University of Nevada, Reno

Precision diet formulation incorporating isoenergetic lipid and carbohydrate supplementations as water intake mitigation strategies for Holstein nursing bull calves

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal and Rangeland Science

By

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THE GRADUATE SCHOOL

We recommend that the thesis
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Thesis Abstract

As resource availability continues to diminish, livestock operations and producers will continue to garner unfounded and poor-evidenced attacks. Regardless of these inconsistencies and poorly evidenced attacks towards livestock production, many producers and scientists worldwide continue pursuing technologies, feedstuffs, and management systems that can even further diminish the environmental footprints of livestock operations. Amongst the most controversial topics, water footprint is often utilized to generate unrealistically negative representations of livestock operations. Though current water footprint models are unrealistic and empirically flawed, researchers and producers can continue to improve production systems and therefore educate the public through the generation of accurate models. Livestock operations are often essential in many communities in the developed and developing world. For instance, dairy operations are often considered a large contributor to the Nevada economy. Dairy operations generally center sustainability efforts in the cow-milking processes, which could disregard a potentially important area for possible improvement, the bull calves. Most bull calves from dairy operations are often sold young for their inability to contribute to dairy production.

Nonetheless, these animals have great potential and present a unique opportunity for filling much-needed niche markets in local economies. Ruminant animals can generate nutrient-dense and balanced feeds for human consumption out of human inedible by-products. The many wonders often achievable by ruminant animals are studied in many branches of science; however, there is great potential in examining ruminant animals at a

young age. That is when their rumen is not fully functional. This unique physiological

and anatomical characteristic of calves opens the door for precision diet formulation

using high lipid and high soluble carbohydrate energy supplementation that could

otherwise be deemed damaging to adult ruminant species. The work presented herein

aims to enlighten the potential voluntary water intake and water footprint reduction

through isoenergetic supplementation without adverse effects on health and performance.

This work, as my life, is dedicated:

To my parents that always taught me to adhere to my values.

To my two brothers, who continuously support me and serve as inspiration to my every doing.

To my best friend who pushed me and believed in me when I wanted to give up.

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Thesis Introduction

As populations continue to increase, the per-capita consumption of animal products alike is expected to increase, potentially adding pressure on freshwater resources (Mekonnen and Hoekstra, 2010). It is therefore imperative that producers and researchers seek for alternative feedstuffs and supplements that further diminish the water footprint.

Nevada's dairy production has a substantial impact on the state's economy –an estimated 7,500 jobs, 280 million dollars in wages, and an economic impact of over $1.2 billion), as well as producing high-quality protein exported worldwide (Nevada Dairymen 2018). An additional income source, often not exploited by farmers worldwide, is the veal market. Which at one point was consumed at rates of 8 lbs. per capita/year to a current low of 0.3 lbs. (USDA, 2013). The potential socioeconomic impact of veal reinstallation in the market could serve as means to allow small agricultural economies to flourish. Veal may be marketed in a variety of ways which include "Bob Veal", "Special-Fed Veal", and generally as meat from a calf (animals under 750 lbs.) or young beef animals (USDA, 2013).

Further increasing pressure for the state of Nevada (**NV**), Northern NV climate is characterized by warm, dry summers and cool, wet winters. High temperatures associated with low humidity directly impact animal health due to distress and discomfort. Cattle exposed to those climate conditions (high temperatures and low humidity) may show higher incidences of dehydration, reduced appetite, reduced growth, decreased immune responses and consequently, increased susceptibility to diseases. Given that more than

50% of the mortalities of calves under 3-weeks old are generally related to digestive and respiratory complications, and understanding that digestive complications generally lead to calf death due to dehydration, mortality complications may be exacerbated through extreme weathers present in NV (USDA-APHIS, 2010). The negative impact of warm weather is maximized by the increase of air temperature, which leads to the loss of temperature compensation ability of calves (Bateman and Hill, 2012). Further, several studies have reported the significant detrimental effects on the immune system due to high temperatures that may cause dehydration and reduced immunoglobulin concentrations in plasma, increased leukocyte levels, erythrocyte destruction, amongst others (Abdel Samee, 1987; Habbeeb, 1987).

Several authors have explored how the inclusion of omega three fatty acids may help reduce the incidence of disease; however, their utilization to minimize voluntary water intake, and therefore, water requirements in animals remains to be explored. The unique anatomical and physiological status of young ruminant animals presents the opportunity for utilization of lipid and soluble carbohydrate supplementation as means to decrease voluntary water intake through metabolic water production increased by higher energy densities in diets. Given that high lipid and soluble carbohydrate diets may be detrimental to adult animals, the pre-ruminant stage presents a unique opportunity that could benefit cow-calf producers of arid regions, as well as assist in sustainability efforts for large-scale producers.

Feeding enriched diets during the first 4 to 5 months after birth during the summer season may improve the health and growth development of the offspring, which direct

fulfills rancher's needs from Nevada and other arid climate states regarding calf production.

References

Abdel-Samee, A.M. 1987. The role of cortisol in improving the productivity of heat-stressed farm animals with different techniques. Ph.D. Thesis, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. 27.

Bateman, G. & Hill, M. 2012. How heat stress impacts the growth of calves. Progressive Dairyman. Available at: https://www.progressivedairy.com/topics/calves-heifers/how-heat-stress-impacts-the-growth-of-calves Assessed 10/11/2017.

Habeeb, A.A.M. 1987. The role of insulin in improving the productivity of heat stressed farm animals with different techniques. Ph.D. Thesis, Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

Mekonnen, M. M., and A. Hoekstra. 2010. The green, blue and grey water footprint of farm animals and animal products. Volume 1: Main Report. Unesco Value Water Res. Rep. Ser. No. 48.

Nevada Dairymen. 2018. Online article access: https://www.nevadamilk.com/nevada-farms

USDA-APHIS. 2010. Mortality of calves and cattle on U.S. beef cow-calf operations. Available at: https://www.aphis.usda.gov/animal_health/nahms/beefcowcalf/downloads/beef008/Beef0708_is_Mortality.pdf Assessed 10/11/2017.

USDA. 2013. Veal from Farm to Table, 2013. USDA-FSIS, Fort Collins, CO.

Chapter I

Effects of lipid and starch supplementation as water intake mitigation techniques on performance and efficiency of Nursing Holstein Calves [I]

Abstract

Exploring alternative supplementation sources capable of maximizing feed and water efficiency in nursing Holstein calves is often ignored. The goals herein involve investigating the effects of two isoenergetic supplements on a non-medicated milk replacer diet on total water intake, milk water intake, fresh water intake, intake parameters, and performance of Holstein nursing bull calves. Twenty-three animals (body weight [BW] = 94.67 ± 12.07 kg, age = 9 weeks old) were randomly assigned to one of three treatments for 68 days: control (CON; ad libitum milk replacer, n = 7), carbohydrate supplement (CHO; corn starch on top of ad libitum milk replacer-based diet, n = 8), or lipid supplement (FAT; menhaden fish oil on top of ad libitum milk replacer-based diet, n = 8). The isoenergetic supplementation consisted of 3% menhaden fish oil addition on a DM basis for FAT. This was matched energetically with corn starch for the CHO group resulting in a 7% composition DM basis. All animals were provided free access to mineral mix and 120 g daily dried microbrewer's spent grains (**BG**). Data were analyzed with the GLMMIX procedure of SAS in a completely randomized design with the diets as a fixed effect. Dry matter intake (**DMI**) in basis of average daily gain (**ADG**; DMI/ADG) displayed significant differences with CON = 2.48, CHO = 2.38, and FAT = 2.27 kg/kg$_{(ADG)}$ (P = 0.033). Energy intake values were lower for CON when analyzing metabolizable energy intake ($P < 0.0001$), net energy intake for maintenance ($P < 0.0001$), and net energy intake for gain ($P < 0.0001$), followed by CHO, and then FAT. Total water intake ($P < 0.0001$), milk water intake ($P < 0.0001$), and fresh water intake ($P < 0.0001$) all resulted in CHO consuming 0.5 l or less water than the other two

treatments. Energy requirements as digestible energy ($P < 0.0001$), metabolizable energy ($P < 0.0001$), net energy for maintenance ($P < 0.0001$), and net energy for gain ($P < 0.0001$) were lower for CHO, followed by CON, and then FAT having the highest requirements. Similar results were observed for residual feed (**RFI**; $P = 0.006$) and residual water intakes (**RTWI**; $P = 0.902$). Ultimately, no performance differences were detected with regards to BW, (CON = 146.71, CHO = 146.25, and FAT = 150.48 kg; $P > 0.1$). These results indicate that energy supplementation could potentially be utilized as means to mitigate water use and potentially increase animal efficiency without adverse effects on performance.

Key words: isoenergetic supplementation, nursing calf, water intake**,** water mitigation strategies, water requirements

Introduction

Water utilization and availability in agricultural production systems are of significant importance to the livestock sector, more so as water shortages and scarcity increase worldwide (Doreau et al., 2012). As water shortages continue to grow, the allocation of water sources may become a future source of conflict. Beef and dairy cattle operations are commonly reported in environmental water footprint studies, accounting for 33% and 19% of the total agricultural water footprint, respectively (Hoekstra, 2012). As the world population continues to grow, the per-capita consumption of animal products alike is expected to increase**,** potentially adding pressure on freshwater resources (Mekonnen and Hoekstra, 2010). It is therefore imperative that conjunctly and proactively, the cattle industry seek ways to accurately account for water usage and for alternative ways to mitigate it.

The majority of veal (milk fed veal calves "Bob-veal" and non-Formula fed veal: generally fed milk/milk replacer until two months of age then transitioned to solid feed or slaughtered; LPM-WIFFS, 2016;) and calf operations are governed by milk-fed (milk replacer, or composited milk from cows for the first eight weeks) management systems (Xiccato et al., 2002). These feeding systems can account for large proportions of water usage, and therefore, highlight a potential region for improvement. This is especially true in the arid areas of the Western US. The state of Nevada is the driest in the US (USGS, 2006 or Western Regional Climate Center, 2021); therefore, minimizing water utilization in livestock operations is a constant concern for the agriculture industry. A potential optimization of the current system could involve precision diet formulation tailored to decrease the fresh water intake of animals. Detailed requirements may be found regarding protein, fat, carbohydrates, minerals, and specific supplements that may increase performance (Fass, 2010; NRC, 2001). To the knowledge of the authors, very few studies have attempted to describe the water requirements of veal Holstein calves (Senevirathne et al., 2018; Wickramasinghe et al., 2019), and there have been no attempts exploring the effects of metabolic water produced in oxidation as a strategy to mitigate water usage by Holstein nursing bull calves. Hence, we aim to compare the influence of lipid-based versus starch-based supplementation on intake, performance, and efficiency of Holstein nursing bull calves fed diets optimized for water consumption mitigation. We hypothesized that targeted supplementation could improve the efficiency of the use of water as well as decrease the fresh water intake without jeopardizing animal performance.

Materials and Methods

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

Animals, diets and facilities

Twenty-three Holstein nursing bull calves were raised from postnatal day 1 to day 135 (9 weeks of adaptation followed by 68 days of experimental diet offering). Calves were acquired from a commercial Dairy Farm located in Northern NV. Upon birth, newborn calves had their umbilicus treated with iodine solution (10%), were weighed and monitored for normal behavior (stand and nurse within 2 hours after birth) and colostrum ingestion. Only singlet bull calves born from non-dystocic parturition that behaved normally and ingested at least 5% of their body weight (BW) in colostrum were selected. Animals were transported to the Dairy barn facilities at the Nevada Agricultural Experimental Station, where animals' BW were recorded and overall health status was evaluated by the clinical veterinarian. Animals averaged 94.67 ± 12.07 kg after the 9-week adaptation period. Housing constituted individual 32 ft^2 galvanized steel pens (Seneca Dairy Systems, LLC; Est. 1978) located inside a barn equipped with heaters, fans, and a swamp cooler for temperature and relative humidity regulation. Weather variables were closely monitored throughout the experimental period to ensure animals remained within their thermoneutral zone at all times. The pens were bedded with wood shavings for the adaptation period, and before the trial start, shavings were replaced with rubber mats. Twenty-three animals (body weight [**BW**] = 94.67 ± 12.07 kg, age = 9

weeks old) were randomly assigned to one of three treatments for 68 days: control (CON; ad libitum milk replacer, n = 7), carbohydrate supplement (CHO; corn starch on top of ad libitum milk replacer-based diet, n = 8), or lipid supplement (FAT; menhaden fish oil on top of ad libitum milk replacer-based diet, n = 8). The isoenergetic supplementation consisted of 3% menhaden fish oil addition on DM basis for FAT. This was matched energetically with corn starch for the CHO group resulting in 7% composition DM basis; all groups received 120 grams of microbreweries spent grains (BG) per day and had free access to a balanced mineral mix (NaCl 96%, manganese 2,400 ppm, iron 2,400 ppm, copper 260 ppm, zinc 70 ppm, cobalt 40 ppm.). The dietary and chemical composition of the diet may be found in Table 1. Animals were fed twice daily at 6h00 and 16h00; milk replacer was reconstituted with warm water (65 $^{\circ}$C) and allowed to cool to 40° C before feeding. Milk replacer and dietary ingredients were mixed on a MILK BAR Cart coupled with a stainless steel whip mixer (MBMk125D and MB126A models, respectively, McInnes Manufacturing Ltd., Waipu, New Zealand). Pre-weighed corn starch and fish oil were incorporated and thoroughly mixed with the milk replacer into separate containers and calves were fed ad-libitum in stainless steel buckets. Orts were collected daily and feeding was adjusted to ensure 10% refusals.

The dry matter (**DM**) intake (**DMI**) was computed as DM of milk replacer before reconstitution + BG + supplements. Samples of milk replacer, BG, supplements, and orts were collected, adequately identified, and stored in a freezer at -20ºC. At the end of each week, a composite sample was prepared and oven dried (60ºC). After that, another

composite sample representing the 28 d period was generated based on the proportion of DMI each week and stored at -20ºC for subsequent chemical analysis.

Chemical Analyses

All samples, except those with less than 15% moisture, were air-dried in a forced draft oven (60°C) and ground to pass a 1-mm screen in a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ 08085) and sent to Cumberland Valley Analytical Services (CVAS; Waynesboro, PA) for chemical analysis of DM (method 930.15; AOAC 2000), ash (method 942.05; AOAC 2000), organic matter (**OM**; AOAC, 2005; method 942.05) calculated as 100 minus ash concentration, neutral detergent fiber (**NDF**) was analyzed according to the technique described by Mertens et al. (2002) without the addition of sodium sulfite, but with the addition of thermostable alpha-amylase. The NDF content corrected to ash (Mertens 2002) and protein (Licitra et al. 1996) content was estimated (**NDFap**), acid detergent fiber exclusive of ash (method 973,18; AOAC, 2000), acid detergent lignin using sulfuric acid (Goering and Van Soest, 1970), crude protein (**CP**; method 990.03; AOAC, 2000) in a Leco FP-528 Nitrogen Combustion Analyzer (Leco Corporation, St. Joseph, MO), non-fibrous carbohydrates (**NFC**) were calculated as NFC (% DM) = 100 − [CP + NDF + EE + ash], ether extract (**EE**; method 2003.05; AOAC, 2006), a complete mineral panel (method 985.01; AOAC, 2000) by a Perkin Elmer 5300 DV ICP (Perkin Elmer, Shelton, CT), the total digestible nutrients (**TDN**) and net energy were computed utilizing empirical equations reported on NRC (2001). The dietary and chemical compositions of the analysis are described in Table 1. Metabolizable energy intake (**ME$_i$**), digestible energy intake (**DE$_i$**), net energy intake for maintenance (**NE$_{im}$**), and net energy intake for gain (**NE$_{ig}$**) were calculated according to the NRC (2001).

Water Analysis

Water was sampled from a single water source that provided water for the animals throughout the experimental period. Water was collected from the cold faucet; the screen and aerator were removed, and water was allowed to run for three minutes. Two samples were collected**:** 100 ml of water was collected and sealed in a sterile bottle with sodium thiosulfate for coliform and *E. coli* bacterial evaluation, and a second sample was placed in a 500 ml sterile sample bottle for water suitability analysis. Water was shipped refrigerated with expedited same-day shipping for analysis at Cumberland Valley Analytical Services 2020 CVAS, Inc. All Rights Reserved. The analysis was performed according to Rice et al. (2017) for pH (method # 4500-H), nitrate (method #4500 NO_3^-), total dissolved solids (method # 2540), sulfates under (method # 4500-SO42), the following minerals: calcium, phosphorus, magnesium, potassium, sodium, iron, manganese, zinc, copper under (method #3500), carbonate hardness with (method #2340), and total coliform and *E. coli* from (method #9223); the results of the analysis are described in Table 2.

Apparent total tract nutrient digestibility

During the trial, two digestibility assays were performed to estimate the nutrient digestibility coefficients. Total fecal collections were performed for four consecutive days on days 28 to 32 and 60 to 64 of the experimental period (after adaptation). Feces were collected immediately after spontaneous defecation and stored in a container. Every morning, feces were weighted, thoroughly homogenized, and a 200 g subsample was

compiled. Fecal samples were oven dried at 55°C for 72 hours for further chemical

analysis.

Water Intake

Animals had free access to clean ad libitum water during the whole trial. Water

was tested for livestock suitability before and during the trial (Table 2). Water data was

collected in total water intake, milk water intake, and fresh water intake. To determine

biological efficiencies and water utilized for tissue deposition amongst treatments, BW

adjustments were made as ratios of water and BW measures. Fresh water intake was

recorded every morning before feeding. Automated individual water systems were

custom-built with 55-gallon plastic barrels. Three holes were drilled on each barrel, two

at the bottom consisting of a line attachment connecting to individual automated floater-

stopper water troughs, and an additional hole for attachment of a translucent food-grade

tubing with a measuring tape attached to the inside, tightly and vertically connected to the

outside of the barrel used as communicating vessels which allowed the measurement of

the volume of water displacement by difference. Individual water pumps helped ensure

water pressure flowing from the 55-gallon barrels to the through was sufficient but not

exceeding the shut off valve regulating the water level in the individual troughs. Barrels

were individually calibrated three times during the experimental period by the same

researcher to minimize calibration errors. Calibrations consisted of water addition using

two and four L graduated cylinders and recording respective volume changes within the

tubing and measuring tape attached inside the clear plastic tubing. The changes were

recorded as mm of water within the tubing and calibrations were converted into the

volume of water respective to the mm change. Calibrations were regressed on volume change and conversion values (distance to water volume) were computed. Barrels were sanitized once monthly, and water troughs were cleaned and disinfected daily to ensure free access to fresh water at all times.

Investigation of metabolic water production and its practical application and effects on fresh water intake are presented in the discussion section. Metabolic water production (**MWP**) was originally postulated and understood as a gram of fat should yield 1.07 grams of water, a gram of carbohydrates should yield 0.6 grams of water, and 1 gram of protein should yield 0.41 grams of water (Brody, 1946). We attempt to elaborate on this theory in the discussion section, where we suggest a more applied equation that best correlates our results.

Slaughter

Animals were withdrawn from feed and water for 16 hours to obtain shrunk body weight and slaughtered at a commercial harvesting plant, Wolf Pack Meats, a USDA inspected facility located at the Nevada Agricultural Experiment Station. Slaughter was performed by trained technicians stunning the animals using a penetrating captive bolt rendering the animal unconscious, followed by exsanguination through the jugular vein. Carcasses were separated into two halves and weighed, then chilled (1 to 4 °C) for 24 h and then re-weighed to obtain the cold carcass weight. By dividing the carcass weights by the shrunk body weight, we obtained the hot and cold carcass yields.

Requirements, efficiency and growth

The tested parameters for feed efficiency were: residual feed intake (**RFI**), residual total water intake (**RTWI**), feed conversion efficiency (**FC**), feed conversion ratio (**FCR**), Kleiber index (**KI**) and relative growth rate (**RGR**). The FCR was obtained by dividing the DMI (kg/d) by the average daily gain (**ADG**, kg/d). The average FC was obtained by the reciprocal of this relationship. To calculate the RGR, the shrunk body weight was taken into account for initial, final shrunk body weight, and d of confinement as RGR = 100 * (log final BW - log initial weight)/d (Fitzhugh and Taylor, 1971). The KI was calculated by dividing the ADG by the average metabolic weight (**$BW^{0.75}$**) (Kleiber, 1936). Residual feed intake and RTWI were calculated as the regression of ADG and the midpoint $BW^{0.75}$ utilized to generate a predicted intake value which was then subtracted from observed DMI, total water intake, to cause RFI and RTWI, respectively, according to Sainz and Paulino (2004).

$$RFI; RTWI = \text{Y}_{12} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \varepsilon_{12}$$

Where Y represents the expected values for feed and water measures to be regressed, β_0 represents the respective equation intercept, β_1 and β_2 represent the coefficients of the equation, X_1 and X_2 represent the midpoint $BW^{0.75}$, and the ADG, respectively, and ε is the respective residuals. Ultimately the fitted regression equations for prediction of TWI and DMI were as follows: $TWI_{predicted} = 5.521 + (0.316)\,(BW^{0.75}) - (0.919)\,(ADG)$ and $DMI_{predicted} = -0.861 + (0.068)\,(BW^{0.75}) + (0.637)(ADG)$

The energy requirements were calculated according to the NRC (2001), assuming dairy calves fed milk replacer and starter at 0.086 $BW^{0.75}$ for net energy for maintenance

(Mcal), 0.84 x $BW^{0.355} \times ADG^{1.2} \times 0.69$ for net energy for growth (Mcal), $0.1 \times BW^{0.75} +$ $(0.84 \times BW^{0.355} \times ADG^{1.2})$ for metabolizable energy (Mcal), and metabolizable energy/0.93 for digestible energy (Mcal).

Biometric measures (**BM**) were taken to assess growth during the trial. The BM was taken alongside BW on days 0, 28, and 56 of the experimental period, with the same technician collecting all BM. Animals were properly adapted to the squeeze chute before the beginning of the trial. Once in the squeeze chute, each animal was erectly positioned. The BM was taken using specific anatomical locations as baseline points by hand palpation as recommended by De Paula et al. (2013) and Fonseca et al. (2017). The measurements were taken with the aid of a large caliper (Hipometro type Bengala with two bars, Walmur, Porto Alegre, Brazil) and a graduated plastic flexible tape. The BM included hook bone width as the distance between the 2 ventral points of the tuber coxae (large calipers); pin bone width as the distance between the two ventral tuberosities of the tuber ischia (large calipers); abdominal width measured as the widest horizontal width of the abdomen (paunch) at right angles to the body axis (large calipers); body length as the distance between the dorsal point of the scapulae and the ventral point of the tuber coxae (tape); rump height as measured from the ventral projection of the tuber coxae, vertically to the ground (large calipers); scapulae as the measure from the humeroscapular joint to the end of the scapula; height at withers measured from the highest point over the scapulae, vertically to the ground (large calipers); pelvic girdle length as the distance between the ventral point of the tuber coxae and the ventral tuberosity of the tuber ischii (large calipers); rib depth measured vertically from the highest point over the scapulae to

the end point of the rib, at the sternum (large calipers); rump depth measured as the vertical distance between the ventral point of the tuber coxae and the ventral line (large calipers); body diagonal length measured as the distance between the ventral point of the tuber coxae and the cranial point of shoulder (tape); and thorax width as the widest horizontal width across shoulder region, at the back (large calipers).

Statistical Methods

Statistical analyses were performed using PROC GLIMMIX of SAS University Edition (SAS Inst. Inc., Cary, NC). All variables were investigated, assuming a completely randomized design with diet as the fixed effect and incorporating the intercept as a random effect with the animal as the subject. Outliers were identified using the plot of studentized residuals against the predicted values and Cook's D coefficients where values exceeding 2.5 studentized t distributions were considered outliers and removed from the data (Neter et al., 2004). Mean comparisons were performed using the LSMEANS statement with the Tukey-Kramer adjustment for all significant effects, assuming significance at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.1$

Results and Discussion

Feed Intake and digestibility

The experimental diets were formulated to simulate two supplementation strategies for Holstein nursing bull calves to evaluate if animals fed ad libitum would be able to decrease their voluntary fresh water intake without jeopardizing performance. The supplements were then designed to be isoenergetic, either carbohydrates-based (i.e.: corn starch) or fat-based (i.e.: menhaden fish oil). Additionally, animals were provided ad

libitum mineral mix so osmotic balance wouldn't interfere with the fresh water intake.

Further, BG was fed to stimulate rumen development (Church, 1988) to provide

additional mechanisms for calves to optimize their body water pool through body water

compartments present in the gastrointestinal tract (King, 1983). Two statistical trends

were observed when adjusting DMI of milk replacer and milk replacer intake per ADG,

DMI of the milk replacer/ADG and milk replacer intake/ADG, respectively ($P = 0.08$;

Table 3). No differences were observed for the daily intake of BG, most likely due to a

slow transition of the animals from a pre-ruminant to a ruminant stage while receiving the

primarily liquid feed. Overall, supplementation of the milk replacer tended to decrease

DMI milk replacer and milk replacer intake per kg of ADG ($P= 0.08$; Table 3). When we

examined DMI accounting for both BG intake and milk replacer, a significant difference

($P = 0.033$; Table 3) was observed between the CON (2.48 kg) and the FAT (2.27 kg),

but not between the CHO (2.38 kg) and CON, or between the FAT and CHO groups. The

measure of DMI/ADG for the whole experimental diets showed a significantly lower

intake for the FAT group when compared to the CON. When examining the partitioned

nutrient intake from the diets, no statistically significant differences were noted on any

nutrient intake values except for EE intake ($P = 0.007$; Table 3). Even though the diets

were formulated to be isoenergetic, the larger crude fat content present in the FAT

increased the overall crude fat intake for this treatment, thus explaining the difference

detected.

　　Interestingly, even though the diets were isonitrogenous, the coefficients of

digestibility of the CP (**CPD**) were significantly different ($P = 0.022$; Table 4). We

observed a decrease in the CPD for the CHO treatment and a decrease in the ether extract

digestibility (**EED**) when compared to FAT. These results indicate that CHO

supplementation significantly affected protein and crude fat digestion, which could

reflect shifts in diet transit within the gastrointestinal tract, hence potentially affecting

water balance (King, 1983). Moreover, upon harvesting, we observed that rumens were

still underdeveloped, reflecting a predominantly liquid diet.

More recently, Amado et al. (2019) investigated the effects of energy source

supplementation in bovine milk, assessing its effects on digestibilities;. However, on their

data, no differences in digestibilities were observed for lactose and fat. Their diets offered

ad libitum hay and starters, which also likely promoted ruminal development in their

animals. A developed rumen could explain the digestibility differences they observed due

to possible changes in digestibility, residence time, and passage rates for their animals

(Church, 1993). Hu et al. (2019) reported higher digestibilities for calves fed moderate

amounts of milk replacer than those fed higher rates of milk replacer. Yet, these authors

fed ad libitum amounts of starter, which could allow from 17 to 20% more storage of

body water in the reticulo-rumen due to the higher ruminal development (King, 1983).

Teixeira et al. (2006) reported that animals who were not restricted-fed had a decreased

CPD, which resulted in lower water intakes compared to the group with higher CPD.

Given that our animals were offered isonitrogenous diets, our findings suggest that the

NFC:CP ratio and their synchronization in precision diet formulation, and not CP intake

alone, could be highly influential on fresh water intake. Regarding EED, a significant

difference ($P = 0.038$; Table 4) was observed, with the FAT group having the highest

digestibility (0.96). Digestible EE intake also shows that the FAT group having a value of 0.54 kg/day consumed higher amounts of lipids than the other two treatments (P = 0.005; Table 4). It is important to notice that the inclusion of fat in the diet was limited to 3% and the amount of fiber in the diet was negligible.

Water Intake

Though evaluation of the effects of dietary supplementation on fresh water intake has been previously reported in the literature (Morrison, 1953; NRC, 2001; Quigley et al., 2006; Santos et al., 2014; Wickramasinghe, 2019), our results are unique in that no other authors have examined precision diet formulation utilizing starch and lipid supplementation regimes as means to mitigate fresh water intake in Holstein nursing bull calves. Fraley et al. (2015) discuss effects on fresh water intake due to mineral supplementation, chiefly, potassium carbonate in lactating dairy cows; the authors observed an increase in potassium supplementation promoted a linear increase in fresh water intake. However, no effects of macronutrients or primary dietary ingredients were reported as drivers to mitigation. Further, other studies investigated the effects of sodium, water temperature, and DMI on fresh water intake but failed to address the specific macronutrient effects or metabolic water production (Murphy, 1992). For this experiment, the availability of ad libitum balanced mineral mix for all animals allows us to control its effects on water consumption.

The total water intake, milk water intake, and fresh water intake showed statistically significant differences *(P < 0.0001; Table 5)*. Starch supplementation significantly decreased total water intake, but no significant differences were observed

between CHO and FAT *(P > 0.1;* Table 5), which had respective means of 17.61 and

17.51 L. The CHO group consumed the least amount of water for total water intake, milk

water intake, and fresh water intake. This reduction can be explained through MWP. A

possible explanation for the lower fresh water intake of CHO and FAT, is that

carbohydrates are expected to have 20% higher MWP Morrison (, 1953). The CON

group, on average producing 1.57 L was not statistically different than the FAT (1.64 L),

but both were statistically lower than the CHO group with estimated values of 1.68 L (*P*

< 0.0001; Table 5). Morrison's (1953) equation better represents the results observed in

this experiment. The increase in MWP observed for the CHO and FAT groups could help

explain the reduced fresh water intake of the animals. Further, though the diets were

isoenergetic, given that the lipids have a higher energy value for more than two-fold, the

quantity of corn starch added to the diets to make them isoenergetic were higher than the

quantity of fish oil, thus serving as an additional explanation for lower water utilization in

the CHO group, and analogously, as a representation in the amount of MWP reducing the

animal requirements for fresh water intake.

For milk water intake, CON and FAT (14.47 and 14.17 L respectively) were not

statistically different *(P > 0.1;* Table 5), but a statistical difference was detected for the

CHO group (*P* < 0.0001; Table 5) who consumed 13.35 L of water through the

reconstituted milk replacer. These results are similar to the DMI of milk replacer, kg,

where the CON consumed 3.06 kg, the CHO consumed 2.82 kg, and the FAT consumed

3.01 kg. According to Allen et al. (2009), glucose and soluble carbohydrates are

ultimately oxidized in the hepatocytes (as propionate in developed ruminants and as

glucose in non-ruminants). Such oxidation of the nutrients in the hepatocytes are said to have a hypophagic response, and therefore, are expected to decrease the feed intake of animals; the same is true for proteins and fats (Allen et al., 2009). Nonetheless, a site-directed increase in the pool of glucose (e.g.: kidneys for young ruminants) or its precursors (i.e.: propionate for the adult ruminant) could be helpful mechanisms for achieving successful fresh water intake mitigation strategies.

A more tangible measure of water usage is fresh water intake, which was decreased by 12% with our supplementation regimes. The CON group consumed 3.73 L and was statistically different ($P < 0.0001$; Table 5) than the CHO and FAT groups with intakes of 3.27 and 3.36 L, respectively. The observed decrease in fresh water intake in addition to the water from feedstuffs is said to approximate the water requirements of cattle (NASEM, 2016). Though throughout the narrative found in NASEM, (2016), it is argued that metabolic water production is of little significance to ruminant animals; however, nursing calves without a fully functional rumen demonstrate that MWP can be of significance in reducing fresh water intake. Wickramasinghe et al. (2019) explain that when milk and water were offered ad-libitum, the fresh water intake could represent the voluntary water intake, and therefore, serve as a representation of the water requirements the animals. Data from our experiment offer an alternative yet important understanding of water requirements for nursing calves. Though MWP may be considered minimal in adult ruminant animals, not accounting for MWP in estimations of fresh water intake or total water intake could carry significant error at the rates of fresh water intake and total water intake observed in younger animals. From our data, we see potential contributions of up

to 30% for fresh water intake and almost 10% in total water intake in terms of water

balance effectively shown as a quantifiable moiety.

Water intake was further explored through BW adjustments to determine water

necessary for BW gain and water intake per BW and $BW^{0.75}$ among treatments. Overall,

statistically significant effects ($P < 0.0001$; Table 5) were detected for fresh water

intake/BW, fresh water intake/$BW^{0.75}$, milk water intake/ADG, milk water intake/BW,

milk water intake/$BW^{0.75}$, total water intake/BW, total water intake/ADG, and total water

intake/$BW^{0.75}$ with respective $\underline{P}$-values of (< 0.0001, < 0.0001, $= 0.001$, < 0.0001, $<$

0.0001, $= 0.007$, $= 0.001$, < 0.0001; Table 5) Least squares means for fresh water intake

adjusted by BW and $BW^{0.75}$ demonstrated the same behavior displaying statistical

differences for CON (fresh water intake/BW = 0.026; fresh water intake/$BW^{0.75}$ = 0.089)

compared to the CHO (fresh water intake/BW = 0.022; fresh water intake/$BW^{0.75}$ =

0.076) and FAT (fresh water intake/BW = 0.022; fresh water intake/$BW^{0.75}$ = 0.078) *(P <*

0.001; Table 5), but no difference between the CHO and FAT groups *(P > 0.1*; Table 5).

These results display an extremely important remark that reductions of fresh water intake

in nursing calves are possible through lipid and carbohydrate supplementation. Texeira et

al. (2006) investigated fresh water intake responses in goats subjected to feed restriction

and noted that animals that were not feed-restricted balanced, fresh water intake and

urinary outputs linearly, while the highest metabolic water production was observed

when animals were not restricted.

Milk water intake and total water intake adjusted by ADG showed statistically

differences between CON (milk water intake/ADG = 8.84; total water intake/ADG =

2.31; $P = 0.001$; Table 5) and FAT (milk water intake/ADG = 8.1423; total water intake/ADG = 1.97), but CHO (milk water intake/ADG = 8.32; total water intake/ADG = 2.12) was not different than the CON and FAT groups ($P > 0.01$; Table 5). The significant decrease in fresh water intake/ADG and total water intake/ADG for the FAT indicates an increased efficiency in water utilization for animals supplemented with lipids. Presumably, increasing dietary energy levels would increase the efficiency of water use per unit of BW produced, indicating that water efficiency increases as animals move into more intensified systems. Not only because less days are required for harvesting, but also, there is a metabolic regulation of water needs. For milk water intake adjusted by BW and $BW^{0.75}$, all treatments were statistically different ($P < 0.05$; Table 5). Lastly, for total water intake, BW and $BW^{0.75}$ adjustments resulted in statistical differences between the CON when compared to the CHO and FAT ($P < 0.001$; Table 5), but the CHO and FAT were not statistically different within themselves (P >0.1; Table 5).

Energy requirements and intake

Animals fed the CHO diet had the lowest energy requirements amongst all treatments (Table 6). The NRC. (2001) shows similar values for energy requirements of animals gaining 1.5 kg per day, all animals in our treatments had higher ADGs which could explain the differences observed with our values. Similarly, the difference observed for the energy intakes is explained through the computation of the increased energy values for supplemented soluble carbohydrates and fat, which in turn help explain the differences that were observed between our supplemented and CON groups.

Performance and efficiencies

No statistically significant differences were detected for BW, total body weight gain, ADG, hot carcass weight, or cold carcass weight (P >0.1; Table 7). Berends et al. (2018) reported similar results in which no significant effects were found regarding BW or FC even though differences were observed on DMI and metabolizable energy intake. With regards to carcass composition, studies have reported increased levels of fat deposition in young calves in response to fat and protein supplementation, which could highlight potential carcass improvement in animals supplemented with soluble carbohydrates and lipids (Tikofsky et al., 2001; Bascom et al., 2007; Hill et al., 2008). The overall efficiency of water use evaluated as RTWI showed no statistical differences (P =0.9024; Table 7), but animals in the CHO group were the only group presenting negative values. Given that the variation in residual intakes was higher than the estimated values, the standard error yielded effects that were not significant. Nonetheless, the only group that appeared to be efficient RTWI was the CHO group (the only group with negative residual values) which would signify that the animal utilized less water to meet its requirements. However, these results should be examined and interpreted carefully, it is important to notice that water efficiency has, until now, not been analyzed in this fashion for Holstein nursing bull calves. Additional experiments are necessary to validate the use of these efficiency indexes when evaluating metabolic water production. Though extremely useful, these models may sometimes over-simplify interactions due to the utilization of mean/median body weights from the experiment for the residual calculations. Future research should further include other metrics and dynamic

interactions in the generation of efficiency metrics. Development of methods and efficiency indexes that additionally allow for the inclusion of water efficiency in addition to feed efficiency will become crucial in regions where water is limiting, such as in the western US rangelands, the Texas panhandle, amongst others.

Regarding RFI, significant differences were noted between the CON group and the supplemented groups (P =0.006; Table 7), where the CON = 0.16 was significantly higher than the CHO = -0.07, and the FAT = -0.07 groups. Negative values of RFI were detected for the CHO and FAT treatments. This suggests that Holstein nursing bull calves supplemented at isoenergetic rates with corn starch and fish oils could potentially be more efficient than animals not supplemented with energy sources on top of milk replacer. Two interesting trends ($P < 0.1$; Table 7) were observed for FC and FCR. For FCR, we noticed that CON = 1.94, CHO = 1.83, and FAT = 1.78 (P = 0.065; Table 7). Our results corroborate with those from Carstens and Tedeschi (2006), who previously described the interaction between RFI and FCR. They should be highly phenotypically correlated, that is, that the animals with low RFI should too have lower FCR values. The FCR values would represent the actual DMI per unit weight of gain, thus reinforcing our hypothesis that supplementation made animals more efficient while not affecting performance.

Conversely, FC values, which may also be termed gross feed efficiency, were slightly higher for the supplemented groups (CON = 0.52, CHO = 0.55, and FAT = 0.56) (Carstens and Tedeschi, 2006). No other significant differences were detected for RGR, or KI, which aligns with the lack of variation in our animal final BW (Table 7) observed

at the end of the trial. When working with Holstein nursing bull calves, energy supplementation in soluble carbohydrates and lipids could help increase both feed and water efficiencies. Given that RFI has been utilized to drive genetic breeding programs, and some success has been seen in selecting for animals with lower RFIs (Carstens and Tedeschi, 2006). Additional studies should continue to evaluate animal water and feed efficiencies in response to different energy supplements, as well as signal the significance of determining the potential genetic merit and heritability of efficiency traits that prove helpful in sustainable systems pursuing feed and water efficiency.

Regarding growth evaluation through biometric measures, no significant differences were detected for biometric measurements of the animals. Biometric measures have proven effective in assessing the body composition of animals (De Paula et al., 2013; Fonseca et al., 2017, Fernandes et al., 2010). A time effect was observed through all of the measures (P <0.001; Table 8), which would be expected given that growth can be modeled and described as a linear allometric pattern, usually through the use of linear components that could help describe the linear/time effect observed in the data (Klingenberg, 1996; Klingenberg, 2016). The linear time effect observed in the growth of animals is extremely important in the assessment of performance, water intake, water footprint, and animal efficiency. Through evaluation of growth and body composition changes through time, we similarly map the change in energy requirements which are paralleled with increased feed and water intakes. Such interactions were most elegantly described in Menendez III et al. (2020); the authors provide a possible framework through systems dynamic methodology that could help explain this

interaction. In Menendez III et al. (2020), the physiological status and age of animals are included in prediction and their contributions to the model were addressed. A big contributor in their casual loop diagrams explaining dynamics of water utilization in livestock operations appeared to be growth and nutrition dynamics which directly influenced the water consumption of the simulated beef supply chain for Texas. Our animals were in the exponential phase of growth, and therefore, were extremely efficient in utilizing the nutrients available (regardless of supplementation); therefore, even when no significant differences were observed when evaluating performances, assessment of other growth stages in response to similar supplementation lines, as well as, evaluation of effects on animals with different frames is necessary.

Conclusion

Increased water and feed efficiencies are achievable through sustainable supplementation procedures. As resource availability becomes more restrictive, increased efficiency of animals and operations will be required. The results of this experiment are the first to show how supplementation of Holstein nursing bull calves through isoenergetic levels of lipid and soluble carbohydrates serve in water intake mitigation in pre-ruminant animals. Though the performance was purposely not altered amongst the experimental treatments, significant increases in feed efficiency were observed for the CHO and FAT groups. A significant increase in water efficiency (noted by a negative RTWI) was observed for the CHO group. Our results expand on the belief that only mineral supplementation affects water intake and its mitigation and further help demonstrate the potential water intake reduction through lipid and carbohydrate supplementation without adversely affecting performance. This represents the beginning

of developing a line of supplements tailored to increase feed and water efficiencies of veal facilities and other livestock operations governed by nursing animals. Overall, as water scarcity continues to increase, accurate assessment of water usage by livestock could benefit from the exploration of water mitigation strategies not only in the early stages of life but throughout different phases of an animal's lifecycle and stages of growth.

Literature Cited

Amado, L., H. Berends, L. N. Leal, J. Wilms, H. Van Laar, W. J. J. Gerrits, and J. Martín- Tereso. 2019. Effect of energy source in calf milk replacer on performance, digestibility, and gut permeability in rearing calves. J. Dairy Sci. 102:3994–4001. doi:10.3168/jds.2018-15847.

Anon. 2012. Twenty-Eighth FAO Regional Conference for Europe: Policies for Food Security in the region: challenges and perspectives - food outlook towards 2050.

Arthur, P. F., G. Renand, and D. Krauss. 2001. Genetic and phenotypic relationships among different measures of growth and feed efficiency in young Charolais bulls. Livest. Prod. Sci. 68:131-139. doi:10.1016/S0301-6226(00)00243-8.

Bascom, S. A., R. E. James, M. L. McGilliard, and M. Van Amburgh. 2007. Influence of dietary fat and protein on body composition of jersey bull calves. J. Dairy Sci. 90:5600–5609. doi:10.3168/jds.2007-0004.

Berends, H., M. Vidal, M. Terré, L. N. Leal, J. Martín-Tereso, and A. Bach. 2018. Effects of fat inclusion in starter feeds for dairy calves by mixing increasing levels of a high-fat extruded pellet with a conventional highly fermentable pellet. J. Dairy Sci. 101:10962–10972. doi:10.3168/jds.2018-15116.

Brody, S. 1945. Bioenergetics and growth with special references to the efficiency

   complex in domestic animals. New York (NY): Hafner Publishing Co., Inc.

Carstens, G.E. and L.O. Tedeschi. 2006. Defining feed efficiency in beef cattle.

   Proceedings of 38th Beef Improvement Federation Annual Research Symposium,

   pp 12-21.

Church, D. C. 1988. The Ruminant Animal: Digestive Physiology and Nutrition. 2nd rev.

   ed. Prentice Hall, Inc., Englewood Cliffs, NJ.

Doreau, M., M. S. Corson, and S. G. Wiedemann. 2012. Water use by livestock: A global

   perspective for a regional issue? Animal Frontiers 2: 9-16.

   http://dx.doi.org/10.2527/af.2012-0036.

Federation of Animal Science Societies (FASS). 2010. Guide for the care and use of

   agricultural animals in research and teaching. Available from:

   https://www.aaalac.org/about/Ag_Guide_3rd_ed.pdf.

Fernandes, H. J., L. O. Tedeschi, M. F. Paulino, and L. M. Paiva. 2010. Determination of

   carcass and body fat compositions of grazing crossbred bulls using body

   measurements. J. Anim. Sci. 88:1442-1453. doi:10.2527/jas.2009-1919.

Fraley, S. E., M. B. Hall, and T. D. Nennich. 2015. Effect of variable water intake as

   mediated by dietary potassium carbonate supplementation on rumen dynamics in

   lactating dairy cows. J. Dairy Sci. 98:3247–3256. doi:10.3168/jds.2014-8557.

Grice, K. D., K. M. Glosson, and J. K. Drackley. 2020. Effects of feeding frequency and

   protein source in milk replacer for Holstein calves. J. Dairy Sci. 103:10048–

   10059. doi:10.3168/jds.2020-19041.

Hill, S. R., K. F. Knowlton, K. M. Daniels, R. E. James, R. E. Pearson, A. V. Capuco,

and R. M.

Akers. 2008. Effects of milk replacer composition on growth, body composition, and nutrient excretion in preweaned Holstein heifers. J. Dairy Sci. 91:3145–3155. doi:10.3168/jds.2007-0860.

Hoekstra, A. Y. 2012. The hidden water resource use behind meat and dairy. Anim. Front. 2:3-8. doi:10.2527/af.2012-0038.

Hu, W., T. M. Hill, T. S. Dennis, F. X. Suarez-Mena, J. D. Quigley, and R. L. Schlotterbeck. 2019. Intake, nutrient digestibility, and growth performance of Holstein dairy calves consuming a milk replacer at moderate or high feeding rates. J. Dairy Sci. 102:7917:7926. doi:10.3168/jds.2019-16282.

King, J. M. 1983. Livestock water needs in pastoral Africa in relation to climate and forage. ILCA Research Report No. 7, Addis Ababa, Ethiopia.

Klingenberg, C. P. 1996. Multivariate Allometry. In: Marcus L.F., Corti M., Loy A., Naylor G.J.P., and Slice D.E., eds, Advances in Morphometrics. NATO ASI Series (Series A: Life Sciences), vol 284. Springer, Boston, MA. https://doi.org/10.1007/978-1-4757-9083-2_3.

Klingenberg, C. P. 2016. Size, shape, and form: concepts of allometry in geometric morphometrics. Dev. Genes Evol. 226:113-137. doi:10.1007/s00427-016-0539-2.

Kutner, M. H., C. J. Nachtsheim, J. Neter, and W. Li. 2004. Applied linear regression. 5th ed.Irwin, Chicago, IL.

LPM-WIFSS. 2016. Livestock Production Manual – Western Institute for Food Safety & Security at the University of California – Davis and the Food and Drug Administration. Cooperative Agreement (1U54FD004327).

Mekonnen, M. M., and A. Hoekstra. 2010. The green, blue and grey water footprint of farm animals and animal products. Value of Water Research Report Series No. 48. Institute for Water Education, Delft, The Netherlands. Available from: www.waterfootprint.org/Reports/Report-48-WaterFootprint-AnimalProducts-Vol1.pdf.

Menendez III, H. M, Atzori, A. S, Tedeshi, L. O. 2020. The conceptualization and preliminary evaluation of a dynamic, mechanistic mathematical model to assess the water footprint of beef cattle production. BioRxiv. 'In press.' doi: https://doi.org/10.1101/2020.04.14.028324

Morrison, S. D. 1953. A method for the calculation of metabolic water. J. Physiol. 122:399-402. doi:10.1113/jphysiol.1953.sp005009.

Murphy, M. R. 1992. Water Metabolism of Dairy Cattle. J. Dairy Sci. 75:326–333. doi:10.3168/jds.S0022-0302(92)77768-6.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Palhares, J. C. P., M. Morelli, and C. C. Junior. 2017. Impact of roughage-concentrate ratio on the water footprints of beef feedlots. Agric. Syst. 155:126–135. doi:10.1016/j.agsy.2017.04.009.

Quigley, J. D., T. M. Hill, L. E. Hulbert, T. S. Dennis, X. F. Suarez-Mena, and E. M. Bortoluzzi. 2019. Effects of fatty acids and calf starter form on intake, growth, digestion, and selected blood metabolites in male calves from 0 to 4 months of age. J. Dairy Sci. 102:8074–8091. doi:10.3168/jds.2019-16688.

Quigley, J. D., T. A. Wolfe, and T. H. Elsasser. 2006. Effects of additional milk replacer

feeding on calf health, growth, and selected blood metabolites in calves. J. Dairy Sci. 89:207-216. doi:10.3168/jds.S0022-0302(06)72085-9.

Rice, E. W., R. B. Baird, and A. D. Eaton. 2017. Standard methods for the examination of water and wastewater. 23nd ed. American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF), Washington, D.C.

Roy, J. 1980. The Calf. 4th. ed. Butterworth Publishing Incorporated, London, UK.

Sainz, R. D., and P. V Paulino. 2004. Residual Feed Inake. Proc. UC Davis: Sierra Foothill Research and Extension Center Field Day. Available from: http://repositories.cdlib.org/anrrec/sfrec/2004_residual_feed_intake.

Santos, F. H. R., M. R. De Paula, D. Lezier, J. T. Silva, G. Santos, and C. M. M. Bittar. 2014.

Essential oils for dairy calves: Effects on performance, scours, rumen fermentation and intestinal fauna. Animal. 9: 958-965. doi:10.1017/S175173111500018X.

Schäff, C. T., J. Gruse, J. Maciej, M. Mielenz, E. Wirthgen, A. Hoeflich, M. Schmicke, R. Pfuhl,

P. Jawor, T. Stefaniak, and H. M. Hammon. 2016. Effects of feeding milk replacer ad libitum or in restricted amounts for the first five weeks of life on the growth, metabolic adaptation, and immune status of newborn calves. PLoS One. 11: e0168974. doi: 10.1371/journal.pone.0168974.

Senevirathne, N. D., J. L. Anderson, and M. Rovai. 2018. Growth performance and health of dairy calves given water treated with a reverse osmosis system compared with municipal city water. J. Dairy Sci. 101:8890-8901.

doi:10.3168/jds.2018-14800.

Teixeira, I. A. M. A., J. M. P. Filho, P. J. Murray, K. T. Resende, A. C. D. Ferreira, and F. L. Fregadolli. 2006. Water balance in goats subjected to feed restriction. Small Rumin. Res. 63:20–27. doi:10.1016/j.smallrumres.2005.01.008.

Tikofsky, J. N., M. E. Van Amburgh, and D. A. Ross. 2001. Effect of varying carbohydrate and fat content of milk replacer on body composition of Holstein bull calves. J. Anim. Sci. 79:2260–2267. doi:10.2527/2001.7992260x.

USDA. 2013. Veal from Farm to Table. United States Department of Agriculture Food Safety and Inspection Service. Available from: https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/meat-preparation/veal-from-farm-to-table/ct_index. Accessed January 25, 2021.

U.S. Geological Survey (USGS). 2006. Quality of Nevada's Aquifers and their susceptibility to contamination, 1990-2004. Scientific Investigations Report 2006-5127. Carson City, NV. Available from: https://pubs.usgs.gov/sir/2006/5127/PDF/SIR2006_5127.pdf.

Wickramasinghe, H. 2019. Effects of Drinking Water on Feed Intake, Growth Performance, Health Status, Nutrient Digestibility and Composition of Gut Microbiota in Young Dairy Calves [thesis]. Iowa State University. Available from: https://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=8616&context=etd.

Wickramasinghe, H. K. J. P., A. J. Kramer, and J. A. D. R. N. Appuhamy. 2019. Drinking water intake of newborn dairy calves and its effects on feed intake,

growth performance, health status, and nutrient digestibility. J. Dairy Sci. 102:377-387. doi:10.3168/jds.2018-15579.

Xiccato, G., A. Trocino, P.I. Queaque, A. Sartori, and A. Carazzolo. 2002. Rearing veal calves with respect to animal welfare: Effects of group housing and solid feed supplementation on growth performance and meat quality. Livest. Prod. Sci. 75:269–280. doi:10.1016/S0301-6226(01)00319-0.

Table 1. Experimental diets for Holstein nursing bull calves fed non-medicated milk replacer only (CON; n=7), non-medicated milk replacer supplemented with 3% menhaden fish oil (FAT; n=8), or non-medicated milk replacer isoenergetically supplemented with corn starch (CHO; n=8)

Item	Treatments [2,3,4]		
	CON	CHO	FAT
	g/l		
Milk Replacer	173.9	173.9	173.9
Fish oil	-	-	5.2
Starch	-	11.8	-
Dried Brewer's spent grain, g [1]	111.1	109.1	104.7
	g/kg		
Dry matter	965.9	966.0	965.9
Organic matter	901.1	901.3	901.0
Crude protein	210.7	210.6	210.7
NDFap2	11.9	12.6	11.4
Acid detergent fiber	6.2	6.5	6.0
Acid detergent lignin	3.5	3.5	3.5
Ether extract	152.0	151.8	181.0
Non fibrous carbohydrates	512.2	576.9	512.6
	Energy available in feed Mcal/kg		
Metabolizable energy	4.7	5.0	5.0
Net energy for maintenance	2.7	2.8	2.8
Net energy for gain	3.5	3.7	3.7

[1] Dried brewer's grain mixture composed of a mixture of dried brewer's grains (Ichytysaurus IPA, Wildhorse German Amber Red Ale, 39 N, Tectonic Event, Great Basin Brewing- Reno, Nevada; Pilsner, Pigeon Head Brewing -Reno, Nevada; Honey Ale, 10 Torr -Reno, Nevada) was offered at a rate of 115.6 grams per day DM basis for to all treatments.

[2] Experimental diets consisted of milk replacer alone for CON, milk replacer supplemented with 3% fish oil for FAT, and milk replacer supplemented with corn starch for CHO to be isoenergetic with FAT.

[3] Commercial mineral mix was also offered ad libitum with a composition (g/kg) of Sodium min. 377.6, Sodium max. 389.4; (ppm) Manganese min. 2400, Iron min. 2400, Copper min. 260, Copper max. 380, Zinc min. 320, Iodine min. 70, and Cobalt min. 40.

[4] Sodium in the form of sodium chloride; manganese as manganous oxide; iron as ferrous carbonate, magnesium as magnesium oxide, copper as copper oxide, zinc as zinc oxide, calcium as calcium iodate, cobalt as cobalt carbonate, and red iron oxide for color

Table 2. Chemical composition of water offered ad libitum to Holstein nursing bull calves fed non-medicated milk replacer only (CON; n=7), non-medicated milk replacer supplemented with 3% menhaden fish oil (FAT; n=8), or non-medicated milk replacer isoenergetically supplemented with corn starch (CHO; n=8)

Water Composition[1]	Collection	Upper tolerable limit Problem Value for Cattle
pH	7.3	< 5.5 or > 8.5
		ppm
Nitrate as Nitrogen	1.7	23
Nitrate	7.4	100
Total Dissolved Solids	441.0	3000
Chloride	86.0	300
Sulfates	44.6	500
Calcium	48.9	150
Phosphorus	<0.1	0.7
Magnesium	20.3	100
Potassium	15.5	20
Sodium	67.5	300
Iron	<0.05	0.4 (taste)
Manganese	<0.05	0.05 (taste)
Zinc	<0.01	25
Copper	<0.01	0.6
Calcium carbonate hardness	205	-
		Colonies per 100 ml
Total Coliform	<1	15
E.coli	<1	10

[1] Water samples collected early morning, preserved in ice and immediately shipped for livestock suitability and total coliform analysis to Cumberland Valley Analytical Services, New York

Table 3. Feed intake of Holstein nursing bull calves fed non-medicated milk replacer only (CON; n=7), non-medicated milk replacer supplemented with 3% menhaden fish oil (FAT; n=8), or non-medicated milk replacer isoenergetically supplemented with corn starch (CHO; n=8)

Item[1]	Treatments			SEM[2]	P-value[2]
	CON	CHO	FAT		Treatment
Dry matter intake					
DMImr, kg	3.06	2.82	3.01	0.14	0.475
DMImr/BW, kg/kg	0.02	0.02	0.02	0.00	0.475
DMImr/ADG, kg/kg	1.88	1.76	1.73	0.04	0.080
DMIbg, kg	0.11	0.11	0.10	0.00	0.644
MRI, kg	17.61	16.23	17.30	0.81	0.475
MRI/BW, kg/kg	0.12	0.11	0.12	0.01	0.475
MRI/ADG, kg/kg	10.79	10.14	9.93	0.25	0.080
DMI, kg	4.05	3.79	3.94	0.15	0.490
DMI/BW, kg/kg	0.03	0.03	0.03	0.00	0.490
DMI/ADG, kg/kg	2.48[a]	2.38[ab]	2.27[b]	0.05	0.033
Nutrient Intake, kg/day					
DMI	3.16	2.92	3.1	0.14	0.476
OM	2.78	2.57	2.73	0.13	0.475
CP	0.65	0.6	0.64	0.03	0.475
EE	0.48[b]	0.44[b]	0.56[a]	0.02	0.007
NFC	1.61	1.67	1.58	0.08	0.671
TDN	2.9	2.8	3.1	0.16	0.217
ADF, g/day	10.2	9.9	10	0.20	0.703
NDFap, g/day	11.5	12.1	11.3	0.66	0.641
NDFi, g/day	0.4	0.4	0.4	0.03	0.614

[1] DMI= dry matter intake; DMImr = milk replacer DMI; DMImr/BW = DMImr relative to body weight, DMImr/ADG = DMImr relative to the average daily gain (ADG); DMIbg = DMI of brewers' grains; MRI = non-medicated milk replacer intake; MRI/BW = MRI relative to BW, MRI/ADG = MRI relative to ADG; DMI/BW = DMI relative to BW; DMI/ADG = DMI relative to the ADG; OM = organic matter, CP = crude protein, EE = crude fat, NFC = non-fibrous carbohydrate, TDN = total digestible nutrients, ADF = acid detergent fiber; NDF_{ap} = neutral detergent fiber assayed with heat stable amylase and expressed exclusive of residual ash and residual CP; NDFi= indigestible neutral detergent fiber; ME_i= metabolizable energy intake; NEim = net energy for maintenance intake; NE_{ig} = net energy for gain intake.
[2] Standard error of the mean.
[3] P-value, <0.1 = trend; <0.05 = significant.

Table 4. Apparent nutrient digestibility coefficients and digestible nutrient intake of Holstein nursing bull calves fed non-medicated milk replacer only (CON; n=7), non-medicated milk replacer supplemented with 3% menhaden fish oil (FAT; n=8), or non-medicated milk replacer with corn starch (CHO; n=8)

Item[1]	Treatments			SEM[2]	P-value[3]
	CON	CHO	FAT		Treatment
Nutrient Digestibility, g/g					
DMD	0.95	0.94	0.95	0.004	0.446
OMD	0.96	0.95	0.96	0.004	0.203
CPD	0.94[a]	0.91[b]	0.93[a]	0.006	0.022
EED	0.95[ab]	0.94[b]	0.96[a]	0.005	0.038
NDFapD	0.63	0.57	0.56	0.063	0.732
NFCD	0.98	0.98	0.98	0.002	0.495
ADFD	0.70	0.62	0.69	0.047	0.465
TDND	1.02[c]	1.07[b]	1.08[a]	0.004	<0.001
Digestible Nutrient Intake, kg/day					
dDM	2.99	2.75	2.93	0.14	0.449
dOM	2.66	2.44	2.62	0.12	0.433
dCP	0.61	0.55	0.60	0.03	0.338
dEE	0.45[b]	0.41[b]	0.54[a]	0.02	0.005
dNFC	0.72	0.74	0.72	0.04	0.874
dNDFap, g/day	0.59	0.62	0.50	0.09	0.660
dADF, g/day	0.01	0.01	0.01	0.01	0.642

[1] DMD = dry matter digestibility; OMD = organic matter digestibility; CPD = crude protein digestibility; EE= ether extract digestibility; NDFapD = digestibility of the neutral detergent fiber assayed with heat stable amylase and expressed exclusive of residual ash and residual crude protein, NFCD = non-fibrous carbohydrates digestibility, ADFD = acid detergent fiber digestibility, TDND = total digestible nutrients digestibility, dDM = digestible dry matter intake, dOM = digestible organic matter intake, dCP = digestible crude protein intake, dEE = digestible ether extract intake, dNFC = digestible non-fibrous carbohydrate intake, dNDFap = digestible neutral detergent fiber assayed with heat stable amylase and expressed exclusive of residual ash and residual, dADF = digestible acid detergent fiber intake
[2] Standard error of the mean.
[3] P-value, <0.1 = trend; <0.05 = significant.
[abc] Means within row without common superscript differ (P ≤ 0.05).

Table 5. Water intake of Holstein nursing bull calves fed non-medicated milk replacer only (CON; n=7), non-medicated milk replacer supplemented with 3% menhaden fish oil (FAT; n=8), or non-medicated milk replacer with corn starch (CHO; n=8)

Item[1]	Treatments			SEM[2]	P-value[3]
	CON	CHO	FAT		Treatment
Water Measure, liters					
TWI	17.61[a]	16.99[b]	17.51[ab]	0.19	<0.0001
MWI	14.47[a]	13.35[b]	14.17[a]	0.14	<0.0001
FWI	3.73[a]	3.27[b]	3.36[ab]	0.10	<0.0001
MWP	1.57[b]	1.68[a]	1.64[a]	0.01	<0.0001
Adjusted by Bodyweights, liters/kg					
FWI/ADG	2.34	2.08	1.94	0.33	0.712
ADG/FWI	0.45	0.61	0.57	0.09	0.678
FWI/BWg	0.04	0.03	0.03	0.01	0.712
FWI/BW	0.03[a]	0.02[b]	0.22[b]	0.00	<0.0001
FWI/BW$^{0.75}$, kg/kg$^{0.75}$	0.09[a]	0.08[b]	0.08[b]	0.00	<0.0001
MWI/ADG	8.84[a]	8.32[ab]	8.14[b]	0.21	0.001
ADG/MWI	0.11	0.12	0.12	0.00	0.083
MWI/BW	0.1[a]	0.09[b]	0.10[c]	0.00	<0.0001
MWI/ BW$^{0.75}$, kg/kg$^{0.75}$	0.34[a]	0.33[b]	0.31[c]	0.00	<0.0001
TWI/BWg	0.17	0.16	0.15	0.01	0.677
TWI/BW	0.12[a]	0.12[b]	0.12[b]	0.00	0.007
TWI/ADG	2.31[a]	2.12[ab]	1.97[b]	0.09	0.001
TWI/ BW$^{0.75}$, kg/kg$^{0.75}$	0.42[a]	0.40[b]	0.41[b]	0.01	<0.0001

[1] TWI = total water intake; MWI = milk water intake; FWI = fresh water intake; MWP = metabolic water production (0.669*Carbohydrate$_{intake}$ + 0.41*Protein$_{intake}$ + 0.532 *Lipid$_{intake}$); FWI/ADG = FWI relative to average daily gain (ADG), ADG/FWI = ADG relative to FWI, FWI/BWg = FWI relative to body weight gain (BWg), FWI/BW = FWI relative to BW, FWI/ BW$^{0.75}$= FWI relative to metabolic body weight (BW$^{0.75}$), MWI/ADG = MWI relative to ADG, ADG/MWI = ADG relative to MWI, MWI/BW MWI relative to BW, MWI/BW$^{0.75}$ = MWI relative to BW$^{0.75}$, TWI/BWg = TWI relative to BWg, TWI/BW TWI relative to BW, TWI/ADG = TWI relative to ADG, TWI/BW$^{0.75}$ = TWI relative to BW$^{0.75}$

[2] Standard error of the mean

[3] P-value, <0.1 = trend; <0.05 = significant.

[abc] Means within row without common superscript differ (P ≤ 0.05).

Table 6. Energy requirements and energy intake of Holstein nursing bull calves fed non-medicated milk replacer only (CON; n=7), non-medicated milk replacer supplemented with 3% menhaden fish oil (FAT; n=8), or non-medicated milk replacer with corn starch (CHO; n=8)

| Item[1] | Treatments | | | SEM[2] | *P-value[3]* |
	CON	CHO	FAT		Treatment
Energy nutrient intake, Mcal/day					
DEi	15.57[b]	15.10[b]	16.08[a]	0.018	<0.0001
MEi	14.92[a]	13.73[b]	14.65[a]	0.144	<0.0001
NEim	8.51[a]	7.83[b]	8.51[a]	0.121	<0.0001
NEig	11.19[a]	10.30[b]	10.99[a]	0.108	<0.0001
Animal Requirements, Mcal/day					
DE, Mcal/day	13.60[b]	13.35[b]	14.43[a]	0.175	<0.0001
ME, Mcal/day	13.06[b]	12.81[b]	13.85[a]	0.162	<0.0001
NEm, Mcal/day	3.64[a]	3.63[a]	3.69[b]	0.041	<0.0001
NEg, Mcal/day	6.09[b]	5.93[b]	6.60[a]	0.061	<0.0001

[1] DE_i = digestible energy intake; ME_i = metabolizable energy intake; NE_{im} = net energy for maintenance intake, NE_{ig} =net energy for gain intake, DE = digestible energy, ME = metabolizable energy, NE_m = net energy for maintenance, NE_g = net energy for gain.
[2] Standard error of the mean.
[3] *P*-value, <0.1 = trend; <0.05 = significant.
[ab] Means within row without common superscript differ ($P \leq 0.05$).

Table 7. Performance and relative efficiencies of Holstein nursing bull calves fed non-medicated milk replacer only (CON; n=7), non-medicated milk replacer supplemented with 3% menhaden fish oil (FAT; n=8), or non-medicated milk replacer with corn starch (CHO; n=8)

Item[1]	Treatments			SEM[2]	*P-value*[3]
	CON	CHO	FAT		Treatment
Weight Measures					
BW, kg	146.71	146.25	150.48	4.47	0.949
TBWg, kg	108	105.75	114.56	4.75	0.414
ADG, kg/day	1.64	1.60	1.74	0.07	0.408
HCW, kg	116.77	119.35	124.74	5.45	0.597
CCW, kg	113.33	116.01	121.39	5.36	0.581
Efficiency Indexes					
RTWI, kg/day	0.25	-0.30	0.08	0.842	0.902
RFI, kg/day	0.16[a]	-0.07[b]	-0.07[b]	0.05	0.006
FCR	1.94	1.83	1.78	0.04	0.065
FC	0.52	0.55	0.56	0.01	0.066
KI, kg/kg$^{0.75}$	0.04	0.04	0.04	0.00	0.253
RGR, %	0.47	0.47	0.45	0.01	0.243

[1] BW = body weight; TBWg = total BW gain; ADG = average daily gain; HCW = hot carcass weight; CCW = cold carcass weight; RTWI = residual total water intake; RFI = residual feed intake; FCR = feed conversion rate; FC = feed conversion; KI = Kleiber index, RGR = residual growth rate
[2] Standard error of the mean.
[3] *P*-value, <0.1 = trend; <0.05 = significant.
[ab] Means within row without common superscript differ ($P \leq 0.05$).

Table 8. Mean biometric measures of Holstein nursing bull calves fed non-medicated milk replacer only (CON; n=7), non-medicated milk replacer supplemented with 3% menhaden fish oil (FAT; n=8), or non-medicated milk replacer with corn starch (CHO; n=8)

Item[1]	Treatment			SEM[2]	P-value[3]			
	CON	CHO	FAT		CON vs.E	CHO vs. FAT	Time	Trt*Time
BW, kg	146.71	146.25	150.48	6.358	0.841	0.643	<0.001	0.3332
Biometric Measures, cm								
TW	33.40	33.46	35.04	0.777	0.406	0.165	<0.001	0.575
AW	27.19	27.65	28.40	0.690	0.359	0.451	<0.001	0.263
HBW	24.57	24.81	25.15	0.624	0.616	0.710	<0.001	0.165
PBW	9.62	10.02	10.02	0.418	0.462	1.000	<0.001	0.331
PGL	32.55	33.00	33.52	0.621	0.403	0.577	<0.001	0.617
BL	48.21	47.85	47.54	1.063	0.638	0.978	<0.001	0.541
Sc	22.57	22.67	22.90	0.464	0.728	0.731	<0.001	0.843
RuDe	40.90	39.75	41.25	0.882	0.724	0.243	<0.001	0.986
RiDe	45.05	44.79	45.06	0.915	0.919	0.836	<0.001	0.245
RuHe	105.02	103.23	103.21	1.210	0.258	0.990	<0.001	0.388
HaW	101.83	100.10	100.63	1.200	0.351	0.762	<0.001	0.666
Diag	77.38	77.25	77.58	0.953	0.977	0.807	<0.001	0.995

[1] BW = body weight, TW = thorax width, AW = abdomen width, HBW = hook bone width, PBW = pin bone width, PGL = pelvic girdle length, BL = body length, Sc = scapula, RuDe = rump depth, RiDe = rib depth, RuHe = rump height, HaW = height at withers, Diag = body diagonal length

[2] Standard error of the mean.

[3] *P*-value, <0.1 = trend; <0.05 = significant.

Chapter II

Effects of Lipid and Starch Isoenergetic Supplementation as Mitigation Techniques on Water Footprint and Health of Nursing Holstein Calves[I]

Abstract

Continued concern for researchers and livestock producers has been to fulfill

production needs sustainably. As these concerns continue to grow, water footprint (WF)

for livestock productions will become a critical concern. Water footprint for dairy

operations is typically centered around milk production; however, a potential significant

decrease is possibly by quantifying and reducing the WF of Holstein nursing bull calves

(HBC). Feedstuffs utilized for production are generally large contributors to WF

calculations. The unique physiological and anatomical status of HBC presents the unique

possibility for diet manipulation that may permit for voluntary water intake decrease;

however, decreasing voluntary water intake raises concerns about the potential adverse

effects on hydration, behavior, and health. The goals herein involve investigating the

effects of two highly digestible isoenergetic supplements on a non-medicated milk

replacer (MR) diet on the health, hydration, behavior, and water footprint of HBC. A total

of 23 HBC weighing 94.67 ± 12.07 kg, two mo. old (post-adaptation), were distributed in

a completely randomized design and received one of three diet supplements for 67 days:

control (CON; n=7), carbohydrate (CHO; n=8), and lipid (FAT; n=8); on top of a MR-

based diet offered ad libitum. The CON was offered MR alone, whereas the FAT was

supplemented with fish oil (3 %), and the CHO was composed of corn starch (matched

isoenergetically with the FAT). All animals were offered mineral mix and water ad

libitum, and 120 g daily dried brewer's spent grains. Data were analyzed with the

GLMMIX procedure of SAS 9.4 in a completely randomized design with the diets as a

fixed effect. Neutrophil count (NC), lymphocyte count (LC), and their ratio (NLR) all

showed statistically significant effects ($P < 0.05$) with the lowest NLR for the FAT

group. Total protein (TP) showed statistically significant differences, but the ranges still were within parameters to consider that animals were healthy (5-6 g/dl). A similar effect was noticed on the fecal fluidity score (FFS), though the values were too within normal ranges for calves. Skin hydration as an assessment of animal hydration was achieved using a skin moisture meter which resulted in the CHO group having a skin moisture value of 5.30, which was significantly higher than CON = 3.76, and FAT =3.99. Blue water footprint (BWF), green water footprint (GWF), grey water footprint (GrWF), and total water footprint (TWF) were measured and resulted in similar results across all three variables ($P < 0.01$); the CHO group had a significantly lower values for all WF measures values than the other treatments, however, when WF values were adjusted by cold carcass weight (CCW) of the animals, both the CHO and FAT groups displayed lower water footprints than the CON group. These results evidence the possibility of increasing animal and water efficiency with precision diet formulation by utilizing isoenergetic supplementation of soluble carbohydrates and lipids without adverse health effects.

Keywords: Holstein calf health, isoenergetic supplementation, water footprint,

Introduction

The pursuit of animal efficiency and selective breeding programs has evolved during the last century. Koch et al. (1963) developed a trademarked simple yet effective assessment of animal efficiency that has influenced selective breeding and animal efficiency studies for decades. The implementation of residual feed intake for increased efficiency and improved sustainability of operations gave light to a new field of research tailored to investigate the interaction between feed and water efficiency. Given that water resources will continue to grow in scarcity, the viability of the livestock industry will

highly depend on not only the feed but the potential water efficiency of livestock animals. Reduction of the water footprint of livestock operations is an essential task that researchers, producers, and policymakers must endeavor in. Mekonnen and Hoekstra (2010) highlighted the significance of the effects of a growing population on livestock producers. They mentioned that as global populations continue to increase, the demand for animal protein will increase, therefore increasing pressure on freshwater resources. As resource depletion continues to limit resources available for livestock operations, alternative methods that have lower environmental footprints will be essential in the near future.

Though the precision diet formulations for many production ruminants are largely governed by foregut fermentation, younger ruminant animals have the potential to withstand supplementation that would be detrimental to adult animals. A significant component of dairy operations comes through the handling of bull calves. Holstein nursing bull calves (**HBC**) provide an excellent opportunity for precision diet formulation using highly digestible and energy-dense feeds, which would likely not be suitable for adult ruminants. Lipid supplementation for ruminants is carefully monitored to prevent potential detrimental effects on rumen microbial populations, lower intestinal absorption at higher fat intakes, nutrient imbalance, amongst others (Palmquist, 1994). Khan et al. (2016) describes the transition from milk to solid feed of dairy heifers and highlights how they are born with a physically and metabolically underdeveloped rumen, thus relying on milk to meet nutrient requirements. The young ruminant animal can be morphologically classified in three phases, the liquid-feeding phase, the transition phase, and the ruminant phase which represent the periods where the animal meets its needs with milk or milk

replacer alone, milk or milk replacer in addition to a starter, and solid feeds for meeting nutrient requirements respectively; as such, young ruminant animals best meets their requirements through high-quality highly-digestible feedstuffs with carbohydrates, proteins, and lipids; such is the case due to the (NRC, 2001). Therefore, supplementation of higher levels of energy supplement as lipids and soluble carbohydrates could reasonably serve as means to improve animal efficiency of young ruminants. The distinction between adult and young ruminant animals is also extended to water intake and absorption. The National Academy of Sciences, Engineering, and Medicine (2016) describe the minor contribution of metabolic water to ruminant animals. However, the values are not quantified for younger ruminant animals.

Given that specific compartments of the rumen, mainly the omasum, of adult ruminant animals serve in water absorption, young ruminant animals with under-developed rumen would need to absorb water through other regions gastrointestinal and urinary tracts. Smith (2009) detailed how increased glucose levels assist in higher water absorption rates in the intestine when the glucose is too absorbed for diarrheic calves; however, unabsorbed glucose reaching the large intestine can increase the amount of VFAs produced, ultimately increasing the severity of the animal dehydration. Such peculiarity was identified for diarrheic calves. However, the osmotic reasoning behind the increased water absorption when higher amounts of glucose are available in the small intestine raises the question of how high energy feeds could potentially increase water absorption in animals without a fully functional rumen (since most of all glucose sources would be utilized by microbes in a functional rumen). We successfully showed that animal water intake could be reduced without altering animal performance

(Unpublished). However, the effects on total water footprint, hydration, and possible adverse health effects have not been investigated thoroughly. The primary objective of this work involves the investigation of isoenergetic supplementation on health, hydration, and water footprint, with secondary objectives of determining the possible correlation of a moisture meter with other animal hydration parameters.

We hypothesize that the animals supplemented FAT will display a water better hydration status and that animals that have been supplemented FAT would have their health parameters improved when compared to the other two treatments; with regards to hydration, we hypothesize that the CHO group will show better values when factoring the water footprint for each treatment.

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, diets and facilities

Twenty-three HBC acquired from a commercial Dairy Farm located in Northern NV were intensively raised in a closed and controlled environment. Power computations using the mean variances and expected mean differences among treatments for similar products and metabolic traits from several earlier published studies indicate that our number of animals is justified to detect significant differences at a level of significance of $P < 0.05$ and levels of 10% difference against the control (Schäff et al., 2016; Senevirathne et al., 2018; Wickramasinghe et al., 2019). The error variance (error mean

square for measurement of interest) of 0.02 would be able to detect differences of 0.29 (e.g., 0.29 kg/d for water intake).

The animals were raised from birth to day 135 and then harvested. The animals were adapted to Dairy barn facilities at the Nevada Agricultural Experimental Station for the first eight weeks of life and received experimental diets for the following 12 weeks. Calf selection was meticulous. They were weighed at birth and monitored for expected behavior, including their ability to stand and nurse within the first two hours. They had their umbilicus treated with an iodine solution (10%).

Further, only singlet male calves from non-dystocic parturitions were selected. The calves ingested five percent of their body weight as colostrum. Animals averaged four ± 3 d of age. Upon arrival, animal body weights (**BW**) were recorded with a 43.5 ± 3 kg of BW. The clinical veterinarian evaluated all animals to ensure they had all arrived healthy at the facilities. Housing constituted of individual 32 ft^2 galvanized steel pens (Seneca Dairy Systems, LLC; Seneca Falls, NY) located inside a barn equipped with heaters, fans, and a swamp cooler for temperature (**TEMP**) and relative humidity (**RH**) regulation; animals were randomly allocated throughout the barn to minimize potential confounders; pens were individually labeled by animal and treatment numbers. Temperature humidity indexes (**THI**) were evaluated twice daily to ensure animals remained within their thermoneutral zone throughout the experimental period. Temperature, RH, wind speed (**WS**), wind direction (**WD**), solar radiation (**SR**) were recorded daily using an 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 Temp/RH Sensor (12-bit; P# S-THB-M002), and

a light sensor level (P# M-LLA) all collecting data points every 30 seconds for generation

of different THI indexes. Temperature humidity indexes were calculated following five

different recommendations for estimation of thermal stress according to NRC (1971),

Yousef (1985), Thom (1959) and NOAA (1976), and Berman et al. (2016). The equations

utilized for the assessment of THI are presented in Table 1.

During adaptation, the pens were bedded with wood shavings and replaced twice

daily to ensure animal welfare. After adaptation, bedding was replaced by rubber stall

mats. Animals were randomly distributed by assignment of random number to each

animal followed by random sorting for assigning to the experimental diets, which

consisted of a control (**CON**), n = 7, which were fed with a commercial non-medicated

milk replacer (**MR**) composed of 20% crude protein, 20% crude fat, 0.15% crude fiber; a

lipid supplemented group (**FAT**), n=8, which consisted of MR + 3% inclusion of fish oil,

and a carbohydrate group (**CHO**), n = 8, which consisted of MR + starch equivalent to

maintaining isoenergetic level as provided by the FAT treatment; additionally, 120 grams

of microbreweries spent grains (**BG**) were offered daily. The supplementation for FAT

and CON consisted of a 3% supplementation of fish oil (fish oil, 5.2 grams per liter of

milk replacer), matched isoenergetically with corn starch (100% PURE CORN STARCH

HODGSON MILL, Effingham Illinois 62401, 11.8 grams per liter of milk replacer).

Feeding occurred twice daily at 6h00 and 16h00 when animals received MR ad libitum

and were supplemented with mineral mix ad-libitum (NaCl 96%, manganese 2,400 ppm,

iron 2,400 ppm, copper 260 ppm, zinc 70 ppm, cobalt 40 ppm). The milk replacer was

reconstituted with warm water (65 $^{\circ}$C) and cool to 40° C before feeding. Milk replacer

and dietary ingredients were mixed on a MILK BAR^{TM} Cart coupled with a stainless steel

whip mixer (MBMk125D and MB126A models, respectively, McInnes Manufacturing Ltd., Waipu, New Zealand). Corn starch and fish oil were individually weighed and thoroughly mixed in separate containers; calves were fed ad-libitum in stainless steel buckets (5 gal.). Orts were collected twice daily and feeding adjustments were performed every two days to ensure up to 10% as fed volume refusal.

Dry matter (**DM**) and dry matter intake (**DMI**) was computed as the individual feedstuffs before reconstitution (milk replacer + spent grains + supplements). Samples of MR, BG, and supplements, as well as orts were collected daily, properly identified, and stored in a freezer at -20 $^{\circ}$C for posterior chemical analysis.

Water Analysis

A single well for groundwater source was utilized for the animals throughout the experimental period. The water source was sampled for analysis by opening the cold outlet letting it run for three minutes; afterward, the screen and aerator were removed and two samples of water (100 mL and 500 mL) were collected into sealed sterile bottles (100 mL contained sodium thiosulfate for *E. coli* bacterial evaluation and total coliform). Samples were sealed correctly and shipped in a cooler with expedited shipping for same day analysis at Cumberland Valley Analytical Services (CVAS, Waynesboro, PA). The total coliform and *E. coli* were evaluated according to method # 2340, the pH from method # 4500-H, nitrate method #4500-NO3^{-}, total dissolved solids method #2540, sulfates method #4500-so42; the following minerals: calcium, phosphorus, magnesium, potassium, sodium, iron, manganese, zinc, copper with method #3500, and carbonate hardness method # 2340 (Rice et al., 2017).

Health and hydration

Animals were monitored twice daily before feeding. Animals were evaluated with regards to fecal fluidity score (**FFS**) according to a scale from 0-4, similar to Larson et al. (1977). Further, respiratory score (**RS**) assessed through observation of nasal discharge, cough or pulmonary auscultation, lethargy (**Le**) scores, were similarly measured as described by Schaefer et al. (2004) and Cortese et al. (1998). In the event of abnormal recordings for FFS, RS, or Le, and during weekly collections, additional health measures were collected and were also utilized to assess hydration. Skin pliability score (**SP**) was measured as the time taken for the skin to return to its initial, non-tented position after tenting the skin at the middle portion of the neck, rotating it at 90°, holding it for 1 s, and releasing. Approximately one inch of skin was pinched for one second. Enophthalmia score (**En**) was measured by a technician trained by the clinical veterinarian. Laser corneal/lens temperature (**LTE**) and rectal temperature (**RTE**) were also collected using a laser temperature gun and through a digital thermometer, respectively. Table 2 describes with regards to the meaning and significance of the health measures.

Assessment of hydration was performed through weekly collections of SP, En, and through the novel use of a moisture reader (**Mo**) (MoistureMeterSC-2, Delfin Technologies Ltd, Kuopio, Finland). Animal moisture was first examined dorsally 2 inches from the nasal cavity and at the cross point between the horns and the eyes. Two days before skin moisture assessment, calves were shaved in the area and thoroughly cleaned. On the day of the collection, after checking for dryness and cleanliness, Mo was collected by holding the moisture meter perpendicular to the skin and holding it with constant pressure for 3 seconds until the reading was done. The skin moisture meter represents epidermal capacitance which is interpreted as the percent of water content in

superficial skin (Palma et al., 2012). Additionally, urine specific gravity (**UG**) was measured with a refractometer to determine its osmolarity compared to water. Similarly, the total protein of the serum (**TP**) was measured with a refractometer and utilized in the assessment of hydration levels.

Spot urine samples were collected 4 hours before and after feeding during spontaneous urination on days 32 and 64 of the experimental period. Samples from each collection were proportionally sampled and filtered through cheesecloth layers, 10 mL aliquots were diluted into 40 mL of H2SO4 (0.036 N) and an additional sample was stored concentrated form. All urine samples were frozen at -20°C for further analysis. Water loss from urine (**WLU**) was evaluated through estimation of urine production through the use of creatinine as a biological marker. The creatinine excretion was estimated according to values from Chizzotti et al. (2008), as well as Costa e Silva et al. (2012), and Lascano and Heinrichs (2011). The ratio of the expected creatinine daily excretion and the measured creatinine in urine were utilized to estimate urinary volume. Creatinine, allantoin, and uric acid were analyzed by high-performance liquid chromatography (HPLC) with an Agilent 1100 liquid chromatograph equipped with a diode array detector (DAD) and a visible lamp; an autosampler with a heated column compartment was utilized with all urine samples run in duplicate with a mobile phase run and three standards run every ten samples to evaluate column performance throughout the analysis. Peak separation was accomplished under isocratic conditions with a 5 um Spherisorb ODS II C$_{18}$ reverse-phase column (300x4.6mm I.D.; Waters, Wilford, MA, USA) addition of a guard column with Spherisorb ODS2 guard cartridges, 80Å, 5 µm, 4.6 mm X 30 mm. The methodology followed was based on Shingfield and Offer (1999)

with modification of the addition of the guard column. Fecal samples were collected and water loss from feces (**WFS**) was calculated as the percent of total feces mass after total water removal from feces. Total water loss (**TWL**) was then calculated as the sum of WLU and WFS.

Weekly jugular venipunctures were performed on the calves. During collections, pen temperature and times were meticulously recorded for assessment of health and blood parameters. Animals were manually restrained and blood was collected into EDTA and red tube vacutainers (BD Vacutainer, Franklin Lakes, NJ). Blood smear slides were prepared with a rapid 3-step staining kit (Hemacolor® Rapid staining of a blood smear, Sigma-Aldrich, St. Louis, MO). They were utilized to determine the white blood cell differential counts and the neutrophil to lymphocyte ratio (**NLR**). For basophil count (**BC**), neutrophil count (**NC**), lymphocyte count (**LC**), eosinophil count (**EC**), and monocyte count (**MC**), three students trained by the clinical veterinarian analyzed the counts. Counts were averaged and the average of the values was utilized for the statistical analysis. Additionally, blood was analyzed for packed cell volume (**PCV**) 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 microhematocrit reader. The remainder of the blood was centrifuged and frozen at -20 °C for later laboratory analysis.

Behavior

The analysis of animal behavior was performed to ensure early detection of abnormal activities that would indicate the early onset of illnesses. The animals were

monitored using 16 Night Owl X Night Owl XHD502-88P-B 8 Channel 5MP Extreme

HD Video Security DVR & Wired Infrared Cameras with 2 TB HDD system. Each

camera had coverage of 3-4 pens and offered different angles for animal monitoring. A

total of 4 days of recording were gathered throughout the experimental period to assess

treatment effects on behavioral patterns. The parameters studied for behavior included the

time spent laying (**TSL**), the time spent standing (**TSS**), the time spent

ruminating/chewing (**TSR**), the time spent eating a mineral mix of brewer's grains

(**TSE**), and the time spent drinking water (**TSD**). Video analysis involved training of 3

laboratory technicians who would evaluate the behaviors and an aggregate of their

observations was combined to determine the ultimate animal behavior and the time spent

in activities. The four days of recordings were analyzed in 5-minute intervals as reported

by Martin and Bateson (1993) and Jensen and Larsen (2014) , continuous animal

observations were made for all 23 calves. We drew our observations on continuous video

recordings alone.

Water balance and footprint

The animals had access to clean ad libitum water. Individual water intakes were

collected every morning before feeding. Individual water troughs with float regulators

were installed inside all pens. The individual troughs were connected to custom-built 55-

gallon plastic tubs with three holes drilled, one adjusted with a hose at the bottom and

individual pumps ensuring water pressure was enough to fill the water troughs. The two

additional holes were drilled, one slightly above the hole for the hose was a translucent

plastic tube with plastic cylinder tape and raised to the top of the barrel. Water intake was

measured as the distance difference seen in this plastic tube. Barrels were calibrated

before and during the trial to ensure measure precision would correlate 100 % with known water volumes; the calibrations were performed by the same technician and consisted of graduated cylinder consecutive additions of water followed by recordings of distance change on the translucent tube. Calibrations were regressed and the sloped utilized to determine the adequate conversion from mm change to volume of water added. Water troughs were washed at least once daily (increased frequency on instances where water was too cloudy), and the water barrels were sanitized and disinfected once monthly.

For analysis of water footprint (**WF**), water was divided into different categories according to Mekonnen and Hoekstra (2010), who classified WF into three main components: blue (**BWF**), green (**GWF**), and grey water footprints (**GrWF**); where the green water is that used for drinking/production of crops from water coming from precipitation (without the inclusion of run-off), grey water refers to the freshwater required to assimilate the load of pollutants given natural background, and lastly blue water refers to blue and groundwater resources. The sum of these WF's constitutes the total water footprint (**TWF**). Water intakes have previously been reported in Macias-Franco et al. (Unpublished); however, the total amount of water, as WF, has not been explored. To assess the WF regardless of the facilities, no grey water footprint was tracked from sanitation. Green water footprint, BWF, and GrWF were estimated from the production of the spent grains and quantified through the intake of grain for the animals, as well as the GWF associated with the production of their MR and supplement intakes. Our BG (mixed from several micro-breweries) was composed of 86% barley, 10% rye, and 4% wheat. Amongst the major components of beer production, barley is commonly

the major component of beer. BWF was computed as the water utilized in the

reconstitution of the milk replacer for feeding in addition to the FWI. The total water

footprint (**TWF**) was calculated as the addition of the BWF, GWF, and GrWF. The

contribution of corn starch for the CHO was accounted for through the values reported in

Mekonnen and Hoekstra (2011), which were GWF = 1295, BWF = 111, and GrWF = 265

m^3/t. The fish oil contribution to the WF calculations for the FAT group was computed

according to Pahlow et al. (2015); the weighted average for fish and crustaceans was

estimated to be GWF = 1629, BWF = 179, GrWF = 166, and TWF = 1974 m^3/t

respectively. Lastly, values from Mekonnen and Hoekstra, (2010) were utilized for WF

quantification of the milk replacer (BWF = 282, GWF =2,065 , GrWF = 464 m^3/t). For

the WF for the BG, values from Mekonnen and Hoekstra (2011) were too adopted. The

summed contributions of the respective grains accounted for a total BWF = 446, GWF =

3,909, and GrWF = 437 m^3/t of water per ton of BG produced. Ultimately, the individual

WF measures were multiplied by the individual animal intakes and were added per

animal for analyses. The WF is further evaluated by examining how the values change by

reporting the ratio with cold carcass weight (**CCW**).

Statistical Methods

Statistical analyses were performed utilizing SAS University Edition (SAS inst.

Inc., Cary, NC) and R Statistical Software v.4.0.1 (R Core Team, 2021, Vienna, Austria).

Assessment of environmental contribution to the parameters evaluated was performed

through principal components analysis (**PCA**) of the correlation matrix (scaled data). For

data analysis, the GLIMMIX procedure of SAS was utilized as a completely randomized

design with the diet as the fixed effect. Mean comparisons were done through the

LSMEANS statement on SAS for least squared means and separated using F-protected t-tests with Tukey-Kramer adjustments to contrast between the means of the variables of interest. Further, the PROC MIXED with COVTEST and a REPEATED command were utilized with orthogonal linear, quadratic, and cubic contrasts to evaluate the treatment effects on health 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). Type I error was established at 5%; trend identification was established at values less than 10%.

Results and Discussion

This experiment was free of extraneous influence due to environmental stressors, overall, out of 135 days, the different assessments of according to NRC (1971), Yousef (1985), Thom (1959) and NOAA (1976). Berman et al. (2016) resulted in just a single day of possible severe heat stress, and on two days according to NRC (1971, THI2). The thermal stress results reported in days, hours, and minutes in mild, moderate, and severe stress are described in Table 3. Assessment of outliers in the data yielded 16 data points for FWI measurements, from a total of 1541. No other outliers were detected or deemed influential, and therefore those were the only data points removed.

Further, an assessment of the influence of environmental variables on the parameters studied was performed using PCA. Principal component eigenvectors for the different THIs were computed with the other parameters examined on this experiment to determine if the variation explained by the respective parameters was correlated. The analysis of the possible contribution of the thermal factors in the parameters evaluated is

reported in the appendix. The appendix (Figs. 1-9) shows the principal components

groupings for the environmental variables and the parameters studied. Overall, through

all of the PCAs performed, the THIs were always grouped into a single PCA-eigenvector,

therefore suggesting that the contributions towards the other parameters were not

significant when analyzed with intake variables (Figs. 1-3), with regards to health (Figs.

4-6), and with regards to blood (Figs. 7-9).

Health and hydration

The results from this experiment serve as evidence of the possible reduction of

water footprint without adverse effects on animal health for livestock operations. The

health of HBC can be assessed through the evaluation of NC, LC, and NLR. Von

Konigslow et al. (2019) reported that NC, LC, and NLR could all be utilized as health

assessment tools for nursing veal calves. They can further serve as good differentiators

between stress, inflammation, and temporary fear or excitement. The blood analysis

yielded NC values that were significantly lower for the FAT group ($P < 0.0001$; Table 4),

the NC values for CON were 34.04, CHO with values of 35.30, and FAT with a value of

29.30. The LC, too yielded, statistically different results ($P < 0.0001$; Table 4), where the

FAT = 63.24 was statistically different than the CON = 59.41, and the CHO = 57.69.

Further, the NLR resulted in values of 0.50, 0.64, and 0.61 for FAT, CHO, and CON

respectively ($P = 0.0029$; Table 4). McDonnell et al. (2019) found similar NC and LC

values during the pre-weaning phase, however, our CHO and CON groups displayed

higher levels of NC and our FAT showed higher LC. Neutrophil to lymphocyte ratio was

similar to the values reported by McDonnell et al. (2019). Higher NLR ratios have been

associated with the immune response to stressors by Swanson and Morrow-Tesch (2001).

Such values 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 group displayed a more desirable NLR value. Thus highlighting the potential benefit to calf health that was originally hypothesized for the FAT treatment. Von Konigslow et al. (2020) reported that elevated LC, even without a statistical difference, was associated with increased ADG, and decreased hazard of morbidity within the 21 days after arrival to the animal facility. This could potentially signal that, in time, the elevated leukocyte number observed for the FAT group could also represent a higher-functioning and more responsive immune system. Regarding MC, EC, and BC, no statistically significant differences were detected ($P > 0.1$; Table 4). Swanson and Morrow-Tesch, (2001) reported increased EC when their animals were introduced to stress; important to note is that his results occurred for younger animals and the source of stress was transportation. Similarly, the lack of increased detectable values in EC indicates that our animals had not been stressed given the dietary treatments provided. The blood parameters were further evaluated through time. Repeated measures analysis for the blood parameters is shown in Table 5. Similar to the least squared means comparisons with Tukey-Kramer discussed prior, statistical differences were observable in the NC, LC, and TP when analyzed through time as repeated measured orthogonal contrasts. Further, all parameters, except for TP, showed a time effect ($P < 0.05$; Table 5), and the LC showed an additional treatment and time interaction trend ($P = 0.066$; Table 5). Such associations and time effects could be explained as the change associated with increased immune function as animals grow. Overall, no adverse effects were detected on leukocyte differential counts,

and the possibility of enhanced immune function on our FAT group could explain the results we observed.

No statistically significant differences were detectable for PTE, LTE, RTE, and RS. Though the animals did not show alarming levels of FFS, the FFS was considered statistically different ($P < 0.0001$; Table 6) with values of 0.3714 for CON, 0.1500 for CHO, and 0.088 for FAT. Similar effects were seen on Bascom et al. (2007), but no significant effects due to increased fat or carbohydrates were observed in their animals, though they had some higher fecal scores signaling looser feces; however, their animals were Jersey bull calves, and they were significantly younger (around six weeks of age). Such results, yet again, reinforce the idea that increasing energy density in the form of lipids and soluble carbohydrates in precision formulated diets do voluntarily decrease water intake have no detectable adverse health effects on HBC.

Related to hydration, no significant differences were observed for PCV. On the other hand, for TP the CON group had a value of 5.57 and was considered statistically different than the other two groups (P < 0.0001; Table 4), the CHO with the value of 5.91, and the FAT with value of 5.88 were not considered statistically different (P > 0.1; Table 4). Increased TP values have been associated with animal dehydration (Marcato et al., 2018). Interestingly, ranges above 6.1 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 in the first weeks the farm (Rea et al., 1996). Though the values are higher for the CHO and FAT groups, no additional assessments of dehydration correlate these results, and given that these results lay within the healthy ranges for HBC, then we can assume that this statistical difference did not

mean the CHO and FAT animals were in a dehydrated state. No significant differences were detected for the UG among the treatments, ($P = 0.526$; Table 4), with CON = 1.018, CHO = 1.019, and FAT = 1.020. According to guidelines mentioned in Peek and Divers (2018), UG values for lactating dairy cows and milk-fed calves normally range in values from (1.004 – 1.015), and dehydration should be considered if values ranged higher than 1.025. According to these measures, no animals in our experiment experienced dehydration. Thus signaling that the isoenergetic supplementation for water intake mitigation had no adverse effects on hydration.

Interestingly enough, Mo displayed a statistical difference ($P < 0.0001$; Table 6). The CON group had a Mo value of 3.76, the CHO of 5.30, and the FAT of 3.99. Given that these values are a direct assessment of skin moisture, several possible explanations exist. First, higher water absorption rates could serve as a representation of more increased skin hydration. Such would occur for animals in the CHO group since higher glucose levels would be expected for animals supplemented with soluble carbohydrates, such hypertonicity, paired with high rates of glucose absorption, would too yield a necessary water absorption (Smith, 2009). As additional support to this claim, Figure 1 shows the distribution, scatterplot, and correlation for Mo with health parameters; a highly significant negative correlation (-0.383; Figure 1) was observed between the Mo measures and the PCV. The observed negative correlation could potentially represent the validity of Mo as an assessment of hydration in HBC.

Additionally, though not examined in this study, if the fat cover were less in the CHO carcass, then water would be eliminated at higher rates due to less insulation; however, if this were true, we would see the animals having to drink more water and the

opposite was true for the CHO group (Unpublished). Though indexes are not yet

available for livestock species, dermal studies can serve as additional health and

hydration biomarkers for livestock producers, as this has been successfully done in

humans. They are extremely non-invasive, and if indexes were available, easy to assess

by non-experienced technicians. Palma et al. (2015) showed that in humans, altered

hydration levels could be detected with the use of the Delfin 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 (though they mention some of the

biomechanics are not fully understood). Such work highlights the potential benefits that

livestock producers could benefit from with future research. Kells et al. (2020) recent

publication highlight the significance of dehydration detection techniques for welfare and

health assessment in HBC; in their work, they mention that dehydration is often cited

among the reasons for calf death. The development of practical, easy-to-use tools for

dehydration detection for livestock producers could assist in determining at-risk calves if

skin hydration indexes were created for livestock species.

Behavior

The analysis of behavior closely resembled the results observed in our health and

blood parameter evaluations. No statistically significant differences were observed for

TSD, TSE, TSL, TSR, TSS ($P < 0.0001$; Table 7). However, it is interesting to note that

the TSE showed a post-hoc Tukey-Kramer grouping effect. It found no difference

between the CHO and CON groups (with mean values of 5 and 4.54 respectively), nor

within the FAT and CON with the FAT having a mean value of 3.97. McDonnell et al.

(2019) similarly reported that fish oil supplemented calves in his trial spent less time in

the concentrate feeder compared to the animals not supplemented. This effect could be

interpreted as the animals in the FAT treatment having higher satiety or feed

aversion/lack of appetite than the other groups, thus not calling them to seek outfeed from

the starter. Allen et al. (2009) reported that satiety in animals is reduced through fatty

acid oxidation in hepatocytes. Other authors have previously investigated the parameters

we utilized in the analysis of calf welfare and health. Hill et al. (2016) utilized data

loggers to determine standing time patterns in animals, Calvo-Lorenzo et al. (2017)

further utilized data loggers to determine time spent standing and lying on the right/left

side post castration. The behavioral assessments from this experiment could represent

significant reductions in the environmental footprint of productions, given that animals

had no noticeable differences in performance, the reduction in feed intake observed in the

FAT and CHO group helps corroborate the idea that isoenergetic supplementation does

not affect health or hydration adversely, and can ultimately decrease WF.

Water balance and footprint

Water balance and footprint are important factors to consider when an increased

policy for sustainable practices haunts agricultural producers. In our study, there was no

significant difference in the WLF, nor any of the three computations of WLU, and for

TWL ($P > 0.1$; Table 8). An interesting remark is that computation of water loss in urine

was made from three different equations. Costa e Silva et al. (2012; WLU_1, TWL_1)

proposed the computation of urinary creatinine excretion for Nellore bulls; Chizzotti et

al. (2008; WLU_2, TWL_2), proposed this similar computation for Holstein heifers around

250kg of BW; lastly, Lascano and Heinrichs (2011; WLU_3, TWL_3) presented a prediction

that was too made in Holstein heifers. Though the animals utilized on this trial were

average around 100 kilograms less than those from Chizzotti et al. (2008), and Lascano

and Heinrichs (2011), the urinary excretion rates were comparable to their results.

Though the results were not statistically different, it is important to highlight that the

estimation of creatinine excretion for nursing male calves should be re-evaluated to

ensure that these computations are valid.

Water footprint evaluation resulted in significant differences for BWF, GWF,

GrWF, and TWF. For BWF, a significant reduction was observed for the CHO group

with daily mean values of 842.08 L. compared to CON and FAT with 889.50 and 889.77

L/day ($P < 0.0001$; Table 8). respectively. Similar results are observed for GWF, GrWF,

and TWF, with the CHO group, always having lower WF ($P = 0.0049$, 0.0087, and

0.0041 respectively; Table 8), and the CON and FAT groups not being statistically

different. Research from our lab previously reported decreased intake and water intake

for animals in the CHO group (Macias-Franco et al., in review 2021). Similar to the

observed decrease in feed and water intakes, the water footprint reductions could be best

explained by Allen et al. (2009) on the hepatic oxidation theory. Regardless of the stage

of ruminant development present in our HBC, supplementation of fish oil and corn starch

would ultimately yield additional substrates that are oxidized by hepatocytes. Membrane

polarization of hepatocytes is thought to signal satiety in animals, and therefore, an

expected decrease in intake would be expected (Allen et al., 2009). Though the pursuit of

WF calculations has helped raise awareness on the amount of water utilized for

anthropogenic activities, the values reported are unrealistically high and controversial.

For instance, one could argue that the high WF values observed for the BG should not be

reported for our animals, given that this was a by-product re-utilized for the feeding of

animals. Removal of the footprint of the BG, would yield overall TWF values that were lower by more than 200 L for all treatments.

A potential alternative to reporting WF values could be to adjust the intakes by the carcass produced. Adjustment of our footprint values as the ratio of WF and cold carcass weight (CCW). When reported as the ratio with CCW, no significant differences were observed amongst the treatments ($P > 0.5$; Table 8). Though not statistically different, values of TWF were decreased by more than 100 L. for the CHO and FAT groups (CON = 2354.33, CHO = 2244.99, FAT = 2200.49 L/kg$_{CCW}$; Table 8). These results could help demonstrate that the utilization of isoenergetic supplementation tailored to decrease voluntary water intake of HBC could help reduce WF substantially. In our experiment, nearly 10% of the estimated WF came from the BG consumed. Given that grain consumption in our experiment was offered at a rate of 120 grams per day, when compared to other experiments and current production scenarios, a possible reduction could come in the replacement of solid feedstuffs for HBC raised for meat. For instance, Wickramasinghe et al. (2019) reported lower water intake values than the ones we observed (their animals were younger); however, the starter intake content was significantly higher than ours; therefore, the water footprint calculation in response to the high starter intake would ensue in their experiment reporting a higher water footprint for the solid intake in their animals. Our experiment displayed potential water reductions of up to 200 L per animal with replacement of WF values of BG intake. The production of HBC raised for meat could be optimized through the replacement of solid feed without adversely affecting animal performance and or health.

Conclusion

Our results display the beneficial reductions on water footprint observed in response to CHO and FAT supplementation on HBC, in addition to the absence of adverse health effects observed in this trial warrants the utilization of these supplements. Our CHO group had the lowest WF and was significantly lower than the CON and FAT groups; further, when adjusted by CCW, though not statistically different, there was still a reduction of more than 100 L. per animal for the CHO and FAT compared to the CON group. Given that increased pressure in the use of natural resources, it is expected to reduce ecological footprints by the livestock industry, and this work presents a potential improvement in the reduction of WF for all livestock species. Additional work is required in the pursuit of a new isoenergetic line of supplements aimed to evaluate the WF reductions observed in this experiment on different life stages. As water scarcity continues to grow, our results enlighten the potential decrease in WF for HBC by replacing starter feeds with milk replacers entirely. As hypothesized, the higher rates of metabolic water production expected on animals in the CHO group displayed higher hydration skin levels, though further investigation is warranted to investigate if tissue deposition could have altered these values; as well as, development of indexes for skin moisture of young ruminants for hydration status should be validated. Lastly, the reported water balances call for models being fit for male nursing calves.

Literature Cited

Allen, M.S., B.J. Bradford, and M. Oba. 2009. Board-invited review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. J. Anim. Sci. 87:3317–3334. doi:10.2527/jas.2009-1779.

Bascom, S.A., R.E. James, M.L. McGilliard, and M. Van Amburgh. 2007. Influence of dietary fat and protein on body composition of jersey bull calves. J. Dairy Sci. 90:5600–5609. doi:10.3168/jds.2007-0004.

Berman, A., T. Horovitz, M. Kaim, and H. Gacitua. 2016. A comparison of THI indices leads to a sensible heat-based heat stress index for shaded cattle that aligns temperature and humidity stress. Int. J. Biometeorol. 60:1453-1462. doi:10.1007/s00484-016-1136-9.

Calvo-Lorenzo, M.S., L.E. Hulbert, M.A. Ballou, A.L. Fowler, Y. Luo, K.C. Klasing, and F.M. Mitloehner. 2017. Space allowance influences individually housed Holstein bull calf innate immune measures and standing behaviors after castration at 3 weeks of age. J. Dairy Sci. 100:2157–2169. doi:10.3168/jds.2016-11833.

Chizzotti, M. L., S. C. Valadares Filho, R. F. D. Valadares, F. H. M. Chizzotti, and L. O. Tedeschi. 2008. Determination of creatinine excretion and evaluation of spot urine sampling in Holstein cattle. Livest. Sci. 113:218–225. doi:10.1016/j.livsci.2007.03.013.

Costa e Silva, L. F., S. C. Valadaris Filho, M. L. Chizzotti, P. P. Rotta, L. F. Prados, R. F. D. Valadares, D. Zanetti, J. M. S. Braga. 2012. Creatinine excretion and relationship with body weight of Nellore cattle. R. Bras. Zootec. 41:807-810. https://doi.org/10.1590/S1516-35982012000300046.

Cortese, V. S., West, K. H., Hassard, L. E., Carman, S. and Ellis, J. A.. 1998. Clinical and immunological responses of vaccinated and unvaccinated calves to infection with a virulent type-II isolate of bovine viral diarrhea virus. J. Am. Vet. Med. Assoc. 213: 1312–1319.

Hill, T.M., J.D. Quigley, F.X. Suarez-Mena, H.G. Bateman, and R.L. Schlotterbeck. 2016. Effect of milk replacer feeding rate and functional fatty acids on dairy calf performance and digestion of nutrients. J. Dairy Sci. 99:6352–6361. doi:10.3168/jds.2015-10812.

Jensen, M.B., and L.E. Larsen. 2014. Effects of level of social contact on dairy calf behavior and health. J. Dairy Sci. 97:5035–5044. doi:10.3168/jds.2013-7311.

Kannan, N., E. Osei, O. Gallego, and A. Saleh. 2017. Estimation of green water footprint of animal feed for beef cattle production in Southern Great Plains. Water Resour. Ind. 17:11–18. doi:10.1016/j.wri.2016.12.002.

Kells, N.J., N.J. Beausoleil, C.B. Johnson, J.P. Chambers, C. O'Connor, J. Webster, R. Laven, and N. Cogger. 2020. Indicators of dehydration in healthy 4- to 5-day-old dairy calves deprived of feed and water for 24 hours. J. Dairy Sci. 103:11820– 11832. doi:10.3168/jds.2020-18743.

Khan, M.A., A. Bach, D.M. Weary, and M.A.G. von Keyserlingk. 2016. Invited review: Transitioning from milk to solid feed in dairy heifers. J. Dairy Sci. 99:885–902. doi:10.3168/jds.2015-9975.

Koch, R.M., L.A. Swiger, D. Chambers, and K.E. Gregory. 1963. Efficiency of Feed Use in Beef Cattle. J. Anim. Sci.. doi:10.2527/jas1963.222486x.

von Konigslow, T.E., D.L. Renaud, T.F. Duffield, V. Higginson, and D.F. Kelton. 2019. Validation of an automated cell counter to determine leukocyte differential counts in neonatal Holstein calves. J. Dairy Sci. 102:7445–7452. doi:10.3168/jds.2019-16370.

von Konigslow, T.E., D.L. Renaud, T.F. Duffield, C.B. Winder, and D.F. Kelton. 2020. Assessing the utility of leukocyte differential cell counts for predicting morbidity, mortality, and growth in a grain-fed veal facility: A prospective single cohort study. J. Dairy Sci. 103:9332–9344. doi:10.3168/jds.2020-18532.

Kutner, M. H., C. J. Nachtsheim, J. Neter, and W. Li. 2004. Applied linear regression. 5th ed. Irwin, Chicago, IL.

Larson, L.L., F.G. Owen, J.L. Albright, R.D. Appleman, R.C. Lamb, and L.D. Muller. 1977. Guidelines Toward More Uniformity in Measuring and Reporting Calf Experimental Data. J. Dairy Sci. 60:989–991. doi:10.3168/jds.S0022-0302(77)83975-1.

Lynch, E. M., M. McGee, S. Doyle, and B. Earley. 2012. Effect of pre-weaning concentrate supplementation on peripheral distribution of leukocytes, functional activity of neutrophils, acute phase protein and behavioural responses of abruptly weaned and housed beef calves. BMC Vet. Res. 8:1-11. doi:10.1186/1746-6148-8-1.

Marcato, F., H. van den Brand, B. Kemp, and K. van Reenen. 2018. Evaluating potential biomarkers of health and performance in veal calves. Front. Vet. Sci. 5:1–18. doi:10.3389/fvets.2018.00133.

Martin, P., and P. Bateson. 1993. Measuring behaviour: an introductory guide. 3rd ed. Cambridge University Press, Cambridge, UK.

McDonnell, R.P., J. V. O' Doherty, B. Earley, A.M. Clarke, and D.A. Kenny. 2019. Effect of supplementation with n-3 polyunsaturated fatty acids and/or β-glucans on performance, feeding behaviour and immune status of Holstein Friesian bull calves during the pre- and post-weaning periods. J. Anim. Sci. Biotechnol. 10:1–17. doi:10.1186/s40104-019-0317-x.

Mekonnen, M. M., and A. . Hoekstra. 2010. The green, blue and grey water footprint of farm animals and animal products. Value of Water Research Report Series No. 48. Institute for Water Education, Delft, The Netherlands. Available from: www.waterfootprint.org/Reports/Report-48-WaterFootprint-AnimalProducts-Vol1.pdf.

Mekonnen, M.M., and A.Y. Hoekstra. 2011. The green, blue and grey water footprint of crops and derived crop products. Hydrol. Earth Syst. Sci. 15:1577–1600. doi:10.5194/hess-15-1577-2011.

Naylor, J. M., D. S. Kronfeld, S. Bech Nielsen, and R. C. Bartholomew. 1977. Plasma total protein measurement for prediction of disease and mortality in calves. J. Am. Vet. Med. Assoc. 171:635-638.

National Research Council. 1971. A guide to environmental research on animals. National Academy of Sciences, Washington, DC.

NOAA. 1976. Livestock hot weather stress. Operations Manual Letter C-31-76. NOAA, Kansas City, MO.

Lascano G. J.Heinrichs A. J. 2011. Effects of feeding different levels of dietary fiber through the addition of corn stover on nutrient utilization of dairy heifers precision-fed high and low concentrate diets. J. Dairy Sci.94:3025–3036. doi:10.3168/jds.2010-3831.

Pahlow, M., P.R. van Oel, M.M. Mekonnen, and A.Y. Hoekstra. 2015. Increasing pressure on freshwater resources due to terrestrial feed ingredients for aquaculture production. Sci. Total Environ. 536:847–857. doi:10.1016/j.scitotenv.2015.07.124.

Palma, L., L.T. Marques, J. Bujan, and L.M. Rodrigues. 2015. Dietary water affects human skin hydration and biomechanics. Clin. Cosmet. Investig. Dermatol. 8:413–421. doi:10.2147/CCID.S86822.

Palma, M.L., C. Monteiro, L. Tavares, M.J. Bujan, and L. Monteiro Rodrigues. 2012. Relationship between the dietary intake of water and skin hydration. J. Biomed. Biopharm. Res.. doi:10.19277/bbr.9.2.39.

Palmquist, D.L. 1994. Conference: regulating lipid metabolism to increase productive efficiency.. J. Nutr. 124:1371S. doi:10.1093/jn/124.suppl.

Peek, S. F., and T. J. Divers. 2018. Rebhun's diseases of dairy cattle: 3rd ed. Elsevier, St. Louis, MO.

Rea, D. E., J. W. Tyler, D. D. Hancock, T. E. Besser, L. Wilson, D. S. Krytenberg, and S. G. Sanders. 1996. Prediction of calf mortality by use of tests for passive transfer of colostral immunoglobulin. J. Am. Vet. Med. Assoc. 208:2047-2049.

Rice, E. W., R. B. Baird, and A. D. Eaton. 2017. Standard methods for the examination of water and wastewater. 23nd ed. American Public Health Association (APHA),

American Water Works Association (AWWA) and Water Environment Federation (WEF), Washington, D.C.

Schaefer, A.L., N. Cook, S. V. Tessaro, D. Deregt, G. Desroches, P.L. Dubeski, A.K.W. Tong, and D.L. Godson. 2004. Early detection and prediction of infection using infrared thermography. Can. J. Anim. Sci. 84:73–80. doi:10.4141/A02-104.

Shingfield, K.J., and N.W. Offer. 1999. Simultaneous determination of purine metabolites, creatinine and pseudouridine in ruminant urine by reversed-phase high-performance liquid chromatography. J. Chromatogr. B Biomed. Sci. Appl. 723:81–94. doi:10.1016/S0378-4347(98)00549-0.

Smith, G.W. 2009. Treatment of Calf Diarrhea: Oral Fluid Therapy. Vet. Clin. North Am. - Food Anim. Pract. 25:55–72. doi:10.1016/j.cvfa.2008.10.006.

Swanson, J.C., and J. Morrow-Tesch. 2001. Cattle transport: Historical, research, and future perspectives. J. Anim. Sci. 79:E102. doi:10.2527/jas2001.79e-supple102x.

Thom, E.C. 1959. The Discomfort Index. Weatherwise. doi:10.1080/00431672.1959.9926960.

Wickramasinghe, H.K.J.P., A.J. Kramer, and J.A.D.R.N. Appuhamy. 2019. Drinking water intake of newborn dairy calves and its effects on feed intake, growth performance, health status, and nutrient digestibility. J. Dairy Sci.. doi:10.3168/jds.2018-15579.

Yousef, M. K. 1985. Stress physiology in livestock. Basic principles. 1st ed. CRC Press, Boca Raton, FL.

Table 1. Equations utilized for calculation of temperature humidity indexes (THI) for determination of thermal stress of Holstein nursing bull calves fed non-medicated milk replacer only (CON; n=7), non-medicated milk replacer supplemented with 3% menhaden fish oil (FAT; n=8), or non-medicated milk replacer isoenergetically supplemented with corn starch (CHO; n=8)

Equation no.	Equation[1]	Author
[1]	THI = (1.8*Temp + 32) − [(0.55 − 0.0055 * RH) * (1.8 + Temp − 26.0]	NRC (1971)
[2]	THI = (0.55 * Temp + 0.2 * DPt) * 1.8 + 32 + 17.5	NRC (1971)
[3]	THI = Temp + (0.36 * DPt) + 41.2	Yousef (1985)
[4]	THI = (0.8 * Temp) + (RH ÷ 100) * (Temp − 14.4) + 46.4	Thom (1959); NOAA (1976)
[5]	THI = 3.43 + 1.058 * Temp - 0.293 * RH + 0.0164 * Temp * RH + 35.7	Berman et al. (2016)

[1] Different equations utilized to calculate temperature humidity index for the experimental period to determine thermal stress

Table 2. Descriptive assessment of clinical health scores used for Holstein nursing bull calves fed non-medicated milk replacer only (CON; n=7), non-medicated milk replacer supplemented with 3% menhaden fish oil (FAT; n=8), or non-medicated milk replacer isoenergetically supplemented with corn starch (CHO; n=8)

Clinical Sign[1]	Score
FFS	0 = normal fluidity
	1 = feces spread slightly, pasty manure (softer than normal)
	2 = moderate spread of feces, mild semi-liquid diarrhea)
	3 = watery, pure liquid feces (severe scours)
RS	0 = no symptoms
	1 = clear nasal discharge or slight cough
	2 = mucopurulent discharge or severe cough with subcrepitant lung sound
	3 = severe pneumonia
Le	0 = normal
	1 = mild depression, suckling but not vigorously
	2 = moderate depression, calf able to stand, suckling is weak or disorganized
	3 = severe depression, calf unable to stand or suckle
SP	2 = any values over 2 seconds reported
En	0 = normal eye position
	1 = slightly sunken eye with no separation between globe and orbit
	2 = separation of globe and orbit
	3 = severe enophthalmia with a 0.5-1.0 cm gap between eye and orbit
Mo	Evaluation of validity to establish thresholds, measured at cross point between horns and eye.
	Temperature recordings, °C
LTE	Evaluation of validity to establish thresholds
	Report temperatures above 103.5° F (39.7°C) or below 100o F (37.8° C) to
RTE	the project manager

[1] FFS = fecal fluidity score, RS = respiratory score, Le = lethargy score, SP = skin pliability, En = enophthalmia score, Mo = skin moisture assessment with moisture meter, LTE = corneal laser temperature, RTE = rectal temperature

Table 3. Hours of thermal stress recorded in different temperature humidity indexes (THI) for Holstein nursing bull calves fed non-medicated milk replacer only (CON; n=7), non-medicated milk replacer supplemented with 3% menhaden fish oil (FAT; n=8), or non-medicated milk replacer isoenergetically supplemented with corn starch (CHO; n=8)

Item[1]	N	Thermal stress[2]				Average % in stress[3]			
Days		Total	Mild	Moderate	Severe	Total	Mild	Moderate	Severe
THI1	67	7.0	5.00	0.0	1.0	10.45	7.46	0.00	1.49
THI2	67	31.0	29.00	0.0	2.0	46.27	43.28	0.00	2.99
THI3	67	2.000	0.0000	1.000	1.000	2.99	0.00	1.49	1.49
THI4	67	2.000	0.0000	1.000	1.000	2.99	0.00	1.49	1.49
THI5	67	2.000	0.0000	1.000	1.000	2.99	0.00	1.49	1.49
Hours									
THI1	1556	347	296	11	33	22.30	19.02	0.71	2.12
THI2	1556	741	675	17	43	47.62	43.38	1.09	2.76
THI3	1556	61	9	10	38	3.92	0.58	0.64	2.44
THI4	1556	52	6	26	17	3.34	0.39	1.67	1.09
THI5	1556	53	7	12	33	3.41	0.45	0.77	2.12
Minutes									
THI1	93290	20591	17636	535	2059	22.07	18.90	0.57	2.21
THI2	93290	44912	40905	1027	2519	48.14	43.85	1.10	2.70
THI3	93290	3622	3622	493	2343	3.88	3.88	0.53	2.51
THI4	93290	3119	277	1615	1046	3.34	0.30	1.73	1.12
THI5	93290	3138	296	754	2041	3.36	0.32	0.81	2.19

[1] $THI1 = (1.8*Temp+32) - [(0.55-0.0055*RH)*(1.8+Temp-26.0]$ (NRC, 1971), $THI2 = (0.55*Temp+0.2xDPt)*1.8+32+17.5$ (NRC, 1971), $THI3 = Temp+(0.36*DPt)+41.2$ (YOUSEF, 1985), $THI4 = (0.8*Temp)+(RH\div100)*(Temp-14.4)+46.4$ (MADER et al., 2006), $THI5 = 3.43+1.058*Temp-0.293*RH+0.0164*Temp*RH+35.7$ (BERMAN et al., 2016)

[2] Thermal stress refers to the THI threshold classified to produce thermal stress on animals; Total = anything greater than 72 THI, Mild ranging from 72 to 79 in THI, moderate from 80 to 89 THI, and severe anything higher than 90 THI

[3] Average time during stress

Table 4. Blood parameter analysis of Holstein nursing bull calves fed non-medicated milk replacer only (CON; n=7), non-medicated milk replacer supplemented with 3% menhaden fish oil (FAT; n=8), or non-medicated milk replacer isoenergetically supplemented with corn starch (CHO; n=8)

Item[1]	Treatments			SEM[2]	P-value[3]
	CON	CHO	FAT		Treatment
Differential counts					
NC	34.04[a]	35.30[a]	29.30[b]	0.9020	<0.0001
LC	59.41[b]	57.69[b]	63.24[a]	0.9114	<0.0001
MC	3.19	3.25	3.52	0.2154	0.5239
EC	1.86	2.31	1.88	0.2254	0.2859
BC	1.47	1.44	1.46	0.1576	0.9882
NLR	0.61[a]	0.64[a]	0.50[b]	0.03001	0.0029
PCV, %	35.70	34.67	35.20	0.8787	0.7263
TP, g/dl	5.57[b]	5.91[a]	5.88[a]	0.04622	<0.0001
UG, %	1.018	1.019	1.020	0.001051	0.526

[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
[2] Standard error of the mean
[3] P-values: significance <0.05, trend: <0.1
[ab] Means within row without common superscript differ (P<0.05)

Table 5. Blood parameters repeated measures analysis of Holstein nursing bull calves fed non-medicated milk replacer only (CON; n=7), non-medicated milk replacer supplemented with 3% menhaden fish oil (FAT; n=8), or non-medicated milk replacer isoenergetically supplemented with corn starch (CHO; n=8)

Item[1]	Treatment			SEM[2]	P-value[3]			
	CON	CHO	FAT		C vs.E	CHO vs. FAT	Time	Trt*Time
Differential counts								
NC	34.04	35.3	29.93	1.7962	0.5414	0.0471	0.0023	0.1509
LC	59.41	57.69	62.24	1.7172	0.638	0.033	<0.0001	0.066
MC	3.19	3.25	3.52	0.2146	0.479	0.387	<0.0001	0.216
EC	1.86	2.31	1.88	0.3248	0.571	0.359	<0.0001	0.173
BC	1.47	1.44	1.46	0.1446	0.909	0.904	<0.0001	0.939
NLR	0.61	0.64	0.50	0.0484	0.527	0.054	0.053	0.278
PCV,%	35.70	34.67	35.20	1.2214	0.630	0.764	<0.0001	0.392
TP,g/dl	5.57	5.91	5.88	0.0910	0.011	0.804	0.393	0.957

[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
[2] Standard error of the mean
[3] P-value, <0.1 = trend; <0.05 = significant

Table 6. Health analysis of Holstein nursing bull calves fed non-medicated milk replacer only (CON; n=7), non-medicated milk replacer supplemented with 3% menhaden fish oil (FAT; n=8), or non-medicated milk replacer isoenergetically supplemented with corn starch (CHO; n=8)

Item[1]	Treatments			SEM[2]	P-value[3]
	CON	CHO	FAT		Treatment
Observations, minutes					
Mo	3.76[b]	5.30[a]	3.99[b]	0.3795	0.0109
PTE	21.13	21.17	51.77	18.2624	0.4014
LTE	38.29	38.31	38.25	0.04413	0.5283
RTE	101.17	101.30	101.34	0.06629	0.1819
Observations measured on 1-5 scale					
RS	0.043	0.013	0.025	0.01789	0.5101
FFS	0.37[a]	0.15[b]	0.088[b]	0.05084	0.0005

[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
[2] Standard error of the mean
[3] P-values: significance <0.05, trend: <0.1
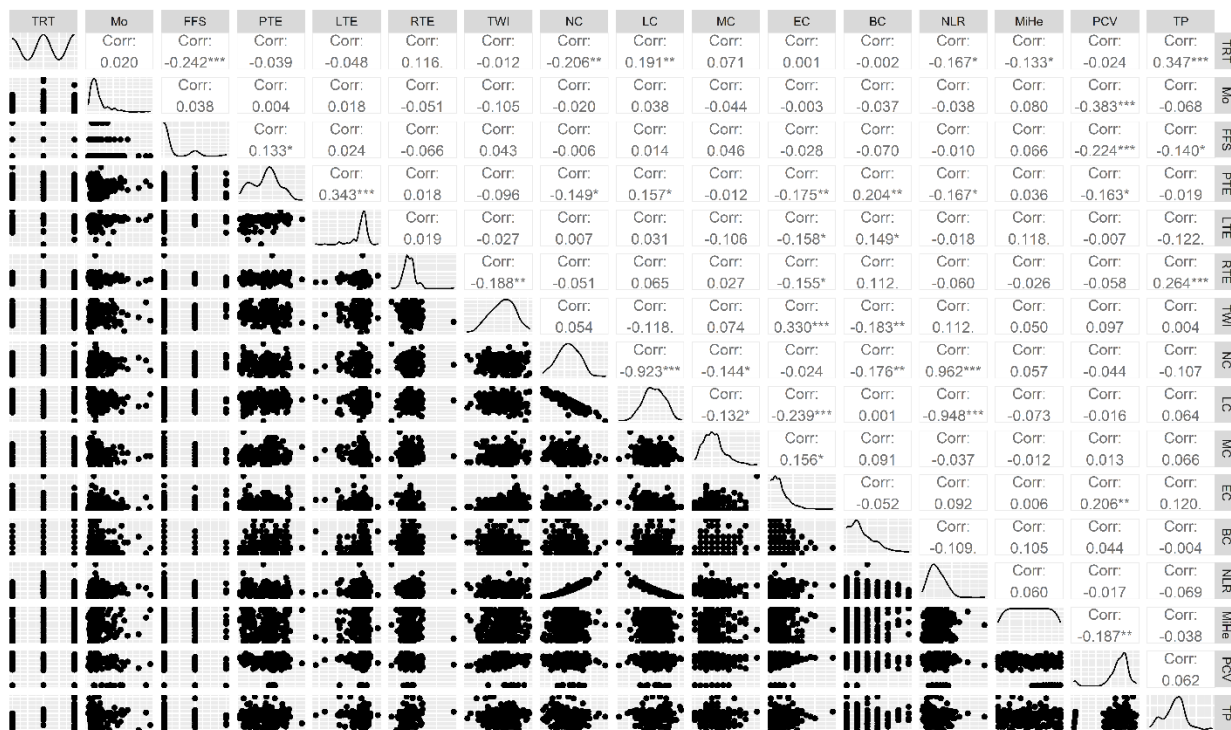[ab] Means within row without common superscript differ (P<0.05)

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

| Item[1] | Treatments | | | SEM[2] | P-value[3] |
	CON	CHO	FAT		Treatment
	Observations, minutes				
TSD	2.250	2.250	2.156	0.3621	0.9784
TSE	4.5357	5.000	3.9688	0.6971	0.3381
TSL	133.25	131.81	139.75	8.1421	0.7663
TSR	28.0714	27.8750	26.2813	5.5856	0.9418
TSS	53.75	54.9063	49.75	5.2960	0.5976

[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
[2] Standard error of the mean
[3] P-values: significance <0.05, trend: <0.1
[ab] Means within row without common superscript differ (P<0.05)

Table 8. Water loss and water footprint of Holstein nursing bull calves fed non-medicated milk replacer only (CON; n=7), non-medicated milk replacer supplemented with 3% menhaden fish oil (FAT; n=8), or non-medicated milk replacer isoenergetically supplemented with corn starch (CHO; n=8)

Item[1]	Treatments			SEM[2]	P-value[3]
	CON	CHO	FAT		
Water loss, L/day					
WLF	0.59	0.61	0.53	0.0470	0.4102
WLU_1	8.11	7.26	6.02	0.5793	0.2721
TWL_1	8.61	7.88	6.48	0.6144	0.2727
WLU_2	7.52	8.29	8.00	0.6969	0.8814
TWL_2	8.03	8.90	8.47	0.7101	0.8389
WLU_3	6.79	7.46	7.22	0.6377	0.8930
TWL_3	7.30	8.07	7.69	0.6517	0.8484
Water footprint daily values, L/day					
BWF	889.50[a]	842.08[b]	889.77[a]	9.0330	0.0001
GWF	6445.66[a]	6193.95[b]	6473.48[a]	93.5982	0.0049
GrWF	1433.85[a]	1372.51[b]	1422.91[a]	14.7285	0.0087
TWF	8769.01[a]	8408.55[b]	8786.16[a]	89.9432	0.0041
Water footprint daily values, L/kg$_{Cold\ Carcass\ Weight}$					
BWF	238.86	224.84	222.82	10.4039	0.5352
GWF	1730.52	1653.72	1621.37	76.2634	0.6155
GrWF	384.95	366.44	356.30	16.9352	0.5182
TWF	2354.33	2244.99	2200.49	103.60	0.5926

[1] WLF = water loss from feces, WLU_1 = water loss from urine (;), TWL_1 = total water loss (;), WLU_2 = water loss from urine (;), TWL_2 = total water loss (;), WLU3 = water loss from urine (;), TWL3 = total water loss (;), BWF = blue water footprint, GWF = green water footprint, GrWF = grey water footprint, TWF = total water footprint
[2] type III standard error of the mean
[3] P-values: significance <0.05, trend: <0.1
[ab] Means within row without common superscript differ (P<0.05)

Figures

Figure 1. Correlogram of parameters evaluated for health, hydration, and moisture of Holstein nursing bull calves fed non-medicated milk replacer only (CON; n=7), non-medicated milk replacer supplemented with 3% menhaden fish oil (FAT; n=8), or non-medicated milk replacer isoenergetically supplemented with corn starch (CHO; n=8) "***" represents $P < 0.001$, "**" represents $P < 0.01$, "*" represents $P < 0.05$, "." represents trends for $P<0.1$
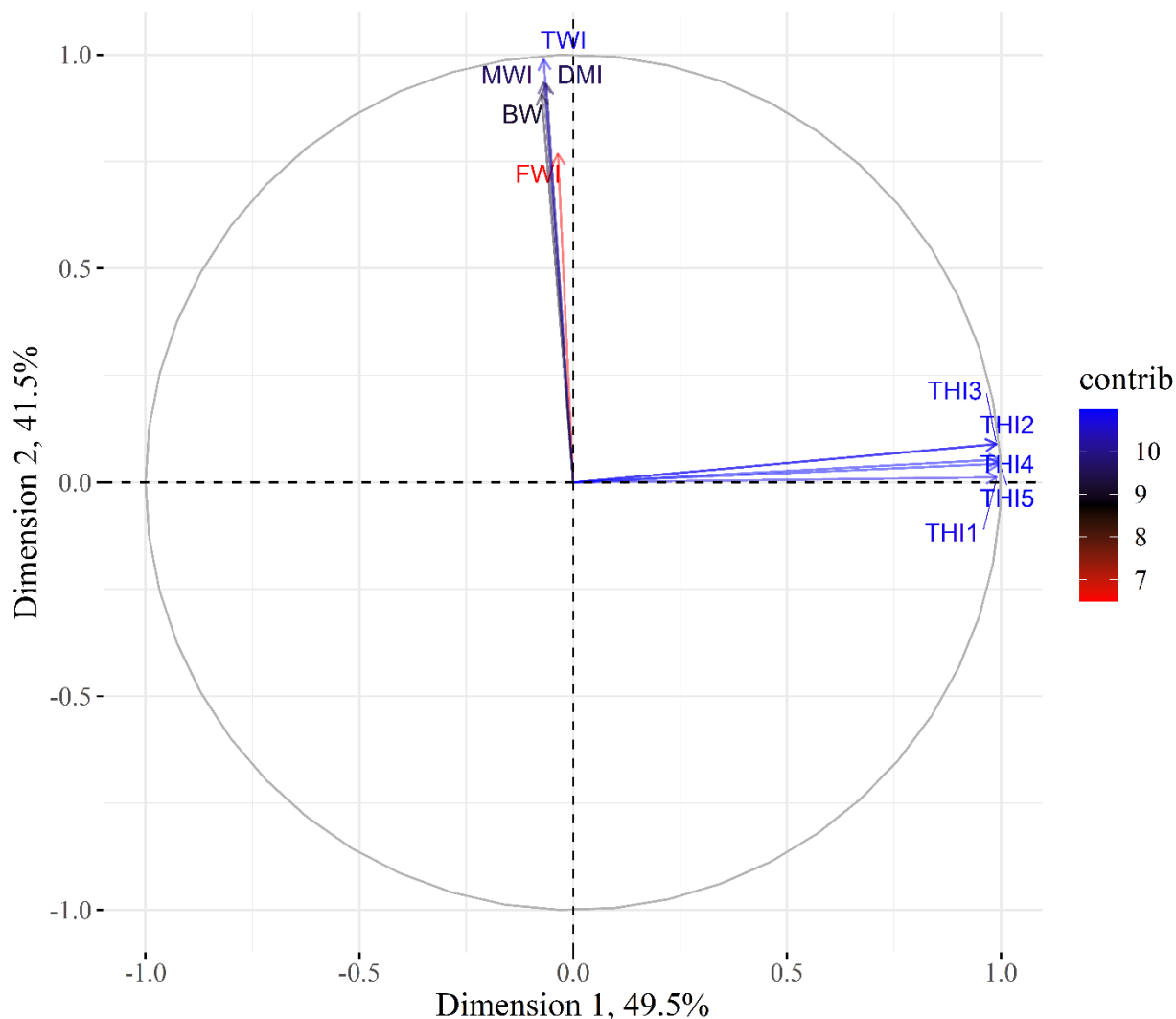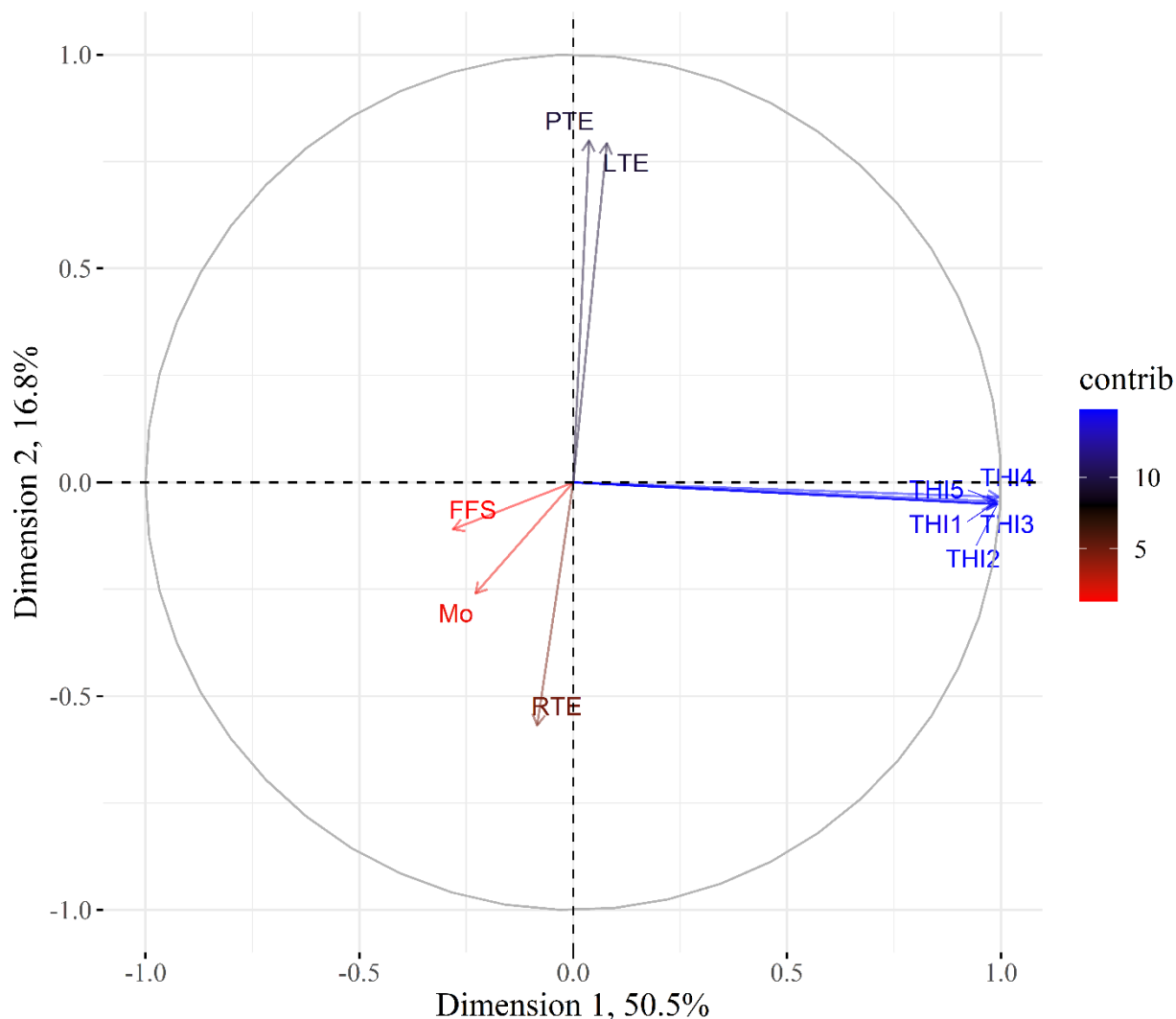
Figure 2. Principal component analysis BIPLOT of intakes and environmental factors for 23 Holstein nursing bull calves fed with a non-medicated commercial milk replacer (Control [CON]; n = 7), supplemented isoenergetically with corn starch (Carbohydrate [CHO]; n = 8), or with fish oil (fat [FAT]; n = 8).

BW = body weight, MWI = milk water intake, TWI = total water intake, DMI = dry matter intake, FWI = fresh water intake, THI1 = (1.8*Temp+32)−[(0.55−0.0055*RH)*(1.8+Temp−26.0], NRC (1971); THI2 = (0.55*Temp+0.2xDPt)*1.8+32+17.5, NRC (1971); THI3 = Temp+(0.36*DPt)+41.2; Yousef 1985, THI4 = (0.8*Temp)+(RH÷100)*(Temp−14.4)+46.4, Thom (1959) and NOAA (1976); THI5 = 3.43+1.058*Temp-0.293*RH+0.0164*Temp*RH+35.7, Berman et al. (2016)

Figure 3. Principal component analysis variable importance of the first principal component intakes and environmental factors for 23 Holstein nursing bull calves fed with a non-medicated commercial milk replacer (Control [CON]; n = 7), supplemented isoenergetically with corn starch (Carbohydrate [CHO]; n = 8), or with fish oil (fat [FAT]; n = 8).

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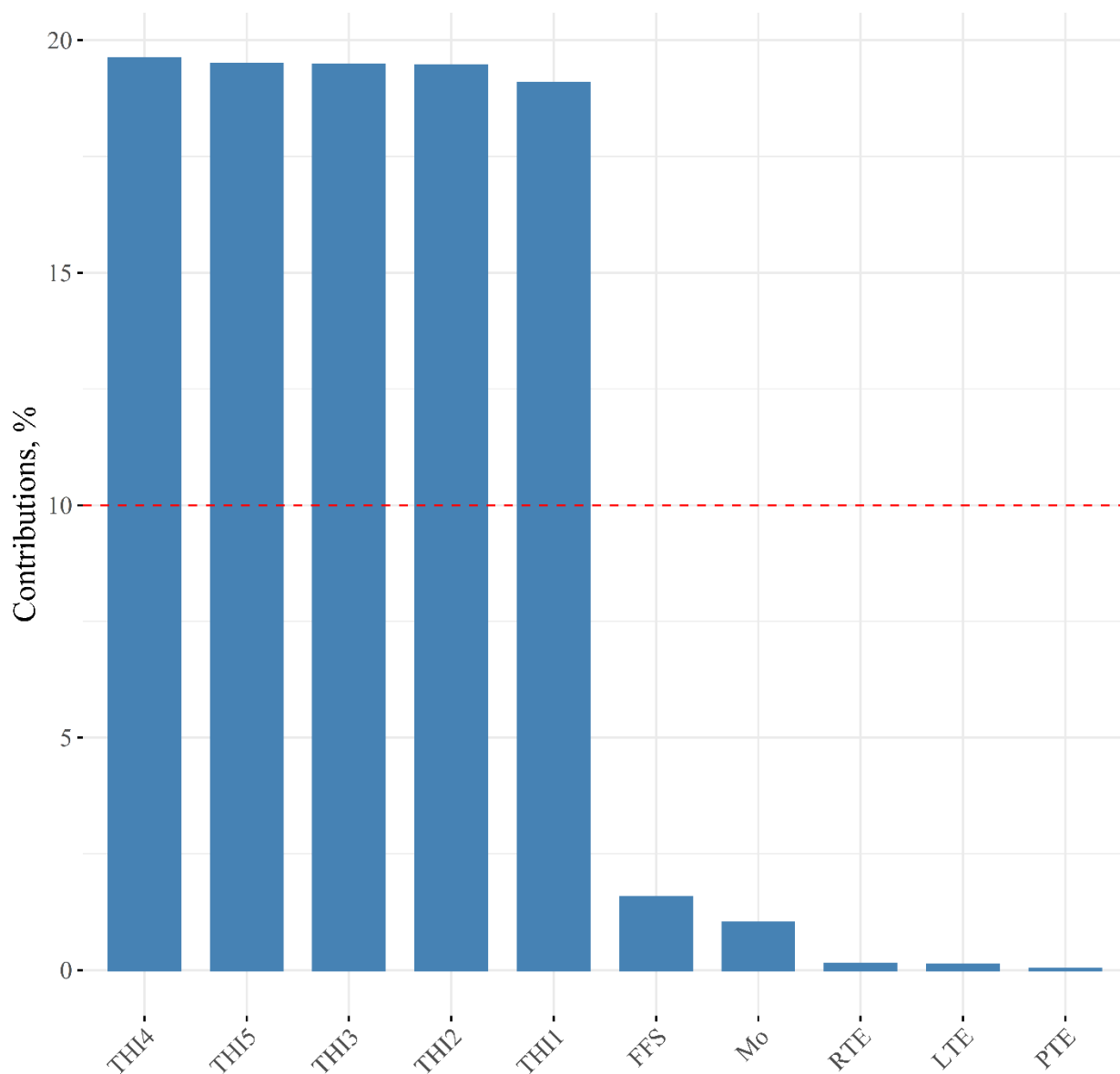
Figure 4. Principal component analysis variable importance of the second principal component of intakes and environmental factors variables for 23 Holstein nursing bull calves fed with a non-medicated commercial milk replacer (Control [CON]; n = 7), supplemented isoenergetically with corn starch (Carbohydrate [CHO]; n = 8), or with fish oil (fat [FAT]; n = 8).
 BW = body weight, MWI = milk water intake, TWI = total water intake, DMI = dry matter intake, FWI = fresh water intake, THI1 = (1.8*Temp+32)−[(0.55−0.0055*RH)*(1.8+Temp−26.0], NRC (1971); THI2 = (0.55*Temp+0.2xDPt)*1.8+32+17.5, NRC (1971); THI3 = Temp+(0.36*DPt)+41.2; Yousef 1985, THI4 = (0.8*Temp)+(RH÷100)*(Temp−14.4)+46.4, Thom (1959) and NOAA (1976); THI5 = 3.43+1.058*Temp-0.293*RH+0.0164*Temp*RH+35.7, Berman et al. (2016)

Figure 5. Principal component analysis BIPLOT of health parameters and environmental factors for 23 Holstein nursing bull calves fed with a non-medicated commercial milk replacer (Control [CON]; n = 7), supplemented isoenergetically with corn starch (Carbohydrate [CHO]; n = 8), or with fish oil (fat [FAT]; n = 8).

PTE = pen temperature, LTE = corneal laser temperature, FFS = fecal fluidity score, Mo = skin moisture, RTE = rectal temperature, THI1 = (1.8*Temp+32)−[(0.55−0.0055*RH)*(1.8+Temp−26.0], NRC (1971); THI2 = (0.55*Temp+0.2xDPt)*1.8+32+17.5, NRC (1971); THI3 = Temp+(0.36*DPt)+41.2; Yousef 1985, THI4 = (0.8*Temp)+(RH÷100)*(Temp−14.4)+46.4, Thom (1959) and NOAA (1976); THI5 = 3.43+1.058*Temp-0.293*RH+0.0164*Temp*RH+35.7, Berman et al. (2016)
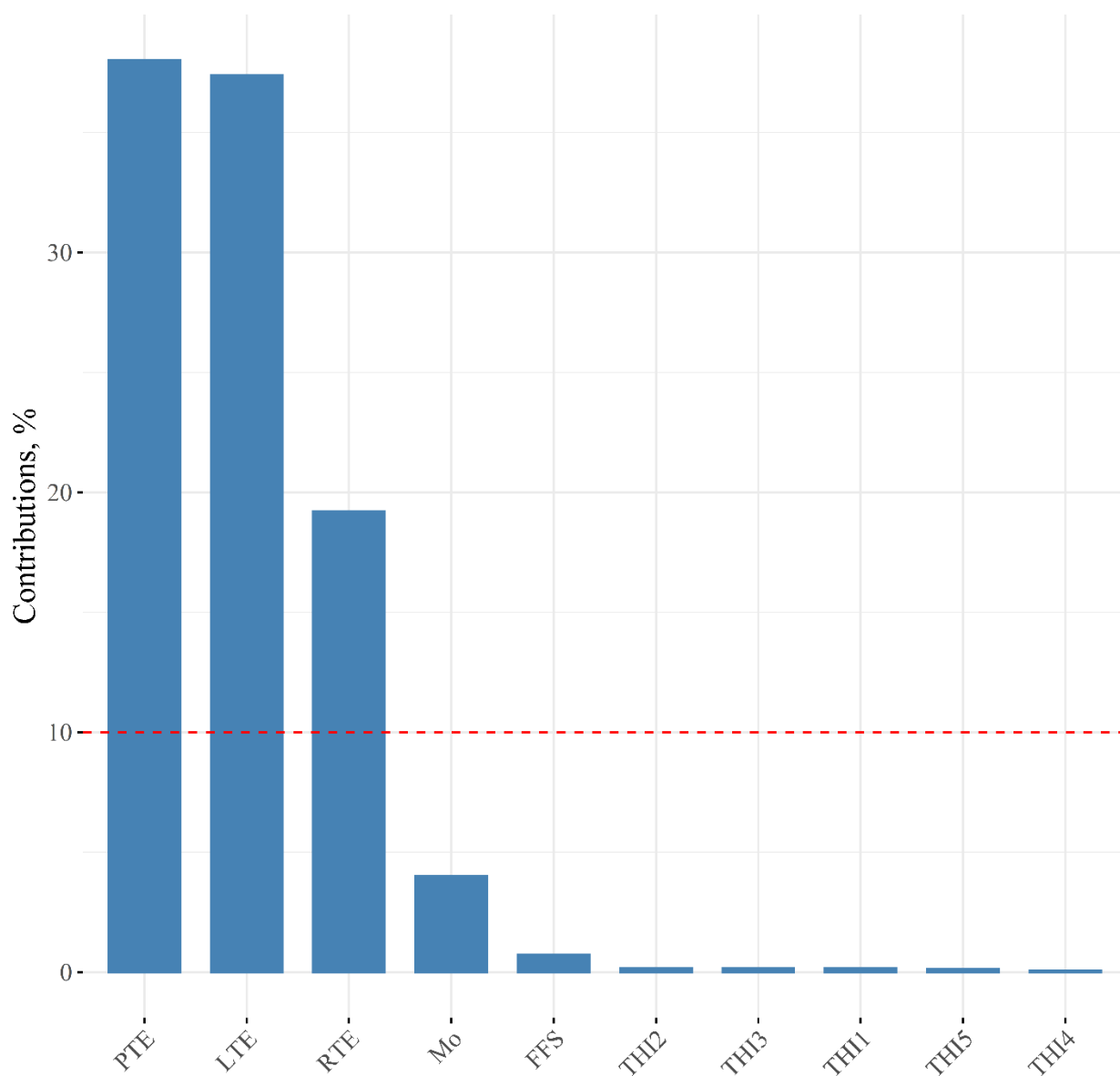
Figure 6. Principal component analysis variable importance of the first principal component of health parameters and environmental factors for 23 Holstein nursing bull calves fed with a non-medicated commercial milk replacer (Control [CON]; n = 7), supplemented isoenergetically with corn starch (Carbohydrate [CHO]; n = 8), or with fish oil (fat [FAT]; n = 8).

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Figure 7. Principal component analysis variable importance of the second principal component of health parameters and environmental factors for 23 Holstein nursing bull calves fed with a non-medicated commercial milk replacer (Control [CON]; n = 7), supplemented isoenergetically with corn starch (Carbohydrate [CHO]; n = 8), or with fish oil (fat [FAT]; n = 8).

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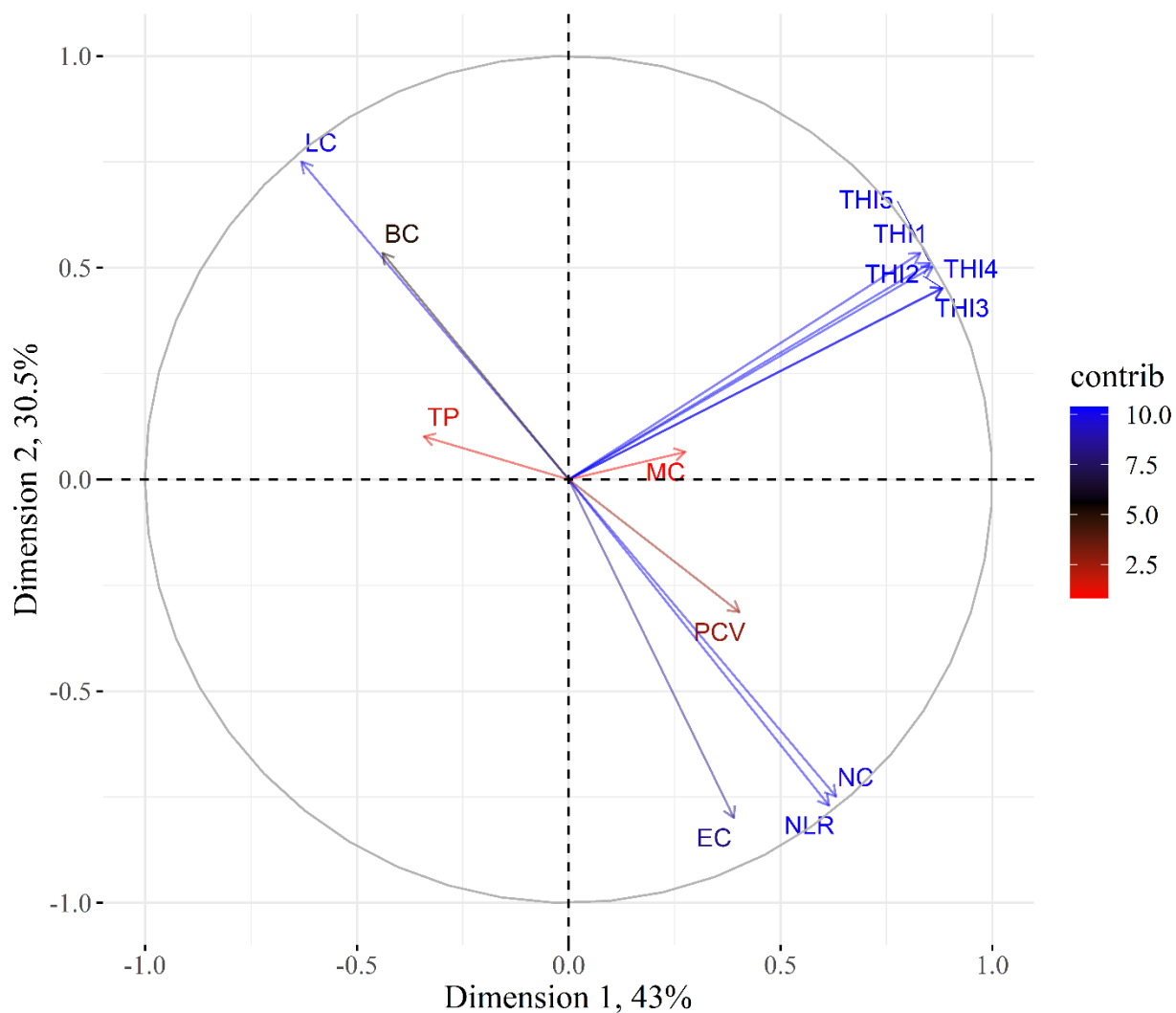
Figure 8. Principal component analysis BIPLOT of blood parameters and environmental factors for 23 Holstein nursing bull calves fed with a non-medicated commercial milk replacer (Control [CON]; n = 7), supplemented isoenergetically with corn starch (Carbohydrate [CHO]; n = 8), or with fish oil (fat [FAT]; n = 8).

LC = lymphocyte count, BC = basophil count, TP = total protein, MC = monocyte count, PCV = packed cell volume, EC = eosinophil count, NLR = neutrophil to lymphocyte ratio, NC = neutrophil count, THI1 = $(1.8*Temp+32)-[(0.55-0.0055*RH)*(1.8+Temp-26.0]$, NRC (1971); THI2 = $(0.55*Temp+0.2xDPt)*1.8+32+17.5$, NRC (1971); THI3 = $Temp+(0.36*DPt)+41.2$; Yousef 1985, THI4 = $(0.8*Temp)+(RH\div100)*(Temp-14.4)+46.4$, Thom (1959) and NOAA (1976); THI5 = $3.43+1.058*Temp-0.293*RH+0.0164*Temp*RH+35.7$, Berman et al. (2016)
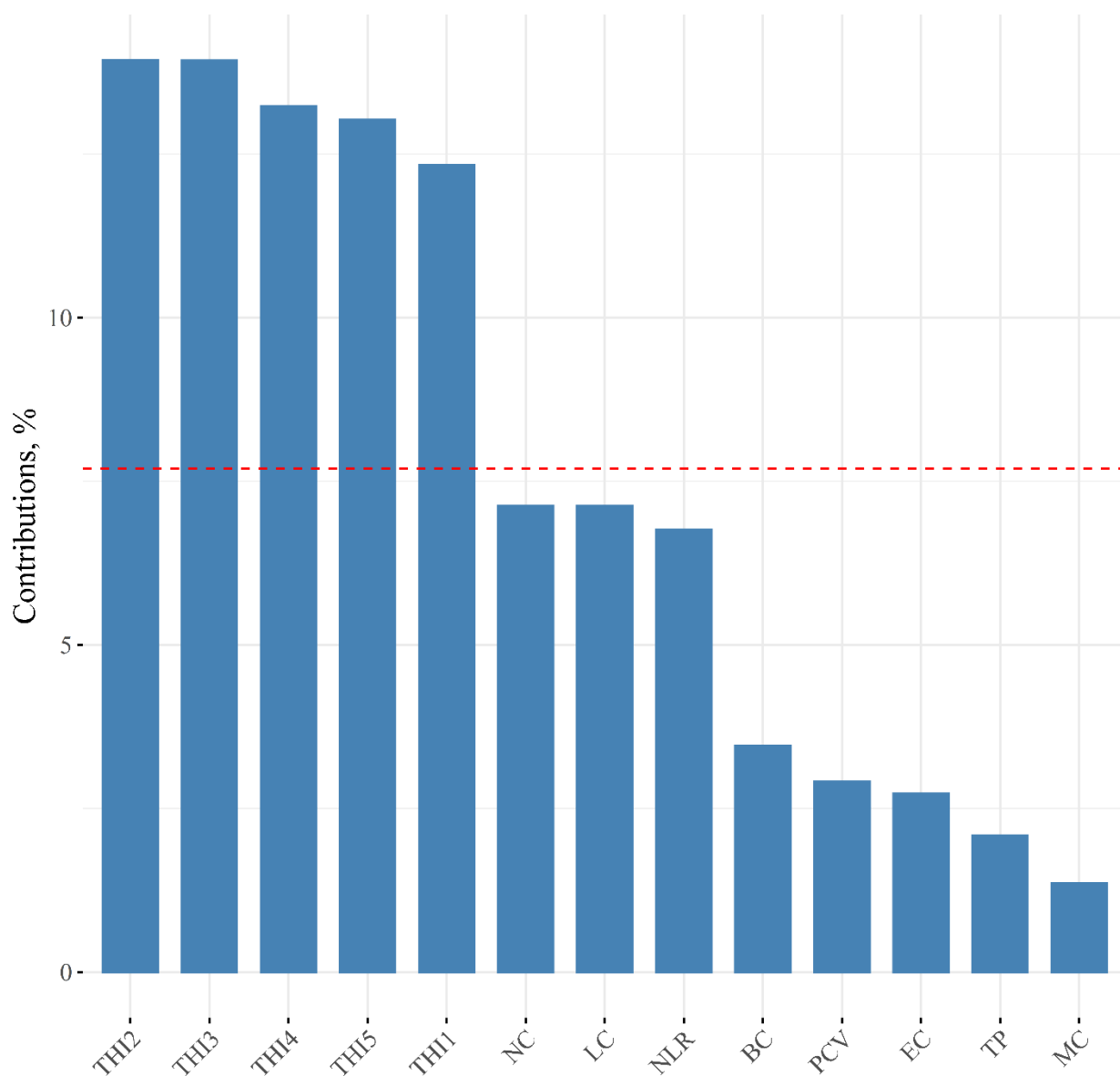
Figure 9. Principal component analysis variable importance of the first principal component of blood parameters and environmental factors for 23 Holstein nursing bull calves fed with a non-medicated commercial milk replacer (Control [CON]; n = 7), supplemented isoenergetically with corn starch (Carbohydrate [CHO]; n = 8), or with fish oil (fat [FAT]; n = 8).

LC = lymphocyte count, BC = basophil count, TP = total protein, MC = monocyte count, PCV = packed cell volume, EC = eosinophil count, NLR = neutrophil to lymphocyte ratio, NC = neutrophil count, THI1 = (1.8*Temp+32)−[(0.55−0.0055*RH)*(1.8+Temp−26.0], NRC (1971); THI2 = (0.55*Temp+0.2xDPt)*1.8+32+17.5, NRC (1971); THI3 = Temp+(0.36*DPt)+41.2; Yousef 1985, THI4 = (0.8*Temp)+(RH÷100)*(Temp−14.4)+46.4, Thom (1959) and NOAA (1976); THI5 = 3.43+1.058*Temp-0.293*RH+0.0164*Temp*RH+35.7, Berman et al. (2016)
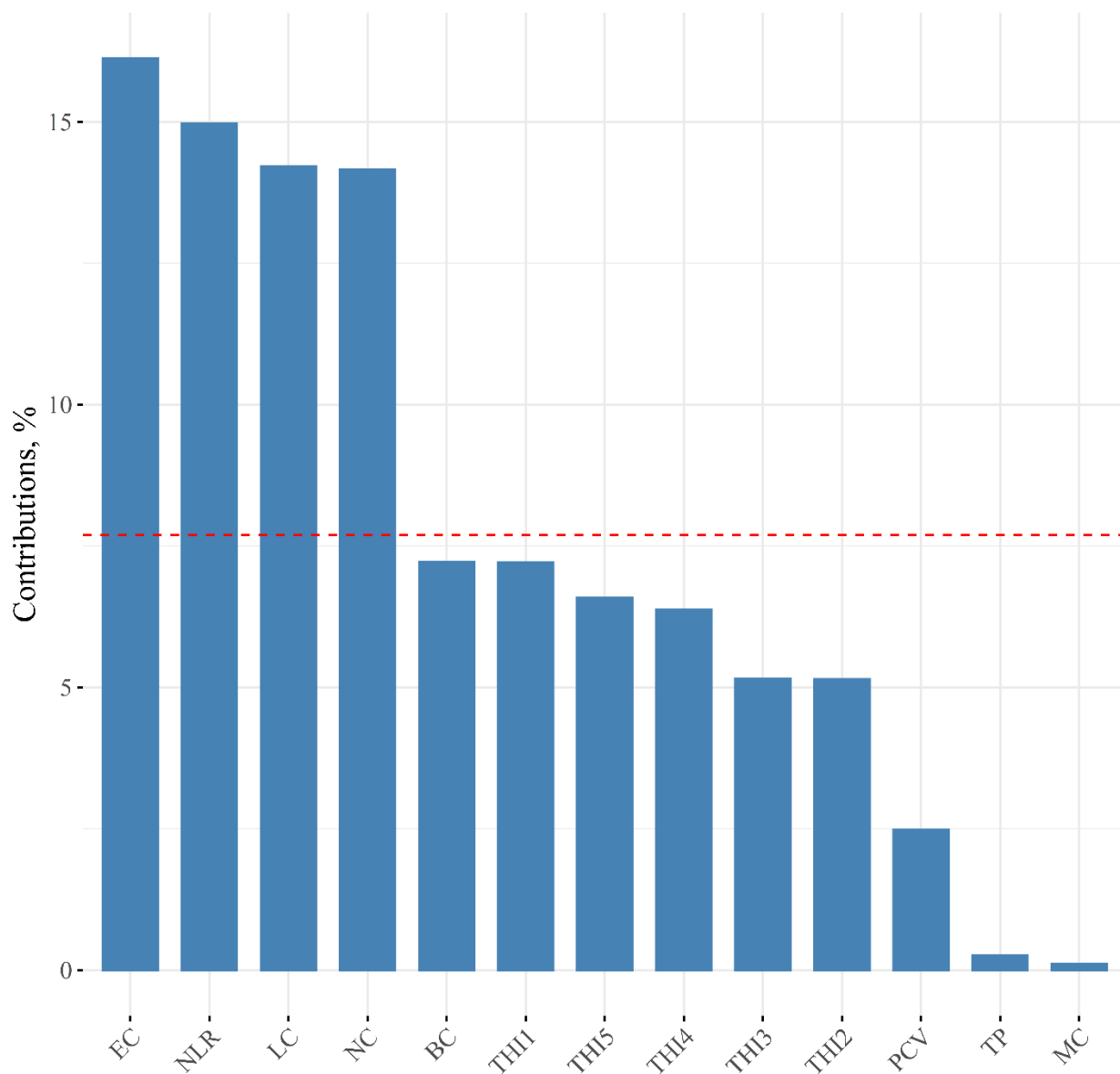
Figure 10. Principal component analysis variable importance of the second principal component of blood parameters and environmental factors for 23 Holstein nursing bull calves fed with a non-medicated commercial milk replacer (Control [CON]; n = 7), supplemented isoenergetically with corn starch (Carbohydrate [CHO]; n = 8), or with fish oil (fat [FAT]; n = 8).

LC = lymphocyte count, BC = basophil count, TP = total protein, MC = monocyte count, PCV = packed cell volume, EC = eosinophil count, NLR = neutrophil to lymphocyte ratio, NC = neutrophil count, THI1 = (1.8*Temp+32)−[(0.55−0.0055*RH)*(1.8+Temp−26.0], NRC (1971); THI2 = (0.55*Temp+0.2xDPt)*1.8+32+17.5, NRC (1971); THI3 = Temp+(0.36*DPt)+41.2; Yousef 1985, THI4 = (0.8*Temp)+(RH÷100)*(Temp−14.4)+46.4, Thom (1959) and NOAA (1976); THI5 = 3.43+1.058*Temp-0.293*RH+0.0164*Temp*RH+35.7, Berman et al. (2016)