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The influence of the previous plane of nutrition on water and nitrogen metabolism of grain versus forage fed beef cattle

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Abstract

The beef cattle production system is mainly segmented into three main sectors corresponding to life stages: cow-calf, backgrounding, and finishing. In each one of those phases, nutritional status of animals can vary greatly according to the feedstuff available within growth phases. In the background phase, for example, the supply of high-quality forage can be very limited at times, which will be followed by periods of reduced performance. In the U.S., once these animals are transitioned into the finishing phase, often upon backgrounding in forage-based systems, they will transition into one of two feeding managements, a grain-fed (conventional beef production) or grass/forage-fed. Growing concerns regarding the environmental impacts of grain-fed systems, often creates a pursuit for grass/forage-fed finishing systems as perceived as a more sustainable alternative for the beef industry goal of being a steward of our natural resources. In order to reach a desirable carcass finishing point within a feasible time frame, cattle will require a high-quality feed, which is usually associated with high levels of energy for grain-based on protein for forage based systems. Increased protein levels in the diet is usually associated with an increase in water requirements – a very limited resource and of high environmental concern for the beef industry. Research on the environmental impact of grass/forage-fed beef vs grain-fed beef is still very limited, and to the best of our knowledge, there is no scientific literature investigating the influence of the previous plane of nutrition on nitrogen and water metabolism at the animal level altogether. This dissertation explores the physiological and molecular mechanisms regulating the water and nitrogen metabolism at the animal level of different background and finishing

systems and their respective interactions in order to address the key role of sustainable use of natural resources. The first chapter provides a literature review about the beef cattle industry in the U.S., as well as the mechanisms that regulate water intake, nitrogen metabolism, and nitrogen recycling in cattle. Then, the second chapter explores how the backgrounding diet can affect the next phase on regards to water intake, animal performance and efficiency of steers under grain or forage-based finishing diets. This study revealed that grain-fed animals are usually more efficient in regard to fresh water use, but that adequate plane of nutrition on earlier stages of life are required to mitigate water requirements/use and ensure the final carcass quality is achieved. From this study, it was observed that the concentration of crude protein was one of the main components controlling water intake, thus fresh water use. Therefore, in the third chapter, it was evaluated how different backgrounding and finishing systems altogether might affect nitrogen metabolism, and consequently water requirements of cattle. The results from this study indicated that animals fed a low plane of nutrition during the background phase were able to reduce their excretion of nitrogen without affecting their water and nitrogen requirements due to a more efficient nitrogen recycling; and once they were transitioned to a grain-fed finishing system, they were still able to carry over those characteristics. Therefore, in the last chapter, it was investigated how the molecular mechanisms controlling water and urea metabolism at the finishing phase can be affected by the previous plane of nutrition. Overall, the results suggest that the previous plane of nutrition can impact gene expression associated with water and urea metabolism during the finishing phase, namely AQP3, AQP7, ATP1B1, and SGK1 in the kidney, and AQP7 and UT-B in the rumen. Our results highlight the often overlooked “elephant in the

room" regarding the carryover effects that previous planes of nutrition may carry in beef cattle production systems. Further, we empirically demonstrate that opposite to common belief, grain-fed and not grass/forage-fed beef is more sustainable in regard to fresh water utilization.

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LITERATURE REVIEW

1. Introduction

Covering over 70% of the earth surface water is often considered an unlimited resource. However, freshwater represents 2.5% of all the available water, with 70% being in the form of glaciers and permanent ice (Thornton et al., 2009). Population growth together with climate change are projected to substantially increase the scarcity of freshwater globally (Heinke et al., 2019). By 2025, it is estimated that 64% of the world population will live in a water-deprived basin, compared to 38% in 2009 (Rosegran et al., 2002). These concerns are directly translated into livestock operations and will likely shift market operations and priorities into a production of animal products that can produce more per unit of water (Nardone et al., 2010).

With beef requiring 80-260% more water than other meat sources (Mekonnen and Hoekstra, 2010), the concern of the consumption of beef by both public and scientific sectors continues to increase (Klopatek et al., 2022a). However, only few studies have been conducted to analyze how efficient cattle could be in utilizing water and which factors could help mitigating its intake (Arias and Mader, 2011; Ahlberg et al., 2019; Macias-Franco, 2021; Wagner and Engle, 2021). Ahlberg et al. (2019) have shown that efficiency on use of water by cattle could be a useful selection tool index without causing any detrimental effects on body gain. However, the authors did not look into how the water use can also shift according to the system that those animals are raised. Recently, Kloplatek et al. (2022b) published a paper on the environmental impacts of grass-fed

versus grain-fed cattle. The authors noticed that due to irrigation and longer finishing periods to reach the desirable finishing weight, grass-fed animals would use 25% more water than grain-fed animals. However, the authors did not consider the previous plane of nutrition of those animals and how that could impact the results observed on water intake under different management systems. Overall, most of the studies conducted examining the effect of previous plane of nutrition of animals provide different levels of nutrients, mainly protein, that could either limit the growth of animals or provide adequate nutrition for the next phase. The differences noticed on those studies are mainly related to the effect of different grazing systems on final body weight and carcass characteristics (Drouillard and Kuhl, 1999), feedlot performance (Mader et al., 1989), metabolites and hormones in the blood (Hancock et al., 1988), among others. However, none of those studies have tried to understand how those diets might impact water requirements and efficiency of cattle.

In the literature, water requirements are often governed by dry matter intake and dry matter content (Vardot et al., 2008); however, the composition of the feeds and the physiological status of the animals also need to be considered. For example, Kloplatek et al. (2022a) showed that grass-fed beef drinks more water due to longer periods on pasture. However, if animals were provided a better quality forage this could be prevented. Higher quality forages could shorten the days on feed and water use but are usually associated with high levels of protein in the diet.

For ruminants, the protein consumed is degraded in the rumen by the microbial population producing ammonia, among other products. Ammonia is absorbed into the blood and metabolized in the liver into urea; urea can then either be recycled back to the

rumen or excreted in the urine when in excess. Therefore, when animals are provided diets with high levels of protein, an increase on water requirements is expected (Winchester and Morris, 1956).

Given all the factors that can affect water requirements and metabolism of cattle and the increase concern on the effects of climate change on water availability, a better understanding of the factors controlling water efficiency of beef cattle production systems is imperative. Furthermore, no studies have shown which molecular mechanisms could be related to the changes on water intake and efficiency in response to diet. Therefore, the objective of this literature review is to elucidate the effects of beef cattle on water usage in the different beef cattle systems and how the use of water can be modulated according to their requirements. Dietary factors and management are explored as a means that can modulate water intake mainly through protein metabolism. Thus, this review will also explore nitrogen metabolism and its relationship with water metabolism, as well as regulatory mechanisms and tissue-selective gene expression.

2. Beef cattle feeding systems

Historically, the beef cattle industry in the U.S. has been highly segmented and operated independently according to the developmental phase of the animal (Drouillard and Kuhl, 1993). The first phase would be cow-calf production, where beef cows are usually maintained to raise calves. Once calves reach weaning age (around 6 to 7 months), cow/calf producers may choose to sell or retain ownership of the calf for the next phases (Duff et al., 2007). In the cow-calf phase, the main source of nutrients are

forages (pasture, hay, ensiled forages, and crop residues) with supplemental energy, protein, vitamins, and minerals as needed to meet their requirements (NASEM, 2016). Commonly, once calves are weaned, they still need to gain more weight before going to the feedlot. Therefore, they move to the second phase: backgrounding/stocker. Systems available for growing cattle are mainly based on grazing, but they can vary tremendously between regions. Most of the nutrients supplied through grazing on rangeland pastures, might have very low levels of protein, limiting fiber utilization. Therefore, low-quality forages, deficient in protein, will usually provide inadequate ruminal nitrogen (N), which decreases microbial growth and, consequently, decrease ruminal fermentation and utilization of fiber that will further decrease the passage rate (Koster et al., 1996).

For the producer obtaining calves for the stocker phase, it is also important to know the source, previous management (vaccination records among others), and age of those animals, since it will be crucial to determine their nutrition and time required to produce the desired product for the feedlots (Duff et al., 2016). Another important consideration in this phase is that it can define the potential of the animal for fat deposition on the subsequent phase. Du et al. (2013) explains that for cattle there is a unique time window to specifically enhance marbling without an overall increase in fatness. This time window coincides with the beginning of the backgrounding phase, and animals need to have their nutritional requirements being supplied to provide sites for lipid accumulation during the finishing phase, resulting in adipocyte hypertrophy and high marbling (Du et al., 2013).

The last phase of the beef cattle production system is the finishing phase. Cattle coming from the backgrounding phase and entering a feedlot are usually placed on a

receiving diet high in forage, and progress through several step-up programs that allow rumen to adjust with the high energy content of feedlot diets (Vasconcelos and Galyean, 2007). The final feed is a high-energy (grain and grain by-product based) diet that is formulated to provide enough energy and protein to optimize growth-rate, feed efficiency, animal health, and carcass quality with the least possible cost (NASEM, 2016). However, due to the increased concern behind the environmental impact of grain-fed beef systems, grass-fed beef is now viewed as a more sustainable alternative (McCluskey et al., 2005; Xue et al., 2010). The USDA (2019) defines grass-fed beef as ruminant animals, and the products derived from those animals, that have solely consumed forages throughout their life, which the exception of the milk consumed before weaning. A limitation of this system is that for the production of grass-fed animals to be acceptable, those animals need to obtain adequate levels of carcass finishing (equivalent to USDA select or Choice) within a feasible time frame. In order to accomplish this, careful attention must be given to the supply of forages, selection of cattle, and production and storage of high-quality forages (NASEM, 2016), which are usually associated with high levels of protein.

3. Water usage in beef cattle production

Robbins (1998) estimated that 20,864 liters of water would be required per kilogram of boneless beef produced. Kreith and Davis (1991) suggested that the actual cost is 20,559 liters of water per kilogram (L/kg). Mekonnen and Hoekstra (2012) suggested that there are large variations according to production systems, estimating 16,353–26,155 L/kg of beef for grazing beef systems, 11,744–16,869 L/kg of beef for mixed systems

(grazing plus grain-based finishing) and 3856–13,089 L/kg of beef for more intense systems where grains are fed on all segments. However, those researchers usually use broad values found in the literature, inconsistent units, and vast regional differences preventing the comparison between models (Menendez and Tedeschi, 2020). Technological and scientific advances have led to significant changes over the last several years due to increased crop yields, better crop irrigation practices, and more efficient animal use (Capper, 2011; USDA-ERS, 2022).

In 1993, Beckett and Oltjen (1993) estimated that beef cattle in the U.S. consume 760 billion liters of water per year, averaging a total of 3,682 L of water per kg of boneless beef. However, a new assessment on water usage of beef cattle, Klopatek et al. (2022b) observed that currently around 2,275 L of water was required to produce one kg of boneless beef in the U.S. Compared with the Beckett and Oltjen (1993) model, there was a reduction of 38% of water usage over the last 30 years. According to Klopatek et al. (2022b), the main reasoning behind this reduction on water usage is due to changes in irrigation practices, crop yields, feed, and animal efficiencies.

Another important point for the observed decrease on the water usage of beef cattle is the change of the diets over the years. The use of byproducts (e.g., bakery waste, potato waste) and coproducts (e.g., DDGs, corn-gluten, tallow) have been widely explored for the diet of beef cattle, decreasing the total water use and lowering the demand for forage and concentrates, and with that, the water use on those systems (Klopatek et al., 2022b). Furthermore, the use of more efficient animals has increased the final body weight of cattle, and consequently, the carcass weight, producing more meat per animal (USDA-ERS, 2022).

Even though water usage from beef cattle has decreased considerably over the years, there is still room for improvement. As an example, animals need to start being selected for not only feed efficiency, but water efficiency. Furthermore, the relationship between feed and water requirements needs to be further explored to understand how different diets can mitigate water intake. Macias Franco et al. (2021) observed that Holstein calves supplemented with corn starch would drink less water than animals receiving a fat supplement or no supplement. The authors attributed this decrease on water intake to the production of metabolic water. However, not many studies have explored the ability of modulating water requirements through feed, and to accomplish that it is important to understand how water metabolism and requirements function in cattle.

4. Water metabolism in cattle

Beef cattle nutrition is largely governed by six essential nutrients to ensure proper body function – carbohydrates, lipids, protein, mineral, vitamins, and water. However, one may argue that from the perspective of maintaining life, water is most important, since cattle can only survive without it for a few days (Wagner and Engle, 2021). Water is distributed in the extracellular and intracellular space within an animal. Intracellular fluid consists of water along with potassium and other inorganic ions, and proteins, whereas extracellular fluid consists of blood plasma (25% of the extracellular water) plus interstitial fluid (75% of the extracellular water) (NASEM, 2016). Water shifts between extracellular and intracellular fluids and their homeostasis is usually regulated by volume sensors, hormones, and water transfer mechanisms involving the hepatic portal system, heart, and kidneys (Macfarlane and Howard, 1972). Overall, the

proportions in each pool vary with feeding practices and environmental conditions and are constantly regulated by concentrations of sodium in the extracellular fluid and potassium in the intracellular fluid (Kleeman and Fichman, 1967; NASEM, 2016).

It is commonly known that transport of water is mainly controlled by osmosis through the lipid bilayer (passive co-transport with ions and solutes) and diffusion (Kleeman and Fichman, 1967). Over the last forty years, the role of water channels known as Aquaporins (AQP) has also been discussed. Aquaporins have exquisite specificity for water and are capable of rapidly transporting water in response to changes in tonicity (Day et al., 2014), making a critical contribution to water flow within an organism.

According to Day et al. (2014), AQPs are expressed in a wide range of tissues: retina — AQP4; olfactory epithelium — AQP4; inner ear — AQP4 and AQP1; brain — AQP4 in astrocytes and AQP1 in choroid plexus; spinal cord — AQP1, AQP4 and AQP8; nucleus pulposus cells of the intervertebral disc — AQP1 and AQP3; osteoclasts — AQP9; blood vessels — AQP1 in endothelial cells; heart — AQP4; kidney — AQP1, AQP2, AQP3, AQP4 and AQP7; salivary glands — AQP5; GIT — AQP3, AQP4, AQP5 and AQP9; liver — AQP1, AQP8 and AQP9; pancreas — AQP1 and AQP8; lungs — AQP3, AQP4, AQP5; fat (adipocytes) — AQP7; skin — AQP1, AQP3, AQP5 and AQP10; female reproductive tract — AQP7, AQP8 and AQP9 in ovaries; and male reproductive system — AQP3 and AQP7 in sperm cells. In the rumen, AQP3, -7, and -10 is highly expressed (Røjen et al., 2011) and plays a role on not only water, but also nitrogen metabolism, since those AQPs are also permeable to urea. Overall, these water channels are usually spatially located within a certain region of the cell allowing the flow of water through tissues and regulating cell volume (Day et al., 2014).

Once ingested, water absorption through the gastrointestinal tract (GIT) is regulated by osmotic gradients, and it can happen paracellularly through tight junctions, transcellularly through cell membranes (Kavouras and Anastasiou, 2010), or via AQPs. Furthermore, movement of water is also linked to ionic movements, where absorption of water is linked primarily to the movement of sodium ions, whereas secretion back into the rumen is linked to the movement of chloride ions (Martinez-Augustin et al., 2009). However, this linkage to ionic movements decreases as it gets into the large intestine, where absorption of even distilled water may occur (Nishinaka et al., 2004). According to Faichney and Boston (1985), in a 3.4 L rumen, water inflow included diffusion (2.86 L/h), saliva (0.38 L/h), and intake (0.1 L/h), whereas absorption of water from the rumen counted for 3.07 L/h and outflow was 0.27 L/h. According to the authors, water absorption was so rapid that the mean residence time of a water molecule in the rumen was only 61 min. Once absorbed, water will be distributed into the intracellular and extracellular compartments of the body.

5. Water requirements of beef cattle

In beef cattle, most of the water requirements can be supplied by the drinking water and the water content in the feed, but small contributions on the water pool can also come from the metabolic water produced by oxidation of organic nutrients (NRC, 1981). The minimum requirement of water for cattle reflects the amount of water required for maintenance, growth, fetal growth, reproduction, lactation, and the loss of water through urine, feces, sweat, and by evaporation from the lungs and skin (NASEM, 2016). Any influence on those factors will influence water requirements. Because of that, estimation

of requirements with accuracy can be extremely hard, but their holistic pursuit can assist in a better understanding of how we will use cattle sustainably in the future.

i. Effect of environment on water intake

Cattle are homeothermic animals that can adjust their body temperature by regulating metabolic heat production (NRC, 1981). However, when the temperature of the environment is higher than 35−37 °C, even Bos indicus cattle, which are adapted to a tropical climate, can increase their water consumption by 40% when compared to a thermo-neutral conditions (Nagarcenkar, 1979). Arias and Mader (2011) concluded that mean ambient temperature, minimum temperature, and temperature-humidity index were the primary factors that could affect water intake. On the other hand, Sexson et al. (2012) using data from four separated feedlots in the U.S., observed that an increase in body weight and relative humidity would decrease water intake, whereas an increase in feed intake, temperature, wind speed and temperature-humidity index would increase water intake.

Furthermore, the thermoregulatory benefit of water during times of low or high temperature are also important. In cold weather, cattle usually increase their water intake if water is in the liquid state, but they will consume ice or snow if water is not available (Young and Degen, 1980). In this last case, cattle can tolerate the stress from ingesting ice or snow by drawing stored body heat and immediately increasing the metabolic rate to compensate the heat required to melt the frozen water and bring it to body temperature (Degen and Young, 1984). However, Brod et al. (1982) reported that ingestion of cold water (0 ^{o}C) might alter fermentation patterns and decrease rumen temperature in sheep.

Conversely, when temperature is high, providing cool water (18.3 ºC) improved ADG in beef cattle (Bond et al., 1976). However, CSIRO (2017) explains that when ambient temperature is high, the thermoregulatory benefits of water are mainly through evaporation (skin and respiration) and not due to the physical intake of cold water.

ii. Animal-related factors influence on water intake

Breed significantly affects water intake of beef cattle. According to Winchester and Morris (1956), Bos taurus cattle have higher water consumption than Bos indicus breeds, mainly when temperature increases. This might happen because the ambient temperature threshold at which Bos indicus cattle start to sweat is higher (28 °C as opposed to in Bos taurus 17 °C) (Horrocks and Phillips 1961). Furthermore, water intakes of Brahman and Romosinuano breeds seems to be comparatively less than Hereford and Belgian blue breeds at the same metabolic body weight (Brew et al. 2011).

Metabolic body size determines nutrient requirements including water requirements of the animals; therefore, higher body weight usually results in higher water consumption (Meyer et al. 2004). Thus, a larger body size usually has a higher intake of water. However, the increase on water intake is not related to water deposition in the body, since as the animal gets heavier, there is a decrease in total body water and an increase in total body fat (Kraybill et al., 1951). The increase on water intake is mostly related with an increase of the digestive tract, since water within the digestive tube accounts for 15 to 35% of total weight (Odwongo et al., 1985).

Another animal-related factor that can affect water intake is the physiological state. Young calves usually have a higher water intake per kilogram of DM, since their main

source of nutrients is milk. In general, they will consume 5 to 7 L of water per kilogram of DM, compared to 3.5 to 5.5 L water per kilogram of DM recommended for older cattle (Pettyjohn et al., 1963; ARC, 1965). As cows get pregnant, they also might consume 30% more water than when not pregnant, and once they calve and start lactating the water intake is estimated to be 0.87 kg water/kg milk produced (Winchester and Morris, 1956; ARC, 1965). As cattle start to get really heavy and more susceptible to heat stress due to the increase in body fat, their predicted water intake is higher than lighter cattle. This behavior can be mainly observed during very hot temperatures, where animals will drink more water due to increased water loss through respiration rate and, to a lesser extent, sweating (Wagner and Engle, 2021). Altogether, this seems to highlight the often-overlooked oversimplification of water requirements in cattle, physiological status, such as negative energy balance and compensatory weight gain are factors that can affect water requirements in cattle and that can carry performance and generational implications.

iii. Water losses effect on water intake

Another factor that can change the water intake of animals is the amount of water lost. For beef cattle, water can be mainly lost through urine, feces, sweat, and by evaporation from the lungs and skin. Urinary water loss is influenced by the hormone vasopressin, which controls reabsorption of water from the kidney tubules and ducts (Bankir et al., 2017). According to NASEM (2016), in conditions of water restriction, the body may reabsorb much more water and concentrate urine decreasing water

requirements. The authors explain that in general, the amount of urine produced vary with the activity of the animal, air temperature, water consumption, etc.

Water lost through feces will depend mainly on the diet, where diets with succulents or high concentration of minerals can contribute to a higher excretion of water in the feces (NASEM, 2016). In general, cattle feces contain 75-85% water, while sheep and goat feces have 60-65% water (NRC, 1981). The variation on water content will depend on the large intestines ability to reabsorb water and excrete drier fecal pellets instead of wet and loose feces, which is presumably one mechanism of water conservation (NRC, 1981).

Water lost through evaporation from the skin and lungs and sweating is extremely important and can even exceed the amount of water excreted in the urine as the temperature and level of activity increases (NASEM, 2016). Evaporation of water through lungs is highly dependent on the environment relative humidity. As the air expired is over 90% saturated, if the relative humidity is low, respiratory losses are high; however, if the relative humidity of air reaches saturation, the losses of water from the lungs decreases (NRC, 1981; NRC, 2007).

iv. Effect of nutritional factors on water intake

Winchester and Morris (1956) suggest a constant relationship of dry matter intake with water intake at thermo-neutral conditions. However, composition of feed can also alter the intake of the water. According to NASEM (2016), feeds such as silage, green chop, or growing pasture are usually high in moisture and will decrease the requirements for drinkable water. The authors also explain that diets high in energy can further

decrease the intake of water due to the production of metabolic water. Diets high in salt, mineral or diuretic substances, on the other hand will increase water intake due to greater water loss though the urine. Furthermore, in systems where very high-quality feeds are required to increase profitability, such as dairy farms and grass-fed finishing systems, the excess of dietary protein can also increase water requirements due to excessive water loss through urine. However, more research still needs to be conducted to understand the relationship between protein in the feed and the mechanisms regulating water requirements of the animals.

6. Effects of protein levels on water nutrition/requirements

Although not usually discussed, diets with excessive levels of proteins are a problem in the dairy and beef cattle industry. For dairy cattle, the exponential increase in genetic potential for milk production has resulted in an increase in dietary crude protein (CP) of diets to ensure a sufficient supply of metabolizable protein to achieve maximal milk production (Law et al., 2009). However, previous studies conducted by Broderick (2003) have shown that diets with concentration above 167 g/kg of CP in dry matter basis has no benefit in terms of yield of milk or milk components.

In the Western U.S., the most common protein forage source available is alfalfa (Putnam et al, 2000). This forage has a high dry matter yield and excellent palatability and is one of the main sources of forages utilized on dairy farms. However, due to the high levels of protein of this forage, producers might be over-feeding protein to animals. Furthermore, in grazing systems where forage is not managed correctly, animals might have a high-quality and lush forage that is young and immature with levels of protein that

can go over 25% (Dobrenz et al., 1969). High protein diets are also a reality in grass-fed beef systems, where animals need to obtain adequate levels of carcass finishing within a feasible time frame.

As previously mentioned, when protein levels exceed the amount of nitrogen required by ruminal microorganisms, there will be an increase on urea excretion in the urine. However, excretion of urinary urea requires water, which inevitably leads to higher water intake and, hence, increased urine output (Katongole and Yan, 2020). Therefore, water intake and urine excretion rates are functions of protein intake.

Although many studies have investigated the effect of protein levels on nitrogen metabolism, very few have looked into its effect on water intake. Ritzman and Benedict (1924) observed that steers on high protein allowances consumed 26% more water than did similar animals on low protein rations. In dairy cattle, raising the CP content from 12 to 13% increased water intake about 0.99 L/day in dry cows, but it was not significant in lactating cows (Holter and Urban Jr. 1992). Rouda et al. (1994) studied the effect of feeding 0 kg/head/d, 0.7 kg/head/d, and 1.4 kg/head/d of supplemental protein for free-ranging cows and observed that animals receiving no supplemental protein had the highest intake of water. Divya et al. (2011) observed that an increase of 10% on rumen degradable protein did not have any significant effect on drinking water intake in crossbred heifers. Therefore, due to the differences found between authors, more research trying to understand the influence of protein on the water metabolism is required, and to accomplish that we need to first understand the protein metabolism on ruminants.

7. Protein metabolism of ruminants

One of the principal contributions of ruminants to humans is the conversion of non-utilizable fibers and nitrogenous compounds available in plants into animal proteins, such as meat, wool, hides, and milk (McDonald, 1968). Ruminants have a complex digestive process, which is composed of two main steps. First, the feed goes through bacterial degradation, and only then are the nutrients available to the host animal. Therefore, when balancing diets of ruminants, it is necessary to consider two entirely separate but interdependent ecosystems (NRC, 1985).

Proteins play an essential role in all biological processes, and they are in a constant flux depending on the balance between synthesis and hydrolysis (Van der Walt and Meyer, 1988). The ruminant cannot use the nitrogen in the protein at the tissue level, but the rumen bacteria can (NRC, 1985). Therefore, the nitrogen is first trapped by the bacteria as bacterial protein, and only then it will be further digested by the animal in the small intestine and used to supply its requirements.

As previously mentioned, the immediate product of protein digestion is ammonia, which might be toxic to the cells depending on the concentrations (Getahun et al., 2019). To avoid ammonia, this product is converted into urea in the liver, which is further excreted in urine or recycled back to the rumen. Back in the rumen, microbes can recycle urea and convert it back into ammonia as a nitrogen source for microbial growth that will be further used by the ruminants as a protein source (Getahun et al., 2019). Despite that, the extent of urea recycling depends mainly on the concentration of intraruminal ammonia, which if high, can inhibit the flux across the rumen wall (Abdoun et al., 2007).

The large intestine is another site for bacterial growth. However, unlike the rumen, bacteria in the large intestine cannot be used by the host animal, since it will not be exposed by the digestive processes in the small intestine (NRC, 1985). In general, the maintenance of optimum nitrogen balance in ruminants depends on the GIT, liver, and kidney. The GIT tissues form an interface between the animal and its diet, which regulates nutrient transfer from the gut to the bloodstream and target organs such as the liver, which after receiving these nitrogenous compounds distributes them to the peripheral tissues (Abdoun et al., 2007). Anything that is not used can be filtered by the kidney and further excreted.

i. Protein degradation in ruminants

Once the feed arrives at the rumen, approximately 70 to 80% of ruminal microorganisms attach to undigested feed particles (Craig et al, 1987), but only 30 to 50% of those have proteolytic activity (Prins et al., 1983). In general, the predominant species of proteolytic bacterium found in the rumen of most animals is *Prevotella ruminicola*, which can consist of up to 60% of the ruminal flora (Van Gyslwyk, 1990); however, when dietary protein seems to be more resistant to degradation, *Butyrivibrio fibrisolvens* seems to have a higher activity (Wallace et al., 1987). Therefore, the rate and extent at which the protein will be degraded will depend on the proteolytic activity of the ruminal microflora and its peptide bonds susceptibility and accessibility (Bach et al., 2005).

The most important factors affecting protein degradation by microbes include the type of protein (true protein vs non-protein nitrogen [NPN]), interactions with other

nutrients (mainly CHO), and the predominant microbial population (dependent on the type of ration, ruminal passage rate, and ruminal pH; Bach et al., 2005). In the rumen, protein degradation of true protein will differ from NPN degradation. Since NPN does not consist of AA, rumen microbes will produce ureases that will hydrolyze NPN to ammonia and CO2, which can be further used in the synthesis of AA by the microbes (Zhu et al., 2022). On the other hand, degradation of true protein is done in two steps. The first step involves bacteria attachment to the feed particles, followed by activity of microbial cell bound proteases (Brock et al., 1982). Due to the numerous amounts of different bonds within a single protein, a synergetic action of different proteases from microbes is necessary to complete protein degradation (Wallace et al., 1996).

Proteolytic activity and the microbial species responsible for degradation are diet-dependent and its mechanism of action among bacteria and protozoa differs greatly (Tamminga, 1979). Ciliate protozoa will assist in the breakdown of feed protein and also bacterial protein, but they are not able to hydrolyze soluble protein (Wallace, 1996). Nevertheless, their complete removal can still cause a decrease in proteolysis (Broderick et al., 1991). On the other hand, bacteria are mainly responsible for protein chain breakdown through hydrolysis of some or all of its peptide bonds outside the bacterial cell (Tamminga, 1979; Wallace, 1996). This process will result in peptides and AAs that can be transported inside of the bacterial cell or further degraded by peptidases into AA and later be absorbed by microbial cells (Nolan and Strachin, 1979; Siddons et al., 1985; Wallace 1996). Once inside the microbial cell, the fate of absorbed AA and peptides will depend on the availability of energy. If energy is available, AA will be transaminated or

used directly for microbial protein synthesis, but if energy is limited, AA will be further fermented into volatile fatty acids (VFA) (Bach et al., 2005).

ii. Ammonia absorption and metabolism in the liver

As previously mentioned, ammonia can be generated from microbial degradation of both true protein and NPN. Ammonia can then disappear from the rumen by incorporation into microbial protein, absorption across the rumen wall (35-65%) or by outflow from the rumen into the omasum (10%) (Nolan and Strachin, 1979; Siddons et al., 1985). In the rumen, ammonia absorption is primarily a function of its concentration and pH, which will play a role in the form of how it will be absorbed (NH3 or NH4). The portion of ammonia that is absorbed across the rumen epithelium, as well as other sections of the GIT, can be in form of NH3 and NH4. According to Abdoun et al. (2006), permeability of the ruminal membrane for NH3 is about 175 times higher than for NH4, but it can be modulated according to the rumen pH. In general, ammonia is usually absorbed in the lipophilic NH3 form by simple diffusion when ruminal pH is 7 or greater however, the pH in the rumen is usually at pH 6.5 or lower, which only allows the absorption of ammonia as NH4 probably via a K^+ channel. Therefore, absorption of ammonia is influenced by pH, and it also interacts with K^+ transport across the apical membrane.

Regulation of rumen pH and consequently the rates of ammonia absorption is highly dependent on diet. Depending on the composition of the diet, an increase or decrease of the pH can be observed. For example, diets with high concentration of urea lead to an increase in the pH and rumen NH3 concentrations, which together with a decrease in

rumen permeability causes a drastic increase in the absorption of ammonia to the bloodstream, leading to ammonia toxicity (Webb et al., 1972; Abdoun et al., 2006). Conversely, forage-based diets lead to a slight decrease in pH that will favor the absorption of NH4. However, if the diet is more concentrate based, this pH might go lower than 6.4, where even the absorption of NH4 can be reduced (Abdoun et al., 2006).

Once absorbed in the rumen, ammonia is transported to the portal drained viscera (PDV). The quantities of absorbed ammonia by the PDV seem to be determined not only by the amount of dietary nitrogen supply, but also by the amount of digestible nitrogen intake (Reynolds et al., 1992). To avoid high levels of ammonia in the blood, all ammonia absorbed by PDV will be then removed by the liver and converted into urea through the ornithine cycle in the periportal hepatocytes, or if they escape urea conversion, they can be converted into glutamine in the perivenous hepatocytes (Abdoun et al., 2006). However, in the subsequent passages through the liver, amid-N of glutamine will be metabolized to urea by the periportal hepatocytes (Hussinger et al., 1992). This process is extremely important for ruminants since increased levels of ammonia in the blood can be toxic. Overall, the primary mechanism of ammonia poisoning is due to inhibition of the Krebs Cycle, which will decrease energy production leading to anaerobic glycolysis and systematic acidosis due to lactate accumulation (Haliburton and Morgan, 1989).

8. Urea metabolism in ruminants

For most mammals, urea is seen as a product from nitrogen metabolism from ammonia detoxification. However, for ruminants, urea is a key metabolite produced

during nitrogen recycling that is essential for the rumen microflora. The quantities of nitrogen recycled can vary widely depending on the rumen environment (e.g., pH, volatile fatty acid profile, ammonia and ammonium concentration, CO2 concentration, Abdoun et al., 2006, Muscher et al., 2010) and dietary characteristics of ruminant rations (e.g., CP content, degradability and digestibility of N sources, fermentability of carbohydrates, forage-to-concentrate ratio; Bach et al., 2005, Batista et al., 2017, Scott et al., 2020). In general, as the N intake is reduced, a greater proportion of total urea production is transferred to the GIT (Reynolds and Kristensen, 2008; Batista et al., 2017). Furthermore, a greater concentration of rumen-fermentable carbohydrates will result in a greater transfer of urea from blood into the rumen when compared with diets where the carbohydrate sources are predominantly high fiber, low rumen-degradable forages, or less processed grains due to higher microbial growth (Huntington, 1989, Delgado-Elorduy et al., 2002, Scott et al., 2020).

i. Urea recycling back to the rumen

Blood urea can be transferred back to the rumen by diffusion through the gap junctions or with the help of urea transporters. Diffusion of urea is usually linearly related to the rumen-blood concentration gradient and permeability of the rumen epithelium (Houpt and Houpt, 1968). Intraruminal hydrolysis of urea by bacterial urease therefore will facilitate the movement of urea through the rumen wall by keeping the concentration low to keep diffusion favorable (Abdoun et al., 2006). Furthermore, damage in the permeability of the rumen wall has been shown to increase urea transport, demonstrating that permeability of the rumen can limit urea diffusion (Houpt and Houpt, 1968). Other

factors that can also affect diffusion of urea through the rumen epithelia are: blood flow supplying the rumen epithelium; plasma urea concentration, which will also be related to the concentration gradient that will allow the passage of urea; epithelial surface area that can be enhanced by ruminal papillae growth; and transepithelial urea permeability of ruminal epithelium, which is mediated by urea transport proteins (Cheng et al., 1979; Cheng and Wallace, 1979; Wallace et al., 1979).

Besides passive diffusion, studies have identified specific transporters for urea in the rumen. Urea Transporter B (UT-B) has been identified in all epithelial layers, with exception of the stratum corneum (Stewart et al., 2005), this transporter allows the chemiosmotic passage of urea across cell membranes (Smith and Rousselet, 2001). In adult cattle its protein abundance and precise localization within the papillae cellular layers are regulated by dietary intake (Simmons et al. 2009), but the outcomes from studies have shown contradictory results about how protein levels might influence the expression of those transporters (Marini and Van Amburgh, 2003; Marini et al., 2004). To prove the function of UT-B, various researchers (Stewart et al., 2005; Abdoun et al., 2010; Doranalli et al., 2011) showed that by adding phloretin (an inhibitor of UT-B function) to ruminal epithelia mounted on a Ussing Chamber there was a reduced serosal-to-mucosal urea flow. This result suggests that a variable portion of the transported urea (up to 50%) was transported via an alternative mechanism.

A possible alternative mechanism for the transport of urea would be the AQPs. Between the AQPs, AQP3, -7, -9, and -10 have been shown to be permeable to urea and potentially related to the mechanism of salvaging urea back to the rumen (Røjen et al., 2011). Among them, AQP7 has the highest urea permeability, similar to UT-B proteins

(Ishibashi et al., 1997). Different from UT-Bs, high levels of nitrogen in the diet upregulates expression of AQP3, -7, and -10 (Røjen et al., 2011). However, there are a limited number of studies looking at AQPs in urea metabolism, and thus more research is needed to understand their exact function(s).

Once in the rumen, either via blood urea across the ruminal wall or via salivary secretions, bacteria will hydrolyze urea for two mains reasons: to use ammonia as a source of N for MCP and as a buffer to rumen (Arioli et al., 2010; Pengpeng and Tan, 2013). This is because ammonia (NH3) produced after urea hydrolysis can be used to buffer acidic conditions formed by high VFA concentrations by combining with the H+ ions in excess in the rumen (Lu et al., 2014). If ammonia is not used by the microbes, it can be excreted in the feces or absorbed again through the PDV and go through the whole cycle again. It is important to mention that small amounts of urea can also be recycled to the intestine (mainly cecum and colon) where there is microbial fermentation (Doranilli et al., 2011).

ii. Urea excreted through the urine

At the whole-body level, the balance of urea excretion and recycling between the kidney and rumen is a key regulatory factor and is highly diet dependent (Reynolds and Kristensen, 2008). A range of 1-71% (mean 29%) of urea can be eliminated through urine (Batista et al., 2017), and this will depend on the relative nitrogen intake and nutritional requirements of the animal (Huntington and Archibeque, 2000). Therefore, whenever there is an increase in nitrogen intake higher than the requirements, instead of being recycled back to the rumen, urea will be transported through the blood to the kidney to be

excreted. However, if levels of nitrogen in the diet are too low, urea is filtrated and re-absorbed into the blood to be recycled (Zhong et al., 2022). In the kidney, transport of ammonia takes place down a concentration gradient with the help of protein transporters.

The main urea transporters in the kidney are UT-A (-1, -2, and -3) and UT-B, but different from the rumen, their localization varies throughout the nephron. Most UT-A isoforms are acutely regulated via phosphorylation and trafficking of the glycosylated transporters to the plasma membranes, this process will be induced by the antidiuretic hormone vasopressin (Stewart, 2011). UT-A2 transporters are localized in the apical and basolateral membrane of the thin descending limbs of Henle's loop, mediating the transport of urea from the lumen to the interstitium (Li et al., 2012). The increase in urea concentration will increase the gradient concentration, increasing the transport of water and forming urine. As urine flows along the collecting duct, urea can be rapidly reabsorbed by UT-A1 and UT-A3, and by UT-B in the descending vasa recta (Stewart, 2011). Further, to avoid excessive loss of water AQPs will be required to concentrate the urine and avoid excessive water loss. AQP-2, -3, and -4 are the main players to avoid excessive water loss in the kidney. Basically, when there is an increase in plasma osmolality, vasopressin is released by the pituitary gland stimulating an increase in the expression of AQP2 (long-term regulation), which will be released from actin vesicles inside of the cell (short-term release), so they can migrate to the apical membrane of the cells and allow free passage of water to inside of the cell (Kwon et al., 2013). AQP-3 and -4, on the other hand, are already located in the basolateral membrane of the collecting ducts of the kidney and they will allow the exit of the water molecules, with that avoiding

excessive loss of water and concentrating the urine with urea and other molecules that need to be excreted (Ikeda and Matsuzaki, 2015).

In general, excretion of urea through the urine and urine concentration in the kidney is a complicated process that not only involves urea and water, but other solutes as well, such as Na^+, K^+, and Cl^- (Chou and Knepper, 1989). Sands and Layton (2009) explains that during the process of concentrating urine, NaCl is actively reabsorbed through the ascending limb by the apical plasma membrane Na-K-2Cl cotransporter and by the basolateral membrane Na/K-ATPase. The Na^+/K^+-ATPase is an important Na^+ and K^+ pump that is usually potentiated by Serum/Glucocorticoid Regulated Kinase 1 (SGK1) and formed by the subunits Alpha 1 (ATP1A1) and Beta 1 (ATP1B1) (Taub et al., 2010; Yang et al., 2020). Meanwhile, Cl^- and water follow the reabsorption of the cations, travelling through the cell either by diffusion or AQP channels (water) or chloride channels (CLC and CLIC family of proteins) (Goodchild et al., 2009; Ikeda and Matsuzaki, 2015). Together, gene expression of all those components can influence gradient concentration and water flow. Therefore, understanding the molecular mechanisms that control the relationship between water and nitrogen metabolism does not only involve water and urea transporters, but solutes such as Na^+, K^+, and Cl^- as well.

9. Conclusion

The increasing concern on water scarcity worldwide might cause a shift in selection of animals in the beef cattle sector toward more water efficient animals. However, due to the different systems that cattle are raised throughout their life, all the different combinations of previous plane of nutrition and finishing systems must be addressed to

understand the factors that might affect water requirements of animals. Furthermore, water requirements can also be affected by the nutrient content of the diet. Thus, understanding the interaction between nutrient content and water metabolism is essential.

The use of high protein diets can be a concern for dairy and beef industries. When protein content in the diet exceeds the use of the microbial population in the rumen, it will be absorbed through the rumen wall as ammonia and metabolized in the liver into urea. Urea is further taken to the kidney and excreted in the urine. Therefore, high dietary protein content can increase water requirements due to excessive water losses through the urine. Based on this literature review, the objective of this dissertation will be to first, understand how different planes of nutrition during the background phase affect water and feed efficiency of beef cattle finished on either grass or grain-based diets. The second goal will be to understand how nitrogen metabolism between the different systems aforementioned change and its relationship with water metabolism. We will investigate what could drive the changes in water and nitrogen metabolism by looking into gene expression of water- and nitrogen-related genes in the rumen and kidney of beef cattle.

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CHAPTER II

NUTRITIONAL CARRYOVER EFFECTS OF THE PREVIOUS PLANE OF NUTRITION OF CROSSBRED ANGUS STEERS FINISHED ON GRAIN OR FORAGE-FED FINISHING SYSTEMS ON FRESH WATER INTAKE, ANIMAL PERFORMANCE, AND EFFICIENCY

Lay Summary

When acquiring stocker cattle at auctions, very limited information is known about the previous plane of nutrition provided to those animals. Previous studies have reported that previous plane of nutrition can affect the performance of cattle during the finishing phase. However, little is known about the effects of the previous plane of nutrition on the water metabolism of those animals. In this study, we investigated the effect of backgrounding animals on a low or moderate plane of nutrition on water intake, performance, and efficiency of animals finished either on a grass/forage-fed or grain-fed finishing system. Our study revealed that there are overlooked carryover effects from the previous plane of nutrition, and that animals backgrounded on a low plane of nutrition and subsequently fed a grain-based diet during the finishing phase had the lowest requirement for water and the highest carcass quality. Further, when only comparing the differences between the finishing systems, it was observed that the requirements for fresh water of grass/forage-finished cattle almost doubled when compared to grain-finished animals.

Highlights

- Animals backgrounded on a moderate plane of nutrition and finished on a grain-based diet had the lowest fresh water intake and the highest carcass quality.
- Grass/forage-finished beef had higher water requirement than grain-finished beef.

1. Abstract

The objective of this study was to evaluate how the backgrounding diet can affect the feedlot water intake, animal performance and efficiency of steers under grain based or forage-based finishing diets. Twenty-four crossbred Angus steers (298.01 ±10.17 kg) were fed either a low plane of nutrition (LP, n =12; triticale only, 9.1 %CP, 0.25 Mcal/kg net energy available for maintenance [NEm], and 0.10 Mcal/kg net energy available for gain [NEg]) or moderate plane of nutrition (MP, n =12; 85% alfalfa and 15% beardless wheat, 12.62 %CP, 0.25 Mcal/kg NEm, and 0.13 Mcal/kg NEg) during the background phase (85d). After this period, steers were then blocked by previous plane of nutrition and transitioned to a finishing diet where they were fed either a forage-based (CP: 20.8% DM, NEm: 1.41Mcal/kg, NEg: 0.83 Mcal/kg) or a grain-based diet (80% whole corn and 20% hay; CP: 10.6% DM, NEm: 1.73 Mcal/kg, NEg: 1.12 Mcal/kg). Differences amongst treatments were compared via orthogonal contrast using the GLIMMIX procedure of SAS (version 9.4). Animals backgrounded on LP were lighter and had a lower dry matter intake (DMI) when compared to MP ($P < 0.01$). However, no differences were observed on the daily water intake (WI) between LP and MP ($P > 0.05$). MP animals were more efficient on water conversion rate and gross water efficiency ($P < 0.01$), but less efficient on residual feed intake ($P < 0.05$), when compared to LP animals.

During the finishing phase, forage fed animals had a higher DMI and WI when compared to grain-fed animals ($P < 0.01$). However, no differences were observed on the final BW between the finishing groups ($P > 0.05$). Grain-fed animals were more efficient for water and feed intake and had a higher marbling score ($P < 0.01$) when compared to forage-fed steers. Effects of previous plane of nutrition were observed for WI, body condition score, rib depth and marbling score, where animals backgrounded on MP and finished on a grain-fed diet had the lowest WI ($P <0.05$) and highest body condition score ($P < 0.05$), marbling score ($P < 0.01$) and rib depth ($P < 0.05$). Altogether, our results highlight the effects of backgrounding and finishing systems on WI and efficiency of animals, as well as how important an adequate plane of nutrition on earlier stages of life are to decrease WI and ensure the final carcass quality of those animals.

Keywords: Backgrounding; Finishing; Gene expression; Nitrogen; Water.

2. Introduction

Beef cattle nutrition is largely governed by six essential nutrients, which ensure proper body function – carbohydrates, lipids, protein, mineral, vitamins, and water. Beef cattle have been documented to survive weeks or months when some of these nutrients are absent; however, in water deprived environments, the survivability of the animals significantly decreases to a few days, making water the most critical and limiting nutrient (NASEM, 2016; Wagner and Engle, 2021).

Although water is considered an unlimited resource, freshwater only represents 2.5% of all water resources, with 70% being in the form of glaciers and permanent ice (Thornton et al., 2009). Given that climate change concerns continue to influence policy,

adequate monitoring of environmental footprints of livestock productions systems is crucial to empirically quantify the impact of livestock production systems on the use of natural resources, specifically freshwater. Environmental changes such as water salination, high chemical contaminants, and warming climatic conditions, will be extremely detrimental on any production system, as they will reduce availability to freshwater resources (Nardone et al., 2010). It is evident, that a new focus for the livestock industry will involve their efficiency and use of freshwater resources. Water scarcity and worsening quality of available water sources will require livestock producers to investigate more sustainable production systems and for selection of animals with increased efficiency for both feed and water.

Few studies have been conducted in beef cattle examining the factors affecting not only water intake (WI), but the efficiency of water use (Ahlberg et al, 2019). Moreover, no studies have tried to understand how carry over effects from the previous plane of nutrition might affect water efficiency. Historically, the beef industry has been highly segmented and operated independently according to the developmental phase of the animal (Drouillard and Kuhl, 1993). However, profitability of each production system is greatly impacted by the interaction with previous segments due to carryover effects of nutrition and management employed during earlier stages of life (Greenwood et al., 2015).

Developing a better understanding of the relationship between WI and previous nutritional management is necessary to ensure that greater sustainability, production efficiency, and proper accountability (i.e.: feedlot) is achieved, while enabling application to animals within sector with different backgrounds. The objective of this study was to

evaluate the influence of the previous plane of nutrition on dry matter intake (DMI), animal performance, WI, hydration levels and water use efficiency of finishing feedlot cattle.

3. Materials and Methods

All experimental and animal husbandry procedures conducted were approved by the Institutional Animal Care and Use Committee of the University of Nevada, Reno, NV (protocol #00845).

i. Experimental design, treatments, and animals

Twenty-four crossbred Angus steers (298.01 ±10.17 kg) were housed in two shaded pens at the research feedlot area of the Main Station Field Laboratory at the University of Nevada, Reno. Each pen was equipped with twelve individual Calan gate feeding systems (American Calan, Nothwood, NH) and four electronic water troughs (Intergado Ltd., Contagem, MG, Brazil) coupled with automatic scale platforms for individual measures of daily DMI, WI and body weight (BW), respectively. The experimental trial lasted 220 days, consisting of two phases: backgrounding and finishing phase. During the backgrounding phase (85 d), animals were randomly assigned to one of the two treatments (n =12 per treatment): low plane of nutrition (LP; 100% Triticale) or moderate plane of nutrition (MP; 85% alfalfa and 15% beardless wheat; Table 1). Following the backgrounding phase, steers were blocked by previous plane of nutrition and transitioned to the finishing phase over a 30-d period and then fed until finishing for 105 d. The diets were designed to mimic two commonly finishing systems, a forage-fed system which

consisted of high quality alfalfa hay only (forage fed, n=12), and a grain-fed system (80% whole corn and 20% alfalfa hay, n =12; Table 2).

All animals had ad libitum access to water and a balanced mineral mix (Table 1 and 2) throughout the testing period. Steers were fed once during the backgrounding phase at 0800 h, and twice during the finishing phase at 0700 h and 1700 h. Orts were collected daily before morning feeding and weighed. Feed intake was adjusted daily to ensure up to 10% of refusals.

ii. Water intake system and behavior

Before the beginning of the experiment, each animal was fitted with a plastic radio-frequency identification tag (FDX-ISO 11784/11785; Allflex, 104 Joinville, Santa Catarina, Brazil) in the left ear. For each visit to the water trough, the system recorded the number of visits per day, visit duration, time, WI, and BW of the individual animals by recording the animal's identification tag and bin number. Thus, drinking water behavior data were recorded as the average time spent drinking water (L/d), drinking rate as the average daily liters of water drunk per minute, and the number of water troughs visited per day was considered the average amount of drinking events per day. All data were continuously recorded and transferred to the cloud and retrieved for WI, BW and drinking behavior. The water bins (0.30 x 0.37 x 0.20 m) were programmed to maintain the water temperature at 25 °C and were automatically and continuously filled to a volume of 115 L after the animals left the weighing platforms. Only one animal was allowed at a time to each individual water trough. A complete description and evaluation of the water system can be found in Oliveira et al. (2018). Each water trough was

manually cleaned and disinfected biweekly or as needed to ensure free access of fresh water at all times. Both BW and WI platforms were calibrated weekly with company-manufactured weights to ensure data accuracy.

iii. Water quality analysis

Water samples were collected monthly throughout the experimental trial, and a composite sample was shipped for chemical analysis at the Cumberland Valley Analytical Services (CVAS; Waynesboro, PA). The chemical analysis performed followed the recommendations of Rice et al (2017) for pH (method # 4500-H), nitrate (method #4500 NO3-), total dissolved solids (method # 2540), sulfates (method # 4500-SO42), minerals (Ca, P, Mg, Na, I, Mn, Zn, Cu; method #3500), carbonate hardness (method #2340), and total coliform and E. coli (method #9223). The results of the chemical analysis are described on Macias Franco et al. (2021).

iv. Feedstuff chemical analysis

Feed samples were collected weekly for bromatological analysis. Feedstuffs were composited into one representative sample for each experimental phase, and a 200 g subsample was shipped to Cumberland Valley Analytical Services (CVAS; Waynesboro, PA). The samples were analyzed for the chemical composition of dry matter (DM; method #930.15 AOAC, 2000), crude protein (CP; method # 990.03; AOAC, 2000), soluble protein (Krishnamoorthy et al., 1982), rumen degradable protein (RDP; Krishnamoorthy et al., 1983), acid detergent fiber (ADF; method # 973.18; AOAC, 2000), acid detergent insoluble CP using ADF residue in a Leco FP-528 Nitrogen

Combustion Analyzer (Leco Corporation, St. Joseph, MO), neutral detergent fiber (NDF; Van Soest et al., 1991) corrected for protein (Leco Corporation, St. Joseph, MO) and ash (apNDFom; method # 942.05; AOAC, 2000), lignin (Goering and Van Soest, 1970), sugar (Dubois at al., 1956), starch (Hall, 2009), ash (method # 942.05; AOAC, 2000) and a complete mineral panel (method# 985.01; AOAC, 2000) in a Perkin Elmer 5300 DV ICP (Perkin Elmer, Shelton, CT). Values for total digestible nutrients and net energy were obtained by empirical equations (Weiss, 1998).

v. Efficiency and performance traits

Body weights were obtained automatically daily and regressed to obtain the estimate for the average daily gain (ADG) discounting differences in rumen fill. The feed conversation ratio (FCR), gross water efficiency (GWE; Pereira et al., 2021), and water conversion ratio (WCR; Pereira et al., 2021) were estimated as the ratio of average DMI to ADG, ADG to average WI and average WI to ADG, respectively.

Residual drinking water (RDWI) and feed (RFI) intake were calculated as the difference between observed and predicted DMI and WI required to meet growth and maintenance energy requirements (Koch et al., 1963). Predicted DMI and WI were estimated as a function of ADG and midpoint metabolic BW ($MidBW^{0.75}$) using the following model recommended by Koch et al. (1963):

$$RFI; RDWI = Y_{12} = \beta_0 + \beta_1 \times MidBW^{0.75} + \beta_2 \times ADG + \varepsilon_{12}$$

Where Y represents the expected values for DMI and WI measures to be regressed, β_0 represents the intercept, β_1 represents the partial regression coefficient of

MidBW$^{0.75}$, β_2 represents the partial regression coefficient for ADG, and ε is the respective residuals for the adjusted model.

Additionally, RDWI was also estimated as a function of observed DMI (RDWIDMI). Expected WI based on DMI was estimated as the regression of observed DMI and MidBW$^{0.75}$ using the following model recommended by Ahlberg et al. (2019):

$$RDWI_{DMI} = Y_{12} = \beta_0 + \beta_1 \times MidBW^{0.75} + \beta_2 \times DMI + \varepsilon_{12}$$

Where Y represents the expected values for WI based on observed DMI to be regressed, β_0 represents the intercept, β_1 represents the partial regression coefficient of MidBW$^{0.75}$, β_2 represents the partial regression coefficient of DMI, and ε is the respective residuals.

Biometric measures (BM) were taken throughout the experimental trial. On days 0, 28, 56, and 85 of the backgrounding phase, and on days 0, 28, 56, 84, and 105 of the finishing phase. For the measurements, animals were normally positioned in a squeeze chute, and the same trained technician was responsible for taking the BM using anatomical locations as reference points. The measurement points were determined by palpation as described by Fonseca et al. (2017) and were taken with the aid of a large caliper (Hipometro type Bengala with 2 bars, Walmur, Porto Alegre, Brazil) and a graduated plastic flexible tape. The BM included hook bone width (HBW) as the distance between the two ventral points of the tuber coxae (large calipers); pin bone width (PBW) as the distance between the two ventral tuberosities of the tuber ischia (large calipers); abdominal width (AW) measured as the widest horizontal width of the abdomen (paunch) at right angles to the body axis (large calipers); body length (BL) 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); scapula 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 (PGL) as the distance between the ventral point of the tuber coxae and the ventral tuberosity of the tuber ischii (large calipers); rib depth (RD) 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 (TW) as the widest horizontal width across shoulder region, at the back (large calipers). On those same days, visual assessments of body condition score (BCS) were also performed only during the finishing phase.

vi. Slaughter

At the end of the finishing phase, all steers were transported approximately 600 km to a USDA inspected commercial abattoir (CS Beef Packers, Kuna, Idaho), where all the animals were slaughtered by trained technicians stunning the animals using a penetrating captive bolt rendering the animal unconscious, followed by exsanguination through the jugular vein. Hot carcass weight (HCW) was obtained immediately after evisceration, and dressing percentage was obtained by dividing HCW by final BW. All carcasses were chilled for 24 h, and qualified personnel measured longissimus dorsi area via direct grid

reading between the 12th and 13th rib, and USDA marbling score and yield grades (USDA, 1997).

vii. Statistical analysis

Data were analyzed as linear mixed models using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC) adopting a $P \leq 0.05$ as significant and $0.05 < P \leq 0.1$ as tendencies. Variables from the background phase were analyzed using a completely randomized design, following the statistical model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where Y_{ij} is the dependent variable taken from experimental unit jth on treatment ith, μ is the overall mean, T_i is the fixed effect of treatment ith, and e_{ij} is the random error associated with ijth data value assuming that $e_{ij} \sim N(0, \sigma^2)$.

For the finishing phase, data were analyzed following a completely randomized block design, where the previous treatment is considered the block. For the finishing phase the statistical model is described in the following equation:

$$Y_{ijk} = \mu + T_i + b_j + Tb_{ij} + e_{ij}$$

Where Y_{ijk} is the dependent variable taken from experimental unit kth in the block jth on treatment ith, T_i is the fixed effect of treatment ith, b_j is the random effect associated with the block jth, Tb_{ij} is the random effect associated with the interaction between treatment ith and block jth, and e_{ijk} is the random error associated with ijkth data value assuming that $e_{ijk} \sim N(0, \sigma^2)$.

Identification of outliers and influential points was performed by plotting the studentized residuals against the predicted values as well as by Cook's distance. Coefficients exceeding 2.5 studentized t distributions were considered outliers and removed from the data (Neter et al., 2004). Mean comparisons were computed using the LSMEANS statement and compared using Tukey-Kramer adjustment. Data from different time points were included as repeated measures in the statistical model, where day was considered the repeated variable. The fixed effect of time and its interactions with treatments was analyzed using the covariance structure that yielded the lowest Bayesian Information Criteria. Plots of effect of previous plane of nutrition data were analyzed using ggplot2 from the Tidyverse package in R version 4.1.2. (R Development Core Team, Vienna, Austria).

4. Results

i. Intake of nutrients in the diet

No effects in the previous plane of nutrition were observed on nutrient intake from feed of animals ($P = 0.2929$). Intake of nutrients during the background phase (Table 3) was higher for animals fed MP ($P < 0.0001$), except for sugars, Fe, Zn, and Cu, which were higher for the LP diet ($P < 0.0001$). Regarding the concentration of energy available for maintenance and gain (Table 3), intake was also higher for the MP diet ($P < 0.0001$). For the finishing phase (Table 4), DMI differed between treatments ($P = 0.0053$), with forage-fed animals having the highest DMI. Forage-fed animals had a higher intake of organic matter (OM), CP, RDP, ADF, apNDFom, lignin, sugars, Mg, K and Na ($P <$

0.0001; Table 4), however, the intake of net energy for maintenance ($P = 0.0171$) and gain ($P = 0.0002$) was higher for the grain-fed animals (Table 4).

Water intake during the backgrounding phase (Table 5) was the same for both treatments when corrected for BW ($P = 0.8205$) or metabolic BW ($P = 0.9197$). However, when corrected for ADG ($P = 0.0002$), DMI ($P = 0.0010$), CP intake (CPi; $P < 0.0001$) and RDP intake (RDPi; $P < 0.0001$) animals on the LP treatment had a higher WI when compared to MP treatment. Although LP animals drank more water, they spent less time drinking water ($P = 0.0054$) and visited less water troughs per day ($P = 0.0185$; Table 5). For the finishing phase, WI was higher for the forage-fed treatment ($P < 0.0001$; Table 6), even when corrected for BW ($P < 0.0001$), ADG ($P < 0.0001$) and DMI ($P < 0.0001$). However, when corrected for CPi ($P < 0.0001$), the intake of water was higher for animals fed a grain-based diet when compared to forage fed animals. Furthermore, it was observed that forage-fed animals visited more water troughs ($P = 0.0016$) and had a higher drinking rate ($P = 0.0115$) and time spent drinking water ($P =0.0016$) when compared to grain-fed animals (Table 6). Interestingly, no effect was observed for nutrient intake from the diet (Table 4), although the previous plane of nutrition affected the drinking WI during the finishing phase (Table 6) when corrected for BW ($P = 0.0106$), metabolic BW ($P = 0.0152$), hot carcass weight (HCW; $P = 0.0294$), DMI ($P = 0.0375$), and tended to influence WI corrected for CPi ($P = 0.0581$). The significant effect is further explored in Figure 1 (A, B, C, and D), where animals on MP treatment during the background phase and finished with grain, had the lowest consumption of water among treatments; whereas animals backgrounded on the LP treatment and finished with forage had the highest WI.

ii. Performance, efficiency, and growth

Regarding performance and efficiency among treatments during the background phase (Table 7), animals on the MP treatment had higher final BW (P = 0.0071), final MidBW (*P* = 0.0074), ADG (*P* < 0.0001), RFI (*P* = 0.0408), and GWE (*P* < 0.0001). MP animals had the lowest FCR (*P* = 0.0351) and WCR (*P* = 0.0002). For the finishing phase (Table 8), the only differences observed were related to efficiency traits, where grain-fed animals were more efficient for FCR (*P* = 0.0309), RFI (*P* = 0.0007), RDWI (*P* < 0.0001), RDWIDMI (*P* = 0.0062), WCR (*P* < 0.0001) and GWE (*P* < 0.0001). The previous plane of nutrition only showed effects on the initial BW (*P* = 0.0116) and $MidBW^{0.75}$ (*P* = 0.0169), where animals backgrounded on the LP treatment and finished on a grain-fed diet were the lightest (Figure 1E and F). Regarding the carcass data, there was only a difference for the marbling score (MS; *P* = 0.0005), where animals in the grain-fed treatment had a higher score when compared to the forage fed animals. The previous plane of nutrition also affected the MS (*P* = 0.0013), where animals fed MP followed by grain finishing diets had the highest score among all the treatments (Figure 1G).

Biometric measurements for the backgrounding phase (Table 10) only differed for the HBW (*P* = 0.0426), TW (*P* = 0.0193) and scapula (*P* = 0.0163); where animals on the MP treatment had larger HBW and TW, but smaller scapula when compared to the animals on LP treatment. All the measurements showed an interaction between treatment and day of collection, except for PBW, AW, rump height and BL. Conversely, for the finishing phase (Table 11), AW (*P* = 0.0373), BL (*P* = 0.0372) and diagonal (*P* = 0.0365)

were the only variables that were different among treatments, where grain-fed animals were larger than forage-fed animals. Previous plane of nutrition affected BCS (P=0.0038) and rib depth (P = 0.0171) of animals, where animals fed MP followed by grain-based diets had the highest values (Figure 1H, I). Interaction between treatment and day of collection was only observed for measurements of rib depth (P = 0.0195) and BL (P = 0.0272).

5. Discussion

Several factors can affect WI, among them, environment, DMI and DM content of the feed are often the most cited (Vardot et al., 2008). In the current trial, however, is assumed that the influence of environmental conditions on animals from different treatments would be similar since all animals were submitted to the same environmental conditions during each phase. Therefore, the differences on climate would not explain much of the difference on WI among treatments.

A positive correlation between DMI and WI has previously been observed regardless of the diet (Meyer et al., 2004; Kume et al., 2010). However, this might not apply to all feeding systems. In this study, even though LP animals ate less, they still have the same or higher WI – when corrected for ADG, DMI, and CPi - when compared to MP, which might be associated with the composition of the ingested feed. The forage provided for LP animals was bulkier and required more water for feed particle hydration. Furthermore, LP animals also visited less water troughs and spent less time drinking water, so they tended to drink water faster than MP animals. For most mammals, water is mostly consumed during or shortly before or after feeding events, and in rats, food-related

drinking can account for almost 70% of the daily WI (Kraly, 1983). For LP animals, the faster drinking rate might be associated with the lower DMI due to lower passage rate, caused by the low CP content in the diet that potentially limited microbial growth (Koster et al., 1996), and consequently led to fewer feeding events, whereas animals on MP would eat more constantly during the day and visit the water troughs more often. This observation is potentially important for management of grazing animals in arid environments that have to walk long distance in search of food and water. Animals on low-quality feed would be willing to walk longer distances when compared to animals grazing a higher quality feed, since they would be drinking water more constantly throughout the day.

During the finishing phase, grain-fed animals had a lower DMI when compared to forage-fed animals, probably due to the higher concentration of energy in the diet. Voluntary intake of ruminants is mainly constrained by intake capacity of the rumen or by chemostatic mechanisms, where the animal only consumes enough DM to supply its physiological demand for energy (Dulphy and Demarquilly, 1994; Montgomery and Baumgardt, 1965). Water intake was the highest for forage-fed animals, which goes back to the diet composition between treatments, where forage-fed animals did not only have a bulkier diet but also double CP content in the diet. Once the CP reaches the rumen, it is degraded into ruminal NH3-N, and whatever is in excess is converted into urinary and fecal N to be excreted out of their bodies (Xia et al., 2018). Although not analyzed in this study, we postulate that the increase of WI of forage-fed animals is due to the high supply of CP through the diet, leading to an increase of N excretion through the urine. However, none of the current equations for WI prediction take into consideration the dietary levels

of CP (Hicks et al., 1988; Meyer et al., 2006; Arias and Mader, 2011; Sexson et al., 2012; Ahlberg et al., 2018; Zanetti et al., 2019).

No carryover effect of feed intake during the previous phase was observed on the subsequent finishing phase. However, a carryover effect was observed for drinking WI of animals during the backgrounding phase when WI was corrected for BW, BW0.75, HCW and DMI. In general, animals fed a MP diet during backgrounding phase and finished with a grain-fed diet drink less water than LP plus grain-fed diet or any of the forage-fed treatments. This remark is of high significance. As freshwater sources continue to decrease, and the policy around them becomes more stringent, these animals could serve as a unique opportunity for producers to maintain production levels while utilizing less water.

It is hypothesized that grass-fed production systems - equivalent to our forage- fed treatment – carry less environmental burden due to the absence of large and confined animal operations, intense use of grains in the diet, reduction in water usage and air pollution (Gwin, 2009; Klopateck et al., 2022); which has increased consumer interest in grass-fed beef often paid with a premium at the grocery store. However, this can vary significantly depending on region, resource availability, and forage quality. For example, Klopateck et al. (2022) compared the environmental impact of grass-fed beef fed for 20 and 25 months on a kg HCW basis. According to her research, animals fed for 20 months used 2.7 times less water than animals fed for 25 months, mainly due to irrigation requirements due to a decrease in forage quality for the extra 5 months. With that, animals finished with 20 months were able to reduce their water footprint by 63%. In this current study, we show that forage quality can play a role on the environmental impact of

grass-fed beef. When comparing grass-fed vs grain-fed animals, our data shows that grass/forage-fed animals double their water requirements when compared to grain-fed animals when their diet is associated with a high CP intake. Furthermore, high CP intake can increase environmental impacts due to increased NH3 excretion. NH3 is volatized from animal waste, which is a major global air quality concern (Dong et al., 2014; Burgos et al., 2007).

According to Ahlberg et al. (2019), WI has no genetic correlation with ADG, moderate correlation with RFI, and strong correlation with water efficiency measurements. Therefore, selecting animals by WI should not inhibit production or efficiency of steers in the feedlot. This is important since cattle are usually sold after background priced on weight, so heavier steers often generate more revenue. Our results show that although animals from LP had a lower RFI, animals on MP had higher ADG, drank less water (when corrected for ADG, DMI, and CPi), and were more water efficient based on WCR and GWE. On the other hand, even though no differences were observed on ADG, animals on grain-fed diets were more feed and water efficient than forage-fed animals. Therefore, selecting those animals based on lower WI would be a great opportunity to sustainably reduce water utilization by cattle.

Carcass characteristics were similar between groups, except for MS. Beef with higher marbling produces higher sensory traits, including tenderness, juiciness, flavor, and overall acceptability of beef samples (Hunt et al., 2014). An increase in net energy intake is an important factor for deposition on marbling in the carcass (Park et al., 2018). Interestingly, in this study, the only treatment that was able to reach scores close to 500 for MS were the animals that were backgrounded on a MP diet and finished on a grain

diet. In beef cattle, adipocytes in the visceral depot occurs during the mid-fetal stage to early postnatal stage (Robelin, 1981); formation of subcutaneous adipocytes occurs between mid- to late fetal stage and the early weaning stage (Hood and Allen, 1973); and formation of intramuscular adipocytes (marbling) is estimated to happen at 250 d of age. As a result, there is a "marbling window" where the requirements of animals need to be supplied to enhance adipogenesis, and later adipocyte hypertrophy and high marbling (Du et al., 2012). In this study, all animals were between the "marbling window" during the background phase. Consequently, this suggests that animals backgrounded on a MP diet and finished on a grain diet had the highest MS score. Therefore, heavier animals at the end of the background phase will not only generate more revenue for the backgrounding producer, but to the owner of the feed yard as well. If those traits are further coupled with a lower WI, together they would allow a more sustainable and economically relevant selection tool for the beef cattle industry.

Although growth typically is measured as the change in live weight, BM is an important tool to help us understand how the pattern of growth and tissue pools can change. In this present study, during the backgrounding phase, the only detectable differences were for HBW, TW and scapula between treatments, where MP animals had higher measurements for HBW and TW, and LP had higher values for scapula measurements. In the finishing phase, however, a previous plane of nutrition effect was only observed for RD, while a trend was noticed for TW, AW and body diagonal. In a study to access body fat composition through BM, Fonseca et al. (2017) observed that among the BM, HBW, RD, AQ and TW were the variables with the highest positive correlations with body fat composition, indicating their importance on fat deposition.

Furthermore, BCS can also be used to assess body reserves of animals as a predictor of fat deposition (Apple et al., 1999). According to the BCS obtained in our finishing phase, animals fed MP during backgrounding and finished with a grain-based diet were able to reach the highest scores. Together with the MS data, we can observe how important the plane of nutrition is in the earlier stages of life to ensure adequate fat deposition of animals at later life stages. Furthermore, as body fat increases, body water decreases (Kraybill et al., 1951), decreasing the animal requirements for water. As previously mentioned, an effect of previous plane of nutrition was also observed for WI of animals, which can indicate a decrease on water requirements of steers coming from a MP due to an increase in fat deposition in earlier stages of life.

6. Conclusion

Our study demonstrates that animals backgrounded in a moderate plane of nutrition and subsequently fed a grain fed diet had the lowest water intake when compared to animals backgrounded in a low plane of nutrition of the finishing system (forage-fed or grain-fed diet). This observation is closely related to fat deposition patterns in the carcass, since only animals coming from a moderate plane of nutrition and finished on a grain-based diet were able to reach the highest carcass marbling score, which consequently would decrease water requirements of animals. This highlights why understanding the interaction among various phases of the beef production system is key to ensure the profitability and sustainability of subsequent phases. Further work is warranted to elucidate the factors modulating water requirements and water metabolism of animals.

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8. Tables

Table 1. Ingredient and nutrient composition of the backgrounding phase for crossbred Angus steers fed a low plane (n = 12) or a moderate plane (n= 12) diets

Item	Treatment	
	Low Plane	Moderate Plane
Ingredient, % of dry matter		
Alfalfa	-	85
Beardless Wheat	-	15
Triticale	100	-
Mineral Mix[1]	*Ad libitum*	
Chemical analysis[2], % of Dry matter		
Dry matter, % as-is	93.70	93.88
Crude protein	9.10	12.62
Organic matter	90.28	92.38
Soluble protein	4.80	5.78
Soluble protein, % CP	52.80	45.62
Rumen degradable protein	7.00	9.20
Rumen degradable protein, % CP	76.40	72.81
Acid detergent fiber	29.28	39.97
NDICP	1.28	1.52
aNDFom	47.78	46.92
apNDFom	46.50	45.40
Lignin	4.07	6.91
Sugar	12.80	7.46
Starch	0.40	0.98
Ash	9.72	7.62
Ca	0.35	1.20
P	0.19	0.21
Mg	0.15	0.32
K	1.41	1.49
Na	0.08	0.16
Fe, ppm	297.00	112.15
Mn, ppm	35.00	27.35
Zn, ppm	22.00	21.40
Cu, ppm	11.00	11.55
Total digestible nutrients	53.00	57.59
Net energy for maintenance, Mcal/kg	0.25	0.25
Net energy for gain, Mcal/kg	0.10	0.13
Non-fiber carbohydrates	25.99	30.40

[1] Mineral mix composition: 18% Ca, 6% P, 18% NaCl, 4% Mg, 0.5% K, 0.36% Mn, 0.0012% Co, 0.12 Cu, 0.006% I, 0.0027% Se, 0.36% Zn; [2] CP: Crude protein; NDICP: Neutral detergent insoluble crude protein; aNDFom: Neutral detergent fiber (NDF) assayed with a heat stable amylase and expressed exclusive of residual ash; apNDFom: NDF assayed with a heat stable amylase and expressed exclusive of residual ash and protein.

Table 2. Ingredient and nutrient composition of crossbred Angus steers backgrounded on different planes of nutrition and subsequently finished on grain (n = 12) or forage-fed (n= 12) finishing systems

Item	Treatment	
	Grain-Fed	Forage-Fed
Ingredient, % of dry matter		
Alfalfa (21% CP)	-	100
Alfalfa (16% CP)	80	-
Corn	20	-
Mineral mix[1]	*Ad libitum*	
Chemical analysis[2], % of dry matter		
Dry matter, % as-is	90.28	94.00
Organic matter	90.8	96.44
Crude protein	10.8	21.3
Soluble protein	3.5	8.2
Soluble protein, % CP	30.46	38.4
Rumen degradable protein	5.07	14.7
Rumen degradable protein, % CP	41.82	69.2
Acid detergent fiber	11.02	26.2
NDICP	0.746	1.93
aNDFom	17.12	32.2
apNDFom	16.374	30.27
Lignin	3.176	5.72
Sugar	3.26	9.5
Starch	56.92	2.2
Ash	3.56	9.2
Ca	0.344	1.82
P	0.288	0.19
Mg	0.168	0.32
K	0.758	1.62
Na	0.092	0.2
Fe, ppm	1283	387
Mn, ppm	33.4	47
Zn, ppm	1107.2	34
Cu, ppm	9.6	13
Total digestible nutrients	80.52	64.8
Net energy for maintenance, Mcal/kg	0.966	0.7
Net energy for gain, Mcal/kg	0.658	0.43
Non-fiber carbohydrates	65.12	36.6

[1] Grain fed mineral mix composition: 26.17% Ca, 10.52% P, 3.35% Na, 2.95% Mg, 6.80% K, 0.17% Mn, 0.0006% Cp, 0.06% Cu, 0.003% I, 0.002 Se, 0.17% Zn, 0.18% Fe. Forage fed mineral mix composition: 18% Ca, 6% P, 18% NaCl, 4% Mg, 0.5% K, 0.36% Mn, 0.0012% Co, 0.12 Cu, 0.006% I, 0.0027% Se, 0.36% Zn; [2] CP: crude protein; NDICP: Neutral detergent insoluble crude protein; aNDFom: neutral detergent fiber (NDF) assayed with a heat stable amylase and expressed exclusive of residual ash; apNDFom: NDF assayed with a heat stable amylase and expressed exclusive of residual ash and protein.

Table 3. Effect of backgrounding on nutrient intake of crossbred Angus steers fed a low (n = 12) or a moderate plane of nutrition (n = 12)

Daily intake, kg/day[1]	Treatment		SEM[3]	*P*-value[4]
	Low Plane	Moderate Plane		
Dry matter	7.58	9.70	0.236	<0.0001
Organic matter	6.84	8.97	0.217	<0.0001
Crude protein	0.69	1.22	0.028	<0.0001
Rumen degradable protein	0.53	0.89	0.020	<0.0001
Acid detergent fiber	2.22	3.88	0.088	<0.0001
apNDFom[2]	3.52	4.41	0.108	<0.0001
Lignin	0.31	0.67	0.015	<0.0001
Sugar	0.97	0.72	0.022	<0.0001
Starch	0.030	0.095	0.002	<0.0001
Non-fiber carbohydrates	1.97	2.95	0.070	<0.0001
Total digestible nutrients	4.02	5.59	0.133	<0.0001
Net energy for maintenance, Mcal/d	9.36	11.74	0.288	<0.0001
Net energy for gain, Mcal/d	3.84	5.92	0.138	<0.0001
Ca, g/d	22.45	31.42	0.245	<0.0001
P, g/d	8.04	8.67	0.049	<0.0001
Mg, g/d	5.54	7.48	0.067	<0.0001
K, g/d	11.24	15.00	0.347	<0.0001
Na, g/d	8.40	9.38	0.034	<0.0001
Fe, g/d	0.22	0.11	0.004	<0.0001
Mn, g/d	0.42	0.42	0.0007	0.9867
Zn, g/d	0.42	0.41	0.0007	<0.0001
Cu, g/d	0.15	0.14	0.0002	<0.0001

[1]Intake is expressed as kg/d unless otherwise specified; [2]apNDFom: Neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash and protein; [3]SEM: Standard error of the mean; [4]*P*-value: <0.1 = trend; <0.05 = significant.

Table 4. Effect of previous plane of nutrition on nutrient intake of crossbred Angus steers backgrounded on low or moderate plane and subsequently finished on grain (n = 12) or forage-fed (n= 12) finishing systems

Daily intake, kg/day[1]	Treatment		SEM[3]	*P*-value[4]		
	Grain-Fed	Forage-Fed		TRT	Previous	TRT x Previous
Dry matter	10.98	12.91	0.331	0.0005	0.9412	0.5617
Organic matter	10.60	11.73	0.318	0.0207	0.9564	0.5728
Crude protein	1.19	2.75	0.040	<0.0001	0.7276	0.4267
Rumen degradable protein	0.56	1.90	0.022	<0.0001	0.5924	0.3577
Acid detergent fiber	1.21	3.38	0.044	<0.0001	0.6609	0.3911
apNDFom[2]	1.80	3.91	0.060	<0.0001	0.7505	0.4397
Lignin	0.35	0.74	0.012	<0.0001	0.7597	0.4450
Sugar	0.36	1.23	0.014	<0.0001	0.5907	0.3569
Starch	6.26	0.28	0.180	<0.0001	0.7999	0.7795
Non-fiber carbohydrates	7.16	4.73	0.209	<0.0001	0.9423	0.6512
Total digestible nutrients	8.85	8.37	0.262	0.2119	0.9923	0.5995
Net energy for maintenance, Mcal/d	34.41	31.33	1.019	0.0450	0.9997	0.6056
Net energy for gain, Mcal/d	23.41	19.94	0.691	0.0020	0.9864	0.6160
Ca, g/d	29.95	30.21	0.213	0.4046	0.4309	0.2929
P, g/d	13.68	4.69	0.093	<0.0001	0.9689	0.6297
Mg, g/d	4.80	5.62	0.062	<0.0001	0.7399	0.4337
K, g/d	15.12	21.11	0.290	<0.0001	0.6989	0.4111
Na, g/d	4.36	5.22	0.035	<0.0001	0.6928	0.4078
Fe, g/d	1.59	0.50	0.041	<0.0001	0.8710	0.7125
Mn, g/d	0.21	0.19	0.001	<0.0001	0.8428	0.4954
Zn, g/d	1.39	0.18	0.035	<0.0001	0.7978	0.7816
Cu, g/d	0.067	0.061	0.001	<0.0001	0.8550	0.5032

[1]Intake is expressed as kg/d unless otherwise specified; [2]apNDFom: NDF assayed with a heat stable amylase and expressed exclusive of residual ash and protein; [3]SEM: Standard error of the mean; [4]*P*-value: <0.1 = trend; <0.05 = significant, TRT: treatment effect, Previous: low versus moderate previous plane of nutrition.

Table 5. Effect of backgrounding on drinking water intake and behavior of crossbred Angus steers fed a low (n = 12) or a moderate plane of nutrition (n = 12)

Item[1]	Treatment		SEM[2]	*P*-value[3]
	Low Plane	Moderate Plane		
Drinking Water Intake, kg	36.53	38.62	1.271	0.2583
Drinking Water Intake, g BW	111.5	110.1	4.36	0.8205
Drinking Water Intake, g $BW^{0.75}$	474.0	476.4	16.75	0.9197
Drinking Water Intake, kg ADG	45.58	31.69	2.164	0.0002
Drinking Water Intake, kg DMI	4.82	4.00	0.153	0.0010
Drinking Water Intake, kg CPi	53.03	31.74	1.551	<0.0001
Drinking water behavior				
Time spent drinking water, min/d	16.58	21.00	1.013	0.0054
Drinking rate, L/min	2.23	1.92	0.107	0.0546
Water trough visits, events/d	5	6	0.198	0.0185

[1]BW: body weight, $BW^{0.75}$: metabolic body weight, ADG: average daily gain, DMI: dry matter intake, CPi: crude protein intake; RDPi: Rumen degradable protein intake; [2]SEM: Standard error of the mean; [3]*P*-value: <0.1 = trend; <0.05 = significant.

Table 6. Effect of previous plane of nutrition on drinking water intake and behavior of crossbred Angus steers backgrounded on low or moderate plane and subsequently finished on grain (n = 12) or forage-fed (n= 12) finishing systems

Item[1]	Treatment		SEM[2]	*P*-value[3]		
	Grain Fed	Forage Fed		TRT	Previous	TRT x Previous
Drinking Water Intake, kg	39.75	67.81	2.158	<0.0001	0.0766	0.5985
Drinking Water Intake, g BW	78.31	132.3	4.35	<0.0001	0.0106	0.6818
Drinking Water Intake, g $BW^{0.75}$	371.5	629.2	19.99	<0.0001	0.0152	0.6535
Drinking Water Intake, g CCW	127.70	223.60	7.137	<0.0001	0.0294	0.5042
Drinking Water Intake, kg ADG	22.54	37.77	1.690	<0.0001	0.7995	0.9280
Drinking Water Intake, kg DMI	3.60	5.24	0.125	<0.0001	0.0375	0.7984
Drinking Water Intake, kg CPi	33.40	24.63	0.935	<0.0001	0.0581	0.6642
Drinking water behavior						
Time spent drinking water, min/d	34.42	45.17	2.842	0.0146	0.7282	0.5018
Drinking rate, L/min	1.20	1.55	0.089	0.0115	0.3841	0.6702
Water trough visits, events/d	5	6	0.276	0.0016	0.1026	0.4502

[1]BW: body weight, $BW^{0.75}$: metabolic body weight, CCW: hot carcass weight, ADG: average daily gain, DMI: dry matter intake, CPi: crude protein intake; RDPi: Rumen degradable protein intake; [2]SEM: Standard error of the mean; [3]*P*-value: <0.1 = trend; <0.05 = significant, TRT: treatment effect, Previous: low versus moderate previous plane of nutrition.

Table 7. Effect of backgrounding on performance, feed and water efficiency of crossbred Angus steers fed a low (n = 12) or a moderate plane of nutrition (n = 12)

Item[1]	Treatment		SEM[2]	*P*-value[3]
	Low Plane	Moderate Plane		
Initial Body Weight, kg	293.84	302.19	10.170	0.5673
Final Body Weight, kg	362.81	405.97	10.237	0.0071
Final Body Weight, kg $BW^{0.75}$	83.05	90.39	1.759	0.0074
Average Daily Gain, kg/day	0.821	1.235	0.0435	<0.0001
Midpoint Body Weight, kg $BW^{0.75}$	77.39	81.34	1.884	0.1525
Feed Conversion Ratio	9.48	8.00	0.467	0.0351
Residual Feed Intake	-0.360	0.360	0.2348	0.0408
Residual Drinking Water Intake	0.079	-0.079	1.2020	0.9265
Residual Drinking Water Intake in function of DMI	0.329	-0.329	1.174	0.6955
Water Conversion Rate	45.59	31.69	2.164	0.0002
Gross Water Efficiency	0.023	0.032	0.0014	<0.0001

[1]BW: body weight, $BW^{0.75}$: metabolic body weight, DMI: dry matter intake, Feed conversion rate: ratio of average dry matter intake to average daily gain, Residual feed intake: difference between observed and predicted dry matter intake required to meet growth and maintenance energy requirements, Residual drinking water intake: difference between observed and predicted water intake required to meet growth and maintenance energy requirements, Water conversion rate: ratio of average water intake to average daily gain, Gross water efficiency: ratio of average daily gain to average water intake; [2]SEM: Standard error of the mean; [3]*P*-value: <0.1 = trend; <0.05 = significant.

Table 8. Effect of previous plane of nutrition on performance and feed and water efficiency of crossbred Angus steers backgrounded on low or moderate plane and subsequently finished on grain (n = 12) or forage-fed (n= 12) finishing systems

Item[1]	Treatment		SEM[2]	*P*-value[3]		
	Grain Fed	Forage Fed		TRT	Previous	TRT x Previous
Initial Body Weight, kg	385.94	383.60	10.797	0.8796	0.0116	0.7289
Final Body Weight, kg	569.95	572.11	8.970	0.8669	0.0885	0.4324
Final Body Weight, kg $BW^{0.75}$	116.61	116.95	1.376	0.8658	0.0886	0.4379
Average Daily Gain, kg	1.786	1.830	0.0842	0.7179	0.1243	0.6998
Midpoint Body Weight, kg $BW^{0.75}$	107.23	108.07	1.468	0.6927	0.0169	0.3394
Feed Conversion Ratio	6.26	7.21	0.288	0.0309	0.0699	0.9052
Residual Feed Intake	-0.792	0.792	0.2811	0.0007	0.3511	0.6377
Residual Drinking Water Intake	-13.30	13.30	2.355	<0.0001	0.0754	0.6234
Residual Drinking Water Intake in function of DMI	-4.72	4.72	2.179	0.0062	0.5588	0.9404
Water Conversion Rate	22.54	37.78	1.690	<0.0001	0.7995	0.9280
Gross Water Efficiency	0.046	0.027	0.002	<0.0001	0.7816	0.9242

[1]BW: body weight, $BW^{0.75}$: metabolic body weight, DMI: dry matter intake; [2]SEM: Standard error of the mean; [3]*P*-value: <0.1 = trend; <0.05 = significant, TRT: treatment effect; Previous: low versus moderate previous plane of nutrition.

Table 9. Effect of previous plane of nutrition on carcass traits of crossbred Angus steers backgrounded on low or moderate plane and subsequently finished on grain (n = 12) or forage-fed (n= 12) finishing systems

Item	Treatment		SEM[1]	*P*-value[2]		
	Grain Fed	Forage Fed		TRT	Previous	TRT x Previous
Hot carcass weight, kg	312.89	304.01	6.249	0.3269	0.2371	0.7454
Dressing percentage, %	54.95	53.19	0.974	0.2181	0.8542	0.7565
Rib eye area, cm^2	11.57	10.98	0.323	0.2097	0.2635	0.9048
Marbling score[3]	457.16	367.79	15.21	0.0005	0.0013	0.1099
Yield grade	3.05	3.07	0.070	0.8416	0.4322	0.4467

[1]SEM: Standard error of the mean; [2]*P*-value: <0.1 = trend; <0.05 = significant, TRT: treatment effect, Previous: low versus moderate previous plane of nutrition; [3]Pratically devoid=100 to 199, slight = 200 to 299, small = 300 to 399, modest = 400 to 499, moderate = 500 to 599.

Table 10. Effect of previous plane of nutrition on body weight and biometric measurements of crossbred Angus steers backgrounded on low (n = 12) or moderate plane of nutrition (n = 12)

Item	Treatment		Days				*P*-value[2]		
	Low Plane	Moderate Plane	0	42	78	SEM[1]	TRT	Day	TRT x Day
Body Weight, kg	330.26	346.55	298.05	341.59	380.06	9.896	0.1820	<0.0001	0.0012
Shrunk Body weight, kg	317.05	335.56	286.13	327.93	364.84	9.501	0.1821	<0.0001	0.0012
Biometric Measurements									
Girth Circumference, cm	155.79	160.04	153.08	157.17	163.5	2.269	0.1991	<0.0001	0.0793
Hook Bone Width, cm	37.02	38.61	35.54	38.97	38.93	0.520	0.0426	<0.0001	0.0040
Pin Bone Width, cm	8.20	8.45	7.54	9.13	8.31	0.199	0.3928	<0.0001	0.3800
Pelvic Girdle Length, cm	39.04	39.65	38.47	40.22	39.33	0.641	0.5077	0.0002	0.0300
Rump Depth, cm	56.44	57.15	56.31	56.37	57.70	0.889	0.5791	0.0007	0.1253
Rib depth, cm	56.30	57.96	55.52	57.47	58.39	0.751	0.1333	<0.0001	0.0062
Thorax Width, cm	33.34	35.69	30.95	35.06	37.54	0.657	0.0193	<0.0001	<0.0001
Abdomen Width, cm	47.31	46.29	43.25	48.20	48.95	0.908	0.4325	<0.0001	0.2716
Scapula, cm	33.26	29.79	31.75	33.22	29.60	0.944	0.0163	0.0113	0.0153
Rump Height, cm	121.38	123.01	119.22	122.29	125.06	1.189	0.3427	<0.0001	0.5795
Height at Withers, cm	115.01	114.00	108.52	116.83	118.17	2.178	0.7452	0.0004	0.0218
Body Length, cm	55.96	57.31	53.47	58.30	58.14	1.296	0.4674	0.1146	0.3315
Diagonal, cm	93.56	93.84	90.27	94.60	96.25	1.159	0.8670	<0.0001	0.0259

[1]SEM: Standard error of the mean; [2]*P*-value: <0.1 = trend; <0.05 = significant, TRT: Treatment effect.

Table 11. Effect of previous plane of nutrition on body weight, body condition score, and biometric measurements of crossbred Angus steers backgrounded on low or moderate plane and subsequently finished on grain (n = 12) or forage-fed (n= 12) finishing systems

Item	Treatment		Days					SEM[1]	*P*-value[2]				
	Grain Fed	Forage Fed	0	28	56	84	103		TRT	PRV	TRT x PRV	Day	TRT x Day
Body Weight, kg	504.16	510.82	429.86	469.92	512.4	554.27	571.03	9.768	0.6349	0.1048	0.4871	<0.0001	0.5177
Shrunk Body weight, kg	484.00	490.39	412.66	451.12	491.90	532.1	548.19	9.38	0.6350	0.1049	0.4874	<0.0001	0.5181
Body Condition Score	6.12	6.09	5.84	5.93	6.02	6.35	6.38	0.146	0.8732	0.0038	0.1400	0.0001	0.0224
Biometric Measurements													
Girth Circumference, cm	183.88	185.18	168.04	178.52	185.21	193.54	197.33	1.960	0.6463	0.2038	0.1329	<0.0001	0.2671
Pin Bone Width, cm	10.70	10.69	9.64	9.75	9.97	10.29	10.83	0.109	0.9153	0.2239	0.2355	<0.0001	0.4744
Pelvic Girdle Length, cm	44.20	43.9	41.70	42.18	43.35	45.95	47.06	0.445	0.6296	0.1101	0.5424	<0.0001	0.5845
Rump Depth, cm	63.26	62.16	60.64	59.97	61.97	61.87	62.08	0.689	0.2690	0.2494	0.3931	<0.0001	0.7731
Rib depth, cm	65.23	64.20	61.27	62.60	65.02	66.89	67.79	0.614	0.2479	0.0101	0.9396	<0.0001	0.0195
Thorax Width, cm	43.73	43.17	41.54	42.56	43.35	45.87	47.93	0.705	0.5815	0.0606	0.1674	<0.0001	0.7084
Abdomen Width, cm	59.19	56.90	54.38	55.94	57.60	57.43	60.87	0.727	0.0373	0.0909	0.0639	<0.0001	0.4395
Scapula, cm	34.99	34.66	31.91	32.14	34.89	37.79	37.39	0.453	0.6173	0.3150	0.6953	<0.0001	0.4419
Rump Height, cm	131.63	129.55	127.69	128.40	132.33	132.56	133.96	0.877	0.1225	0.3820	0.8926	<0.0001	0.0665
Height at Withers, cm	126.05	123.18	120.96	123.00	125.12	125.17	128.83	1.044	0.0665	0.2685	0.6883	<0.0001	0.1016
Body Length, cm	65.46	62.39	61.58	62.93	65.41	66.50	66.20	0.974	0.0372	0.1344	0.2188	<0.0001	0.0272
Diagonal, cm	104.13	101.40	100.19	99.93	102.83	103.52	107.33	0.901	0.0365	0.0811	0.1435	<0.0001	0.0652

[1]SEM: Standard error of the mean; [2]*P*-value: <0.1 = trend; <0.05 = significant, TRT: treatment, PRV: low versus moderate previous plane of nutrition.

9. Figures

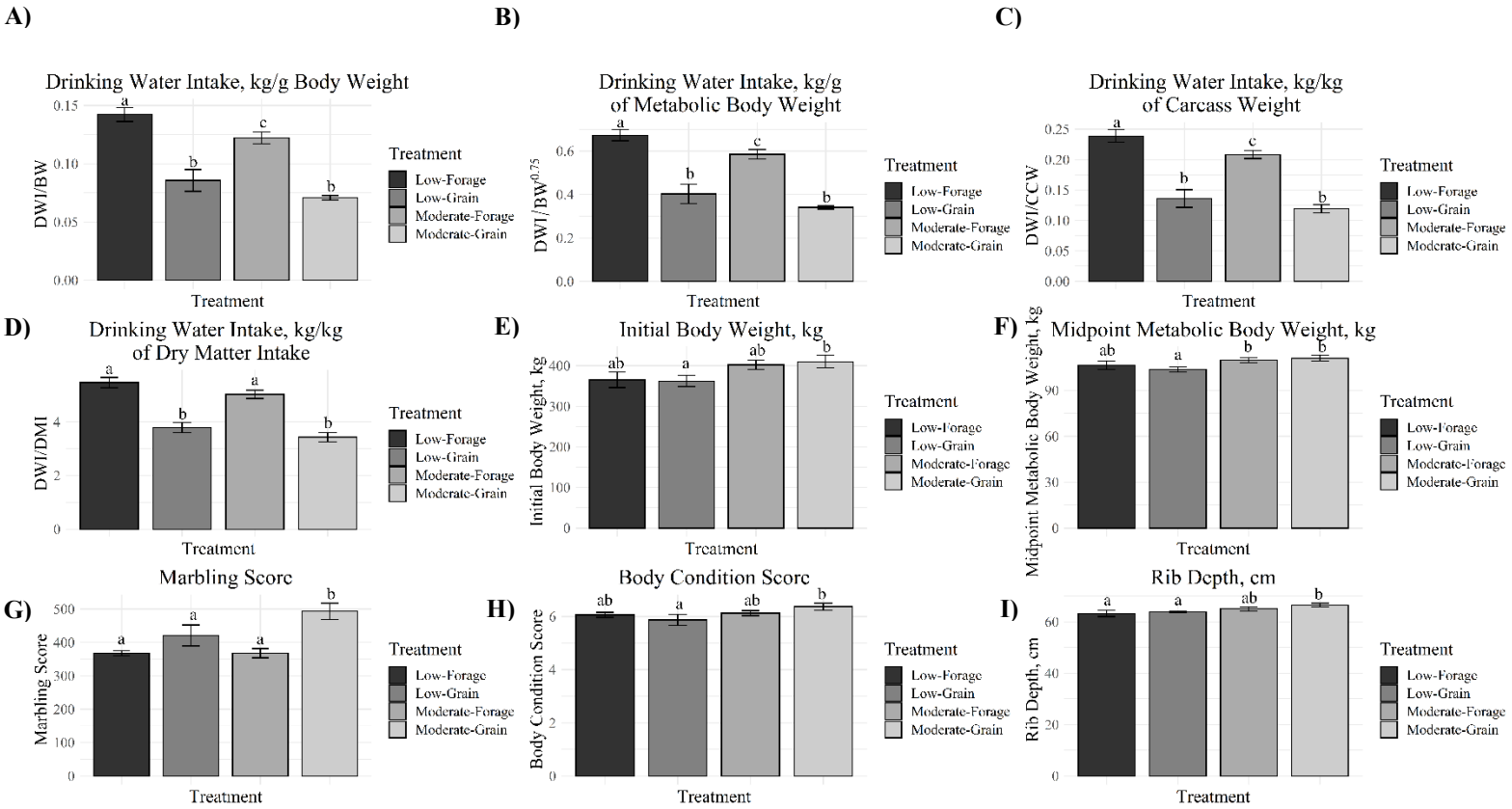
A)
Drinking Water Intake, kg/g Body Weight
DWI/BW
Treatment
B)
Drinking Water Intake, kg/g of Metabolic Body Weight
DWI/BW^0.75
Treatment
C)
Drinking Water Intake, kg/kg of Carcass Weight
DWI/CCW
Treatment
D)
Drinking Water Intake, kg/kg of Dry Matter Intake
DWI/DMI
Treatment
E)
Initial Body Weight, kg
Initial Body Weight, kg
Treatment
F)
Midpoint Metabolic Body Weight, kg
Midpoint Metabolic Body Weight, kg
Treatment
G)
Marbling Score
Marbling Score
Treatment
H)
Body Condition Score
Body Condition Score
Treatment
I)
Rib Depth, cm
Rib Depth, cm
Treatment
Treatment
Low-Forage
Low-Grain
Moderate-Forage
Moderate-Grain

Figure 1. Influence of a low (n = 12) or moderate (n = 12) previous plane of nutrition of crossbred Angus steers subsequently finished in a grain (n = 12) or a forage-fed (n = 12) finishing system.
A) Influence of previous plane of nutrition on drinking water intake corrected for body weight (DWI/BW); B) Influence of previous plane of nutrition on drinking water intake corrected for metabolic body weight ($DWI/BW^{0.75}$); C) Influence of previous plane of nutrition on drinking water intake corrected for carcass weight (DWI/CCW); D) Influence of previous plane of nutrition on drinking water intake corrected for dry matter intake (DWI/DMI); E) Influence of previous plane of nutrition on initial body weight (IBW); F) Influence of previous plane of nutrition on midpoint metabolic body weight ($Mid\ BW^{0.75}$); G) Influence of previous plane of nutrition on marbling score; H) Influence of previous plane of nutrition on body condition score (BCS); I) Influence of previous plane on rib depth. ^{ab} Means within each separately plot without common letter differ ($P \leq 0.05$).

CHAPTER III

CARRYOVER NUTRITIONAL EFFECTS ON NITROGEN METABOLISM AND WATER REQUIREMENTS OF LOW OR MODERATE PLANE OF NUTRTION DURING BACKGROUNDING ONTO GRAIN OR FORAGE-FED FINSIHED CATTLE

Lay Summary

Due to the increasing concern over climate change, the demand for grass/forage-fed beef has increased considerably. However, in order to reach adequate levels of carcass finishing, the production of forage-fed animals requires very high-quality forage, which is usually associated with high levels of protein. In order to address how those diets could increase nitrogen excretion and water requirements of animals, we compared the effects of grain versus forage-fed system on water and nitrogen metabolism. Also, we further investigated if the previous plane of nutrition of those animals could interfere their water and nitrogen usage during the finishing phase. Overall, animals finished on a forage-based diet doubled their nitrogen excretion and also required more fresh water when compared to grain-based finishing systems. Furthermore, animals fed a low plane of nutrition during the backgrounding phase were more efficient on nitrogen usage, which was carried over to the finishing phase only when they were finished in an appropriate level of protein during with a grain-based diet.

Highlights

- Forage-fed finishing animals doubled nitrogen excretion and required more fresh water than grain-fed animals.
- Low plane during background will increase efficiency on protein usage which can be carried over during the finishing phase when animals receive moderate levels of protein

1. Abstract

The objective of this study was to examine how different backgrounding and finishing systems might affect nitrogen (N) metabolism, and consequently water requirements of cattle. Further, we investigated if the previous plane of nutrition might affect the results obtained in the subsequent phase. Twenty-four (n = 24) animals were randomly distributed into either a low (LP; crude protein (CP): 9.10%) or moderate (MP; CP: 12.62%) plane of nutrition during the background phase for 85d. Animals were then blocked by their previous plane of nutrition and were moved onto a 105-d finishing phase. The forage-finished group received only high-quality alfalfa hay (CP: 21.3%), whereas the grain-finished group received a high grain diet (80% whole corn and 20% alfalfa hay; CP: 10.8%). Animals on LP diets excreted less N through urine ($P < 0.01$) and feces ($P < 0.01$), while retaining more N ($P < 0.01$), which resulted in an increased N efficiency ($P < 0.01$) when compared to MP animals. No differences were observed for water intake or microbial N being observed ($P > 0.05$). Grain-fed animals consumed less N and water ($P < 0.01$), but still had a higher amount of N being digested when compared to forage-fed animals ($P < 0.01$), as well as the lowest excretion of N through feces ($P <$

0.01) and urine ($P < 0.01$), and consequently a higher rate of N being retained ($P < 0.01$) and a higher efficiency on utilizing the ingested N ($P < 0.05$). Further interaction was also observed between treatment and previous plane of nutrition, where animals coming from a LP had the lowest dry matter intake, highest amount of N being digested and lowest N excretion, fecal output, and excretion of N through the feces ($P < 0.05$). These results indicate that animals receiving LP will reduce their excretion of N without modifying the required amount of water while producing the same amount of microbial N due to more efficient N recycling. Extremely important is the fact that efficiency is carried over after these animals are transitioned to grain-fed finishing systems.
Keywords: Nitrogen recycling; Urinary excretion; Water.

2. Introduction

High protein diets are a reality for both the dairy and beef industries. For dairy cattle, the inclusion of high levels of protein in the diet aiming to achieve high yields has become a common practice (Salo, 2018), while in the beef industry this can also be very common particularly when by-products (e.g., dried distillers' grains) are included in high levels in the diet (Koenig and Beauchemim, 2018) or when high-quality and lush forage that is young and immature is fed to grazing (and grass-finished) animals. Protein is an important limiting nutrient for ruminants, and it becomes necessary when an animal attains its optimum growth and peak of production (Ali et al., 2009). However, when in excess, high protein is metabolized in the rumen and the excess of nitrogen (N) is excreted as urea in urine or in the feces (Koenig and Beauchemim, 2018). Urinary urea excreted is rapidly hydrolyzed to ammonium by microbial ureases and converted to

ammonia which is volatile and easily diffused to the environment (Mobley and Hausinger, 1989). Volatized ammonia carries concerning environmental effects related to human health effects, eutrophication of surface waters, acidification of ecosystems, and fine particulate matter formation in the atmosphere (Lee et al., 2012). Additional environmental concerns related to ammonia are its effects on livestock water use. Excretion of urinary urea requires water, which inevitably leads to higher water intake and, hence, increased urine output (Katongole and Yan, 2020). Therefore, water intake and urine excretion rates are functions of protein intake. Ritzman and Benedict (1924) observed that steers on high protein allowances consumed 26% more water than did similar animals on low protein rations. In dairy cattle, raising the crude protein (CP) content from 12 to 13% increased water intake about 0.99 L/day in dry cows, but it was not significant in lactating cows (Holter and Urban Jr. 1992). None of the previous studies have investigated how previous management and nutrition may affect nitrogen levels and water efficiency in later phases.

Historically, the beef cattle industry in the U.S. has been highly segmented and operated independently according to the developmental phase of the animal (Drouillard and Kuhl, 1993). The first phase would be the cow-calf production, where beef cows are usually maintained to raise calves. Once calves are weaned, they move to the backgrounding phase where nutrients supplied through grazing on rangeland pastures, might have very low levels of protein, limiting fiber utilization (Koster et al., 1996). Finally, animals are transferred to the finishing phase, where animals can either receive a diet high in grains or forage. However, for the production of forage-fed animals to be

acceptable within a feasible time frame, those animals are fed very high-quality forage (NASEM, 2016), which is usually associated with high CP.

Therefore, taking in consideration the wide diversity of production system in which beef cattle are raised in the U.S., different combinations of backgrounding and finishing planes of nutrition also need to be addressed to fully understand what factors are regulating nitrogen and water metabolism of those animals. This study aimed to examine how different backgrounding and finishing systems affect nitrogen metabolism, and consequently water requirements of cattle while examining potential carryover effects from previous planes of nutrition/management.

3. 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, NV (protocol #00845).

i. Experimental design, treatments, and animals

Twenty-four crossbred Angus steers (298.01 ± 10.17 kg) were housed in the research feedlot area of the Main Station Field Laboratory at the University of Nevada, Reno. The experimental trial lasted 220 days, consisting of two phases: backgrounding and finishing phase. During the backgrounding phase (85 d), animals were randomly assigned to one of the two treatments (n=12 per treatment): low plane of nutrition (LP, CP: 9.10%, net energy available for maintenance [NEm]: 0.25 Mcal/kg, net energy available for gain [NEg]: 0.10 Mcal/kg) or moderate plane of nutrition (MP, CP: 12.62%, NEm: 0.25

Mcal/kg; NEg: 0.13 Mcal/kg). At the end of the backgrounding phase, steers were blocked by previous plane of nutrition (LP or MP) and transitioned to the finishing phase during 30-d. After the transition period, animals were fed either alfalfa hay only (forage-fed, n=12; CP: 21.3%, NEm: 0.32 Mcal/kg; NEg: 0.20 Mcal/kg) or predominantly whole grain (grain-fed, n=12; CP: 10.8%, NEm: 0.40 Mcal/kg; NEg: 0.30 Mcal/kg) for 105-d. All animals had free access to clean water and a commercial balanced mineral mix throughout the experimental period.

ii. Water intake system

Before the beginning of the experiment, each animal was fitted with a plastic radio-frequency identification tag (FDX-ISO 11784/11785; Allflex, 104 Joinville, Santa Catarina, Brazil) in the left ear. For each visit to the water trough, the system recorded the water intake of individual animals and data were continuously transferred to the cloud. The water bins (0.30 x 0.37 x 0.20 m) were programmed to maintain the water temperature at 25 °C and were automatically and continuously filled to a volume of 115 L after the animals left the weighing platforms. Only one animal was allowed at a time to each individual water trough at a time. A complete description and evaluation of the water system can be found in Oliveira et al. (2018). Each water trough was manually cleaned and disinfected biweekly or as needed to ensure free access of fresh water at all times. Water intake platforms were calibrated weekly with company-manufactured weights to ensure data accuracy.

iii. Samples collection

Steers were fed once during the backgrounding phase at 0800 h, and twice during the finishing phase at 0700 h and 1700 h. Feeds offered and orts were collected daily before morning feeding and weighed, and feed intake was adjusted daily to ensure up to 10% as-fed refusals. Daily orts were then mixed into a composite on a weekly basis, identified, oven dried, and ground mill through a 2 mm to determine the indigestible neutral detergent fiber (iNDF) concentration and 1 mm for further analysis.

To evaluate the apparent digestibility of nutrients, on the 28th and 56th day of the background phase and on the 28th, 56th and 84th day of the finishing phase, spot fecal samples were taken during spontaneous defecation over 4-d periods at 0600 h on day 1, 1000 h on day 2, 1400 h on day 3, and 1800 h on day 4 to obtain a proportional and representative sample. A composite sample from each animal was created per period, identified, oven dried, and ground mill as described for orts and feeds. On the day following the 4-d period of fecal collection of each phase, urine spot samples were collected four hours before and four hours after the morning feed to evaluate the microbial protein production. Urine samples were proportionally sampled and diluted in 40 mL H_2SO_4 (0.036 N), and frozen for analysis (-20°C).

iv. Laboratory analysis

Samples of offered feed, orts, and feces processed in a 1 mm screen were analyzed for the chemical composition of dry matter (DM; method #930.15 AOAC, 2000), CP (method # 990.03; AOAC, 2000), and ash (method # 942.05; AOAC, 2000). Indigestible neutral detergent fiber concentrations in all samples were determined after a 288-h in situ

incubation (Huhtanen et al., 1994) in the rumen using three canulated steers fed alfalfa hay. After removal from the rumen, the bags were rinsed clean, boiled in a neutral detergent solution (100 mL/g of sample; Mertens et al., 2002), thoroughly rinsed, dried at 60°C for 24 h, and weighed. Concentrations of iNDF were used to determine total fecal excretion by dividing the intake of iNDF by the fecal iNDF concentration.

On urine samples, concentrations of allantoin, uric acid, and creatinine were determined via high-performance liquid chromatography (Agilent 1100-HPLC System) as described by (Shingfield and Offer, 1999). Since concentrations of xanthine and hypoxanthine are rarely detected in cattle urine, total purine derivatives (PD) were calculated by summing allantoin and uric acid and expressed as mmol/d (González-Ronquillo et al., 2004). The absorbed PD was then calculated using the equation proposed by Chen and Gomes (1992). Urinary volume was computed according Chizzotti et al. (2008) assuming a constant rate of creatinine excreted in the urine.

v. Statistical analysis

Data were analyzed as linear mixed models using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC) adopting a $P \leq 0.05$ as significant and $0.05 < P \leq 0.1$ as tendencies. Mean comparisons were computed using the LSMEANS statement and compared using Tukey-Kramer adjustment. Data from different time points were included as repeated measures in the statistical model, where day was considered the repeated variable. The fixed effect of time and its interactions with treatments was analyzed using the covariance structure that yielded the lowest Bayesian Information Criteria.

Variables from the background phase were analyzed using a completely randomized design, following the statistical model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where Y_{ij} is the dependent variable taken from experimental unit jth on treatment ith, μ is the overall mean, T_i is the fixed effect of treatment ith, and e_{ij} is the random error associated with ijth data value assuming that $e_{ij} \sim N(0, \sigma^2)$.

For the finishing phase, data were analyzed following a completely randomized block design, where the previous treatment is considered the block. Therefore, the statistical model for the finishing phase is described in the following equation:

$$Y_{ijk} = \mu + T_i + b_j + Tb_{ij} + e_{ij}$$

Where Y_{ijk} is the dependent variable taken from experimental unit kth in the block jth on treatment ith, T_i is the fixed effect of treatment ith, b_j is the random effect associated with the block jth, Tb_{ij} is the random effect associated with the interaction between treatment ith and block jth, and e_{ijk} is the random error associated with ijkth data value assuming that $e_{ijk} \sim N(0, \sigma^2)$.

Identification of outliers and influential points was performed by plotting the studentized residuals against the predicted values as well as by Cook's distance. Coefficients exceeding 2.5 studentized t distributions were considered outliers and removed from the data (Neter et al., 2004). Principal component analyses were analyzed using the prcomp function from the stats package and all plots were plotted using either ggbiplot, ggplot2 or ggboxplot from the Tidyverse and GGally packages in R version 4.1.2. (R Development Core Team, Vienna, Austria).

4. Results

i. Nitrogen Metabolism

During the backgrounding phase, no treatment effect was observed for daily water intake (L/d) and water intake corrected to microbial N efficiency for animals fed either LP or MP (Table 1). However, when corrected to the amount of N ingested, water intake was higher for LP animals when compared to MP animals ($P < 0.01$; Table 1), whereas when corrected to N digested, it was higher for MP ($P < 0.01$; Table 1). In general, MP animals ingested more N but digested it to a lesser extent than LP animals ($P < 0.01$; Table 1). A treatment effect for N intake was also observed between collections, where intake of N was higher during the second collection for both treatments (Figure 1A). Further, LP animals excreted less N through urine ($P < 0.01$) and feces ($P < 0.01$), and retained more N ($P < 0.01$), which made them more efficient at utilizing N ($P < 0.01$) when compared to MP animals (Table 1). The main route of N excretion also changed according to the diet, and MP animals excreted more N through the urine when compared to LP ($P < 0.01$; Table 1). An interaction between the volume of urine and day of collection was also observed, and MP animals had the highest urinary volume at the second collection when compared to LP animals ($P < 0.01$; Figure 1B).

The nitrogen metabolism data for the finishing phase is presented on Table 2. Water intake of forage-fed animals was much higher than grain-fed animals ($P < 0.01$), apart from when water intake was corrected for the amount of N ingested, which was higher for grain-fed animals ($P < 0.01$). Furthermore, an interaction between treatment and time was observed for water intake corrected for grams of N digested, where water intake

peaked at the second collection and then decreased for the third collection for grain-fed animals; whereas for the forage-fed animals, it increased at the second collection and stayed high during the following collection ($P < 0.01$; Figure 2A). As shown in Table 2, dry matter intake ($P < 0.05$) and N ingested ($P < 0.01$) were both higher for forage-fed animals, and further interaction between previous plane and treatment was also observed for dry matter intake, where animals backgrounded on LP and finished on a grain-based diet had the lowest dry matter intake (Figure 3A). Grain-fed animals had a higher amount of N being digested when compared to forage-fed animals ($P < 0.01$), as well as the lowest excretion of N through feces ($P < 0.01$) and urine ($P < 0.01$), and consequently a higher rate of N being retained ($P < 0.01$) and a higher efficiency in utilizing the ingested N ($P < 0.05$; Table 2).

An interaction between treatment and day of collection was also observed for the N digested, N excreted, fecal excretion of N, and excretion of urinary N as a % of total N excreted (Figure 2B, C, D, and E, respectively; $P < 0.05$). For N digested, no differences were observed for the grain-fed treatment, but for the forage-fed animals, there was a decrease on digestion of N for the second and third collection. Excretion of N in the feces and total N excreted had a quadratic response for grain-fed animals, peaking at the second collection, whereas for forage-fed animals, it increased linearly. Urinary N excretion as a % of total N excretion increased linearly for grain-fed animals, but for forage-fed animals, it was constant during the finishing phase. An interaction was also observed between treatment and previous plane of nutrition on the amount of N digested and excreted, as well as on the excretion of feces (Figure 3B, C, D, and E). In general,

animals coming from a LP had the highest amount of N being digested and lowest N excretion, fecal output, and excretion of N through the feces ($P < 0.05$).

ii. Microbial crude protein synthesis

The N synthesis of crude microbial protein was unaffected ($P > 0.05$) by the experimental diets during the background phase (Table 3). However, during the finishing phase (Table 4), forage-fed animals were more efficient in the production of microbial N ($P < 0.05$) and also had a higher excretion of uric acid per day ($P < 0.05$).

iii. Relationship between water and nitrogen metabolism

The relationship between water intake and nitrogen metabolism for the backgrounding phase are presented in Figure 4. For the LP animals water intake appears to be positively correlated to ingested N, and negatively correlated to N digested (Figure 4A). However, when the animals are backgrounded on MP, water intake seems to be positively correlated mainly to N ingested and microbial N efficiency (Figure 4B).

The relationship between nitrogen metabolism variables and water intake during the finishing phase are in Figure 5. For grain-fed animals (Figure 5A), water intake is positively correlated to N ingested, urine volume, microbial N efficiency, and microbial N, but negatively correlated to digested N and urinary N excretion. On the other hand, for forage-fed animals, water intake was positively correlated mainly to excretion of fecal N, but negatively correlated to ingested N and urinary N excretion.

iv. Urinary and fecal N excretion through time

Animals backgrounded on LP near-doubled their urinary N excretion from the first collection compared to the second collection (Figure 6A). However, even though excretion of N from urine increased in the second phase for MP animals, the decrease on N excretion in the feces was much lower compared to LP animals (Figure 6B). Steers finished on a grain-based diet linearly increased their excretion of N through urine over the trial (Figure 7A), whereas animals finished on a forage-based diet increased their excretion of N via feces (Figure 7B).

5. Discussion

In general, ruminants convert about 20% to 30% of dietary N into animal protein, while the rest is excreted in urine and feces (Doranalli et a., 2011), which has adverse economic and environmental implications. Therefore, there is a growing critical concern to better understand how nitrogen levels in the diet can interfere with the nitrogen metabolism of ruminants. Herein, our results indicate that dietetic nitrogen intake has a direct impact on nitrogen metabolism, as well as water requirements. During the backgrounding phase animals on MP ingested more N, but had a lower digestion rate of N, indicating that animals excreted more N via feces when compared to LP animals. Furthermore, MP animals also excreted more N via urine than LP animals. With that, LP animals were more efficient in retaining the N from the feed. According to Sampaio et al. (2010), to optimize utilization of fiber by microbes, diets should have 100 g/kg DM of CP. Therefore, in cases where protein levels are below this threshold, as for LP diets, rumen microbes need to be more efficient at utilizing dietary N and increase urea

recycling in the rumen. When growing cattle were fed very low protein prairie hay (4.7% CP), 98% of all urea entering the blood pool was returned to the gut and only 2% was excreted in the urine (Wickersham, 2006). The recycling of urea in animals fed low-protein diets emphasizes the importance of the ruminant ability to salvage urea N for recycling and anabolic purposes when dietary protein is in short supply (Reynolds and Kristensen, 2008). Therefore, in the present trial, the lack of differences on microbial N between LP and MP is probably due to a more efficient recycling of urea in the rumen of LP animals.

During the finishing phase, ingestion of N was over 2-fold higher for forage-fed animals compared to grain-fed animals. Similarly, to the backgrounding phase, animals consuming more N, as in the forage-fed treatment, also had lower digestion of N and higher excretion of N in the feces and urine. Therefore, retention rates of N were much lower for forage-fed animals since most of the N was excreted and not recycled. In the rumen, bacterial urease activity facilitates urea-N transfer into the rumen by maintaining a favorable concentration gradient (Remond et al., 1996). However, urease activity is negatively correlated with ruminal ammonia concentration; therefore, high concentrations of ammonia in the rumen will decrease ruminal epithelium permeability to urea-N (Cheng and Wallace, 1979; Egan et al., 1986). Thus, with an increase of urea-N in the blood, urea will need to be filtered in the kidney and excreted in the urine. Overall, urea is more concentrated in the urine than in the blood, but when diets have really high levels of protein, the kidney capacity for handling urea can be limited and consequently augment the volume of urinary liquid (French, 1956). Increased urinary volume means increased water loss through the urine, which will further increase the water requirements

of animals. However, in the present trial, water intake was negatively correlated with urine volume for forage-fed animals, which might indicate that significant amount of water was being lost through a different pathway.

During the background phase, an increase in the % of urinary excretion of N with a decrease on fecal N excretion was observed throughout the collection days. However, even though water intake would be expected to increase in those conditions, no correlation was found between those variables. An increase in the % urinary N excreted through time was also observed for grain-fed animals; however, a negative correlation between water intake and % urinary N excreted was observed, as well as a positive correlation between water intake and urinary volume, which might indicate that the urine of those animals was more diluted during the collections. Urinary N excretion are a big concern to the environment since it is more volatile than fecal N and is rapidly converted to ammonia by ureases present in soil and on pen floors (Lee et al., 2014). On the opposite side, forage-fed animals seem to increase the concentration of N excreted in the feces through time and have a high correlation with water intake, which might indicate a decrease on ruminal permeability. Once microbes digest the protein in the rumen into ammonia, absorption of ammonia is mainly dependent on the pH; ammonia is usually absorbed in the lipophilic NH3 form by simple diffusion when ruminal pH is 7 or greater; however, the pH in the rumen is usually at pH 6.5 or lower, which only allows the absorption of ammonia mainly as NH4 via K^+ channels (Abdoun et al., 2007). In the present trial, the higher expected pH for the forage-fed diet would allow most of the NH4 produced in the rumen to be absorbed. However, our data indicates that throughout the trial, due to the increase in N excreted in the feces, ammonia potentially accumulated

inside of the rumen and was excreted in the feces. This novel observation indicates a possible change in the permeability in the rumen as a defense mechanism against ammonia toxicity due to the high levels of N in this diet.

Regarding potential carryover effects involving the previous plane of nutrition, it was observed that when animals were finished on a forage-based diet, which had high levels of CP, no differences were observed regarding the previous plane of nutrition of those animals. However, when animals were fed a grain-based diet, which had half of the CP content of the forage-based diet, animals that came from LP ate less and were more efficient at utilizing N by decreasing fecal excretion. This observation might indicate an adaptation mechanism developed by those animals due to a better recycling of N during the previous phase. Therefore, for systems where animals are kept in low quality pastures and rangelands during the backgrounding phase, a subsequent feeding with moderate levels of CP would be more adequate to maximize their potential for protein utilization during the finishing phase. Increasing both N and water efficiency is both economically and environmentally relevant for livestock production systems. Our work is the first to demonstrate that preferential mechanisms, significant environmentally and economically, differentially regulate N and water metabolism dependent on previous planes of nutrition.

6. Conclusion

The supply of diets with high levels of protein is a reality for various sectors of the cattle industry, and when in excess, it can be detrimental to the environment due to an increase in water requirements and N excretion of animals. However, excretion levels of N might change according to the production system that animals are raised. Our work

shows that during the backgrounding phase, animals receiving low protein will reduce the excretion of N while still requiring the same amount of water and producing the same amount of microbial N due to a more efficient N recycling. Further, when comparing different finishing systems, animals finished on a high-quality forage with high protein levels, excreted two times more nitrogen and required much more water than animals finished on a grain-based diet. Furthermore, the proportion of fecal N on forage-fed animals seems to increase thorough time while the proportion of urinary N decreases, suggesting some adaptative mechanism to decrease the permeability of the rumen wall and the risk of ammonia toxicity. When investigating the interaction between previous plane of nutrition and finishing systems, animals backgrounded on LP and finished on diets with moderate levels of CP, such as grain-fed diets, were more efficient in the use of N and able to decrease fecal excretion of N when compared to animals that were backgrounded on a diet with moderate levels of CP. Indicating that if animals were moderately deprived of protein earlier in their life, they might be more efficient in using protein in subsequent phases. More studies are required to better understand mechanisms underlying the processes discovered herein and how the previous plane of nutrition might affect them.

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8. Tables

Table 1. Effect of low (n = 12) or moderate (n = 12) plane of nutrition at the backgrounding phase on drinking water, feed, and nitrogen (N) intake and metabolism of crossbred Angus steers

Item	Treatment		Collection		SEM[1]	*P*-value[2]		
	Low Plane	Moderate Plane	1	2		TRT	Day	TRT x Day
Water intake, L	42.49	42.80	39.16	45.14	1.590	0.8891	<0.0001	0.1254
Water Intake, L/g N ingested	0.36	0.30	0.32	0.336	0.009	<0.0001	0.0504	0.5283
Water Intake, L/g N digested	0.70	1.30	0.97	1.018	0.058	<0.0001	0.4155	0.8965
Water Intake, L/g microbial N efficiency	-0.37	-1.52	-4.47	2.07	2.007	0.6876	0.0377	0.9919
Dry matter intake, kg	7.97	10.21	8.74	9.42	0.236	<0.0001	<0.0001	0.2426
N intake, g/d	117.31	142.85	124.24	135.85	3.614	<0.0001	<0.0001	0.0459
N digested, %	61.16	33.36	46.01	48.44	1.363	<0.0001	0.1161	0.5396
N excreted, g/d	72.49	178.74	116.75	132.82	10.120	<0.0001	0.2331	0.9198
Fecal output, kg/d	3.33	5.02	3.99	4.34	0.106	<0.0001	0.0025	0.5377
Fecal N g/d	45.68	96.01	68.61	73.14	2.170	<0.0001	0.0634	0.1795
Urine volume, L/d	9.29	17.08	10.09	16.85	2.370	0.0198	0.0335	0.0426
Urine N g/d	26.80	93.12	56.64	62.93	11.24	<0.0001	0.7186	0.6689
Urine N, % of excreted N	33.87	44.54	35.13	42.85	3.881	0.0510	0.1965	0.2888
Retained N, g/d	44.81	-35.49	6.83	2.84	9.217	<0.0001	0.7636	0.8417
N retention rate, %	38.32	-24.69	9.01	4.73	6.614	<0.0001	0.6655	0.6085
Efficiency of N utilization, g/g ingested N	0.383	-0.247	0.090	0.47	0.066	<0.0001	0.6659	0.9089

[1]SEM: Standard error of the mean; [2]*P*-value: <0.1 = trend, <0.05 = significant, TRT: treatment effect.

Table 2. Effect of previous plane of nutrition on drinking water, feed, and nitrogen (N) intake and metabolism of crossbred Angus steers backgrounded on low or moderate plane and subsequently finished on grain (n = 12) or forage-fed (n= 12) finishing systems

Item	Treatment		Collection			SEM[1]	*P*-value[2]				
	Grain Fed	Forage Fed	1	2	3		TRT	PRV	TRT x PRV	Day	TRT x Day
Water intake, L	43.86	68.83	51.69	62.60	54.98	2.319	<0.0001	0.1052	0.7447	0.0022	0.3782
Water Intake, L/g N ingested	0.207	0.149	0.173	0.195	0.164	0.007	<0.0001	0.9331	0.1994	0.0001	0.1994
Water Intake, L/g N digested	0.582	1.042	0.691	0.926	0.829	0.040	<0.0001	0.1008	0.6301	0.0007	0.0046
Water Intake, L/g Microbial N efficiency	2.54	3.13	2.702	3.182	2.576	0.185	0.0247	0.2532	0.3948	0.1240	0.7312
Dry matter intake, kg	12.18	13.25	12.37	12.83	13.19	0.335	0.0233	0.0628	0.0183	0.1413	0.2365
N intake, g/d	214.22	462.81	332.43	336.51	352.78	10.141	<0.0001	0.2961	0.0601	0.0946	0.1983
N digested, %	77.09	65.43	75.02	69.53	68.42	1.525	<0.0001	0.5730	0.0116	0.0014	0.0486
N excreted, g/d	179.42	441.22	263.41	316.12	362.51	13.353	<0.0001	0.5291	0.0335	<0.0001	0.0047
Fecal output, kg/d	4.76	6.82	5.14	6.10	6.25	0.268	<0.0001	0.2464	0.0004	0.0039	0.2801
Fecal N g/d	105.04	159.04	112.69	139.20	149.74	11.458	<0.0001	0.3074	0.0043	0.0002	0.0143
Urine volume, L/d	21.73	27.19	22.22	22.25	29.15	1.544	0.0133	0.8890	0.8536	0.0041	0.5918
Urine N g/d	77.16	282.19	151.37	175.88	149.74	15.894	<0.0001	0.9073	0.6640	0.0026	0.6104
Urine N, % of excreted N	41.83	63.39	49.80	51.30	56.30	2.236	<0.0001	0.2358	0.0760	0.1950	0.0248
Retained N, g/d	32.01	21.58	67.20	20.77	-12.01	11.251	0.5275	0.8988	0.2939	<0.0001	0.0861
N retention rate, %	59.83	4.34	41.95	29.85	23.57	2.686	<0.0001	0.9671	0.1439	<0.0001	0.0976
Efficiency of nitrogen utilization, g/g ingested N	0.159	0.043	0.230	0.065	-0.003	0.036	0.0240	0.7135	0.1635	0.0002	0.5447

[1]SEM: Standard error of the mean; [2]*P*-value: <0.1 = trend, <0.05 = significant, TRT: treatment effect, Previous: low versus moderate previous plane of nutrition.

Table 3. Effect of low (n = 12) or moderate (n = 12) plane of nutrition backgrounding phase on nitrogen (N) microbial and microbial crude protein synthesis of crossbred Angus steers

Item[1]	Treatment		Phase		SEM[2]	*P*-value[3]		
	Low Plane	Moderate Plane	1	2		TRT	Day	TRT x Day
Microbial N, g/d	68.01	69.65	47.79	88.89	13.069	0.9272	0.0319	0.9896
Microbial N efficiency, g N/kg DOMI	13.48	13.72	9.43	17.80	2.870	0.9522	0.0653	0.6552
Uric Acid mmol/d	16.37	19.54	12.45	23.39	2.591	0.3808	0.0038	0.1154
Allantoin, mmol/d	95.44	97.96	73.91	120.46	13.915	0.8953	0.0360	0.8387
Purine derivatives, mol/d	112.75	115.91	88.74	138.77	15.355	0.8811	0.0257	0.9174
Absorbed purines, mol/d	93.55	95.80	65.74	122.27	17.975	0.9272	0.0319	0.9896

[1]DOMI: Digestible dry matter intake; [2]SEM: Standard error of the mean; [3]*P*-value: <0.1 = trend, <0.05 = significant, TRT: treatment effect.

Table 4. Effect of previous plane of nutrition on nitrogen (N) microbial and microbial crude protein synthesis of crossbred Angus steers backgrounded on low or moderate plane and subsequently finished on grain (n = 12) or forage-fed (n= 12) finishing systems

Item[1]	Treatment		Phase				*P*-value[3]				
	Grain Fed	Forage Fed	1	2	3	SEM[2]	TRT	PRV	TRT x PRV	Day	TRT x Day
Microbial N, g/d	140.22	155.92	144.19	142.32	158.37	5.780	0.0523	0.1350	0.3368	0.2003	0.4592
Microbial N efficiency, g N/kg DOMI	18.55	24.14	19.60	20.67	24.42	1.534	0.0107	0.5500	0.1307	0.2423	0.3019
Uric Acid mmol/d	34.77	41.11	37.61	32.81	44.36	2.234	0.0430	0.7310	0.2034	0.0016	0.0970
Allantoin, mmol/d	171.95	181.72	170.81	174.47	184.66	5.954	0.2363	0.0954	0.5503	0.0954	0.5503
Purine derivatives, mol/d	206.72	222.84	168.04	178.52	185.21	6.964	0.0969	0.1242	0.3581	0.2682	0.7228
Absorbed purines, mol/d	192.86	214.46	198.33	195.75	217.82	7.950	0.0523	0.1350	0.3368	0.2003	0.4592

[1]DOMI: Digestible dry matter intake; [2]SEM: Standard error of the mean; [3]*P*-value: <0.1 = trend, <0.05 = significant, TRT: treatment effect, Previous: low versus moderate previous plane of nutrition.

9. Figures

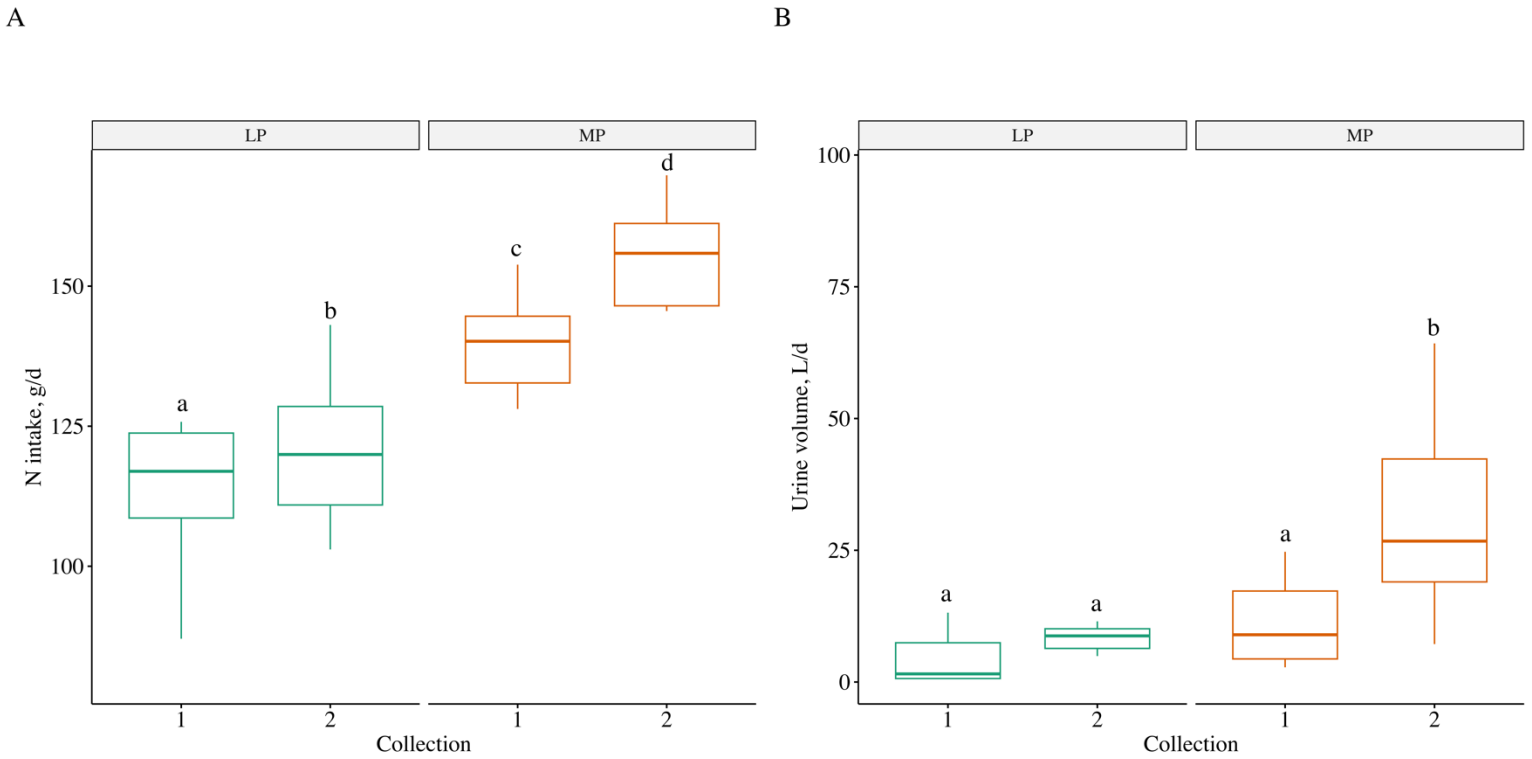

Figure 1. Effect of a a low (LP; n = 12) or a moderate plane of nutrition (MP; n = 12) on nitrogen (N) intake (g/d) and urine volume (L/d) of crossbed Angus steers.
A) Effect of collection and treatment interaction on N intake; B) Effect of collection and treatment interaction on urine volume.

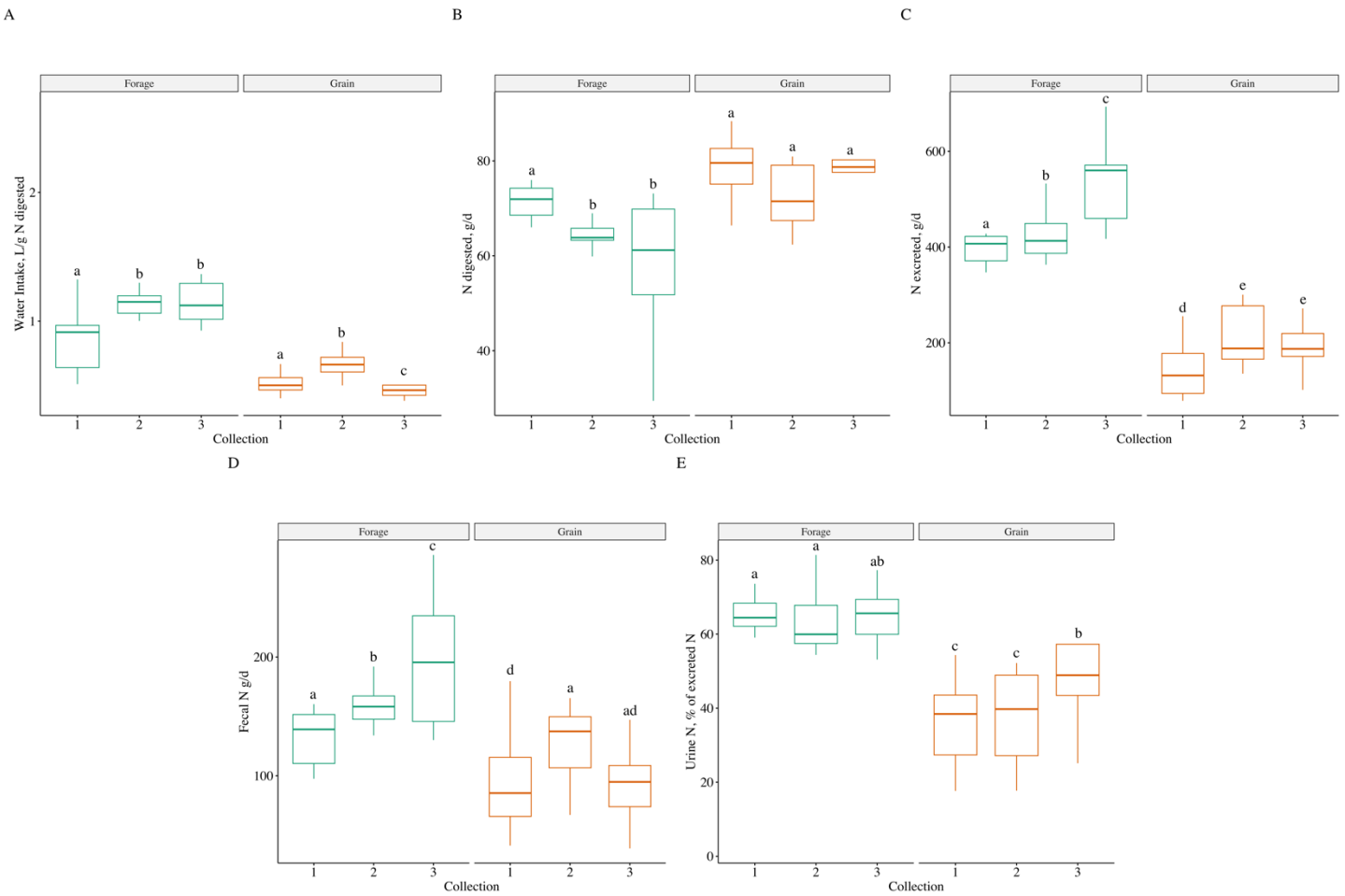

Figure 2. Effect of a grain (n = 12) or forage-fed (n= 12) finishing systems on water intake (L/g N digested), nitrogen (N) digested (g/d), N excreted (g/d), fecal N (g/d) and urine N (%excreted) of crossbred Angus steers.
A) Effect of collection vs treatment interaction on water intake (L/g N ingested); B) Effect of collection vs treatment interaction on % of N excreted on urine; C) Effect of collection vs treatment interaction on fecal excretion of N; D) Effect of collection vs treatment interaction on N digested; E) Effect of collection and treatment interaction on N excreted.

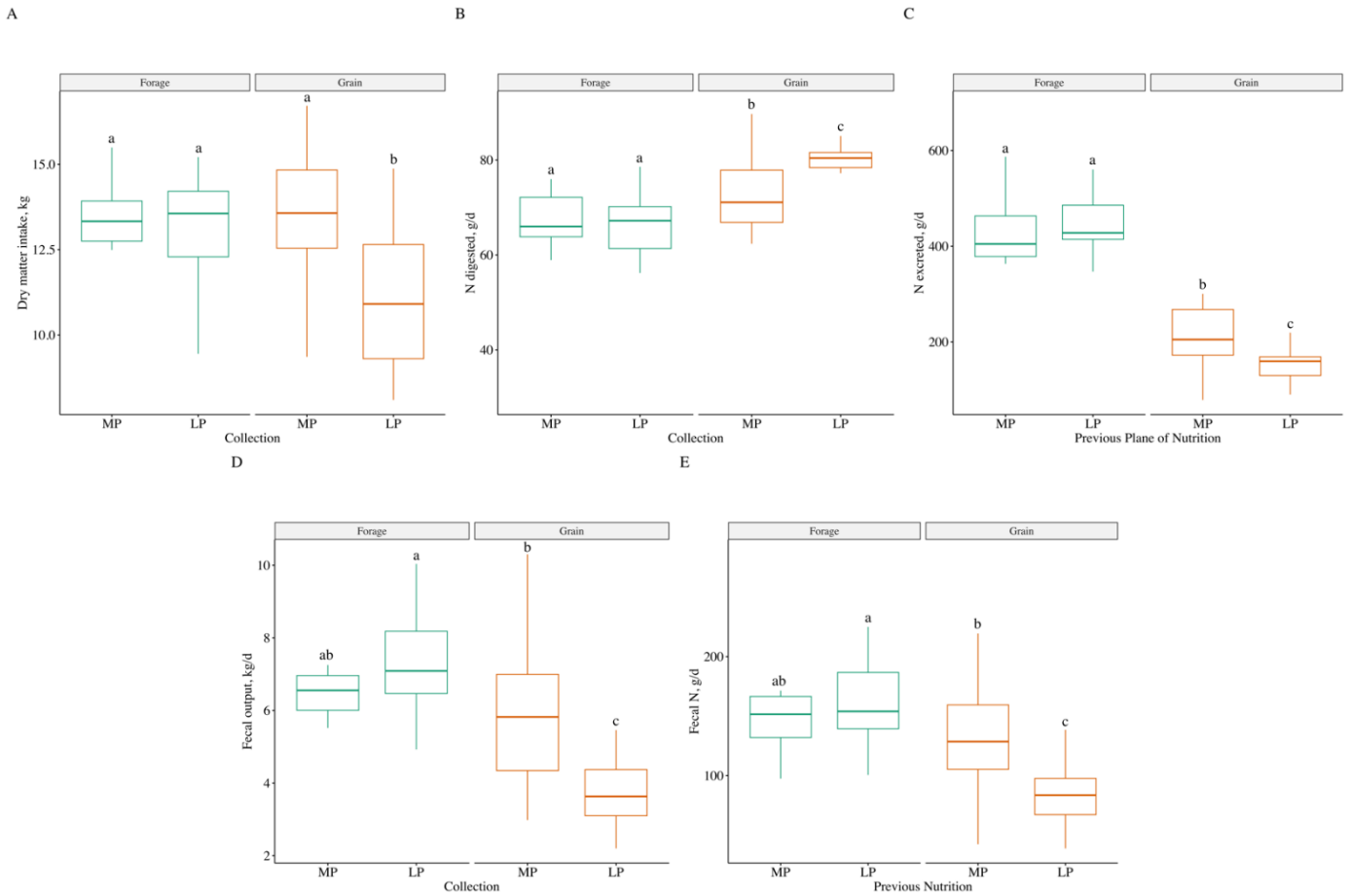

Figure 3. Effect of low (LP) or moderate (MP) plane of nutrition on dry matter intake (kg/d), nitrogen (N) digested (g/d), N excreted (g/d), fecal output (kg/d), and Fecal N (g/d) of crossbred Angus steers finished on grain (n = 12) or forage-fed (n= 12) finishing systems.
A) Effect of previous plane vs treatment interaction on N digested; B) Effect of previous plane vs treatment interaction on N excretion; C) Effect of previous plane vs treatment interaction on total fecal excretion; D) Effect of collection vs treatment interaction on fecal excretion of N.

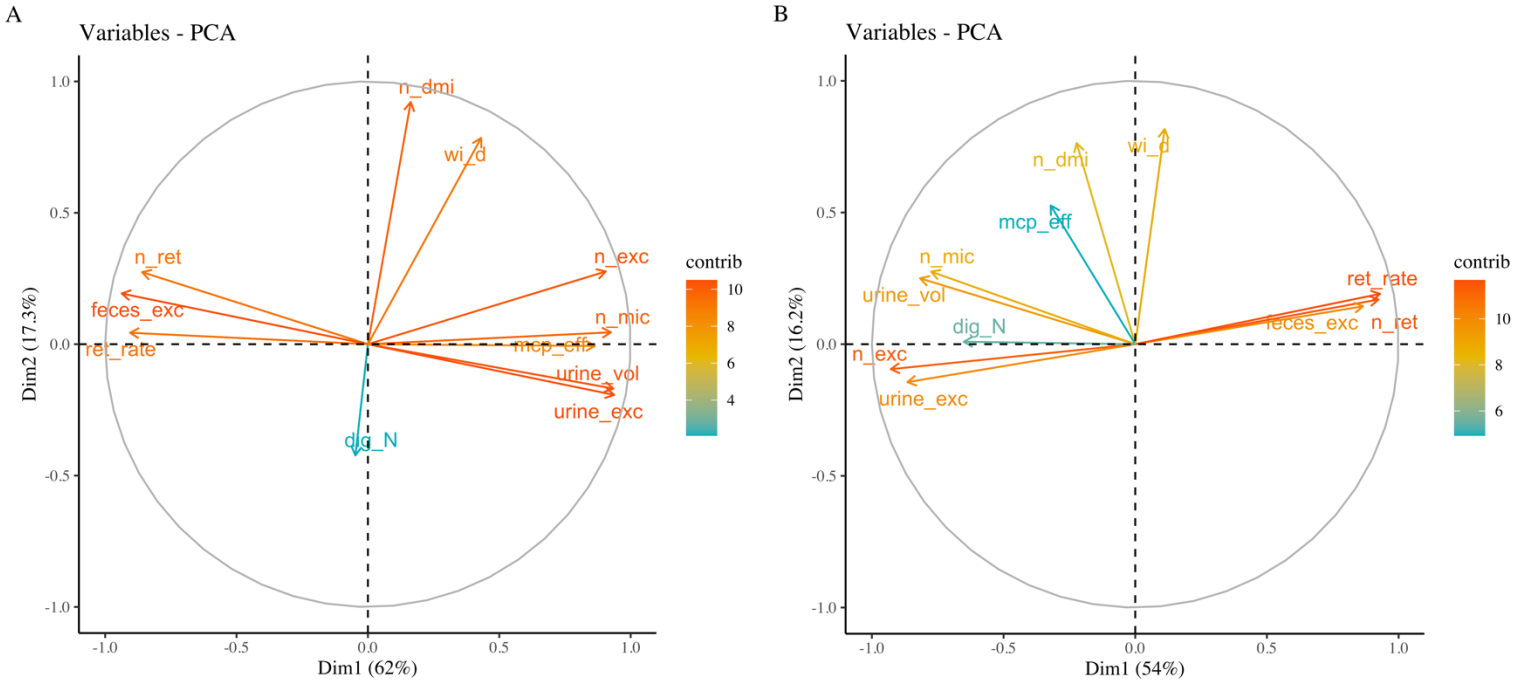

Figure 4. Principal component analysis (PCA) biplot of water intake and nitrogen metabolism variables of crossbred Angus steers backgrounded on a low (n = 12) or a moderate plane of nutrition (MP; n = 12).
A) PCA for animals on a low plane of nutrition; B) PCA of animals on a moderate plane of nutrition.
n_dmi: Nitrogen intake, kg/d on dry matter basis ; dig_N: N digested, g/d; n_exc: N excreted, g/d; urine_exc: Urinary N, % N excreted; feces_exc: fecal output of N, % N excreted; n_mic: microbial N, g/d; mcp_eff: Microbial N efficiency, g N/kg digestible organic matter intake; ret__rate: N retention rate; n_ret: retained N; wi_d: daily water intake.

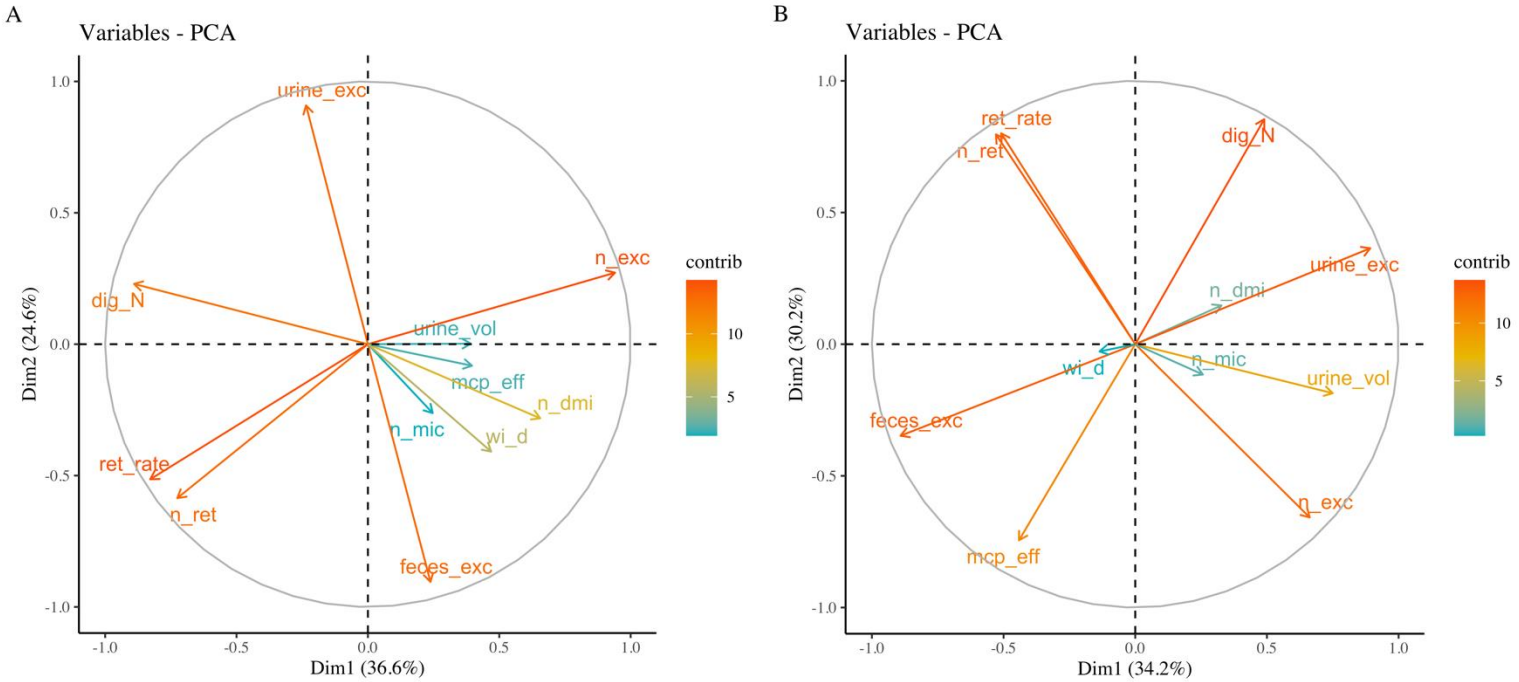

Figure 5. Principal component analysis (PCA) biplot of nitrogen metabolism and water intake variables of crossbred Angus steers finished on a grain (n = 12) or forage (n = 12) based system.
A) PCA for animals on a grain-fed based finishing; B) PCA for animals on a forage-fed based finishing.
n_dmi: Nitrogen intake, kg/d on dry matter basis ; dig_N: N digested, g/d; n_exc: N excreted, g/d; urine_exc: Urinary N, % N excreted; feces_exc: fecal output of N, % N excreted; n_mic: microbial N, g/d; mcp_eff: Microbial N efficiency, g N/kg digestible organic matter intake; ret__rate: N retention rate; n_ret: retained N; wi_d: daily water intake.

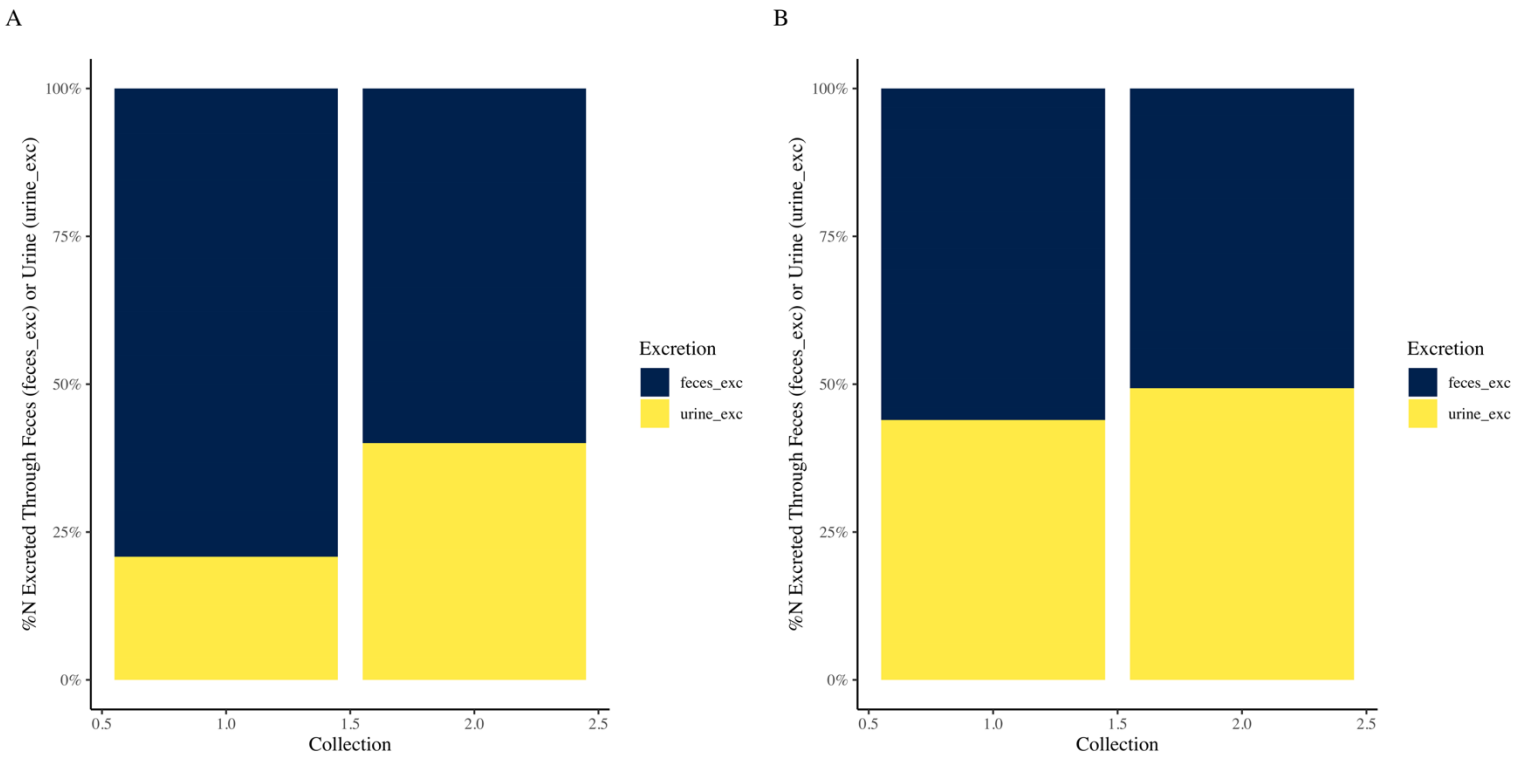

Figure 6. Urinary (urine_exc) and fecal (feces_exc) N excretion pattern through time of crossbred Angus steers backgrounded on a low (n = 12) or a moderate plane of nutrition (MP; n = 12).
A) Excretion pattern of urinary and fecal excretion of animals on a low plane of nutrition; B) Excretion pattern of urinary and fecal excretion of animals on a moderate plane of nutrition.

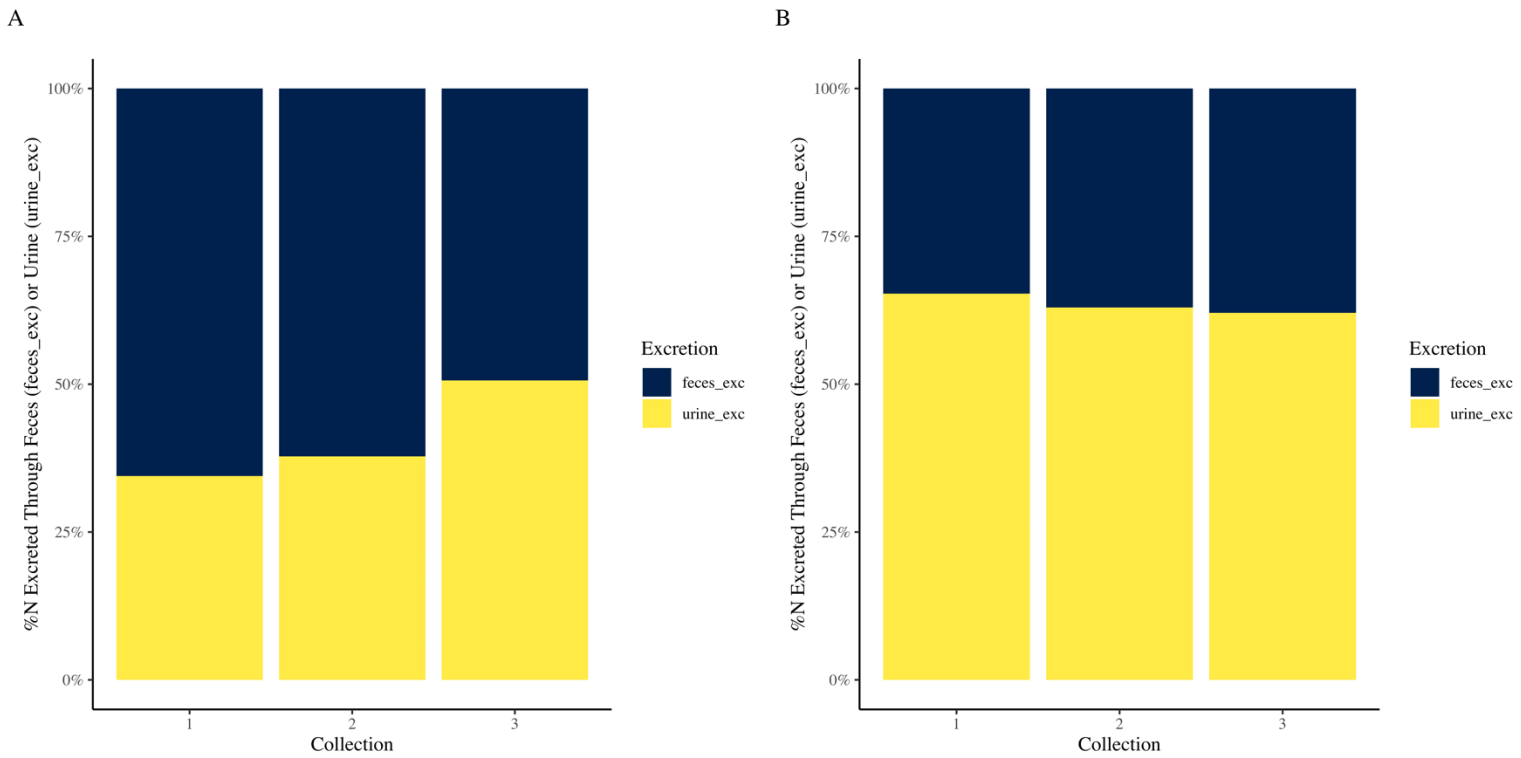

Figure 7. Urinary (urine_exc) and fecal (feces_exc) excretion pattern through time of crossbred Angus steers finished on a grain (n = 12) or forage (n = 12) based system.
A) Excretion pattern of urinary and fecal excretion of animals on a grain-fed based system; B) Excretion pattern of urinary and fecal excretion of animals on a forage-fed based system.

CHAPER IV

MOLECULAR MECHANISMS REGULATING GENE EXPRESSION OF UREA AND WATER METABOLISM IN THE RUMEN AND KIDNEY OF CROSSBRED ANGUS STEERS ON DIFFERENT BACKGROUNDING-FINISHING SYSTEMS

Lay Summary

Water and solutes transport across the cell wall are one of main factors affecting the molality of fluids in the body. Therefore, this study aimed to evaluate how a low or moderate plane of nutrition during the backgrounding phase can affect the water and urea metabolism of cattle at the end of the finishing phase in both grain and forage-fed systems. Tissues from the rumen and kidney were analyzed for gene expression of water, sodium, urea, and chloride channels. Overall our results indicate that a low plane of nutrition have a greater influence on the subsequently finishing phase, and that forage-fed animals control the overload of nitrogen in the system mainly with the help of water and sodium transporters in the kidney. In the rumen, water transporters also might play a role on the overload of nitrogen of forage-fed animals only when they are also related with the nitrogen metabolism. Animals finished on a grain-fed system but previously fed a low plane of nutrition, might increase urea recycling back to the rumen with the help of urea transporters.

Highlights

- Low plane of nutrition during the backgrounding phase can influence the water and urea metabolism of animals fed a grain or forage-based diet during the finishing phase.
- Animals backgrounded on a low plane of nutrition and finished on a forage-based diet increased the expression of AQP3, AQP7, ATP1B1 and SGK1 in the kidney and AQP7 in the rumen when compared to grain-fed animals.

1. Abstract

The objective of this study was to understand how the molecular mechanisms controlling water and urea metabolism at the finishing phase can be affected by previous plane of nutrition (PPN) of crossbred Angus beef steers. Twenty-four (n = 24) animals were randomly distributed into either a low or moderate plane of nutrition during the background phase for 85d. Animals were then blocked by their PPN and were moved onto a 105-d finishing phase. The forage-finished group received only high-quality alfalfa hay, whereas the grain-finished group received a high grain diet (80% whole corn and 20% alfalfa hay). By the end of the finishing phase, animals were harvested, and tissue samples from the rumen and kidney were collected. Changes in gene expression of Aquaporins (AQP) -2, -3, -4, -7, ATP1A1, ATP1B1, SGK1, CLIC1 (kidney and rumen) and UT-B (rumen only), were assayed via real-time qPCR; and 18S rRNA was used as an endogenous control. One-way ANOVA followed by Tukey's post hoc analysis was conducted and statistical significance was declared at $P < 0.05$, whereas statistical tendency was declared at $0.05 < P \leq 0.10$. When animals were forage-finished, the

relative abundance of AQP3 (P = 0.0289), AQP7 (P = 0.0260), ATP1B1 (P = 0.0239), and SGK1 (P = 0.0411) in the kidney had a higher expression when animals were prevenient from a low PPN when compared to grain-fed animals also from a low PPN. In the rumen, AQP7 was differentially expressed between all treatments during the finishing phase (P = 0.0011), with steers coming from a low PPN and subsequently finished on a forage-fed system, having the highest expression of AQP7. When comparing only animals from the low PPN, UT-B had a tendency (P = 0.0752) of presenting a higher expression on grain-finished animals. Overall, these results suggest that PPN can impact gene expression of genes associated with water and urea metabolism during the finishing phase, namely AQP3, AQP7, ATP1B1, and SGK1 in the kidney, and AQP7 and UT-B in the rumen. The greatest impact on gene expression that happened during the finishing phase occurred in animals backgrounded on a low PPN.

Keywords: Backgrounding; Finishing; Gene expression; Nitrogen; Water.

2. Introduction

Stocker/backgrounding production occurs year-round in various forage systems, which might vary widely in quality and availability depending on the time of the year (Brown, 1985). Therefore, there are very few locations where stocker grazing systems are available and offer high-quality forage year-round. However, a relatively small percentage of the backgrounding production may occur in confinement or semi-confinement systems, where forage available have a higher quality and, thus, stocker productivity (Pell, 2003). Once animals transition into the finishing phase, animals are mainly fed a diet high in grain. However, due to the increasing concern over the

environmental impacts of conventional beef, grass/forage-fed beef is now viewed by consumers as a more sustainable alternative (Xue et al., 2010). Conversely, Klopatek et al. (2022), has shown that grass-fed beef had 150% greater water footprint than grain-fed animals, indicating a great influence of diet on water metabolism of those animals.

In cattle, water contained in the intracellular space constitutes on average two-thirds of total body water pool, whereas the remaining one-third consists of water surrounding the cells and connective tissue, water in the blood plasma and the gastrointestinal track (Woodford et al., 1984). Once consumed, the rumen serves as a giant reservoir for water that can be utilized when water is not available (Shkolnik et al., 1980). Synchronously, the regulation of extracellular fluid volume and composition is be controlled by the kidney (Chosniak et al., 1984).

At the cellular level, the maintenance of the correct molality of fluids and proper distribution of water within body compartments will depend upon the environment, where cells will adapt to changes by altering their patterns of gene transcription and protein modification as well as their cytoskeletal structure (Bell et al., 2000). Some of the most important water-related cell components that will be prone to be modified upon hydric stress are the aquaporins (AQP), a specialized group of water channels that allow the passage of water and other small molecules (Michalek, 2016) to and from the cytosol. Water transport also affects the balance of water in the body, being driven by the creation of an osmotic gradient across an epithelium through active ion/solute transport (Verkman, 2008). Therefore, other cellular components related to solute transport are also important and involved in the balance of body water, such as Na^+/K^+-ATPase, sodium channels, and chloride channels. All those channels are regulated by several genes including

ATPase Na+/K+ Transporting Subunit Alpha 1 (ATP1A1) and Beta 1 (ATP1B1), Serum/Glucocorticoid Regulated Kinase 1 (SGK1), and Chloride Intracellular Channel 1 (CLIC1), which are related to the ability of the cell to sense and appropriately respond to environmental changes in osmotic balance through an integrated network of intracellular signaling pathways (Bell et al., 2000). In ruminants, another factor that plays a role in osmotic balance is the level of urea in the blood due to their ability of recycling dietetic nitrogen as urea (Lapierre and Lobley, 2001). High levels of blood urea, which is associated with high protein diets, need to be excreted in the urine, which will require proper regulation of ion/solute transport to avoid excessive loss of water through the urine (Bankir et al., 1996). Altogether, taking in consideration the molecular mechanisms that might regulate water in the body, the objective of this study is to understand how the previous plane of nutrition can affect water and urea metabolism of cattle at the end of the finishing phase in both grain and forage-fed systems.

3. Material and Methods

All experimental and animal husbandry procedures conducted were approved by the Institutional Animal Care and Use Committee of the University of Nevada, Reno, NV (protocol #00845).

i. Experimental design, treatments, and animals

Twenty-four crossbred Angus steers (298.01 ± 10.17 kg) were housed in the research feedlot area of the Main Station Field Laboratory at the University of Nevada, Reno. The experimental trial lasted 220 days, consisting of two phases: backgrounding and finishing

phase. During the backgrounding phase (85 d), animals were randomly assigned to one of the two treatments (n=12 per treatment): low plane of nutrition (LP, CP: 9.10%, net energy available for maintenance [NEm]: 0.25 Mcal/kg, net energy available for gain [NEg]: 0.10 Mcal/kg) or moderate plane of nutrition (MP, CP: 12.62%, NEm: 0.25 Mcal/kg; NEg: 0.13 Mcal/kg). By the end of the backgrounding phase, steers were blocked by previous plane of nutrition (LP or MP) and transitioned to the finishing phase which included a 30-d adaptation period and a 105-d finishing period. The finishing period consisted of either alfalfa hay only (forage-fed, n=12; CP: 21.3%, NEm: 0.32 Mcal/kg; NEg: 0.20 Mcal/kg) or predominantly whole grain (grain-fed, n=12; CP: 10.8%, NEm: 0.40 Mcal/kg; NEg: 0.30 Mcal/kg). Therefore, we had a factorial composed of four treatments: LP + Grain (animals from LP and finished on grains), LP+ Forage (animals from LP and finished on forages), MP+ Grain (animals from MP and finished on grains), MP + Forage (animals from MP and finished on grains). All animals had access to ad libitum water and a balanced mineral mix throughout the experimental period.

ii. Sample collections

By the end of the finishing phase, all steers were transported near to a USDA inspected commercial abattoir (CS Beef Packers, Kuna, Idaho), where all the animals were harvested. Steers were stunned and exsanguinated immediately. Kidney and ventral sac rumen wall tissue samples were collected from each steer immediately upon evisceration (within 10 minutes from slaughter). Collected samples were placed in a 2-mL cryotube and flash frozen in liquid nitrogen. Samples were then transferred to a −80 °C freezer for storage and subsequent RNA extraction and analysis.

iii. Real-Time qPCR

Total RNA was extracted from kidney and rumen samples using TRIzol Reagent (Invitrogen, Carlsbad, California). RNA samples were diluted to 100 ng/µl (500 ng) and then converted to complementary DNA (cDNA) using the Verso cDNA Synthesis Kit (ThermoFisher Scientific, Waltham, MA). Total mRNA expression quantitative real-time polymerase chain reaction (qPCR) was performed using Apex qPCR Master Mix (Genesee Scientific Corp., San Diego, CA; 42-120) on a BioRad CF96X qPCR instrument (Bio-Rad Laboratories, Hercules, California), and primers purchased from IDT (Table 1). Target genes (Table 2) included for both tissues were: Aquaporin -2 (AQP2), -3 (AQP3), -4 (AQP4), and -7 (AQP7), ATPase Na^+K^+ Transporting Subunit Alpha 1 (ATP1A1) and Beta 1 (ATP1B1), Serum/Glucocorticoid Regulated Kinase 1 (SGK1), Chloride Intracellular Channel 1 (CLIC1), and Solute Carrier Family 14 Member 1 (rumen only; codes for Urea Transporter B [UT-B]). The PCR amplification protocol consisted of enzyme activation at 95°C for 20 s, followed by 40 cycles of denaturation at 95°C for 3 s combined with annealing/extension at 60°C for 30 s. Expression levels of target genes were normalized to 18S ribosomal RNA (18S), which was validated as a suitable reference gene under these experimental conditions. The $2^{-\Delta\Delta}$CT method was used to determine relative abundance of mRNA (Livak and Schmittgen, 2001; Ferguson et al., 2010) and expressed as fold change relative to MP + Grain treatment when both previous planes were considered or LP + Grain when only LP was considered.

iv. Statistical Analyses

Data were analyzed as a completely randomized block design following the statistical model:

$$Y_{ijk} = \mu + T_i + b_j + Tb_{ij} + e_{ij}$$

Where Y_{ijk} is the dependent variable taken from experimental unit kth in the block jth on treatment ith, μ is the mean, T_i is the fixed effect of treatment ith, b_j is the random effect associated with the block jth, Tb_{ij} is the random effect associated with the interaction between treatment ith and block jth, and e_{ijk} is the random error associated with ijkth data value assuming that $e_{ijk} \sim N(0, \sigma^2)$.

GraphPad Prism software (GraphPad InStat Software, San Diego, CA) was used to analyze data and produce graphs. When comparing the four treatment groups, one-way ANOVA followed by multi comparison Tukey post hoc analysis was conducted. When only LP animals were compared, differences were analyzed through a Student's t-test. Statistical significance was declared at $P \leq 0.05$, whereas statistical tendency was declared at $0.05 < P \leq 0.10$. Identification of outliers was performed by plotting the studentized residuals against the predicted values as well as by Cook's D. Coefficients exceeding 2.5 studentized t distributions were considered outliers and removed from the data (Neter et al., 2004). Linear model assumptions were examined on the residuals.

4. Results

i. Aquaporins

The mRNA expression of AQP2, -3, -4, and -7 in the rumen and kidney are presented in Figure 1 and 2. Previous plane of nutrition had no effect on AQP expressed in the kidney at the finishing phase regardless of system. However, in the rumen (Figure 1B), animals from the LP + Forage diet had a higher expression of AQP7 than animals from the MP + Forage ($P < 0.05$), MP + Grain ($P < 0.01$) and LP + Grain ($P < 0.01$). Next, we analyzed if only animals coming from the LP treatment would have differences when their relative mRNA abundance was compared. Interestingly, the expression of AQP3 and AQP7 were both higher for forage-fed animals when compared to the grain-fed animals in the kidney ($P < 0.05$; Figure 2A), whereas for the rumen, the only differences found were still for the AQP7, which was still higher for the forage-fed animals ($P < 0.01$; Figure 2B).

ii. Na^+/K^+ ATPase subunits

As shown in the Figure 3A, relative abundance of Na^+/K^+ ATPase subunits were only different for ATP1B1 in the kidney between animals coming from LP, where forage-fed animals had a higher expression of ATP1B1 when compared to grain-fed animals ($P < 0.05$). The same behavior can also be observed in Figure 4A. However, no differences were observed in the rumen for ATP1A1 and ATP1B1 (Figure 3B and 4B).

iii. Genes related to osmotic balance

As shown in Figure 5, no differences were observed when the combination of previous plane and finishing diets were explored, for both kidney and rumen. However, when comparing only animals coming from LP (Figure 6), the expression of SGK1 in the kidney was higher for forage-fed animals when compared to grain-fed animals ($P < 0.05$; Figure 6A). No differences were observed in the rumen (Figure 5B and 6B).

iv. Urea Transporter

Differences in UT-B were observed only when animals were backgrounded in LP (Figure 7B). Animals that were backgrounded in lower planes of nutrition and finished in grain-based systems tended to have a higher gene expression of UT-B ($P = 0.0752$) in the rumen compared to forage-fed animals.

5. Discussion

One of the main proteins required in the process of regulating water balance and proper acid-base balance are the AQPs (Michalek, 2016). The finishing diets that we utilized in this study were inherently different on protein levels (10.8% vs 21.3% for grain-finished and forage-finished, respectively). Previous studies have shown increased water intake when animals were fed diets with increased protein levels (Ritzman and Benedict, 1924; Holter and Urban Jr. 1992), however, no differences were found on mRNA fold change expression of AQP of those animals in the kidney. These results suggest that the differences on protein levels of the finishing diet, independent of the

previous nutrition plane of animals, did not affect the capacity of the kidney in concentrating the urine.

On the other hand, when we analyzed animals that were backgrounded in a lower plane of nutrition, we observed a higher mRNA expression of AQP3 and AQP7 in the kidney of forage-fed animals, when compared to grain-finished steers. Once ingested, protein will be degraded by ruminal bacteria into ammonia. This ammonia will be absorbed through the rumen wall and go to liver, where it will be metabolized into urea and either recycled to the rumen or transported to the kidney. When levels of protein in the diet are low, urea arriving in the kidney needs to be reabsorbed, which will decrease its excretion in the urine and increase its recycling back to the rumen (Marini et al., 2004). However, as protein levels in the diet increase, excretion of urea in the urine also increases (Huhtanen et al., 2008). In the kidney, reabsorption of urea can also be done through aquaglyceroporins (AQGP), which are AQPs that are not only permeable to water, but to glycerol, ammonia, as well as urea (Rojek et al., 2008). Aquaporin -3 and -7 are both also considered AQGP, and the observed increase on their expression in the kidney of animals backgrounded in a lower plane of nutrition and subsequent fed a forage-based diet indicates that after a period of restricted protein supply, a subsequent overload of protein will increase the reabsorption of urea even if those animals do not have a limitation of protein in their diets anymore. The reabsorbed urea is then expected to go back to the rumen. In the rumen, bacterial urease will hydrolyze urea into ammonia, which can be excreted in the feces or, once more, absorbed in the rumen wall and metabolized into urea in the liver. However, this process of producing urea repeatedly can be a major energy consuming event. According to McBride and Kelly (1990), each

mole of urea produced in the liver has an energetic cost of 4 moles of ATP. Furthermore, Huntington and Archibeque (2000) estimated that 2.5% to 5% of whole-body oxygen consumption was attributable to ureagenesis in the liver. Similarly, Jennings et al. (2018) also noticed that animals fed high protein diets increased their energy requirements by 3 to 4.5%. Therefore, energy available for tissue deposition would be expected to decrease for LP + Forage animals when compared to LP + Grain Animals.

On the water side, AQP3-deficient mice were shown to be severely polyuric, demonstrating that basolateral membrane water transport can also be a rate-limiting factor for water reabsorption (Ma et al., 2000). However, AQP7 null mice appear to lack clear defects in urinary concentration abilities or in water balance abnormality (Sohara et al., 2005), indicating that AQP7 might have a bigger role on absorption of solutes (such as glycerol, ammonia, and urea) rather than water. Therefore, these data suggest that the overload of protein from LP + Forage animals lead to an increase in filtration of water in the kidney when compared to LP + Grain animals, mainly due to AQP3.

Rojen et al. (2011) investigated the mRNA expression of AQP in the rumen and observed that mRNA abundances of AQP-3, 7, and 10 were significantly upregulated when lactating Holstein cows were fed 17% CP compared to cows fed 12.9% CP. Our results indicate that animals on LP + Forage diets had the highest expression of AQP7 in the rumen when compared to MP + Forage, MP + Grain and LP + Grain fed animals, but no differences were observed for AQP3. Although there is still limited information regarding the function and localization of AQP7 in the rumen epithelium, in the brush border cells of the intestine, AQP7 expression is more intense at the apical side of brush

border membranes (Tritto et al., 2007). Ultimately, these data indicate that AQP7 could act in transporting excessive ammonia from the rumen to the bloodstream.

Despite large variations of feed and water intake, body fluid homeostasis can be maintained mostly due to reabsorption and secretion processes that happens on the kidney tubules (Summa et al., 2001; Feraille and Dizin, 2016). In the kidney, reabsorbed solutes first cross the apical membrane and then are extruded from intracellular medium to the interstitium, whereas secreted solutes are taken from the interstitium across the basolateral membrane and are then extruded into the lumen after crossing the apical membrane (Feraille and Dizin, 2016). Both processes preserve the balance between the intake and loss of water and ions and can be energized by the Na+ gradient generated by the Na^+/K^+-ATPase, a Na^+/K^+ pump required for the establishment of electrochemical gradients driving cellular transport and substrate flow across epithelia (Zouzoulas et al., 2005; Feraille and Dizin, 2016). The Na^+/K^+-ATPase comprises two subunits, a large catalytic α subunit (coded by the gene ATP1A1) and a smaller highly glycosylated β subunit (coded by the gene ATP1B1) necessary for the proper folding, insertion, and maturation of the α subunit in the plasma membrane (Zouzoulas and Blostein, 2006). ATP1A1 and ATP1B1, are two distinct, differentially regulated genes, where expression of α1 subunits are usually present in excess when compared to β1 subunits, which might limit the formation of the αβ heterodimer that will compose the Na^+/K^+-ATPase (Taub, 2018). Interestingly, in this current study, only ATP1B1 was higher for the forage-finished animals that were fed a lower plane of nutrition during the backgrounding phase. This might be related to an overload of urea in the blood caused by the higher content of protein in the finishing diet, which might have increased only ATP1B1 since it is the

limiting subunit for the formation of Na^+/K^+-ATPase. In ruminants, excessive dietary protein is degraded into ammonia in the rumen and metabolized to urea in the liver (Lu et al., 2014). Excess of blood urea needs to be excreted through urine to avoid toxicity; however, when protein intake is higher than the requirements to avoid massive water loss, a huge amount of plasma needs to be filtered. Such filtration is driven by the sodium chloride gradient in the kidneys that would allow for water to be conserved (Knepper and Roch-Ramel, 1987; Bankir et al., 1996). In rats, Bouby and Bankir (1987) observed that diets with higher concentration of protein increased Na^+/K^+-ATPase activity enabling an enhanced NaCl transport pipeline. We did not observe differences among the animals that were backgrounded in a MP of nutrition, which might suggest that adequate levels of protein during the background phase will decrease the effect of an overload of protein in the subsequent phases.

Although the rumen epithelium has a high expression of ATP1A1 (Graham and Simmons, 2005; Albrecht et al., 2008), no differences were observed in the rumen level for either ATP1A1 or ATP1B1. According to López et al. (1994), water exchange between the rumen contents and the plasma can occur in both directions depending on the osmolality pressure, where net movement of this water will define the balance in the rumen pool. However, when studying the flux of water in the rumen, the authors noticed that the rumen seemed not to be very permeable to water since the net extent of the transepithelial movement of water into or out of the rumen observed by them was not very high. López et al. (1994) explained that to keep the animal hydrated, most of the water seems to be absorbed and recycled post-ruminally. Thus, since not much water is

absorbed in the rumen, an increase on Na^+/K^+-ATPase activity might not be required in order to create a gradient for water absorption in the rumen.

Besides the Na^+,K^+-ATPase, the epithelial sodium channel (ENaC) is another important transporter of sodium. Regulation of sodium channels can be done by SGK1. Upregulation of SGK1 is usually stimulated by aldosterone when blood sodium levels are low, SGK1 will then stimulate sodium transport by the ENaC and Na^+/K^+-ATPase and increase transport of sodium to the cell (decreases sodium urinary excretion), leading to increased water uptake (concentration gradient), and thereby inducing a regulatory cell volume increase (Hills et al., 2008). In the current study, effects of diet on SGK1 expression were only observed in the kidney when comparing just the LP animals, where animals finished on a grain-based diet had a lower expression of SGK1 when compared to forage fed animals. This result corroborates with previous data, indicating that water is shifted to urine excretion and the kidney increases the transport of sodium as an alternative to save urinary water.

Lastly, since the levels of protein appear to play a role in the mRNA expression of the aforementioned genes, we investigated the expression of UT-B in the rumen. Once ammonia is converted into urea in the liver, it can be excreted in the urine or recycled back to the rumen. Blood urea can then cross the rumen mucosa by simple diffusion, AQGP or facilitative urea transporters (UT-Bs), which mediate the movement of urea down a concentration gradient (Stewart et al. 2005; Abdoun et al. 2007; Walpole et al., 2015). In this current study, a trend was observed when comparing animals coming from LP, where animals finished on a grain-based diet tended to have a higher expression of UT-B. This result indicates that due to the lower levels of protein in the diet during the

backgrounding phase and subsequent moderate levels of protein in the finishing phase – which also correspond to the conventional beef production in the U.S.— animals had to recycle more urea back to the rumen to optimize microbial growth and maximize nutrient utilization. Although previous studies have reported no effect of dietary protein levels on the expression of UT-B in the rumen (Ludden et al., 2009; Rojen et al., 2011; Sacca et al., 2018), the differences between protein levels in these studies ranged from 1.5 to 5%, whereas in this current study the levels of protein almost double between the finishing diets.

6. Conclusion

In conclusion, this study is the first to show that changes in the diet from earlier ages can influence the future water and urea metabolism of animals, and those effects can be further evidenced between different finishing systems. In the kidney, mRNA expression of AQP3, AQP7, ATP1B1 and SGK1 were higher for animals backgrounded in a lower plane of nutrition and finished in a forage-based diet, whereas in the rumen only AQP7 was different between groups. UT-B tended to be higher for animals backgrounded in a lower plane of nutrition and finished in a grain-based diet. In the U.S. most animals are backgrounded in a low-quality forage and then finished either on a grass/forage-based or grain-based diet. Our results suggest that the decreased supply of protein earlier in life might cause some adaptative mechanism to cope with the lower nitrogen supply. However, further differences will also depend on the finishing diets of those animals. If animals are finished on a diet with moderate protein, such as the grain-based diet, they will tend to be better recyclers of protein; whereas if they have an overload of protein in

the next phase, due to a more efficient reabsorption of nitrogen in the kidney, those animals might have a higher energy and water cost related to urea recycling and excretion. Therefore, the molecular mechanisms regulating gene expression of urea and water metabolism on those animals are dependent on not only the present diet, but also the previous diet. Future investigations in protein expression and translocation are needed to improve our overall understanding for beef cattle diets in water, urea and ammonia regulation

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8. Tables

Table 1. Primer sequences for gene transcripts analyzed by quantitative real-time reverse transcription polymerase chain reaction (qPCR)

Gene[1]	Primer design	Primer sequence
Gene control		
18S	FWD	5'-GCC GCT AGA GGT GAA ATT CTT A-3'
	REV	5'-CTT TCG CTC TGG TCC GTC TT-3'
Target genes		
AQP2	FWD	5'-CAA TGC CCT CAA CAA CAA CTC-3'
	REV	5'-GTC AGT GGA GGC GAA GAT AC-3'
AQP3	FWD	5'-GTC CAG GTA CAG GCA TTT CTC-3'
	REV	5'-CCT CCT CCT AGC CCT ACT TAT ATT-3'
AQP4	FWD	5'-TTC GGT GCT AGG AAA GGA ATG-3'
	REV	5'-CCA AAG GGA CCT GGG ATT TAG-3'
AQP7	FWD	5'-CTC TTA GCC ATC GCA GAC AA-3'
	REV	5'-GAG TTC ATG CCC AGG GAT ATT-3'
ATP1A1	FWD	5'-GGA GAT CTG GTG GAA AAA G-3'
	REV	5'-TCC CGT GAG TGA GGA GTT AT-3'
ATP1B1	FWD	5'-GAA CTC GGA GAA GAA GGA GTT T-3'
	REV	5'-TGG ATG GTT CCG ATG AAG ATG-3'
SGK1	FWD	5'-TCT CCT GGC AAG ACA CAA AG-3'
	REV	5'-AAC ATT CCG CTC CGA CAT AAT A-3'
CLIC1	FWD	5'-CAG CTG GGC TGG ACA TAT T-3'
	REV	5'-ACT TTC AGG GCT TTC AGG AG-3'
SLC14A1	FWD	5'-CTC CTT CAG ACT CCA GAA CAT C-3'
	REV	5'-CTT AGT GCC AAT GCC CTA CT-3'

[1]18S = eukaryotic 18S ribosomal; Aquaporin -2 (AQP2), -3 (AQP3), -4 (AQP4), and -7 (AQP7); ATPase Na^+/K^+ Transporting Subunit Alpha 1 (ATP1A1) and Beta 1 (ATP1B1); Serum/Glucocorticoid Regulated Kinase 1 (SGK1); Chloride Intracellular Channel 1 (CLIC1); Solute Carrier Family 14 Member 1 (SLC14A1; codes for Urea Transporter B [UT-B]); [2]FWD = forward primer (anti-sense strand); REV = reverse primer (sense strand); [3]Optimal temperature (Annealing, extension, and read fluorescence)

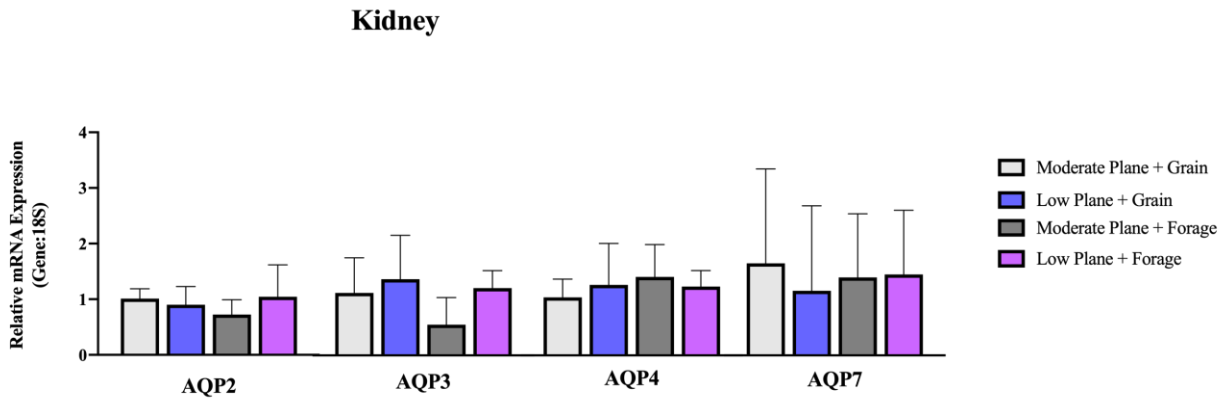
Table 2. Target gene related to water and urea metabolism and its respective functions

Target genes[1]	Function	Reference
AQP2	Located in the cytosol, but when in need of increased water absorption, it will migrate to the membrane and allow free passage of water	Kwon et al. (2013)
AQP3/ AQP4	Transports water, urea, ammonia, and glycerol, and represent exit pathways for water reabsorbed via AQP2	Rojek et al. (2008); Ikeda and Matsuzaki, (2015)
AQP7	Allows movement of water, glycerol ammonia, and urea across cell membranes down a gradient concentration	Rojek et al. (2008)
ATP1A1	Encodes the large catalytic α subunit of Na^+,K^+-ATPase pump	Zouzoulas and Blostein (2006)
ATP1B1	Encodes a smaller highly glycosylated β subunit of Na^+,K^+-ATPase pump, which is necessary for the proper folding, insertion and maturation of the α subunit in the plasma membrane	
SGK1	Phosphorylated in response to aldosterone, and it stimulates sodium transport by the epithelial sodium channels (including Na^+,K^+-ATPase pump and ENaC) and increase transport of sodium to the cell (decreases sodium urinary excretion). This will also lead to an increase in water uptake (concentration gradient).	Feraille and Dizin (2016)
CLIC1	Chloride channel - carry out transepithelial transport of salt and water according with the concentration gradient.	Ulmasov et al. (2007)
SLC14A1	This gene will code for urea transporter B (UT-B) in the rumen, but its exact pathway in the rumen is still being studied. However, it is believed that the UT-Bs located on the luminal and basolateral membrane of the ruminal epithelium are responsible for facilitate urea transport from the blood to the rumen epithelium.	Zhong et al. (2022)

[1] Aquaporin -2 (AQP2), -3 (AQP3), -4 (AQP4), and -7 (AQP7); ATPase Na^+/K^+ Transporting Subunit Alpha 1 (ATP1A1) and Beta 1 (ATP1B1); Serum/Glucocorticoid Regulated Kinase 1 (SGK1); Chloride Intracellular Channel 1 (CLIC1); Solute Carrier Family 14 Member 1 (SLC14A1).

9. Figures

A)



B)

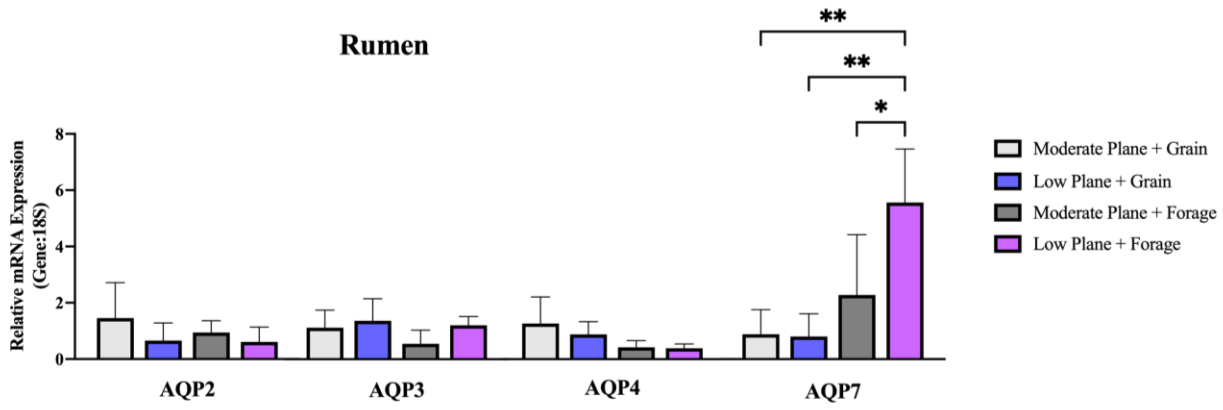
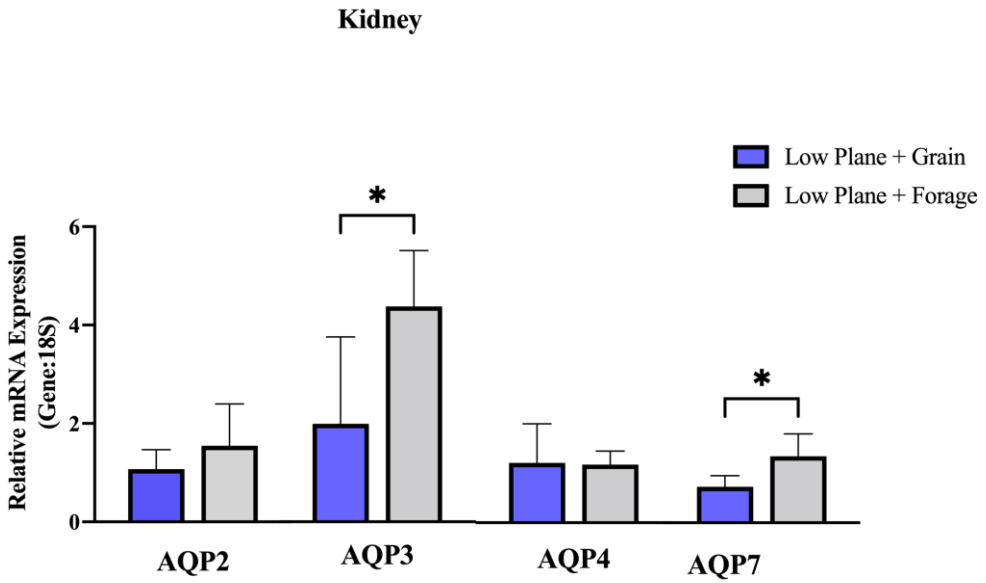


Figure 1. Gene expression of Aquaporins (AQP) 2, 3, 4 and 7 in the kidney and rumen at the end of finishing phase of crossbred Angus beef steers previously backgrounded in either a low or moderate plane of nutrition. During the finishing phase animals were either grain (n = 12) or forage-finished (n = 12).
Asterisks indicate statistical significance (*: $P \leq 0.05$; **: $P \leq 0.01$) between groups indicated by brackets. Error bars show the standard error of the mean. A) Aquaporins expression in the kidney; B) Aquaporins expression in the rumen.

A)



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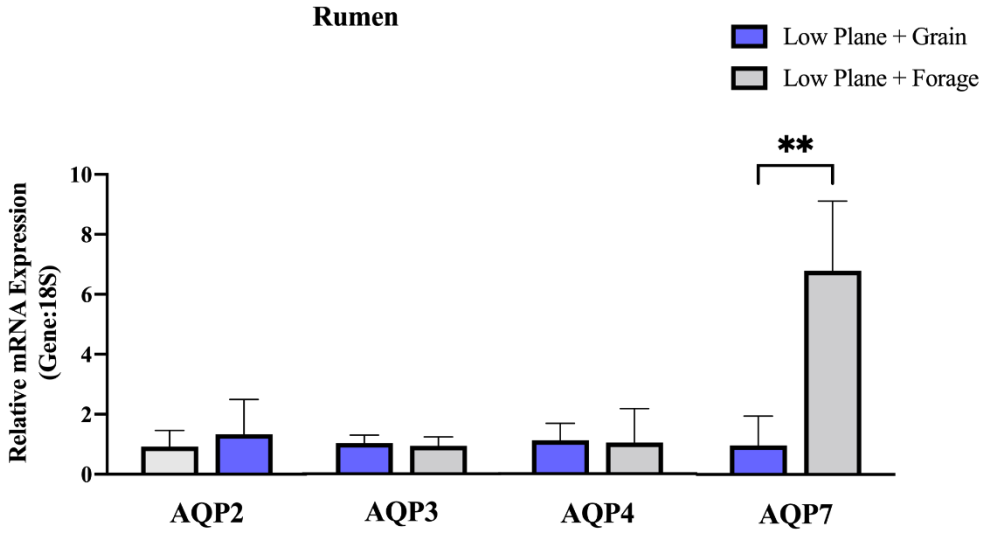
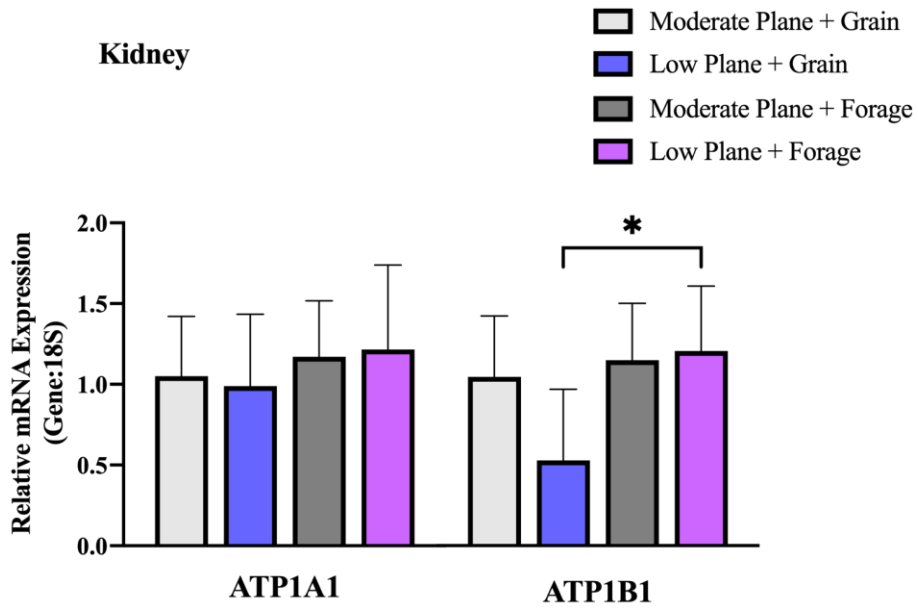


Figure 2. Gene expression of Aquaporins (AQP) 2, 3, 4, and 7 in the kidney and rumen at the end of finishing phase of crossbred Angus beef steers previously backgrounded in a moderate plane of nutrition prior to the finishing phase animals when animals were either grain (n = 6) or a forage-fed (n = 6).
Error bars show the standard error of the mean. Asterisks indicate statistical significance (*: $P \leq 0.05$; **: $P \leq 0.01$) between groups indicated by brackets. A) Aquaporins expression in the kidney; B) Aquaporins expression in the rumen.

A)



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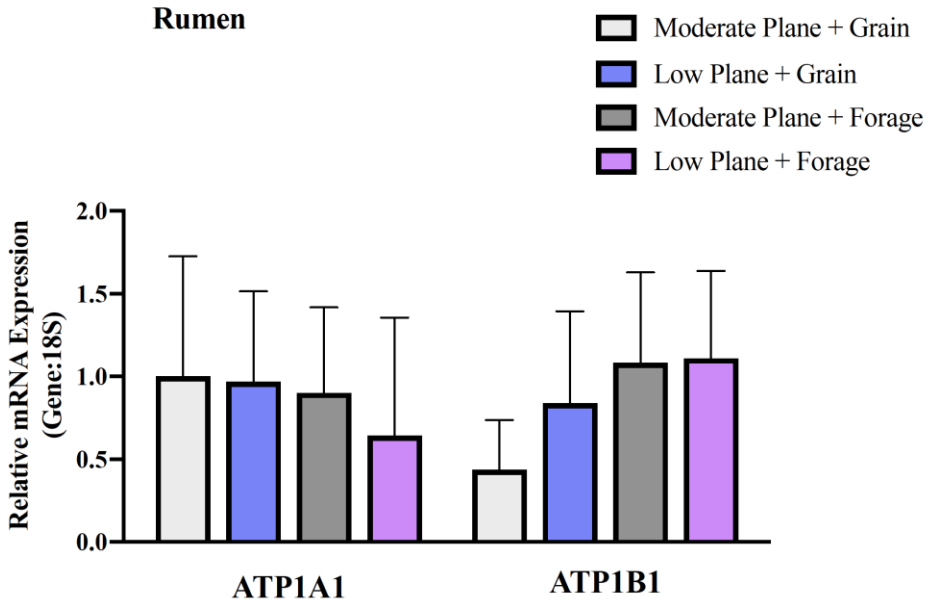
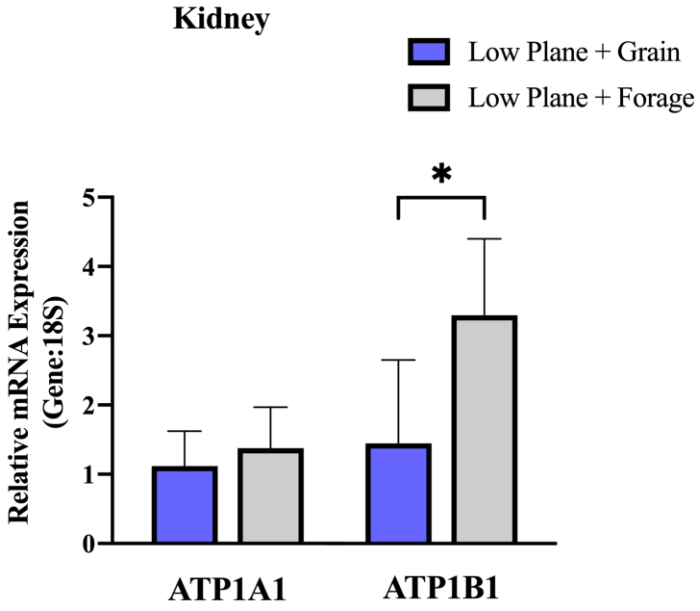


Figure 3. Gene expression of Na^+/K^+ ATPase subunits A1 (ATP1A1) and B1 (APT1B1) in the kidney and rumen of crossbred Angus beef steers previously backgrounded in either a low or moderate plane of nutrition. During the finishing phase animals were either grain (n = 12) or forage-fed (n = 12).
Error bars show the standard error of the mean. Asterisks (*) indicate statistical significance ($P \leq 0.05$) between groups indicated by brackets. A) ATP1A1 and ATP1B1 expression in the kidney; B) ATP1A1 and ATP1B1 expression in the rumen.

A)



B)

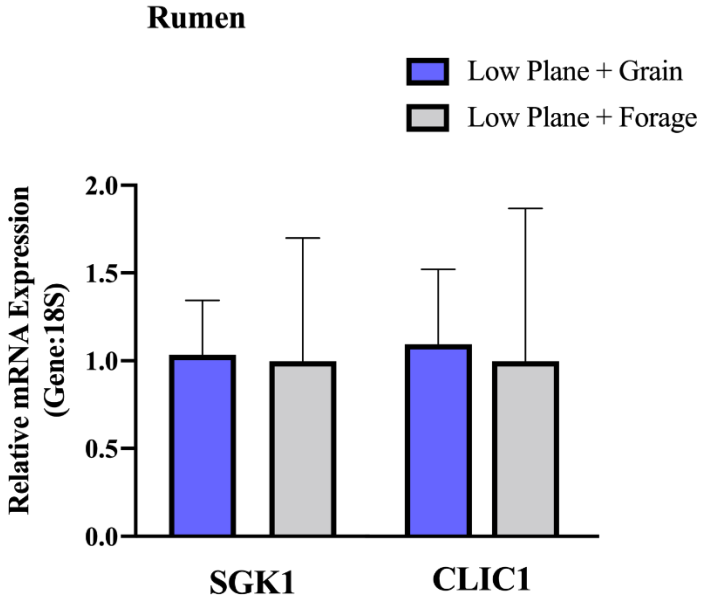
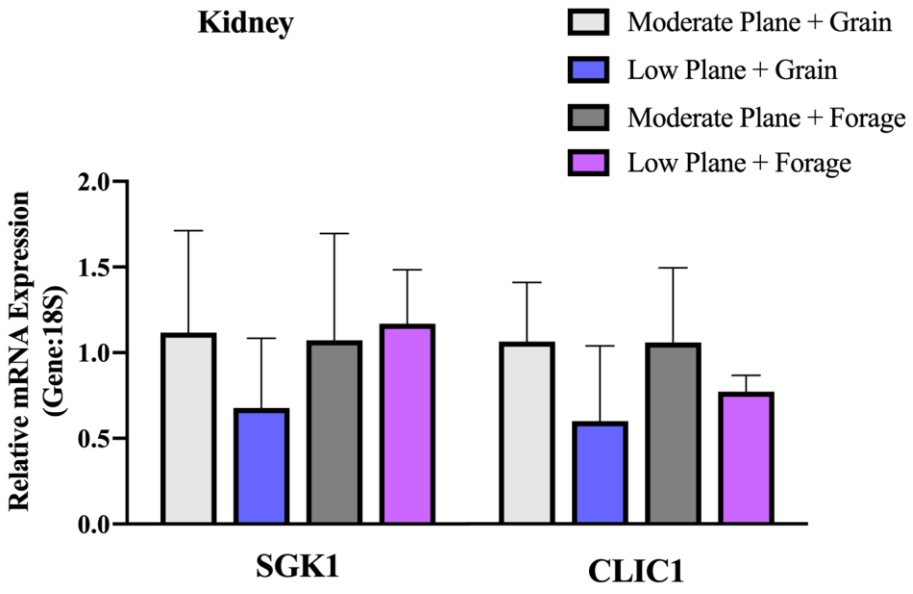


Figure 4. Gene expression of Na^+/K^+ ATPase subunits A1 (ATP1A1) and B1 (APT1B1) in the kidney and rumen at the end of finishing phase of crossbred Angus beef steers previously backgrounded in a moderate plane of nutrition prior to the finishing phase animals when animals were either grain (n = 6) or forage-fed (n = 6).
Error bars show the standard error of the mean. Asterisks (*) indicate statistical significance ($P \leq 0.05$) between groups indicated by brackets. A) ATP1A1 and ATP1B1 expression in the kidney; B) ATP1A1 and ATP1B1 expression in the rumen.

A)



B)

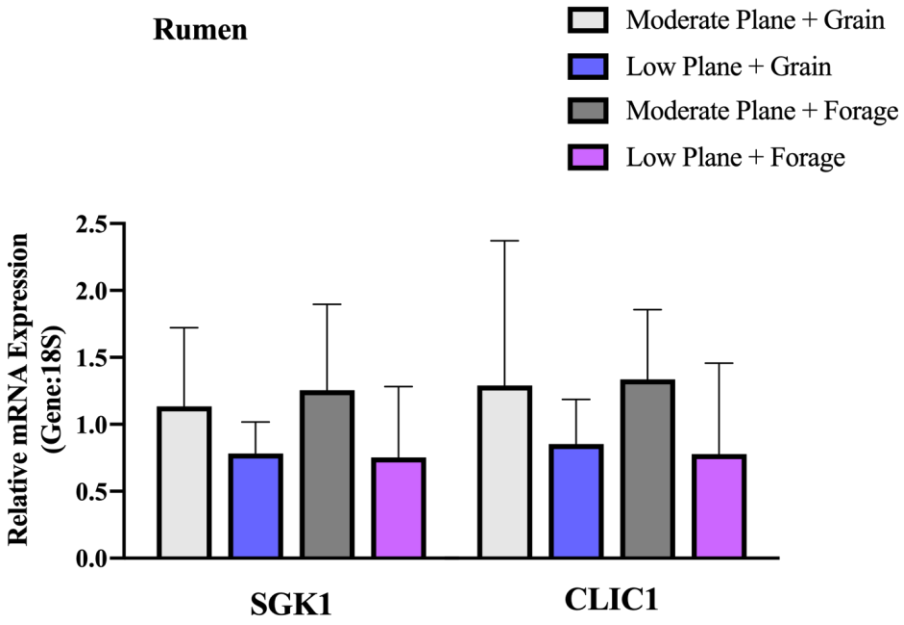
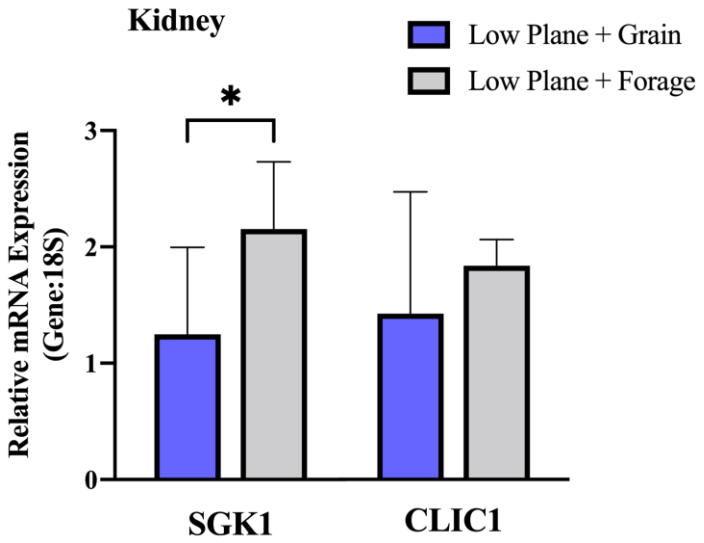


Figure 5. Gene expression of Serum/Glucocorticoid Regulated Kinase 1 (SGK1) and Chloride intracellular channel protein 1 (CLIC1) in the kidney and rumen at the end of finishing phase of crossbred Angus beef steers previously backgrounded in either a low or moderate plane of nutrition. During the finishing phase animals were either grain (n = 12) or forage-fed (n = 12).
Error bars show the standard error of the mean. A) SGK1 and CLIC1 expression in the kidney; B) SGK1 and CLIC1 expression in the rumen.

A)



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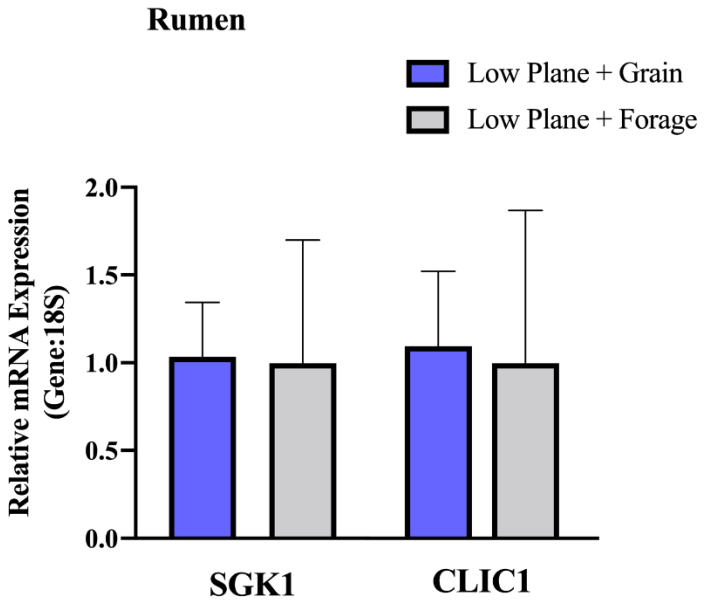
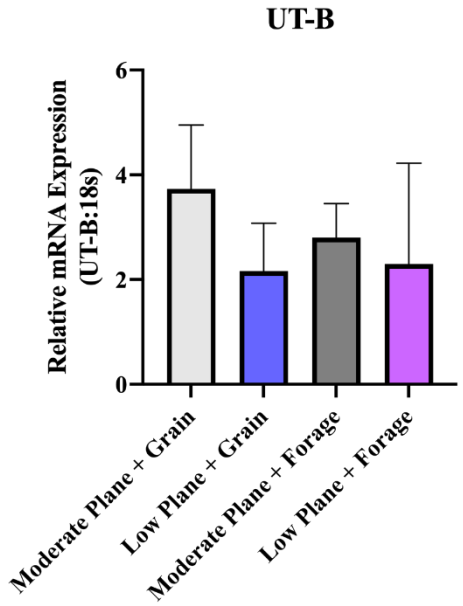


Figure 6. Gene expression of Serum/Glucocorticoid Regulated Kinase 1 (SGK1) and Chloride intracellular channel protein 1 (CLIC1) in the kidney and rumen at the end of finishing phase of crossbred Angus beef steers previously backgrounded in a moderate plane of nutrition prior to the finishing phase animals when animals were either grain (n = 6) or forage-fed (n = 6).
Error bars show the standard error of the mean. Asterisks (*) indicate statistical significance ($P \leq 0.05$) between groups indicated by brackets. A) SGK1 and CLIC1 expression in the kidney; B) SGK1 and CLIC1 expression in the rumen.

A)



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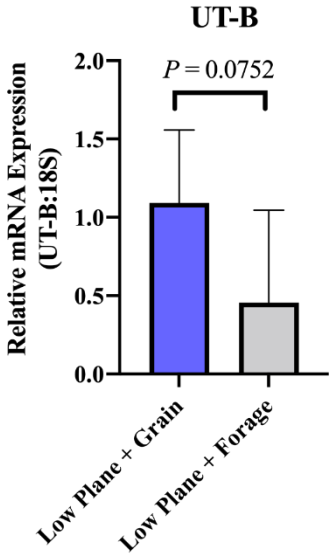

Figure 7. Gene expression of urea transporter B (UT-B) in the rumen at the end of finishing phase of crossbred Angus beef steers previously backgrounded in either a low or moderate plane of nutrition. During the finishing phase animals were either grain (n = 12) or forage-finished (n = 12).
Error bars show the standard error of the mean. A) UT-B gene expression of animals from a low or moderate plane of nutrition finished on a grain versus forage diet; B) UT-B gene expression of animals from a moderate plane of nutrition finished on a grain versus forage finishing diet.

IMPLICATIONS

With the increasing concern of the impacts that beef cattle production may cause in the environment, it is imperative the use of feeding strategies that can reduce cattle's footprint. Altogether, from the results obtained in this dissertation, it was observed that the diet offered for cattle can play a significant role on reducing the use of fresh water and nitrogen excretion. Production systems where diets cannot be timely managed nor precisely fed are often considered as environmentally friendly (i.e.: grass/forage-fed finishing systems), however, because of lack of control on dietary provision, they actually require much more water and animals within these systems excrete more nitrogen back to the environment. These results indicate of a need to tailor diets into precision formulation and management approaches, which encompasses key challenges not only for the forage-systems, but for systems that overfeed nitrogen in attempting to reach higher levels of productivity such as the dairy sector and/or the grain-fed finishing systems for beef. High levels of protein have been associated with high quality feeds and the use of by-products, such as dried distiller's grains. Furthermore, our results indicate that the previous plane of nutrition can also be a key component carrying its own share of the footprint for the next stages of the life of those animals. For instance, a moderate plane of nutrition during the backgrounding phase can ensure the highest carcass quality and lowest water intake, whereas animals backgrounded on low plane of nutrition can adapt to better utilize the nitrogen consumed when levels of protein are moderate in the finishing diet. This information is extremely valuable not only in an environmental perspective, but from a producer standpoint as well since communication between production systems is key to solve a challenge that reaches to broadness of the entire beef

cattle industry. From an environmental perspective, we showed that improving the forage utilization during backgrounding is critical for the beef cattle industry to mitigate water use, but also, that finishing animals in a grain-based system is also key to continue to mitigate the same water use, not only from the time needed to finish an animal, but also from the dietary influence on water requirements. Interestingly, throughout the years, most of these strategies have been pursued by the beef industry already, but maybe, not documented well enough to demonstrate that sustainable use of natural resources has been on industry's portfolio for decades. From a producer standpoint, the results outline herein can indicate how important it is to ensure proper nutrition earlier in life of cattle, and how this information should be integrated with the next sector of interest. Proper nutrition will not only guarantee higher carcass quality, but it will also change tissues deposition within the animal's body further reducing water requirements. In regions where supply of fresh water is limited, the use of animals that are more water efficient is key to ensure the sustainability of the production system.