WATER AND FORAGE INTAKE, DIET DIGESTIBILITY, AND BLOOD PARAMETERS FOR BEEF COWS AND GROWING HEIFERS CONSUMING WATER WITH VARYING CONCENTRATIONS OF TOTAL DISSOLVED SALTS

By

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Abstract: As water becomes a more limited resource, efficient water utilization is needed to continue animal production during water scarcity and declining water quality. The objective of this study was to investigate the effects of water quality on water intake (WI), forage intake, diet digestibility, and blood constituents in beef cows and growing beef heifers. The experimental design was two simultaneous 5 × 5 Latin squares with five drinking water treatments within each square: 1) control (fresh water-FRW); 2) brackish water (100 BRW treatment) with approximately 6,000 mg/kg TDS; 3) same TDS level as 100 BRW achieved by addition of NaCl to fresh water (100 SLW); 4) 50% brackish water and 50% fresh water to achieve approximately 3,000 mg/kg TDS (50 BRW); and 5) same TDS level as 50 BRW achieved by addition of NaCl to fresh water (50 SLW). Each of the five 21-d periods consisted of 14 d of adaptation and 5 d of data collection. Animals were housed individually, fed alfalfa cubes and provided one of the five water sources for ad libitum feed and WI. Feed and water intake were recorded daily, with recorded WI adjusted for vaporization. The PROC MIXED procedure of SAS 9.4 was used for data analysis where animal was the experimental unit. Age, treatment, and age x treatment were fixed effects, and animal ID within age was the random variable for intake, digestibility, and blood parameter data. Significance was declared at $P \leq 0.05$, and a tendency was declared at $P > 0.05$ and $P \leq 0.10$. Compared to previously published data, water and feed intake were extremely elevated regardless of age or water treatment. No treatment x age interactions were identified for WI ($P = 0.71$), WI expressed as g/kg body weight (BW; $P = 0.70$), or dry matter intake (DMI; $P = 0.21$). However, there was an age x treatment tendency for DMI when scaled to BW ($P = 0.09$) in cows consuming 100 BRW compared to fresh water. No differences were found for the other three treatments. Heifers consuming 50 SLW showed a significant difference ($P < 0.05$) for lower feed intake (g/kg BW) compared to fresh water and 100 BRW. No statistical differences ($P > 0.05$) in water, feed intake or diet digestibility were found due to water quality treatment. In conclusion, under these conditions neither absolute WI, absolute feed intake, nor diet digestibility were influenced by the natural brackish or saline water used in this experiment. These results suggest that further research is necessary to determine thresholds for TDS or salinity concentration resulting in reduced water and/or feed intake and diet digestibility.

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FORMAT OF THESIS

This Thesis is presented in Journal of Animal Science style and format. Use of this format allows the individual chapters to be suitable for submission to scientific journals. One paper has been prepared from the data collected and includes an abstract, introduction, materials and methods, results and discussion, and literature cited section. This paper is Chapter II.

CHAPTER I

REVIEW OF LITERATURE

Introduction

Historically, renewable freshwater sources have been essential for all life from plants and animals to human sustainability. Most large cities were established around water sources from ancient times (e.g. Mesopotamia situated between the Tigris and Euphrates, Phoenicia along the Mediterranean Sea, etc.). Life as we know it is not possible without water. In the Sixth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC), it is anticipated that as the earth temperature increases, water vapor concentrations will follow synonymously which will eventually alter the hydrological cycle and therefore regional freshwater resources (Jiménez Cisneros et al., 2014). Dependent upon global location, some areas will see an increase in rainfall and others a decrease. Naturally, increased atmospheric temperatures and decreased rainfall will reduce surface and groundwater resources and will increase vaporization of rivers, lakes, etc. Conversely, an increase in rainfall will lead to surface runoff, flooding and nutrient removal, and a reduction in groundwater recharge (Jiménez Cisneros et al., 2014). Regardless of rainfall, drought, vaporization, and water availability, a warming climate will exacerbate the existing pressures on freshwater sources in the world and

result in increased competition for water between plants, livestock, and humans (Jiménez Cisneros et al., 2014).

Water availability is expected to be a common challenge across animal agriculture as the demands of agriculture increases and the climate continues to change. In addition to water scarcity, water quality is expected to become poorer due to increased salination and contamination with, chemicals, heavy metals, and biological substances (Nardone et al., 2010). As concern over the availability of drinking water grows, understanding how livestock can utilize sources of water other than fresh, without sacrificing animal performance, is imperative for creating water management techniques for future livestock production. Furthermore, it is equally important to measure how ingesting moderate to poor-quality water will affect forage intake and diet digestibility in order to forecast production implications.

Water Quality

Brackish water is defined as water that contains 1,000 to 10,000 mg/L of total dissolved or soluble salts (**TDS**) and can be classified as one of four groups (USGS, 2013; Stanton et al., 2017). Water group definitions of Stanton et al. (2017) are Group 1: sodium-bicarbonate-dominant water type in which sulfate contributes about one-third of the total anion equivalents; Group 2: calcium-sulfate-dominant water type in which sodium and magnesium each contribute about one-quarter of the total cation equivalents; Group 3: sodium-chloride-dominant water type and has a high mean concentration of TDS (8,440 mg/L); and Group 4: mixture of cations and anions with low TDS (1,360 mg/L) and a high percentage of silica (1.7 percent of the total moles of cations and

anions). One characteristic of a water's quality is the concentration of TDS, often termed as the level of salinity. Drinking water with TDS above 1,000 mg/kg (i.e., mg/kg or liter) is termed 'saline,' and brackish water usually refers to water with TDS between 1,000 and 10,000 mg/kg but can be much higher (USGS, 2013; Stanton et al., 2017; Stanton and Dennehy, 2017).

Specifically, ruminants frequently consume water moderate to high in TDS globally. Brackish and saline ground water sources are widespread, including countries like Egypt (Assad and El-Sherif, 2002), Australia (McGregor, 2004), India (Sharma et al., 2017), Tunisia (Yousfi et al., 2016), Brazil (Castro et al., 2017), Asia, the eastern Mediterranean, Africa (Masters et al., 2005), and most of the central U.S. (Androwski et al., 2011; USGS, 2013). In regards to the United States (U.S.), Figure 1 shows depths of brackish water between 500 and 3,000 feet throughout a large portion of the country.

There are a number of factors suggesting increasing reliance on saline or brackish water by ruminant livestock. In many parts of the U.S., rates of groundwater withdrawal are greater than those of recharge, resulting in decreased groundwater supplies, lower stream and lake levels, and/or land subsidence, with an increased reliance on water concentrated in TDS (Masters et al., 2005; El Shaer, 2010). As previously mentioned, climate change may exacerbate these conditions, with decreasing and less consistent precipitation in some regions and increased evaporation (Beach et al., 2015).

Because of the increasing importance of drinking water with high TDS likely in the foreseeable future, it is important to gain a better understanding of factors affecting the utilization of brackish/saline water by ruminant livestock. If adverse effects of

consumption of a source of drinking water moderate to high in TDS are sufficiently severe, such as in decreased water intake (WI), forage intake, or diet digestibility, then potential changes in management practices can be considered. For example, it may be possible to change to another water source altogether or dilute the source with fresh water to minimize the level of TDS. Moreover, differences among species exist, such as greater salt tolerance by goats than sheep and older than young small ruminants (McGregor, 2004) and perhaps lower tolerance of cattle vs. goats and sheep (Squires, 1988; SCA, 1990). Since the highest quality water, low in TDS, will continue to be used primarily for consumption by and food production for humans, ruminant livestock will increasingly rely upon saline drinking water, which necessitates research to identify optimal methods of usage for highest levels and efficiencies of production.

Apart from the TDS level in water consumed by ruminants, estimates of the amount of fresh water required and consumed by ruminant livestock species and methods of prediction vary considerably. For cattle, the latest data available for estimating fresh water requirements for beef cows was published in 1956 (Winchester and Morris, 1956). The National Academies of Sciences, Engineering, and Medicine's (NASEM, 2016) recommendation of the water requirement of beef cows was based on this data with 900-pound cows. Today, a mature beef cow averages between 1,075 pounds (Walker et al., 2015) and 1,400 pounds (McMurry, 2008), furthering the necessity of current water consumption research.

Factors Affecting Water Intake

Many factors affect daily WI by cattle including feed dry matter intake (DMI), breed, body weight (BW) and age, water quality, temperature, and other environmental factors. Consequently, water requirements (Arias and Mader, 2011) can be difficult to determine or predict. Collecting *ad libitum* WI data can also be challenging, resulting in most data consisting of growing or feedlot cattle with the only current mature cow data collected in 1956 (Winchester and Morris, 1956).

Temperature and DMI

Winchester and Morris (1956) conducted the earliest research on WI in cattle, which had been used as the basis for the daily water requirements for cattle published by the National Research Council (NRC, 2000). This research is still currently used for calculating mature cow requirements (NASEM, 2016), although recent research has been conducted with growing animals (Brew et al., 2011) and feedlot animals (Hicks et al., 1988; Arias and Mader, 2007, 2011; Sexson et al., 2012, Zanetti et al., 2019, Ahlberg et al., 2018, 2019).

Winchester and Morris (1956) studied the ratio of WI to DMI at varying temperatures to predict WI in beef cattle. They reported WI for beef animals based on the amount of feed they consumed at different temperatures. Their data suggest that WI of differing classes of cattle (i.e. mature cows, growing animals, mature bulls, etc.) is ultimately a function of DMI and ambient temperature. Water intake was constant up to 4.4^{o}C (Winchester and Morris, 1956) indicating a constant relationship between WI and DMI at thermal neutral conditions. Since WI generally increases and DMI generally

decreases in warmer months of the year, with the opposite relationship occurring in the cooler months of the year, the prediction of WI from DMI is not consistent (NASEM, 2016). Conversely, other researchers have found that WI increases with DMI (Murphy et al., 1983; Hicks et al., 1988; Loneragan et al., 2001) indicating that ambient temperature may not be as influential on WI as DMI. Diet quality and particle size (Martz, 1985) can also affect the relationship between DMI and WI.

Arias and Mader (2011) found that the primary factors that dictate daily WI for feedlot cattle are the average ambient temperature, temperature humidity index (THI), and minimum temperature after looking at mean ambient temperature, maximum and minimum temperature (°C), precipitation, relative humidity, wind speed, solar radiation, and THI. A relationship between ambient temperature and DMI does exist (NRC, 1981). However, since DMI is also influenced by breed, BW, body composition and frame size, in addition to management, diet type, and various environmental factors, the strength of the relationship between ambient temperature and DMI alone is questionable (Mader et al., 2010).

Sexson et al. (2012) evaluated variables similar to those of Arias and Mader (2011) and also found several environmental and temperature-related factors were related to WI, but DMI alone had minimal impact on daily WI. The relationship between WI and DMI in beef cattle is not fully understood, and the impact that environmental variables have on this relationship may differ between animals.

Breed, Body Weight, and Age

Winchester and Morris (1956) identified that *Bos taurus* breeds have higher WI in liters than *Bos indicus* cattle, especially as outside temperature increases. In 2011, Brew et al. further confirmed these results with Charolais x Angus cross steers consuming more water (42.8 kg; $P < 0.05$) than Brangus (30.8 kg), Angus x Brangus (30.8 kg), Charolais x Brangus (29.7 kg), Charolais x Romosinuano (20.7kg), and Brangus x Romosinuano (24.1 kg). Although differences were found in WI between breeds in this study, there were no differences in WI per kg of metabolic BW (MBW) between heifers, steers, and bulls. This indicates that BW and age can also play a complex role on water consumption.

Current estimates for beef cattle WI established in the NASEM (2016), from results of Winchester and Morris (1956), are based on animal BW and, consequently, age, as well as ambient temperature. Data collected by Winchester and Morris (1956) was completed over short periods of time with animals in individual pens. This has been criticized by some because of potential effects of individual housing on DMI and WI behavior (Da Haer and Mercks, 1992; Nielsen et al., 1995; Guiroy et al., 2001; Beatty et al., 2006). The average WI observed by Brew et al. (2011) on bulls, steers, and heifers, aligned with the range predicted by Winchester and Morris (1956) of 29.98 L ± 8.56 L/head/d. When adjusted for MBW, cattle drank an average of 0.38 L ± 0.11 L/ kg of MBW even though animals were from a large population with data collected in groups and for a long duration. Results of Sexson et al. (2012) agree with those of Winchester and Morris (1956) in that BW and MBW are important predictors of WI. Cattle weighing less than 500 kg show increased water consumption (from 22 to 38 liters per animal per

day or 0.04 to 0.08 L/kg of BW) as BW increases. Cattle that weigh greater than 500 kg show decreased WI as BW increases (Sexson et al., 2012). The decline in WI associated with increasing BW could be explained by the change in composition of gain, with an increasing proportion of fat and decreasing levels of protein and water (NRC, 2000). Little research has been completed focusing solely on the impact of age on WI; however, BW is highly confounded with age. The studies discussed in this section reflect this relationship between age and BW.

Seasonal Effects and Environment

Hoffman and Self (1972) examined the effects of seasonality on WI in feedlot cattle. They found approximately a 12-liter difference in WI by cattle with those fed in the summer consuming 31.2 liters of water, and those fed in the winter consuming only 19.0 liters. Hicks et al. (1988) reported an average daily WI of 35.9 liters per day during the summer months when animals were housed in confinement. Arias and Mader (2011) confirmed these two studies and reported that cattle finished during the summer drink 87.3% more water than cattle finished during the winter (32.4 liters vs 17.3 liters). This is explained by the primary way cattle reduce heat stress; through evaporative cooling (Morrison, 1983), which increases daily WI (Beede and Collier, 1986).

In the study of Hoffman and Self (1972), a portion of the cattle were provided with an overhead structure allowing airflow and shade. There was a tendency for cattle to consume less water (30.1 vs 32.6 liters) than cattle that did not have access to shade. However, this trend was not observed for cattle fed during the winter, with effect of access to shade (Hoffman and Self, 1972). Not all shade structures are created equal,

however, and those that are closed on multiple sides restrict airflow and would likely increase heat stress and WI (Mader et al., 2006).

Ittner et al. (1951) explored the effects of water temperature on WI in beef cattle. The study was conducted from June to September and included two groups of four animals each with three steers and one heifer per group. Calibrated water meters on pen waterers were used to detect water temperature. The cattle that drank water at 18ºC showed decreased WI compared to cattle that consumed water around 31.2ºC (58.14 liters vs 62.87 liters). Simply adding shade over pen waterers will aid in decreasing water temperature and heat stress in cattle presumably decreasing WI (Ittner et al., 1951).

Water Quality

Cattle regularly drink from ponds, streams, and lakes as well as groundwater sources like wells, where water quality consistency is lacking. However, very little research has been conducted on what components of water quality are most influential in impacting WI and how they affect cattle performance and well-being.

Research completed by Ray (1986) evaluated various levels of TDS in drinking water for feedlot cattle. He indicated that cattle drinking water containing 6,000 mg/kg had lower weight gains than those drinking "safe" water (1,300 mg/kg) when energy content of the diet was low and animals were experiencing heat stress. The effects of the high TDS water were negated during the winter months and when energy content of the diet was high.

According to NASEM (2016), water with a TDS of < 1,000 to 3,000 mg/kg has historically been deemed safe for beef cattle and no negative side effects should be expected other than potential initial diarrhea (NRC, 2001). From 3,000 to 5,000 mg/kg, diarrhea should be expected and intake may be lower. From 5,000 mg/kg and greater, water should be avoided especially for pregnant and lactating animals, but anything past 7,000 mg/kg should be avoided entirely (NRC, 2001). Further research is needed to substantiate these recommendations and address other factors that may have influence.

High levels of nitrates can cause nitrate poisoning which can lead to abortions, infertility, and other complications. Safe levels of nitrate-nitrogen (NO_3-N) should be less than 10 mg/kg, and levels of nitrate should be less than 44 mg/kg (NASEM, 2016). Finally, Suttle (2010) stated that the toxicity of saline water depends on the specific salts present, with NaCl being the least harmful, and the greatest effects coming from magnesium sulfates and carbonates. Conversely, publications such as Paiva et al. (2017) have stated that effects of saline water sources are due solely to the level of TDS without impact of whether the total dissolved salts consist of a simple salt or a complex (Boyles, 2009).

Intake of sulfate (SO_4) is often of concern as sulfur is required for rumen microbial fermentation (NASEM, 2016), but sulfur can also inhibit NDF digestibility at high concentrations (López et al., 2016). High concentrations can result in hydrogen sulfide (H_2S) gas production, leading to S-toxicity-induced polioencephalomalacia (Olkowski, 1997; Drewnoski et al., 2014). Sulfur is also known to interact with many trace minerals (Smart et al., 1986; NRC, 2000; Drewnoski et al., 2014) and can reduce their availability to the animal. Research has shown that cattle can be very tolerant of

water with SO_4 levels up to 2,500 mg/kg (Weeth and Hunter, 1971; Weeth and Capps, 1972; Digesti and Weeth, 1976; Grout et al., 2006). Weeth and Capps (1972) reported no reduction in WI by young cattle with SO_4 levels up to 2,814 mg/kg. However, feed intake was reduced at 2,814 mg/kg of SO_4 and growth rate was reduced at 1,462 mg/kg of SO_4 or higher. Weeth and Hunter (1971) found that concentrations of 3,493 mg/kg SO_4 reduced feed intake and resulted in weight loss in cattle. Other studies reported tolerances of cattle to concentrations of SO_4 up to 4,732 mg/kg, although WI was depressed by 20 percent at concentrations of 6,760 mg/kg or above (Embry et al. 1959).

Finally, where overall water TDS was at 7,000 mg/kg or greater and SO_4 concentration was 3,000-4,000 mg/kg, WI and feed intake were reduced (Weeth and Hunter, 1971; Digesti and Weeth, 1976; Loneragan et al., 2001; Grout et al., 2006). These levels of TDS and sulfate were confirmed by Lopez et al. (2014 and 2016) in growing cattle.

Influence of Water Quality on Feed Intake

As mentioned previously, the relationship between WI and DMI in beef cattle is not fully understood; however, it is clear from the literature how certain aspects of water quality affect feed intake. Sulfate is one of the most influential components of water that can affect WI. It can also affect DMI and has been demonstrated to be more negatively influential than all other components of water quality including but not limited to water hardness, pH, sodium, and nitrates.

Embry et al. (1959) found that beef cattle seemed tolerant of water concentrations of Na_2SO_4 up to 7,500 mg/kg. Conversely, when Weeth and Hunter (1971) introduced

animals to water at this level of Na_2SO_4, some animals refused to drink the water for 24 hours. Therefore, the study was restarted with the water level of 5,000 mg/kg Na_2SO_4. Over the summer, nine Hereford heifers, weighing an average of ~250 kg, were offered one of three *ad libitum* water treatments: tap water, 4,110 mg/kg NaCl water, or 5,000 mg/kg Na_2SO_4 water. Heifers were arranged in a 3x3 Latin square with three replicates per treatment. Heifers were also fed grass hay to achieve *ad libitum* intake for the duration of the trial. The length of each period was 30 d with the last 7 d for collection of feed and WI data. Heifers consuming the Na_2SO_4 treatment showed a 30 percent decrease in feed intake compared to heifers on tap water or salt water. As a consequence, those heifers lost weight while on trial as compared to heifers on the other two treatments, who gained weight. These results are conflicting with earlier research (Embry et al., 1959) where growing animals did not show signs of toxicity at 7,500 mg/kg of sulfate or at 10,000 mg/kg TDS (6,817 mg/kg Na_2SO_4).

Harper et al. (1997) conducted an experiment in Australia on the effects of drinking coal mine pit water with high mineral content on feed intake and health in beef steers. Steers were assigned to one of three treatments: control (~30 mg/L sulfate), 2,000 mg/L sulfate (4,000-6,000 mg/kg TDS), and 4,000 mg/L sulfate (8,600 mg/kg TDS). The diluted pit water (2,000 mg/L sulfate) did not compromise the animals' health or negatively impact performance. However, the high concentration of sulfate (4,000 mg/L) depressed DMI by 14 percent and WI by up to 40 percent although DMI increased slightly the longer the animals were on the treatment. They concluded that any water higher than 2,000 mg/L of sulfates could cause health complications to animals and should be avoided.

Patterson et al. (2003) reported data that showed growing steers receiving water with over 4,800 mg/kg TDS and 3,000 mg/kg of that TDS being sulfate caused a reduction in average daily gain (ADG), DMI, gain to feed (G:F) ratio, and WI. A year later, they repeated the study to identify at what threshold do these performance parameters begin to decline (Patterson et al., 2004). From May to September, 84 crossbred steers, averaging 290 kg each, were blocked by weight and randomly assigned to one of 12 pens with 7 steers per pen. Pens were randomly assigned to one of four water treatments (3 pens/treatment) based on targeted TDS concentrations (mg/kg): 1) 1,000 (average = 1,226 mg/kg TDS; 441 mg/kg sulfates); 2) 3,000 (average = 2,933 mg/kg TDS; 1,725 mg/kg sulfates); 3) 5,000 (average = 4,720 mg/kg TDS; 2,919 mg/kg sulfates); and 4) 7,000 (average = 7,268 mg/kg TDS; 4,654 mg/kg sulfates). Water was sourced from natural wells. Steers were fed a diet of ground grass hay and wheat middlings. Average daily gain, DMI, and G:F declined quadratically with increasing TDS and sulfate levels in the water. Water intake declined linearly with increasing TDS and sulfates. As water with 7,268 mg/kg TDS and 4,654 mg/kg sulfates caused marked reductions in steer performance and health, they concluded that animal performance should be expected to decline with increased sulfates in water. Furthermore, water with greater than 3,000 mg/kg of sulfate could potentially cause death.

Lopez et al. (2016) conducted two experiments to evaluate the impact of early life exposure to high salt water on later cattle performance on saline water. In experiment one, 24 cow-calf pairs were randomly assigned to a high saline treatment (HSW: 7478 mg/kg TDS; 3,103 mg/kg sulfate) or a low saline treatment (LSW: 512 mg/kg TDS; 146 mg/kg sulfate), although they found that their target TDS measures were consistently

higher throughout the experiment. All pairs were fed alfalfa hay to achieve *ad libitum* intake for 60 days until the calves were weaned. The calves were then fed a total mixed ration (TMR) during the last 45 d of the exposure period. They found that during the last 45 days, animals on the HSW treatment showed a 10 percent decrease in DMI and a 22 percent decrease in WI compared to those on the LSW treatment. For experiment two, 24 pregnant heifers were assigned to either LSW or HSW treatments. The concentration of TDS in the HSW treatment was 10,827 mg/kg of which 146 mg/kg was sulfate. Values stayed the same in experiment one for the LSW treatment. The exposure period lasted until the heifers' calves were 3 months old. These calves continued to drink LSW for 95 days and then HSW for 30 days. During the last 30 days, no differences between treatments were identified for DMI and WI. This may be a result of animals not being exposed to HSW for long enough time. No results were reported for mature cow or heifer intakes in either experiment.

Most recently, Penner et al. (2020) conducted a study at the University of Saskatchewan with 16 Hereford-cross heifers using a randomized complete block design with four treatments: 1) 0 mg/L sodium sulfate (NaS), 2) 1,000 mg/L NaS, 3) 2,000 mg/L NaS, and 4) 3,000 mg/L NaS. They found that as NaS water concentration increased, DMI increased initially and then decreased similar to findings by Loneragan et al. (2001) but water quality did not affect ADG or final BW. Penner et al. (2020) concluded that sulfate concentrations above 2,000 mg/L may decrease DMI which would have a significant effect on long term performance.

Influence of Water Quality on Diet Digestibility

While several studies have been conducted on the effects of water quality on WI and DMI, fewer have included a diet digestibility component. This section will include information available on the effect of water quality on diet digestibility in cattle as well as goats.

Lopez (2016) measured diet digestibility of calves consuming LSW or HSW. In the first experiment, calves exposed to the HSW treatment showed no negative effects on total tract digestibility. Calves consuming a TMR ration and either LSW or HSW had a feed digestibility of ~ 73 percent. In the second experiment, similar results were observed with no differences in feed digestibility between LSW and HSW calves. No specific diet component digestibilities such as neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), or ether extract (EE) were reported. However, 2 years before Lopez et al. (2014) reported a reduction in total tract digestible organic matter intake (DOMI) when steers consumed water containing 6,473 mg/kg TDS with 2,026 mg/kg sulfate compared with steers consuming water containing 786 mg/kg TDS.

A digestibility trial conducted in Brazil of Alves et al. (2017) on 24 Red Sindhi heifers (*Bos indicus* dairy breed) showed results similar to Lopez et al. (2016). These heifers were blocked by weight (~ 200 kg at the start of trial) and were randomly assigned to four treatments of: 1) 640 mg/L TDS, 2) 3,187 TDS, 3) 5,740 TDS, and 4) 8,326 TDS, with the target TDS achieved by adding NaCl to drinking water. Heifers were housed in individual pens and were given a TMR *ad libitum* while consuming water *ad libitum*. The heifers were given 15 days to adapt to treatments followed by a 5-day

collection period for DMI, WI, and apparent fecal output. Fecal output was determined by the internal marker, LIPE™. Water intake increased in proportion to the TDS inclusion rate. Dry matter intake, ADG, feed to gain (F:G), and digestibility were mainly unaffected by the TDS inclusion rates. Heifers consumed DM within the limits recommended by the NRC (2001). However, intake and digestibility of NDF were affected when TDS increased. Neutral detergent fiber intake and digestibility was reduced by 30 percent for heifers drinking treatment one and by 10 percent for heifers drinking treatment four. It is presumed that the increase in WI of these animals caused the decrease in intake and digestibility of NDF. With higher WI, physical distension of the reticulo-rumen may have occurred decreasing the rates of fiber removal from these compartments (Mertens 1987; Dado and Allen 1995).

Tsukahara et al. (2016) conducted a study with 20 Boer goats and 20 Spanish goats using various levels of brackish water to determine the effects on DMI, WI, and digestion among other parameters. Both breeds were divided into five groups with four animals per group, and one group assigned per treatment for a 2 x 4 factorial design. Treatments were 100 percent brackish water, 67 percent brackish water, 33 percent brackish water and a control treatment of solely fresh water while animals consumed grass hay *ad libitum*. Animals were adapted to each treatment for 14 days before a 5-day total DMI, WI, and fecal collection period. Total tract digestibility of organic matter decreased for all treatments containing brackish water. Trends for similar differences in digestion of feed DM, total DM, and gross energy were also detected; however, effects of brackish water on digestibility of nitrogen and NDF were numerical.

Influence of Water Quality on Blood Parameters

Blood mineral composition may be difficult to interpret, particularly related to consumption of water with high concentrations of contaminants. However, it is generally accepted the best way to identify the status of many trace minerals is through liver biopsy (Penner et al., 2020). Osmolality can be used to reflect the balance between electrolytes in the blood and whether or not the animal is experiencing anhydremia. Packed cell volume (PCV) or hematocrit, hemoglobin levels, methemoglobin levels, and oxygen levels in the blood can be significant health indicators of animal well-being.

Previously mentioned research conducted by Weeth and Hunter (1971) on nine Hereford heifers consuming one of three water treatments (tap water, 4,110 mg/kg NaCl-water, or 5,000 mg/kg Na_2SO_4 water) with three replicates per treatment examined blood parameters to assess animal health. Blood samples were taken during the trial although time of sampling was not given. No differences in total hemoglobin concentration were noted; however, heifers consuming sulfate water had a 450 percent increase in methemoglobin concentration. As the NaCl treatment had no effect on methemoglobin, the authors concluded that the sulfate ion was involved in the increased formation of methemoglobin. Wintrobe (1967) established that no symptoms of hypoxia occur in cattle until methemoglobin comprises more than 20 percent of total hemoglobin, which is much larger a percentage than seen in the heifers on this study. An observation in humans (Harris et al., 1968) showed that there is a significant reduction in oxygen transport when methemoglobin comprises 7.6 percent of total hemoglobin. However, in the work of Weeth and Hunter (1971) non-functional hemoglobin was well below this threshold.

Blood was sampled and subjected to various analyses in the two studies of Lopez et al. (2016), with high saline water treatments 7,478 mg/kg TDS with 3,103 sulfur in Experiment 1 and 10,827 mg/kg TDS with 146 mg/kg sulfur in Experiment 2 compared with fresh water. Samples were collected at the beginning of the experimental period and again 30 days later. In the first experiment with 24 calves, there was no treatment x day interaction for hemoglobin, PCV, plasma glucose, or mineral serum concentrations except for Na. None of the blood variables were affected by treatments, except for Na on day 0. However, concentrations of glucose and PCV were numerically higher at the end of the trial, and serum concentrations of Ca, P, Mg, and K were higher at the beginning of the trial for both high-TDS treatments relative to control. For experiment two, there were no treatment x day interactions for hemoglobin, PCV, plasma glucose or serum mineral concentrations except for Mg. None of the blood variables were affected by either of the two treatments, but there was a day effect as hemoglobin, PCV, and serum concentration of Na, Ca, and K were higher at the end of the experiment. Previous research (Harper et al., 1997) reported results similar to Lopez et al. (2016). Harper et al. (1997) found no significance or effect of coal mine water (8,600 mg/kg TDS with 4,000 mg/kg sulfate) compared with PCV, hemoglobin, and other hematological indices. Results on macromineral levels found in the Harper et al. (1997) and Lopez et al. (2016) were reported in the study of Penner et al. (2020) with micromineral serum concentrations (magnesium, manganese, iron, cobalt, zinc, and molybdenum), except copper (Cu) and selenium (Se), being unaffected by water sulfate concentration. The level of Cu in the blood linearly decreased with increasing water sulfate concentration potentially indicating a negative interaction between Cu and sulfate. Even though trace mineral

serum concentrations may not be the most sensitive indicator of whole-animal Cu status as compared with liver Cu levels (Claypool et al., 1975), the decline in serum Cu may still indicate reduced whole-animal Cu levels (Van De Weyer et al., 2011). Nevertheless, Cu concentrations were within normal range (Puls, 1994) throughout the experiment. Serum Se was affected quadratically, increasing first and then decreasing as water sulfate concentration increased.

Conclusion

Water availability is expected to continue to be a common challenge in animal agriculture as the demands of agriculture increases and the climate continues to change. Water scarcity and quality are expected to continue to decline due to increased salinization, chemical contaminants, heavy metal contamination, and biological contamination (Nardone et al., 2010). Understanding how livestock can utilize contaminated water efficiently without sacrificing animal health or performance is not well known especially in beef cattle. Furthermore, it is equally important to measure how ingesting poor-quality water can affect WI, feed intake, and diet digestibility in order to forecast production implications in the future.

If the water is concentrated enough in sulfur and TDS, growing steers and heifers as well as finishing cattle have shown reductions in feed and water consumption. No research has been completed on mature beef cows or beef cows in a state of lactation or gestation. It is difficult to ascertain the long-term implications of this, but certainly, it could affect proper body condition, fetus growth, maturity, animal health, and productivity.

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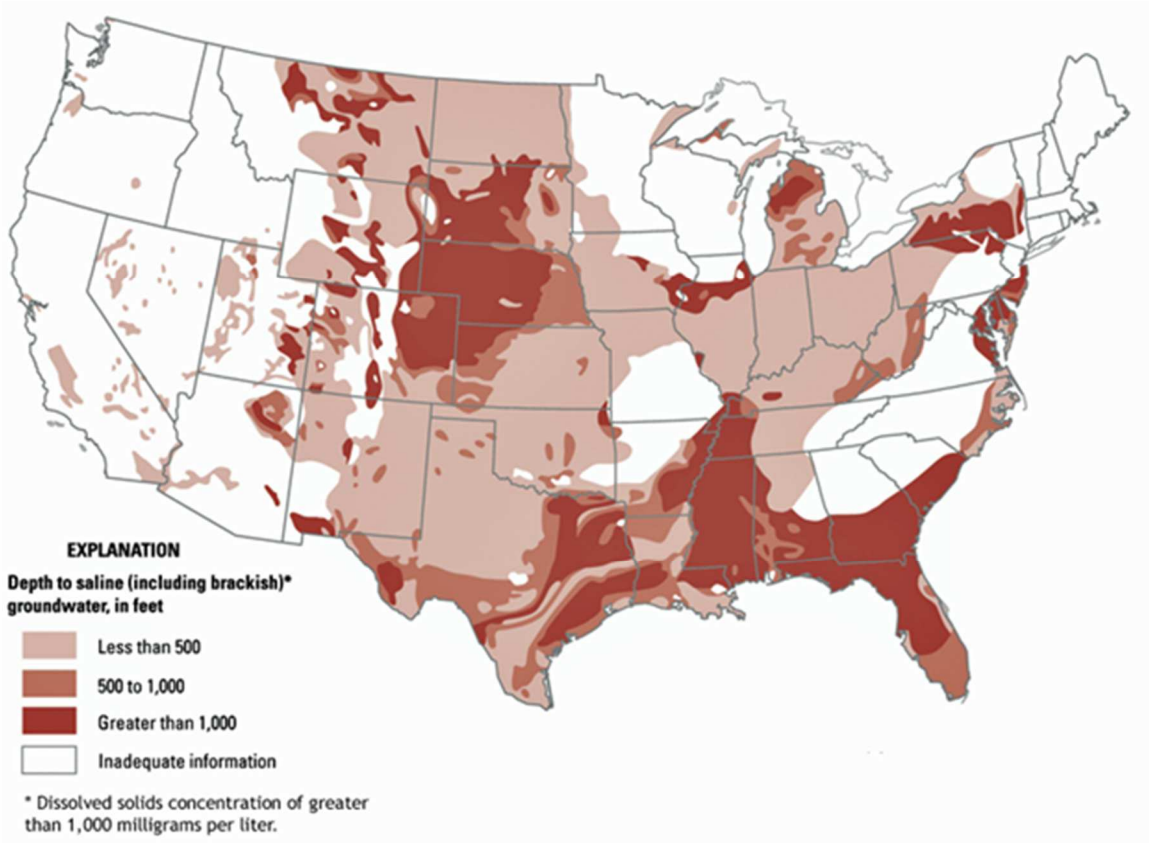
Figure 1. Depth to saline and brackish water in the USA.



CHAPTER II

Running head: Effects of water quality on cows and heifers

Water and forage intake, diet digestibility, and blood parameters of beef cows and growing heifers consuming water with varying concentrations of total dissolved salts

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ABSTRACT:

As water becomes a more limited resource, efficient water utilization is needed to continue animal production during water scarcity and declining water quality. The objective of this study was to investigate the effects of water quality on water intake (WI), forage intake, diet digestibility, and blood constituents in beef cows and growing beef heifers. The experimental design was two simultaneous 5 × 5 Latin squares with five drinking water treatments within each square: 1) control (fresh water-FRW); 2) brackish water (100 BRW treatment) with approximately 6,000 mg/kg TDS; 3) same TDS level as 100 BRW achieved by addition of NaCl to fresh water (100 SLW); 4) 50% brackish water and 50% fresh water to achieve approximately 3,000 mg/kg TDS (50 BRW); and 5) same TDS level as 50 BRW achieved by addition of NaCl to fresh water (50 SLW). Each of the five 21-d periods consisted of 14 d of adaptation and 5 d of data collection. Animals were housed individually, fed alfalfa cubes and provided one of the five water sources for ad libitum feed and WI. Feed and water intake were recorded daily, with recorded WI adjusted for vaporization. The PROC MIXED procedure of SAS 9.4 was used for data analysis where animal was the experimental unit. Age, treatment, and age x treatment were fixed effects, and animal ID within age was the random variable for intake, digestibility, and blood parameter data. Significance was declared at $P \leq 0.05$, and a tendency was declared at $P > 0.05$ and $P \leq 0.10$. Compared to previously published data, water and feed intake were extremely elevated regardless of age or water treatment. No treatment x age interactions were identified for WI ($P = 0.71$), WI expressed as g/kg body weight (BW; $P = 0.70$), or dry matter intake (DMI; $P = 0.21$). However, there was an age x treatment tendency for DMI when scaled to BW ($P = 0.09$) in cows consuming 100 BRW compared to fresh water.

No differences were found for the other three treatments. Heifers consuming 50 SLW showed a significant difference ($P < 0.05$) for lower feed intake (g/kg BW) compared to fresh water and 100 BRW. No statistical differences ($P > 0.05$) in water, feed intake or diet digestibility were found due to water quality treatment. In conclusion, under these conditions neither absolute WI, absolute feed intake, nor diet digestibility were influenced by the natural brackish or saline water used in this experiment. These results suggest that further research is necessary to determine thresholds for TDS or salinity concentration resulting in reduced water and/or feed intake and diet digestibility.

Key words: beef cattle, digestibility, feed intake, water quality

INTRODUCTION

In many parts of the U.S., rates of groundwater withdrawal are greater than those of recharge, resulting in decreased groundwater supplies, lower stream and lake levels, and/or land subsidence, with an increased reliance on water other than fresh (Masters et al., 2005; El Shaer, 2010). Low rainfall can cause an increase in TDS in the soil and groundwater (Yousfi et al., 2016), potentially affecting the cattle drinking the groundwater. Understanding how the quality of water available for drinking can impact water consumption and efficiency of livestock production is critical for current and future livestock production enterprises as well as the safety and security of the food supply. One characteristic of water quality is the concentration of total dissolved or soluble salts (**TDS**), often termed as the level of salinity. Drinking water with TDS above 1,000 mg/kg (*i.e.*, mg/L) is termed 'saline,' and brackish water usually refers to water with TDS between 1,000 and 10,000 mg/kg but can be much higher (USGS, 2013; Stanton et al., 2017; Stanton and Dennehy, 2017).

Cattle frequently consume water moderate to high in TDS globally. Brackish and saline ground water sources are widespread, including in countries like Egypt (Assad and El-Sherif, 2002), Australia (McGregor, 2004), India (Sharma et al., 2017), Tunisia (Yousfi et al., 2016), Brazil (Castro et al., 2017), Asia, the eastern Mediterranean, Africa (Masters et al., 2005), and most of the central U.S. (Androwski et al., 2011; USGS, 2013).

Because of the increasing importance of drinking water with high TDS likely in the foreseeable future, it is important to gain an understanding of factors affecting the utilization of brackish/saline water by ruminant livestock. If adverse effects of consumption of drinking water moderate to high in TDS are documented, management

practices could be altered to lessen the effects. Moreover, differences among species exist, such as generally greater salt tolerance by goats than sheep and older than young small ruminants (McGregor, 2004) and perhaps lower tolerance of cattle versus goats and sheep (Squires, 1988; SCA, 1990). However, little research has been conducted in beef cattle.

Apart from the TDS level in water consumed by ruminants, estimates of the amount of fresh water required and consumed by ruminant livestock species and methods of prediction vary considerably. While more recent data is available for growing (Brew et al., 2011) and finishing (Arias and Mader, 2011; Sexson et al., 2012, Zanetti et al., 2019, Ahlberg et al., 2018 and 2019) cattle, the latest data available for estimating fresh water requirements for mature beef cows was published in 1956 (Winchester and Morris, 1956). The NASEM (2016) recommendation of the water requirement of beef cows was based on these data with 900-pound cows despite today's mature beef cows averaging between 1,075 (Walker et al., 2015) and 1,400 pounds (McMurry, 2008). Therefore, the objective of this study was to determine the effects of age and BW of beef cattle and water quality on water intake (WI), feed intake, digestion, and blood constituent concentrations.

MATERIALS AND METHODS

All procedures for animal use were approved by the Oklahoma State University Institutional Animal Care and Use Committee (AR-18-RS-270). This experiment was conducted at the Nutrition and Physiology Research Center (NPRC) of Oklahoma State University (Stillwater, OK, USA).

Animal and diet management

The experimental design was two simultaneous 5 × 5 Latin squares using cows and growing heifer calves similar to the design employed by Yirga et al. (2018) as described by Snedecor and Cochran (1967). Each of the five periods consisted of 14 d of treatment adaptation and 5 d of data collection (Harris, 1970; Kaufmann et al., 1980; Van Soest, 1982; Merchen, 1988). Five Angus cows and five Angus growing heifers were alternatingly placed and housed in individual 2.44 m x 4.73 m partially-enclosed concrete stalls with rubber mats covering the slats over a manure pit to ensure total fecal collection and improve animal comfort (Horn et al., 1954). Temperature (ºC) and relative humidity (%) were subject to environmental temperatures from September to December 2019 (Table 1).

Both alfalfa cubes (Table 2) and water (Table 3) were offered *ad libitum*. The level of feeding was approximately 120% of consumption of the preceding day's intake. Feed and water were delivered once daily at 0700 with additional feed and water delivered in the afternoon as needed to ensure *ad libitum* intake.

Brackish water was brought from a well of the American Institute for Goat Research at Langston University (Langston, OK, USA) as needed and was stored in a large water tank at the research facility. Water treatment mixtures were made every other week or as needed and stored individually in clearly labeled intermediate bulk containers (IBC), including fresh water (FRW), so that conditions were similar among water treatments. Conductivity, pH, TDS, and salinity were measured with a Pocket Pro+ (Hach, Loveland, CO) during mixing treatments to ensure consistency. Before delivery to

animals, water treatments were stirred to ensure no solids stayed separated at the bottom of the tank. During the collection period, 19-liter buckets of water for each treatment were set near the stalls and weighed at the beginning and end of the collection period to account for water vaporization. A mineral block was offered free choice for cattle (American Stockman, Stillwater Milling Co., Stillwater, OK, USA). The mineral block contained between 96 and 99% NaCl, 2,400 ppm of manganese (Mn) and iron (Fe), 260-380 ppm of copper (Cu), 320 ppm of zinc (Zn), 70 ppm of iodine (I), and 40 ppm of cobalt (Co). Animals were weighed at four hours post feeding every 3 wk.

Feed and fecal collection

Feed was sampled daily during the collection period and composite samples were formed for each week. Feed was dried over a 72-h period in forced-air drying ovens at 55°C or until no weight change was detected.

Total excretion of feces was determined over a 5-day collection period with total fecal material manually gathered at 0700 h and 1900 h each day. A pooled 10% sample of the daily excretion of feces was used to form composite samples, once for 5% at 0700 h and once for 5% at 1900 h each collection day. Samples were immediately dried in a forced-air oven at 55°C for 72 h or until no weight change in the sample was detected. Samples were mixed two to three times daily for even drying. Samples were stored in re-sealable plastic bags to avoid moisture before and after grinding. All samples were ground to pass through a 1-mm screen with a Fritsch Pulverisette 19 Cutting Mill (Markt Einersheim, Germany). Once ground, feed samples were pooled to create a single feed sample per animal for periods 1-3 and periods 4-5 according to two shipments of feed for

a total of 20 samples. Fecal samples were pooled to create one fecal sample per animal per period for a total of 50 samples.

Blood collection

Blood samples were collected via coccygeal venipuncture or jugular venipuncture if the latter was not reliable at 4 h post feeding on day 14 and the last day of each period. A portion of blood was collected in 5-mL tubes coated with sodium fluoride and potassium oxalate, another portion of blood was collected in 9-mL tubes coated with sodium heparin, and a third portion was collected in 10-mL dry tubes for serum. Immediately after sampling, whole blood in green-top tubes with sodium heparin was used to determine packed cell volume (PCV) with a Micro-hematocrit centrifuge (PSS Select; Model DSC-030MH) and hemoglobin, oxygen saturation, and Met- and CO-hemoglobin with an OMS3 Hemoximeter (Radiometer America, Westlake, OH, USA). Plasma was collected from sodium heparin coated tubes after centrifuging for 20 min at approximately 3,000 × *g* at 10 °C and was used to determine osmolality with a model 2020 Osmometer (Advanced Instruments, Inc., Norwood, MA, USA). Serum and plasma were frozen at -20°C, and plasma from tubes with sodium fluoride and potassium oxalate was thawed and used to determine glucose and lactate concentrations with a YSI 2300 Plus Glucose & Lactate Analyzer (YSI Inc., Yellow Spring, OH). Thawed serum was analyzed for albumin (ALB), alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatine kinase (CK), gamma-glutamyl transferase (GGT), calcium (Ca), total cholesterol (CHOL), chloride (CL-), creatinine (CREAT), glucose, lactate, magnesium (Mg), potassium (K), sodium (Na), total triglycerides (TRIG), and blood urea nitrogen (BUN) with a Vet Axcel Chemistry

Analyzer (Alfa Wassermann Diagnostic Technologies, West Caldwell, NJ) according to manufacturer's instructions.

Feed and fecal analysis

All ground samples were analyzed for analytical dry matter (DM) by drying at 105 °C for 24 h. Following DM analysis, feed and fecal samples were combusted at 500 °C for at least 5 h to determine the ash content. Organic matter (OM) content was calculated by subtracting ash % from 100. Samples were also analyzed for acid detergent fiber (ADF) and neutral detergent fiber (NDF), with amylase and sodium sulfite used during NDF determination (ANKOM, 2020; methods 12 and 13). Acid detergent insoluble ash (ADIA) was measured as the residue from ADF after ashing at 500ºC for at least 5 hours (Van Soest et al., 1991). Crude fat (CF) was determined using ANKOM (2020; solvent extraction method for XT^{15}) with ether extraction (EE, $ANKOM^{XT15}$ Extractor). Total nitrogen (N) was analyzed by the Soil, Water, and Forage Analytical Laboratory at Oklahoma State University, and crude protein (CP) was determined by multiplying N values for each sample by 6.25. Both feed and fecal samples were analyzed simultaneously to avoid potential run error. Apparent total tract digestibility of DM (DMD) was based on feed DM intake and fecal DM output. Organic matter digestibility (OMD) was based on feed OM intake and fecal DM output. Intake of digestible OM (DOMI) was calculated by multiplying OM intake (OMI) by OM digestibility and is reported as g/kg BW.

Statistical analysis

Data collected for WI, DM intake (DMI), body weight (BW), blood constituents, total fecal output, digestibility, and water components were analyzed using the MIXED procedure in SAS (SAS 9.4; SAS Institute Inc., Cary, NC). Data were analyzed as a side by side 5 x 5 Latin square where animal was the experimental unit. Animal within age was used as a random variable for intake, digestibility, and blood parameters data, while week and load were used as the random variable for analyzing water components and diet components, respectively. Period was used as a repeated measure for all analysis, and animal within age was the subject for intake, digestibility, and blood variable analysis. For analysis of intake, digestibility, and blood constituents, treatment means were separated using least square means and were analyzed for age x treatment interaction. For analysis of water and diet components, treatment means were separated using least square means and were analyzed for treatment or load interactions, respectively as treatment x age interactions were irrelevant. Significance was declared if $P \leq 0.05$ and tendencies were declared at $P > 0.05$ and $P \leq 0.01$.

RESULTS AND DISCUSSION

Water composition

Means for water treatment chemical composition and electrical conductivity are presented in Table 3. Criteria used to classify water quality include: organoleptic properties (odor and taste), physiochemical properties (TDS, pH, hardness, and oxygen), toxic compounds, excess mineral compounds, and bacteria (NASEM, 2016). The United States has set National Primary and Secondary Drinking Water Standards for humans to

ensure safe, agreeable drinking water. Water should have a pH between 6.5 to 8.5, less than 500 mg/L TDS, and less than 1 mg/L of nitrites and 10 mg/L of nitrates, among other components. The fresh water (control) used on this experiment fell within both primary and secondary water standards with chlorine falling between the primary and secondary standards (4mg/L maximum residual disinfectant level goal; 250 mg/L maximum contaminant level).

Brackish water is defined as water that contains 1,000 to 10,000 mg/L of TDS and can be classified as one of four groups (USGS, 2013; Stanton et al., 2017). Water group definitions of Stanton et al. (2017) are Group 1: sodium-bicarbonate-dominant water type in which sulfate contributes about one-third of the total anion equivalents; Group 2: calcium-sulfate-dominant water type in which sodium and magnesium each contribute about one-quarter of the total cation equivalents; Group 3: sodium-chloride-dominant water type and has a high mean concentration of TDS (8,440 mg/L); and Group 4: mixture of cations and anions with low TDS (1,360 mg/L) and a high percentage of silica (1.7 percent of the total moles of cations and anions). In this experiment, the 100 BRW treatment would be classified in Group 3. Additionally, treatment silica concentrations were much lower in this experiment than suggested for Group 4.

Water with a TDS of < 1,000 to 3,000 mg/kg has historically been deemed safe for livestock consumption and no negative side effects should be expected other than potential initial diarrhea (NRC, 2001). Stanton et al. (2017) defines fresh water or safe water to be < 1,000 mg/L. From 3,000 to 5,000 mg/kg, diarrhea should be expected and intake may be suppressed, inhibiting maximum performance (NASEM, 2016). From 5,000 mg/kg and greater, water should be avoided (NRC, 2001) for pregnant and

lactating animals. Anything beyond 7,000 mg/kg should be avoided entirely (NRC, 2001). Therefore, the 100 BRW and 100 SLW treatments would be considered "unsafe" for livestock according to these guidelines (NRC, 2001). Similarly, according to these guidelines, the 50 BRW and 50 SLW would be considered safe for livestock. Hardness is defined as the sum of calcium and magnesium reported in addition to other cations such as zinc, iron, selenium, etc. (NASEM, 2016). According to water hardness guidelines in NASEM (2016), hardness (mg/L) from 0-60 is considered soft, from 61-120 mg/L is considered moderately hard, from 121-180 mg/L is considered hard, and ≥ 181 mg/L is considered "very hard". Both brackish water treatments employed in this experiment would be classified as "very hard".

All water treatments were below nitrate-nitrogen (NO_3-N) standards recognized as safe for beef cattle (less than 10 mg/kg; NASEM, 2016). Sulfur can be detrimental to water and feed intake and general recommendations are less than 500 mg/L for calves and less than 1,000 mg/L for adult cattle. Water sulfur concentration means for both the 50 BRW and 100 BRW treatments were well beyond these recommended maximum concentrations although the remaining three treatments were similar in sulfur concentration and well below these recommendations. Additionally, concentrations of sodium, calcium, magnesium, chlorine, and level of electrical conductivity aid in classifying both 50 BRW and 100 BRW as brackish. Both treatments were within normal range of pH and residual carbonates were negligible.

Boron was considered at a toxic level (above 5 mg/L) for the 100 BRW treatment (EPA, 1997). Sodium chloride-based treatments (50 SLW; 100 SLW) contained high concentrations of sodium and chloride, as expected; however, no other concentrations

were abnormally high with the exception of electrical conductivity.

Water intake, feed intake, and digestion

Least square means for WI, feed intake and feed component digestibility are shown in Table 4. At 120 g/kg BW, water consumption for cows in this study was high compared to current predicted WI (Spencer et al., 2017) for mature cows and previous observed WI (Winchester and Morris, 1956) for mature cows. However, Spencer et al. (2017) estimated WI requirements for non-lactating cows consuming 22 g/kg BW in DMI compared to this study's 36 g/kg BW in DMI. Winchester and Morris (1956) suggested a linear relationship between DMI and WI, which could account for a portion of the increased daily water consumption observed in this study.

Similarly, WI in this study was high compared to previous reports for growing cattle. Sexson et al. (2012) reported growing steers to consume 21 g/kg BW in DMI of a TMR and 79 g/kg BW in WI with an average temperature of 21.96 ºC. Arias and Mader (2011) found heifers consuming 21 g/kg BW in DMI consumed 72 g/kg BW in WI with average ambient temperature of 21.4 ºC. With heifers consuming twice as much DMI g/kg BW in this experiment at an average temperature of 13.6 ºC, it is not surprising to see WI g/kg BW to be double if WI and DMI have a constant relationship suggested by Winchester and Morris (1956). Winchester and Morris (1956) also reported WI to be constant up to 4.4 ºC. Other researchers have also reported a constant relationship between DMI and WI (Murphy et al., 1983; Hicks et al., 1988; Loneragan et al., 2001). Additionally, animals on a similar high-quality diet of alfalfa hay (Williams et al., 2018) consumed an average DMI of 34.0 g/kg BW and 35.0 g/kg BW, which is very close to

the DMI consumption seen in this study. Diet quality could be another possible explanation for the large WI seen in this study.

As expected, heifers consumed less water on an absolute basis (kg/d) than did cows. However, heifers consumed 24.2 g/kg BW more water ($P = 0.0063$) compared to cows. This finding is in agreement with previous research of Sexson et al. (2012). Cattle weighing less than 500 kg showed increased water consumption (22 to 38 liters per animal per day) as BW increased. Cattle that weighed greater than 500 kg showed decreased WI as BW increased (Sexson et al., 2012). The decline in WI associated with greater BW could be explained by the change in composition of gain, with an increasing proportion of fat and decreasing levels of: protein and water (NRC, 2000).

Daily consumption of 50 BRW and 100 BRW did not differ ($P = 0.97$). However, there was a tendency ($P = 0.1$) for a treatment effect. Cattle consuming either the 50-BRW or 100 BRW treatments drank less than cattle consuming either fresh water or 100-SLW ($P < 0.05$). Patterson et al. (2004) also described lower consumption of brackish water containing ~ 3,000 mg/kg TDS up to ~ 7,000 mg/kg TDS compared to water with ~1,000 mg/kg TDS. There is no other published literature comparing consumption of brackish and salt water in cattle.

Like WI, consumption of alfalfa cubes was copious in this experiment with an average of 38 g/kg BW. For example, in a recent experiment using Angus cows from the same herd, nonlactating cows consumed 28 g/kg BW of a grass hay and molasses-based liquid supplement diet (55.7% TDN; Andresen et al., 2020). Williams et al. (2018) also reported copious feed intake when lactating Angus beef cows consumed alfalfa cubes

(34-35 g/kg BW, DM basis). As expected, cows consumed more DM, OM and digestible OM, kg/d ($P < 0.001$) than heifers. However, heifers consumed more feed DM than cows when expressed per unit of BW ($P = 0.02$).

Cows consuming 100 BRW showed a significant difference ($P = 0.05$) for lower feed intake compared to fresh water, and no differences were found for the other three treatments. Heifers consuming 50-SLW showed a significant difference ($P < 0.05$) for lower feed intake compared to fresh water and 100-BRW. The NRC (2005) estimates 1 g of NaCl/kg of BW can be consumed by ruminants with no effect on feed intake. Animals consuming the 100 SLW treatment averaged 1.86 g of NaCl per 1 kg of WI (0.19 g/kg BW) and 1.39 g of NaCl per kg of WI (0.13 g/kg BW) while on the 100 BRW treatment. Additionally, the highest sulfur level was found to be 1985 mg/kg in the 100 BRW treatment which is below the threshold (2,500 mg/kg) reported to cause a reduction in feed intake in several experiments (Weeth and Hunter, 1971; Weeth and Capps, 1972; Digesti and Weeth, 1976; Loneragan et al., 2001; Grout et al., 2006; Lopez et al., 2014, 2016). There was no difference among water treatments for OMI.

Neither age nor water treatment affected DOMI, DMD, OMD, NDF, ADF, or EE ($P = 0.17$). Similar results were reported by Lopez et al. (2016). Alves et al. (2017) recorded a decrease in NDF digestibility when cattle consumed water containing ~ 3,000 mg/kg TDS to 8,000 mg/kg TDS. Nevertheless, under these conditions, up to 5,878 mg/kg TDS did not influence diet or component digestibility. An age effect was found for CP digestibility ($P = <.0001$) for heifers to have greater CP digestibility than cows. Martz (1985) established that processing forage like the alfalfa cubes used on this study will

increase voluntary intake and depress digestibility due to increased passage rate. All digestibility components follow this pattern except for CP, and the reason is not clear.

Serum mineral concentrations, blood metabolites, and hematological indicators

Least squared means for blood constituent concentrations are shown in Table 5. Concentrations of Cl, Ca, K, Mg, and Na, were all within normal range for cattle (Fielder, 2015) although, according to Zelal et al. (2017), Mg concentrations were bordering on suspicion of hypomagnesemia in heifers at 1.96 mg/dL. However, Harvey and Bruss (2008) consider 1.8 mg/dL to be low and close to or below 1 mg/dL being common with no clinical signs. An age effect was found for Ca ($P = 0.007$) with cows having lower serum Ca than heifers. An age x treatment interaction ($P = 0.03$) was found for blood Mg concentration. Heifers consuming 50 SLW and 100 SLW had lower serum Mg, whereas water treatment did not influence serum Mg concentration in cows. An explanation for this interaction is unclear. Perhaps the slightly elevated K concentration in the SLW treatments resulted in reduced Mg absorption (Zelal et al., 2017). Neither age or treatment effects nor age x treatment interactions were found significant for serum Na concentration.

Heifers had lower ($P = 0.03$) serum ALB concentration compared to cows. However, according to Harvey and Bruss (2008), ALB concentrations were within normal range for cattle (3.0-3.55 g/L). The difference in ALB concentrations between heifers and cows is not considered to be biologically significant.

A trend ($P = 0.09$) for age x treatment interaction was found for hepatic ALT although ALT concentration was within normal range for cattle (11-40 U/L; Ingvartsen,

2006, Fielder, 2015) and thus, the interaction was not deemed to be biologically significant. The main and interaction effects were not significant for hepatic AST. Nevertheless, AST concentrations were lower than the normal range reported for cattle (78-132 U/L; Ingvartsen, 2006, Fielder, 2015). High activity for hepatic AST and ALT is most often indicative of acute or chronic liver disease. Increased serum AST activity is considered a sensitive marker for identifying liver damage, even if the damage is subclinical (Kauppinen, 1984; Meyer and Harvey, 1998). In contrast, ruminant liver cells do not show high ALT enzyme activity, and increased serum activity from liver damage, even in necrosis, is insignificant (Forenbacher, 1993). The most sensitive marker to diagnose acute liver damage is ALT, while AST is more sensitive in reflecting the degree of damage (Kew, 2000).

An age effect for ALP ($P = 0.0003$) was found with heifers having over twice the concentration (92.1 U/L) of cows with the normal range reported between 7-43 U/L (Putnam et al., 1986). Often, there is a linear relationship between activity of serum ALP and GGT in cholestatic liver injury (Meyer, 1983). Although GGT concentrations in this study are not increased as dramatically as ALP, they are still elevated overall. This relationship is seen in Table 5 with GGT overall being slightly above the normal range for cattle (Fielder, 2015). Greater serum ALP concentration is often observed in normal growing or adult animals with increased osteoblastic activity (Sun et al., 2015). Higher serum ALP concentration was also observed in young beef calves compared to their dams (Hidiroglou and Thompson, 1980). The skeletal growth of calves is the primary site of ALP activity and likely contributes most of the difference documented in this experiment (Moog, 1946). Hidiroglou and Thompson (1980A) also suggested increased

concentrations of serum ALP in young animals were the result of bone growth and development.

An age effect ($P = 0.0016$) was found for CK with heifers having higher serum concentrations than cows, but CK levels were within the normal range for both heifers and cows (0-350 U/L). A tendency for a treatment effect from the 50 SLW treatment was noted for CREAT ($P = 0.0524$), although concentrations were within the normal range for both cows and heifers (0.5-2.2 mg/dL).

There was a significant age ($P = 0.02$), treatment ($P = 0.0018$), and age x treatment interaction ($P = 0.04$) for BUN with the lowest concentrations in heifers consuming 100 BRW or 100 SLW treatments. Blood urea nitrogen was within the normal range (20-30 mg/dL) for both cows and heifers (Harvey and Bruss, 2008).

No effects or interactions were identified for total serum TRIG, but concentrations for total TRIG in both cows and heifers were twice the concentration considered normal (0-14 mg/dL; Harvey and Bruss, 2008). Cows had higher serum CHOL concentration than heifers creating an age effect ($P = 0.02$); however, concentrations for both ages were elevated compared to standard levels (58-88 mg/dL; Harvey and Bruss, 2008).

No effects on age, treatment, or age x treatment interactions were found for serum glucose concentrations in both cows and heifers with both ages within the normal range reported for cattle (0.45-0.75 g/L; Fielder, 2015). Similarly, no effect on age or age x treatment interaction was found for serum lactate concentrations; however, there was a tendency ($P = 0.01$) for a treatment effect for the 100 BRW treatment even though

concentrations stayed within the normal range for cattle (0.05-0.2 g/L, Harvey and Bruss, 2008).

There were no age or treatment effects and no age x treatment interactions for osmolality, PCV, O_2, tHb, HbO_2, and HbCO. Osmolality (270-300 mOsm/kg; Harvey and Bruss, 2008), PCV (24-46 %; Fielder, 2015), and tHb (8-15 g/dL; Fielder, 2015) were all within normal range for cattle. A treatment effect occurred for MetHb (P = 0.008) due to both BRW treatments being depressed and both SLW treatments being elevated, but no other effects or interactions occurred. Concentrations of MetHb, HbCO, HbO_2, tHb, and O_2 were all higher in cows compared to heifers potentially due to greater blood volume and circulation due to greater body and organ size.

CONCLUSION

Based on the results of the present study, brackish or saline water up to 6,000 mg/kg TDS, had little to no effect on WI, feed intake, or diet digestibility in cows or heifers. Animal response to water quality in blood constituents was varied in blood concentration of ALP and GGT. Additionally, total serum TRIG and CHOL were both elevated, indicating subclinical response from brackish water. Further research is necessary to determine thresholds for TDS or salinity concentration in beef cattle water sources as well as the potential effects of long-term exposure to high-TDS water.

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APPENDICES

Table 1. Monthly rainfall, ambient temperature and relative humidity[1]

		Temperature, °C			Relative humidity, %		
Month/Year	Rainfall (cm)	Mean	Min.	Max.	Mean	Min.	Max.
September 2019	2.56	26.2	19	35	72.6	33	100
October 2019	1.53	13.5	-5	33	69.1	17	99
November 2019	0.82	8.22	-10	25	67.2	14	100
December 2019	0.23	6.56	-7	22	65.5	16	100

[1]Source: Oklahoma Climatological Survey and the Oklahoma Mesonet- Stillwater, Oklahoma

Table 2. Chemical composition of alfalfa cubes[1]

Item [2]	Load 1 (Period 1-3)[3]	Load 2 (Period 4-5)[3]	SEM	*P*-value
Nutrient				
DM, %	95.4	95.2	0.49	0.06
OM, %	88.7	87.8	0.13	<0.0001
CP, %[4]	16.8	17.3	0.23	0.03
NDF, %[4]	49.5	47.3	0.43	0.0018
ADF, %[4]	31.7	31.5	0.25	0.35
EE, %	1.85	1.13		

[1]McCracken Hay, Elgin, OK, USA.

[2] Fed ad libitum throughout each of the five 21-d periods.

[3]Feed was delivered in two loads. Load 1 was fed during periods 1 through 3 and Load 2 was fed during periods 4 and 5.

[4]CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; EE= ether extract.

Table 3. Composition of water consumed by growing Angus heifers and Angus cows

	Treatment[1]					
Item	Control	50 BRW	50 SLW	100 BRW	100 SLW	SEM
TDS, mg/kg[2]	276.3	2852	3309	5263	5878	130.5
Sodium, mg/kg	38.9	755.1	1001	1394	1860	42.4
Sodium, %	4.07	7.47	9.48	7.58	9.71	0.76
Calcium, mg/kg	34.5	162	34.3	285.3	35.8	3.87
Magnesium, mg/kg	8.25	36.9	7.79	62.6	6.57	0.62
Potassium, mg/kg	4.77	4.69	6.54	5.69	9.54	0.59
Nitrate-N, mg/kg	0.91	0.42	0.76	0.63	0.80	0.11
Chloride, mg/kg	33.2	627.9	1585	1243	2782	58.6
Sulfate, mg/kg	36.7	1038	37.1	1985	41.2	17.2
Boron, mg/kg	0.10	4.34	0.09	8.27	0.12	0.07
Bicarbonate, mg/kg	137.6	184.2	144.2	210.4	143.7	4.46
Alkalinity, mg/kg[3]	112.8	151.0	118.2	172.5	117.8	3.65
Hardness, mg/L[4]	119.9	556.0	117.1	969.5	116.5	10.9
SAR[5]	1.54	13.9	40.2	19.5	75.5	1.96
PAR[6]	0.10	0.06	0.15	0.02	0.23	0.02
EC, dS/m[7]	404.7	3722	4846	7055	8700	217.9
pH	8.00	8.70	7.96	8.01	7.98	0.29

[1] Treatment: Control = fresh water; 50 SLW = 50% NaCl and 50% fresh water (3,000 mg/kg TDS); 50 BRW = 50% brackish water and 50% fresh water (3,000 mg/kg TDS); 100 BRW = 100% brackish water (6,000 mg/kg TDS); 100 SLW = 100% NaCl water (6,000 mg/kg TDS).

[2] TDS = total dissolved solids or salts

[3] Alkalinity as $CaCO_3$

[4] Hardness guidelines = Soft: 0-60 mg/L; Moderately hard: 61-120 mg/L; Hard: 121-180 mg/L; Very hard: ≥ 181 mg/L.

[5] SAR= sodium adsorption ratio

[6] PAR= potassium adsorption ratio.

[7] EC= electrical conductivity

Table 4. Effects of age and water treatment on water intake, feed intake, and apparent total tract diet digestibility

	-------Age------		----------Treatment[1]----------						----------P-value----------		
Item	Heifer	Cow	Control	50 BRW	50 SLW	100 BRW	100 SLW	SEM[2]	Age	Treatment	Age x Treatment
Intake											
BW, kg	310.4	604.2	457.5	458.1	460.1	454.4	456.5	14.0	<.0001	0.81	0.83
Water, kg/d	44.2	72.6	60.9	55.4	59.0	55.5	61.2	2.29	<.0001	0.10	0.71
Water, g/kg BW	144.9	120.7	139.2	126.9	132.6	126.8	138.6	5.72	0.0063	0.28	0.70
DM, kg/d	12.6	22.1	18.0	17.5	17.3	16.8	17.0	0.59	<.0001	0.37	0.21
DM, g/kg BW	40.5	36.4	40.1	38.5	37.4	38.0	38.2	1.08	0.02	0.31	0.09
OM, kg/d	11.1	19.5	15.9	15.4	15.2	14.8	15.0	0.52	<.0001	0.37	0.20
DOM, kg/d	6.83	12.1	9.76	9.50	9.45	9.3	9.3	0.40	<.0001	0.83	0.69
Digestibility											
DM, %	60.9	61.5	61.3	60.9	61.1	61.4	61.4	0.32	0.11	0.77	0.44
OM, %	61.0	61.7	61.1	60.9	61.6	61.9	61.4	0.80	0.51	0.87	0.83
NDF, %	55.9	57.6	57.9	55.2	55.0	58.7	57.0	2.48	0.50	0.77	0.40
ADF, %	52.2	53.9	54.3	51.1	51.3	55.2	53.3	2.71	0.56	0.75	0.43
CP, %	91.5	86.1	88.9	88.4	88.4	89.3	89.1	0.48	<.0001	0.61	0.28
EE, %	41.9	46.1	49.1	38.9	37.0	46.5	48.4	4.34	0.30	0.18	0.16

[1] Treatment: Control = fresh water; 50 SLW = 50% NaCl and 50% fresh water (3,000 mg/kg TDS); 50 BRW = 50% brackish water and 50% fresh water (3,000 mg/kg TDS); 100 BRW = 100% brackish water (6,000 mg/kg TDS); 100 SLW = 100% NaCl water (6,000 mg/kg TDS).

[2] SEM= average across treatments

Table 5. Effects of age and water treatment on blood constituent concentrations

	Age		Treatment						P-value		
Item	Heifer	Cow	Control	50 BRW	50 SLW	100 BRW	100 SLW	SEM[6]	Age	Treatment	Age x Treatment
Cl[3], mmol/L	103.6	104.3	103.5	104.3	104.0	103.4	104.6	0.49	0.17	0.29	0.88
Ca[3], mg/dL	10.4	10.03	10.1	10.2	10.2	10.3	10.3	0.12	0.0070	0.65	0.28
K[3], mmol/L	4.59	4.47	4.40	4.57	4.49	4.54	4.65	0.09	0.19	0.43	0.18
Mg[3], mg/dL	1.96	2.13	2.11[a]	2.10[a]	2.09[a]	2.05[a,b]	1.90[b]	0.06	0.02	0.08	0.03
Na[3], mmol/L	142.2	143.2	142.3	143.3	142.6	142.6	143.0	0.42	0.07	0.40	0.35
ALB[3], U/L	3.07	3.21	3.17	3.23	3.08	3.14	3.10	0.06	0.03	0.47	0.88
ALT[3], U/L	19.4	19.1	19.3	20.3	19.2	17.7	19.6	1.63	0.89	0.71	0.09
AST[3], U/L	62.2	57.4	61.5	58.8	61.5	54.5	62.7	4.21	0.44	0.46	0.65
ALP[3], U/L	92.1	42.3	71.6	61.3	64.3	66.1	72.7	6.14	0.0003	0.49	0.81
GGT[3], U/L	16.9	21.3	18.7	19.2	18.9	19.8	19.1	1.09	0.06	0.61	0.15
CK[3], U/L	185.3	144.4	179.9	166.6	158.9	162.4	156.6	9.54	0.0016	0.45	0.82
CREAT[3],mg/dL	0.95	0.94	0.93[b]	0.99[a]	0.92[b]	0.96[a,b]	0.96[a,b]	0.02	0.62	0.05	0.27
TRIG[3], mg/dL	30.8	29.7	29.8	31.0	29.6	31.4	29.6	1.39	0.59	0.67	0.82
CHOL[3], mg/dL	106.3	133.3	125.0	124.4	119.3	114.1	116.2	6.17	0.02	0.36	0.62
BUN[3], mg/dL	20.1	23.9	23.2[a]	22.4[a,b]	22.2[a,b,c]	21.0[d]	21.3[c,d]	0.76	0.02	0.0018	0.04
Glucose, g/L	0.46	0.46	0.46	0.47	0.46	0.45	0.46	0.02	0.79	0.98	0.19
Lactate, g/L	0.17	0.15	0.14	0.16	0.15	0.13	0.20	0.02	0.49	0.10	0.78
Osmolality	286.8	284.5	282.1	287.1	290.0	285.9	283.3	2.29	0.36	0.12	0.72
PCV[3], %	34.4	35.7	34.7	36.4	35.0	35.5	33.7	0.81	0.12	0.23	0.52
O_2, mmol/L	12.1	13.7	13.3	13.6	13.0	12.3	12.2	0.75	0.07	0.64	0.87
tHb[3], g/dL	12.9	13.5	13.2	13.5	13.4	13.2	12.7	0.35	0.16	0.44	0.66
HbO_2[3], %	66.9	72.1	71.4	69.8	68.9	68.2	69.3	3.39	0.13	0.97	0.73
HbCO[3], %	1.94	2.13	2.11	2.02	1.96	1.97	2.13	0.19	0.29	0.95	0.69
MetHb[3], g/dL	0.45	0.58	0.50[b,c]	0.43[b,c]	0.66[a]	0.41[c]	0.57[a,b]	0.05	0.08	0.008	0.59

[a,b,c,d] Main effect water treatment means without a common superscript letter differ ($P < 0.05$).

[1] Treatment: Control = fresh water; 50 SLW = 50% NaCl and 50% fresh water (3,000 mg/kg TDS); 50 BRW = 50% brackish water and 50% fresh water (3,000 mg/kg TDS); 100 BRW = 100% brackish water (6,000 mg/kg TDS); 100 SLW = 100% NaCl water (6,000 mg/kg TDS).

[2] SEM= average across treatments

[3] Cl^-= chloride; Ca= calcium; K= potassium; Mg= magnesium; Na= sodium; ALB= Albumin; ALP= alkaline phosphatase; ALT= alanine aminotransferase; AST= aspartate aminotransferase; CK= creatine kinase, CREAT= creatinine; TRIG= triglycerides; BUN= blood urea nitrogen; CHOL= cholesterol; GGT= gamma-glutamyl transferase; PCV= packed cell volume or hematocrit; tHb= total hemoglobin; HbO_2= hemoglobin oxygen saturation; HbCO= carboxyhemoglobin; MetHb = methemoglobin.

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