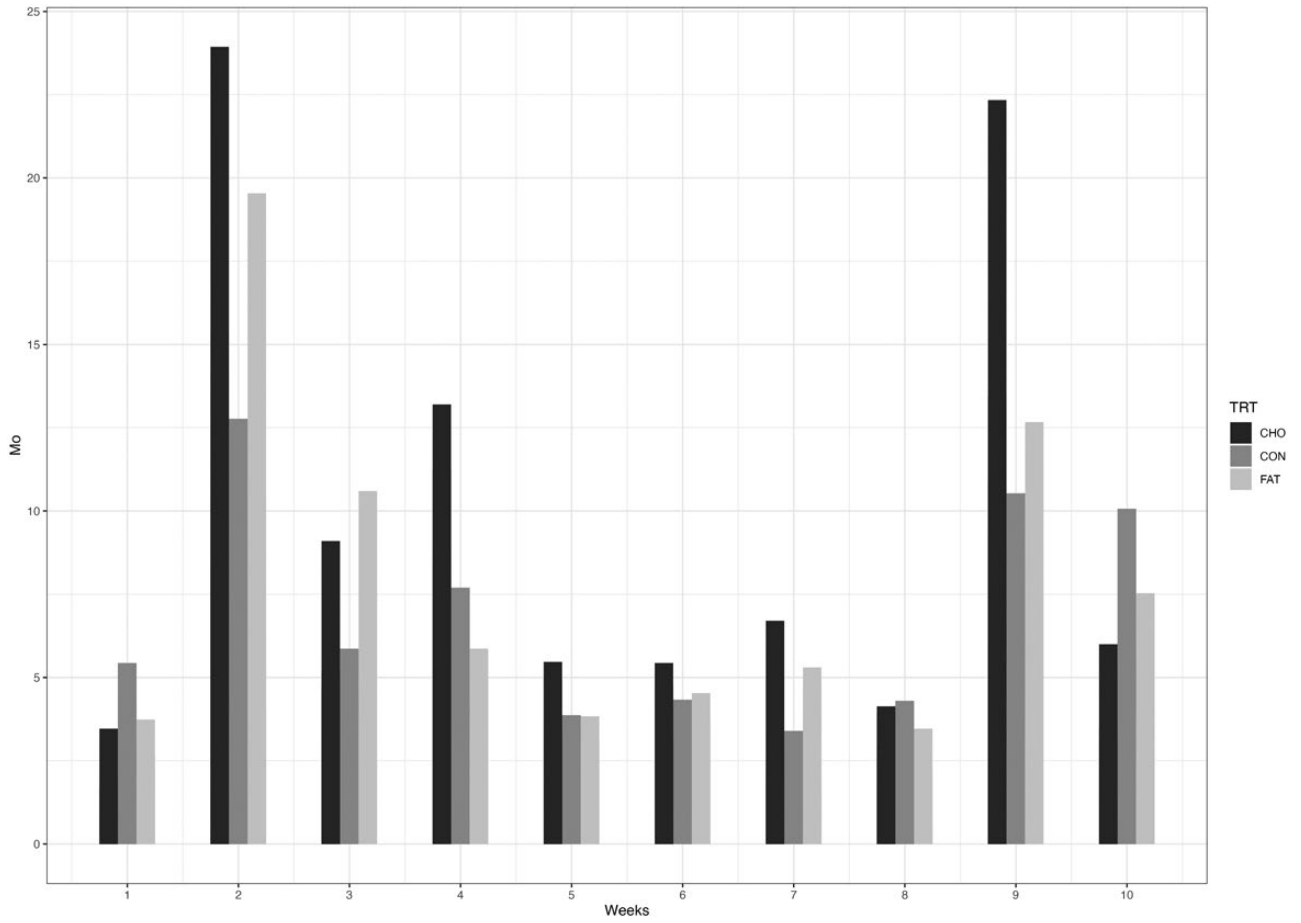

Figure 4. Treatment and time interactions for Skin Moisture (Mo) for 23 nursing Holstein bull calves fed with a non-medicated commercial milk replacer (Control [CON]; *n* = 7), supplemented isoenergetically (gross energy) with cornstarch (Carbohydrate [CHO]; *n* = 8), or with fish oil (fat [FAT]; *n* = 8).

health, the inverse relationship with skin moisture highlights the potential incorporation of this easy-to-use tool to assess hydration and health in livestock species.

High total protein values have been associated with animal dehydration (Marcato et al., 2018). However, values above 6.1 g/dL have been associated with increased survival for animals under five weeks of age (Naylor et al., 1977), while values under 4.5 g/dL have been associated with higher risks of death (Rea et al., 1996). Though counterintuitive, the values observed in this experiment lied within healthy thresholds and therefore appear to represent higher immune response instead of dehydration. Further reinforcing this idea, urine specific gravity can indicate dehydration Peek and Divers (2018) where values for lactating dairy cows and milk-fed calves normally range in values from (1.004 to 1.015), and dehydration should be considered if values were 1.025 and higher. Corroborating with our previous hydration assessment, urine specific gravity showed that animals in our experiment were not dehydrated.

Pertaining to health, neutrophil and lymphocyte counts, as well as neutrophil:lymphocyte have proven effective as a robust health assessment in conditions where animals are prone to stress, inflammation, and temporary fear or excitement (Von Konigslow et al., 2019). Similarly, these values could help evaluate when an animal suffers from a suboptimal dose of a nutrient such as water. The fat-supplemented animals on this experiment showed lower neutrophil and higher lymphocyte counts than the control and carbohydrate-supplemented groups. McDonnell et al. (2019) found similar neutrophil and lymphocyte counts during the pre-weaning phase of calves. Higher neutrophil:lymphocyte have been associated with a compromised immune response (Swanson and Morrow-Tesch, 2001) and could be a result of increased levels of neutrophils and lowered lymphocytes in response to stressors. In our case, though no major complications were observed, the fat-supplemented group displayed significantly more desirable neutrophil:lymphocyte values and therefore could be assumed to have a more desirable health status. Von Konigslow et al. (2020) reported that elevated lymphocyte counts were associated with increased ADG and decreased hazard of morbidity within 21 d upon animal receiving. The elevated leukocyte count observed for the fat-supplemented group could represent a higher functioning and more responsive immune system (Von Konigslow et al., 2020) at older ages and corroborate on the use of leukocyte differential counts as a health biomarker for water-mitigated animals. These results warrant the additional investigation of fat-based supplementation for longer time periods as well as for older animals; especially during periods of high mortality, such as ones observed at early receiving in the finishing phase for beef and beef-on-dairy systems. Eosinophil count values have been associated to stress (Swanson and Morrow-Tesch, 2001), our results indicate that our animals did not experience major stressors that affected their eosinophil counts. This

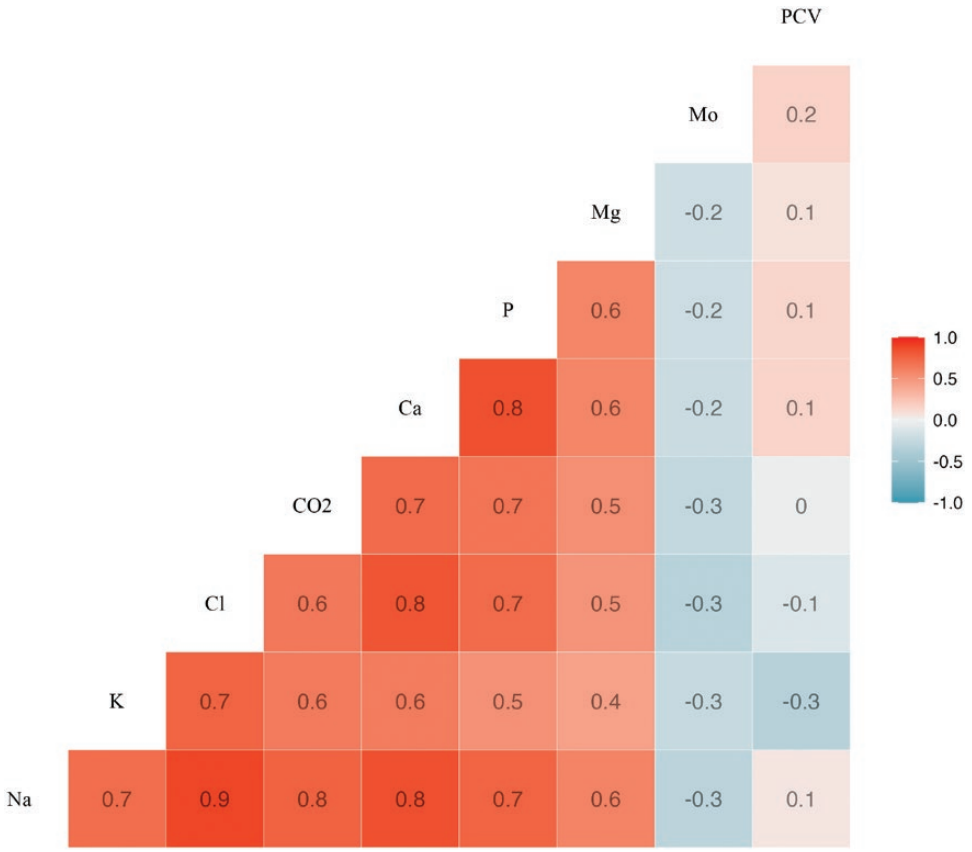

Figure 5. Correlation values for Skin Moisture (Mo) measured as skin capacitance for 23 nursing Holstein bull calves fed with a non-medicated commercial milk replacer (Control [CON]; *n* = 7), supplemented isoenergetically (gross energy) with cornstarch (Carbohydrate [CHO]; *n* = 8), or with fish oil (fat [FAT]; *n* = 8) and serum electrolytes.

indicates that fat and carbohydrate-supplemented calves fed to mitigate water use and water footprint may also promote an improved immune function. The observed time effects on the white blood cell differential counts represents variations laying within healthy intervals (Figure 1).

Rectal and corneal temperatures, as well as respiratory and fecal fluidity scores were evaluated to assess animal health. No effects were observed for respiratory scores, again reinforcing no deviations from normality (health) were detectable in respiration. The observed time effects for rectal temperature represent normal variations within healthy temperature limits and could be attributed to the animal's aging that would represent physiological and anatomical developments (rumen function) that allow them to better regulate temperature and hydration status (Roland et al., 2016). Regarding fecal fluidity scores, under fat and carbohydrate supplementation, Bascom et al., (2007) reported similar effects except for higher fecal scores for their animals, albeit their animals were younger (around 6 wk of age). The observed decreases in fecal fluidity scores indicate a better utilization of water through mechanisms of reabsorption (Church, 1976) for fat and carbohydrate-supplemented steers.

In conclusion, water use mitigation through dietary supplementation is possible for nursing bull calves. More importantly, these reductions are possible without adverse effects on hydration, health, and ingestive behavior. Given that societal and governmental pressures will likely increase for livestock production systems to become more environmentally sustainable, supplementation strategies like the ones examined herein will become more prevalent across other life stages. These results highlight the significant decrease attainable in current veal and cow-calf operations that could benefit from these supplementation strategies. Additionally, though these results only investigated one life-stage, the life cycle assessment and increased pressure warrant the betterment and increasing sustainability of operations on all life stages of growth. Our carbohydrate-supplemented group had the lowest water footprint compared to the control and fat-supplemented groups. Future research should invest in examining similar supplementation lines in other life stages. Supplementation lines for reduction of water footprint of livestock production systems are promising nutritional management strategies that increase overall sustainability of cattle production systems.

Supplementary Data

Supplementary data are available at *Translational Animal Science* online.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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