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

A. Macias-Franco*; A. E. M. da Silva*; F. H. de Moura*; A. B. Norris*; K. Van Den Broek*; M. Valcheck*; A. de Mello*; M. Fonseca**;

*Department of Agriculture, Veterinary, & Rangeland Sciences, University of Nevada-Reno, Reno, Nevada; United States of America, 89557.

# Department of Natural Resources Management, Texas Tech University, Texas; United States of America, 79409.

||Corresponding author: mfonseca@unr.edu
Acknowledgements: This research was partially funded by USDA-NIFA HATCH project # NEV00761A and USDA-NIFA award #: 2019-69006-29329. The first author’s scholarship was funded by the Richard Kleberg Agricultural Scholarship Endowment from the College of Agriculture Biotechnology and Natural Resources of the University of Nevada, Reno. We would like to extend our most sincere gratitude and acknowledgement to the undergraduate student workers that assisted during facility preparation, cleaning and on the daily husbandry of calves, as well as in laboratory analysis and data entry, including: Sarah Quiñonez, Serena Roloson, Heather Peterson, and Lucilaine Veline. Additionally, we would like to thank the brew masters, breweries, and microbreweries that provided us with grain for the experiment, especially: Bryan Holloway at Pigeon Head Brewing, Joe Baldwin from BJ’S brewing, Jazz Aldrich and Nick from Great Basin Brewing, Jason Green from IMBIB brewing, and the 10 Torr crew. Conflict of interest statement: The authors declare no conflicts of interest.
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, feed intake parameters, and performance of Holstein nursing bull calves. Twenty-three animals (body weight [BW] = 94.67 ± 12.07 kg, age = 67 days 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 a 7% composition in 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) adjusted by average daily gain (ADG; DMI/ADG) resulted in significantly lower values for supplemented groups 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 lipid-based and starch-based supplementation can potentially increase feed efficiency and decrease voluntary water intake without adversely affecting 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 for 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 livestock 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 Holstein nursing bull calves (Senevirathne et al., 2018; Wickramasinghe et al., 2019), and there have been no attempts exploring the effects of metabolic water produced from 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 (67 days of adaptation and 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% w/v), 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 67-day adaptation period. Housing constituted individual 32-ft² 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 = 67 days 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 BG were fed to stimulate rumen development to provide additional mechanisms for calves to optimize their body water pool through body water compartments present in the gastrointestinal tract (Church, 1988; King, 1983).

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 °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 as fed basis.
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 -20°C. At the end of each week, a composite sample was prepared and oven dried (60°C). After that, another composite sample representing the 28 d period was generated based on the proportion of DMI each week and stored at -20°C 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) calculated as 100 minus ash concentration, neutral detergent fiber (NDF) was analyzed according to 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), 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), the total digestible nutrients (TDN) and net energy were computed utilizing empirical equations reported on NRC (2001). Metabolizable energy intake (MEi), digestible energy intake (DEi), net energy intake for maintenance (NEim), and net energy intake for gain (NEig) 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 livestock suitability analysis. Water was shipped refrigerated, in the same day, for analysis at Cumberland Valley Analytical Services 2020 CVAS, Inc. The analyses were performed according to Rice et al. (2017) for pH (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 (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 apparent digestibility assays were performed to estimate the nutrient digestibility coefficients. Total fecal collections were performed for four consecutive days of the experimental period: 28 to 32 and 60 to 64 of (after adaptation). Feces were collected immediately after spontaneous defecation and stored in a plastic 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 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 trough 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/day) by the average daily gain (ADG, kg/day). The average FC was obtained by the reciprocal of this relationship. To calculate the RGR, the shrunk body weight was considered for initial, final shrunk body weight, and d of confinement as RGR = 100 * (log final BW - log initial weight)/ (number of days) (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, or total water intake, to generate RFI and RTWI, respectively, according to Sainz and Paulino (2004).

\[
RFI; RTWI = Y_{12} = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \varepsilon_{12}
\]

Where \(Y\) represents the expected values for feed and water measures to be regressed, \(\beta_0\) represents the respective equation intercept, \(\beta_1\) and \(\beta_2\) represent the coefficients of the equation, \(X_1\) and \(X_2\) represent the midpoint BW^{0.75}, and the ADG, respectively, and \(\varepsilon\) is the respective residuals. Ultimately the fitted regression equations for prediction of TWI and DMI and their standard errors were as follows: 

\[
TWI_{predicted} = (5.520 \pm 6.058) + (0.317 \pm 0.255)(BW^{0.75}) - (0.919 \pm 4.645)(ADG) \text{ with } R^2 = 0.164 \quad \text{and} \quad DMI_{predicted} = (-0.861 \pm 0.36) + (0.068 \pm 0.015)(BW^{0.75}) + (0.637 \pm 0.272)(ADG) \text{ with } R^2 = 0.873.
\]

Though standard errors of RTWI were high, multicollinearity, expected in residual calculations, increases standard errors. Further, when fitting the regression to predict for total water intake, the distribution of our residuals and data didn’t show biologically abnormal values; statistically, no significant leverage or studentized residuals higher than 2.5 were found, and therefore, no datapoints were removed from these computations.

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} x ADG^{1.2} x 0.69 for net energy for growth (Mcal), 0.1 x BW^{0.75} + (0.84 x BW^{0.355} x 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 were taken by the same technician alongside with the BW on days 0, 28, and 56 of the post-
adaptation experimental period. 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 were taken using specific anatomical locations as baseline points by hand palpation (De Paula et al., 2013; 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 point 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 projection 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 (SAS Inst. Inc., Cary, NC). All variables were investigated assuming a completely randomized design with diet as the fixed effect with the animal as the subject. Outliers were identified using the plot of studentized residuals against the predicted values as well as by 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. With regards to feed intake, two statistical trends were observed when adjusting DMI of milk replacer as well as milk replacer intake per ADG, DMI of the milk replacer/ADG and milk replacer intake/ADG, respectively, where FAT and CHO displayed lower intake than the CON group ($P = 0.08$; 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 of 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. When
examining the partitioned nutrient intake from the diets, no statistically significant differences were noted on any nutrient intake values except for EE intake \( (P = 0.007; \text{Table 3}) \). 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.

Even though the diets were isonitrogenous, the coefficients of digestibility of the CP (CPD) were significantly different \( (P = 0.022; \text{Table 4}) \). 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 apparent digestibility. However, on their data, no differences in digestibility 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, 1988). Hu et al. (2019) reported higher digestibility 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 that 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, and therefore, permits examination of macronutrient supplementation effects on water intake.
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/day. 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; therefore, this could serve 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/day 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). 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 significant 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 representation of the water requirements of 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 $P$-values of $< 0.0001$, $< 0.0001$, $= 0.001$, $< 0.0001$, $< 0.0001$, $< 0.0001$, $< 0.0001$, $< 0.0001$ (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 significant 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 ($P > 0.1$; 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. The lack of statistical significance for RTWI is not overly-alarming; a possible explanation for the high standard errors could be the multicollinearity of the predictor variables (correlation between ADG and BW^{0.75} > 0.8); high multicollinearity decreases model sensitivity to change and therefore increases the standard errors (Yoo, 2014).

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 strategically supplemented Holstein nursing bull calves could potentially be more efficient than non-supplemented animals. Two interesting trends (P < 0.1; Table 7) were observed for FC and FCR; for FCR, 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 found 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). 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 use of BM, no significant differences were detected, indicating that any potential changes to intake did not affect the overall growth curve of supplemented animals. Biometric measures have already been 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); this is expected given that growth can be modeled, and described, as a linear allometric pattern through the use of principal component analysis which explains 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 over 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
frame sizes 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. The lipid and carbohydrate supplementation we investigated helps demonstrate the potential water intake reduction without adversely affecting performance. This represents the beginning of developing a line of supplements tailored to increase feed and water efficiencies of livestock operations governed by nursing animals. 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.

AOAC International. 2000. Official methods of analysis of AOAC International. 17th ed. AOAC Int., Gaithersburg, MD.

AOAC International. 2006. Official methods of analysis of AOAC International. 18th ed. AOAC Int., Gaithersburg, MD.

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.

Goering, H.K., and P.J. Van Soest. 1970. Forage fiber analysis. USDA Agricultural Research Service. Handbook number 379. U.S. Dept. of Agriculture. Superintendent of Documents, US Government Printing Office, Washington, D.C. 20402.

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.
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.

Licitra, G., T. M. Hernandez, and P. J. Van Soest. 1996. Feedbunk management evaluation techniques. Anim. Feed Sci. Technol. 57:347–358.

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

Mertens, D. R. 2002. Measuring fiber and its effectiveness in ruminant diets. Proc Plains Nutr Conf, San Antonio, TX, USA, pp. Available from: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.727.5023&rep=rep1&type=pdf#page=47

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/anrec/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.

Yoo, W., Mayberry, R., Bae, S., Singh, K., He, Q. P., & Lillard Jr, J. W. 2014. A study of effects of multicollinearity in the multivariable analysis. International journal of applied science and technology, 4(5): 9-19.
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 |  
|-----------------------------|------------|
|                             | CON (g/l)  | CHO (g/l) | FAT (g/l)  |
| Milk Replacer               | 173.9      | 173.9     | 173.9      |
| Fish oil                    | -          | -         | 5.2        |
| Starch                      | -          | 11.8      | -          |
| Dried Brewer's spent grain  | 111.1      | 109.1     | 104.7      |

| 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† | Collection | Upper tolerable limit for Cattle (ppm) | Problem Value |
|--------------------|------------|--------------------------------------|----------------|
| pH                 | 7.3        | < 5.5 or > 8.5                       |                |
| 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      | <0.05 (taste)                        | 0.4 (taste)    |
| Manganese          | <0.05      | <0.05 (taste)                        | 0.05 (taste)   |
| Zinc               | <0.01      | <0.01                                | 25             |
| Copper             | <0.01      | <0.01                                | 0.6            |
| Calcium carbonate hardness | 205 | Colonies per 100 ml                 |
| Total Coliform     | <1         |                                       | 15             |
| E.coli             | <1         |                                       | 10             |

†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  | Treatments | SEM  | P-value |
|-------|------------|------|---------|
|       | CON | CHO | FAT |      | Treatment |
| Dry matter intake | | | | | |
| DMIr, kg  | 3.06 | 2.82 | 3.01 | 0.14 | 0.475 |
| DMIr/BW, kg/kg | 0.02 | 0.02 | 0.02 | 0.00 | 0.475 |
| DMIr/ADG, kg/kg | 1.88 | 1.76 | 1.73 | 0.04 | 0.080 |
| 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<sup>a</sup> | 2.38<sup>ab</sup> | 2.27<sup>b</sup> | 0.05 | 0.033 |

Nutrient Intake, kg/day

| Item  | Treatments | SEM  | P-value |
|-------|------------|------|---------|
| 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<sup>b</sup> | 0.44<sup>b</sup> | 0.56<sup>a</sup> | 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 |
| NDF<sub>ap</sub>, 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; DMIr = milk replacer DMI; DMIr/BW = DMIr relative to body weight; DMIr/ADG = DMIr 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<sub>ap</sub> = neutral detergent fiber assayed with heat stable amylase and expressed exclusive of residual ash and residual CP; NDFi= indigestible neutral detergent fiber; ME<sub>i</sub> = metabolizable energy intake; NEim = net energy for maintenance intake; NE<sub>ig</sub> = 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 | Treatments | SEM | P-value<sup>3</sup> |
|------|-------------|-----|---------------------|
|      | 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<sup>a</sup> | 0.91<sup>b</sup> | 0.93<sup>a</sup> | 0.006 | 0.022 |
| EED  | 0.95<sup>ab</sup> | 0.94<sup>b</sup> | 0.96<sup>a</sup> | 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<sup>c</sup> | 1.07<sup>b</sup> | 1.08<sup>a</sup> | 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<sup>b</sup> | 0.41<sup>b</sup> | 0.54<sup>a</sup> | 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 |

<sup>1</sup>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.

<sup>2</sup>Standard error of the mean.

<sup>3</sup>P-value, <0.1 = trend; <0.05 = significant.

<sup>abc</sup>Means within row without common superscript differ (\(P \leq 0.05\)).
### 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 | Treatments | SEM | P-value |
|------|------------|-----|---------|
|      | CON | CHO | FAT |       |
| TWI  | 17.61<sup>a</sup> | 16.99<sup>b</sup> | 17.51<sup>ab</sup> | 0.19 | <0.0001 |
| MWI  | 14.47<sup>a</sup> | 13.35<sup>b</sup> | 14.17<sup>a</sup> | 0.14 | <0.0001 |
| FWI  | 3.73<sup>a</sup> | 3.27<sup>b</sup> | 3.36<sup>ab</sup> | 0.10 | <0.0001 |
| MWP  | 1.57<sup>b</sup> | 1.68<sup>a</sup> | 1.64<sup>a</sup> | 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<sup>a</sup> | 0.02<sup>b</sup> | 0.22<sup>b</sup> | 0.00 | <0.0001 |
| FWI/BW<sup>0.75</sup>, kg/kg<sup>0.75</sup> | 0.09<sup>a</sup> | 0.08<sup>b</sup> | 0.08<sup>b</sup> | 0.00 | <0.0001 |
| MWI/ADG | 8.84<sup>a</sup> | 8.32<sup>ab</sup> | 8.14<sup>b</sup> | 0.21 | 0.001 |
| ADG/MWI | 0.11<sup>b</sup> | 0.12<sup>ab</sup> | 0.12<sup>a</sup> | 0.00 | 0.083 |
| MWI/BW | 0.1<sup>a</sup> | 0.09<sup>b</sup> | 0.10<sup>c</sup> | 0.00 | <0.0001 |
| MWI/BW<sup>0.75</sup>, kg/kg<sup>0.75</sup> | 0.34<sup>a</sup> | 0.33<sup>b</sup> | 0.31<sup>c</sup> | 0.00 | <0.0001 |
| TWI/BWg | 0.17 | 0.16 | 0.15 | 0.01 | 0.677 |
| TWI/BW | 0.12<sup>a</sup> | 0.12<sup>b</sup> | 0.12<sup>b</sup> | 0.00 | 0.007 |
| TWI/ADG | 2.31<sup>a</sup> | 2.12<sup>b</sup> | 1.97<sup>b</sup> | 0.09 | 0.001 |
| TWI/BW<sup>0.75</sup>, kg/kg<sup>0.75</sup> | 0.42<sup>a</sup> | 0.40<sup>b</sup> | 0.41<sup>b</sup> | 0.01 | <0.0001 |

<sup>1</sup>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<sup>0.75</sup> = FWI relative to metabolic body weight (BW<sup>0.75</sup>), MWI/ADG = MWI relative to ADG, ADG/MWI = ADG relative to MWI, MWI/BW = MWI relative to BW, MWI/BW<sup>0.75</sup> = MWI relative to BW<sup>0.75</sup>, TWI/BWg = TWI relative to BWg, TWI/BW = TWI relative to BW, TWI/BW<sup>0.75</sup> = TWI relative to BW<sup>0.75</sup>

<sup>2</sup>Standard error of the mean.

<sup>3</sup>P-value, <0.1 = trend; <0.05 = significant.

<sup>abc</sup>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 | Treatments | SEM² | P-value³ |
|------|------------|------|----------|
|      | CON        | CHO  | FAT      |          |
| DEi  | 15.57b     | 15.10b| 16.08a   | 0.018    | <0.0001  |
| MEi  | 14.92a     | 13.73b| 14.65a   | 0.144    | <0.0001  |
| NEim | 8.51a      | 7.83b | 8.51a    | 0.121    | <0.0001  |
| NEig | 11.19a     | 10.30b| 10.99a   | 0.108    | <0.0001  |
| DE, Mcal/day | 13.60b | 13.35b| 14.43a   | 0.175    | <0.0001  |
| ME, Mcal/day | 13.06b | 12.81b| 13.85a   | 0.162    | <0.0001  |
| NEm, Mcal/day | 3.64a  | 3.63a | 3.69b    | 0.041    | <0.0001  |
| NEg, Mcal/day | 6.09b  | 5.93b | 6.60a    | 0.061    | <0.0001  |

¹ DEi = digestible energy intake; MEi = metabolizable energy intake; NEim = net energy for maintenance intake, NEig = net energy for gain intake, DE = digestible energy, ME = metabolizable energy, NEm = net energy for maintenance, NEg = 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 ≤ 0.05).
Table 7. Performance and relative efficiencies of Holstein nursing bull calves fed non-medicating milk replacer only (CON; n=7), non-medicating milk replacer supplemented with 3% menhaden fish oil (FAT; n=8), or non-medicating milk replacer with corn starch (CHO; n=8).

| Item | Treatments | SEM | P-value |
|------|------------|-----|---------|
|      | 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<sup>a</sup> | -0.07<sup>b</sup> | -0.07<sup>b</sup> | 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<sup>0.75</sup> | 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.
4<sup>ab</sup> Means within row without common superscript differ (P ≤ 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 | Treatment | SEM² | P-value³ | Time | Trt*Time |
|------|-----------|------|----------|------|----------|
|      | CON       | CHO  | FAT      | CON vs.E | CHO vs. FAT |       |          |
| BW, kg | 146.71 | 146.25 | 150.48 | 6.358 | 0.841 | 0.643 | <0.001 | 0.3332 |

Biometric Measures, cm

| Item | Treatment | SEM² | P-value³ | Time | Trt*Time |
|------|-----------|------|----------|------|----------|
| 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.