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Identification of Genes Expressed in the Anterior Pituitary Associated With Water Restriction in Beef Cattle

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IDENTIFICATION OF GENES EXPRESSED IN THE ANTERIOR PITUITARY ASSOCIATED WITH WATER RESTRICTION IN BEEF CATTLE

BY

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ABSTRACT

IDENTIFICATION OF GENES EXPRESSED IN THE ANTERIOR PITUITARY ASSOCIATED WITH WATER RESTRICTION IN BEEF CATTLE

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Water is an essential nutrient and is required for growth, metabolism, lactation, and reproduction. Without water, livestock production would not be possible. Despite its importance, water requirements in livestock have not received much attention from the research community since the 1950s. The most recent edition of the Nutrient Requirements of Beef Cattle (8th edition) relies on literature from the 1950s or earlier for defining water requirements in beef cattle. Since the time these requirements were estimated, much has changed with regards to body size, genetics, and productivity of our livestock herds, including beef cattle. Global surface temperatures have increased in the last 20 years leading to climate variability, drought conditions, and desertification. Range animals may be exposed to more variable water supplies. Nutrigenomics research in livestock has largely focused on interactions between dry matter components of the diet and genomics. Transcriptome and genomic variation in response to changes in water intake have not been studied to our knowledge. Given the importance of water in livestock diets, this gap in our understanding of livestock nutrigenomics is significant. RNA-seq analysis was carried out on total RNA from anterior pituitary of beef cattle followed by water restriction (control, n = 3 and water restriction, n = 3). 14,280 genes were deemed to be expressed (>4 reads per gene per animal), 40 of which were shown to be statistically significantly differentially expressed (SDE) at a false discovery rate (FDR) of 0.1% with a ≥2-fold change between

control and water restricted animals. GOseq/ KEGG pathway analysis showed that SDE genes with ≥2- fold change was associated (P <0.05) with one KEGG pathway and six Curated Reactomes pathways associated with G protein-coupled receptor incorporated hormone secretion and transport. Most interestingly, all these pathways were related to Arginine Vasopressin (AVP) which is one of the major regulators of water balance.

RNA-seq analysis suggested that, to regulate plasma osmolarity from water restriction, the major changes in anterior pituitary gene expression in water deprivation cattle were related to release of AVP into the circulation and transportation to the kidney where AVP binds to the vasopressin type 2 receptor. This indicates the crucial role of AVP in regulating plasma osmolarity in response to water restriction. This study provides valuable insights into the genomic response of beef cattle to water restriction, highlighting the significance of AVP-related pathways in maintaining water balance. The findings contribute to advancing our understanding of water requirements in modern livestock and emphasize the need for updated guidelines in the Nutrient Requirements of Beef Cattle. Ultimately, this research can aid in the development of more efficient management strategies to ensure optimal livestock health and productivity in the face of climate variability and limited water availability.

Keywords: RNA-seq, Arginine Vasopressin, Bovine, anterior pituitary

CHAPTER I: REVIEW OF LITERATURE

LIVESTOCK WATER REQUIREMENT

Water is required by animals for both maintenance and growth. Water accounts for more than 98% of all molecules in the body and approximately 60-80% of animal's total body weight at maturity. Water molecules affect cellular, developmental and organ functions (Lorenzo *et al.,* 2019) and hydration and brownian movement of water is important for protein function (Iwaki *et al.,* 2009). Water acts as a solvent to dissolve molecules (Shirahata *et al.*, 2012). Water is required for regulation of body temperature, reproduction, and growth (Jéquier & Constant, 2010). According to the findings by Miglierina *et al.* (2018), the quantity and quality of water to dairy cows are important for milk production and composition. The amount of water consumed by the animal was directly associated with milk yield and milk fat percentage where reduced frequency of water intake resulted in decreased milk yield and milk fat percentage (Williams *et al*., 2016).

Water consumption by an animal greatly varies with the breed and size of the animal, physiological state, dry matter intake, activity level, water quality, water temperature and environmental temperature (Table 1.1; Beede, 2005). The minimum requirement of water intake is reflected by the amount needed for body growth, fetal growth or lactation and that lost by excretion in urine and feces. The water requirement is provided by drinking water as feeds that are high in moisture such as silage which provide part of this requirement (Sileshi *et al.,* 2003). Lactating cows require more water than non-lactating animals. Hotter temperatures increase water intake. Animals consume less water from pastures that have dried out, increasing the need to consume water from other sources (Babinszky *et al*., 2011).

Dry matter intake has been directly associated with water intake (NRC 2000). Poor water intake leads to poor feed intake and consequently reduced weight gain, milk production and animal performance (Lukas *et al.*, 2008). Poor quality water could serve as a carrier for spreading diseases where the acceptability or safety of animal products for human consumption may be affected (WHO, 2011). Water quality problems affecting livestock are often caused by high concentrations of minerals, high nitrogen content, bacterial contamination, heavy growth of toxic blue green algae or accidental spills of petroleum, pesticides or fertilizers (Wright, 2007). Tolerance of minerals in water is determined by factors such as age, diet, and species. High levels of nitrogen in surface water are caused by decaying plant or animal protein, nitrogen fertilizer and silage runoff (Carson, 2000).

Water requirements of beef cattle

Water requirements of beef cattle are a function of stage of production, lactation, and environmental temperature. A beef cattle's water requirement increases with weight, during pregnancy, during lactation, and when temperatures are elevated (Petersen *et al.*, 2016). A significant amount of water can be lost through excretion in the form of urine, feces or sweat. Therefore, the minimum requirement of beef cattle depends on the amount needed for body and fetal growth, lactation, and replacement of water that is lost by excretion as urine, feces, sweat, and evaporation from lungs or skin. Any factor influencing water gains and losses will influence water needs by the animal (Murphy, 1992).

When water supply is restricted, the animal reabsorbs a greater amount of water than usual from the kidney which increases the concentration of urine (Sands & Layton, 2009). As this capacity for urine concentration is limited, water needs can be reduced but not eliminated. When the animal is fed a high protein or salt diet which contains substances

having a diuretic effect, excretion of urine increases, resulting in increased water consumption (Chan & Swaminathan, 1994). Water requirements of beef cattle can be difficult to assess as feeds contain water and the oxidation of certain nutrients in feed produces water. Not all the water required by the animal must be provided as drinking water (Jéquier & Constant, 2010). When cattle consume feeds high in water content (moisture) such as silage, green chop, or pasture, water intake is reduced. Feeds such as grain and hay are usually low in moisture (Felton & DeVries, 2010). High energy feeds produce more metabolic water than low energy feeds (Rutkowska *et al.*, 2016). Fasting animals or those on a low protein diet may generate water from the destruction of body protein or fat.

Total water uses of U.S beef herd

The U.S. beef industry is the world's largest fed-cattle industry in the world (USDA, 2011). The beef cattle industry is largely separated from the dairy cattle industry in the U.S. USDA mentioned that the United States is the world's largest consumer of beef. According to Beckett and Oltjen (1993), beef cattle in the United States directly consume approximately 760 billion liters of water per year. Beef has a large water footprint, requiring 6,800 liters of water to produce one pound of beef (Mekonnen & Hoekstra, 2012) which is much higher than that of vegetables or grains (Water Footprint Network, 2017).

Total water used for beef production above included the amount of water consumed by animals, water used for producing feed, and water used for processing cattle into boneless beef at harvesting facilities. In the United States, irrigation of crop feedstuffs for beef cattle required 12,991 billion L and Irrigated pasture for beef cattle production required an additional 11,243 billion L of water . Most of the water for beef cattle production is used

to produce crops consumed by beef cattle (Oltjen, 1991). Environmental conditions can significantly affect the amount of water required for crop production.

Beef cattle raised in grain-fed and pasture-based systems have a very different water footprint. The first six to nine months of the lives of grain-fed cattle are typically spent with their mothers, who usually live in a relatively open, sometimes pasture-raised environment, in what's called a calf-cow operation. After weaning, they eat grass and forage. They are then transitioned to grain feed before being transported to a concentrated animal feeding operation (CAFO). Compared to grass fed beef production, grain-fed beef production has a higher blue water footprint, which is the amount of surface and groundwater required, where grain fed beef production has a higher green water footprint, which is the amount of rainwater required (Gerbens-Leenes *et al.,* 2013). In pasture-based cattle systems, the green footprint is larger as the primary source of forage for these cattle is rainfed grasses and forage cultivated on rangelands. Pasture raised cattle typically take 24 to 28 months to reach market weight where grain-fed cattle typically reach slaughter weight between 12 to 18 months (Provenza *et al.,* 2019). These cattle have a higher green water footprint as they rely on predominantly non-irrigated grass. Beef production on pasture may be affected by droughts that affect the availability of grass.

In the United States, 80% of beef cattle are conventionally raised, where they spend six months grazing on pasture and then are moved to a feedlot for four to six months where they are fed a feed mix made from corn, soy, and other grains (Mathews & Johnson 2013). Approximately 29 million cattle in the United States consumed large amounts of grain as of 2012 (National Agricultural Statistics Service 2012). Raising thousands of cattle in feedlots has increased the efficiency of beef production per hectare of land. Most of the

grains used for cattle production are from irrigated crops and most of the irrigated acreage is in the American plains and western states that experience frequent droughts (US Economic Research Service 2021). Cattle in US grazing systems are also fed large amounts of grains, predominantly maize, which is irrigated and fertilized (Anderson 2011). Feed conversion efficiency in beef production varies among production systems. Conventional beef production systems use 3.7 times less feed than grazing beef production systems to produce the same amount of beef (Gerbens-Leenes *et al.* 2013). In conventional systems, the fraction of concentrates in the total mix ration is higher (18%) than a mixed system which combines livestock and crops. Concentrate percentages range from 2% for grazing systems to 4% for mixed systems (Gerbens-Leenes *et al*. 2013).

Beef cattle in the United States directly consumed for drinking 760 billion L of water per year (Beckett and Oltjen 1993). Beckett and Oltjen (1993) used the data presented by Winchester and Morris (1956) to predict total water intake by class of cattle. Based on regression equations (SAS, 1985), those data were analyzed as follows:

$$Water\ Intake = b_0 + b_1.Weight + b_2.Temperature + b_3.Temperature^2$$

The yearly predicted total water intake for cows is calculated as the sum 1) of all cows with calves (85% of all cows) times the water consumption for lactating cows for 4 months of the year, 2) of all cows without calves (15 %) times the water consumption for maintenance for 4 mo of the year, 3) of all pregnant cows (92%) times the water consumption for pregnant cows for 8 mo of the year, and 4) of all nonpregnant cows (8%) times the water consumption for maintenance for 8 mo of the year. The equations for predicting water intake include both the water consumed directly by the animal and the water contained in the feed. The total water intake prediction was subtracted from the water in the feed to

determine direct water consumption. Beckett and Oltjen (1993) calculated the amount of water in feed based on a 75% DM average of the feedstuffs that the animals consume. This feedstuff-bound water is subtracted from the total water intake predictions to estimate the amount of water consumed from drinking. Feedlot animals consumed an estimated 37.9 liters of water per day (Winchester and Morris 1956). Irrigation of crop feedstuffs for beef cattle required 12,991 billion L of water in the United States. Irrigated pasture for beef cattle production required an additional 11,243 billion L of water (Beckett and Oltjen 1993). Therefore, irrigation of crops and pasture for beef cattle production consumes more water than they consume for drinking.

According to the USDA Livestock Slaughter 2009 Summary, 33.3 million cattle were harvested in 2009, requiring about 51 billion / L of water for processing. Beckett and Oltjen (1993) showed that carcass processing required 79 billion L of water and about 3,682 L of developed water per kilogram of boneless meat was used for beef cattle production in the United States. The amount of water uses to process beef cattle from slaughter to trimmed boneless beef was calculated as 1,533 L/carcass in a harvest facility in California (Beckett and Oltjen 1993). According to Kreith (1991), 20,559 L of water per kg boneless beef was required and Beckett and Oltjen (1993) suggested that the amount of water required was 20,864 L to produce one kilogram of boneless beef. However, Beckett and Oltjen (1993) stated that those reports contain several erroneous assumptions and oversimplifications that result from basing estimates on a single production scenario.

Dry matter intake (DMI) and daily water intake (DWI)

Dry matter intake is the amount of feed a cow consumes per day on a moisture-free basis (Østergaard & Gröhn 2000). An animal's daily water intake is the amount of water

consumed daily from liquid water and feed (Arias & Mader 2011). DWI can be increased or decreased depending on the amount of total energy and energy density in a diet. According to Brosh *et al.* (1998), cattle fed a high-ME diet of 10.6 MJ/kg of DM, which consisted of a concentrate: sorghum hay ratio of 80:20 on a DM basis, had higher water intake, metabolizable energy intake, energy expenditure and conserved more energy compared to cattle fed a low ME roughage diet of 7.2 MJ/kg of DM, which consisted only of sorghum hay. DWI is affected by weather, type of diet, breed, body weight (BW), and animal physiological status. Little information is available about how these factors interact with each other to affect DWI of cattle. The DMI increases and DWI decreases when ambient temperature decreases, whereas increased temperature increases DWI and decreases DMI (Arias & Mader 2011). DMI is influenced by cattle breed and body condition, management, and other environmental factors (Mader *et al.* 2010).

According to Arias & Mader (2010) several climate variables were used to calculate temperature–humidity index (THI), including wind speed, temperature, relative humidity, and estimated solar radiation. Arias & Mader (2011) showed that DWI was positively influenced by THI in the summer within season analysis. During winter, daily maximum ambient temperature (T_{max}) and THI were the most reliable predictors of DWI. However, daily minimum ambient temperature (T_{min}) and THI appear to have the greatest influence on DWI across all seasons. The Livestock Weather Safety Index (LWSI; LCI 1970) is widely used to manage livestock in general, and feedlot cattle, when temperatures are high which is also a benchmark commonly used to assign heat stress levels to normal, alert, danger, and emergency categories (Mader *et al*. 2007). THI values greater than 75 are considered "alerts" and indicate potential heat stress in cattle. The LWSI measures

environmental conditions using the THI based on temperature and humidity only (Thom 1959). The THI inflection points of 67.2 represents an environmental threshold that causes animals to activate physiological mechanisms to cope with the additional heat load. Generally, a THI of 70 or 72 is considered a low threshold, though there are differences among animals, with animals with high metabolic heat loads having lower thresholds (Mader 2003). Although THI has always been an excellent indicator of heat stress, T_{min} has been shown to be an indicator of reproductive success in beef cattle (Amundson *et al*. 2006).

Influence of heat stress on water intake

Heat stress is caused by thermal radiation, minimal cloud cover, little or no air movement, high relative humidity, and overnight low temperatures above 70°C. Heat stress in beef cattle is often less severe than in dairy cattle as beef cattle have a higher average temperature-humidity index threshold resulting from a lower metabolic rate and lower body heat production (St-Pierre *et al*. 2003). Excessive heat load was associated with decreased cattle health and performance in feedlots in the United States during the summer season (Gaughan *et al.* 2004; Mader *et al*. 2006) and decrease animal well-being. Heat stress may reduce weight gain and feed intake, increase morbidity and mortality, decrease milk production, and decrease reproductive performance. Increased morbidity in cattle resulted from a combination of high temperature, high relative humidity, low wind speed, and high solar radiation (Mader *et al.* 2006). High heat and humid conditions increase individual water requirements, such as often experienced by calves in feedlots during the summer (Mader *et al.* 2006). Heat waves in 1995 and 1999 resulted in an economic loss to cattle feeders in Iowa and Nebraska exceeding $20 million dollars each.

Different environmental conditions across the United States influence heat stress occurrence, potential, and severity. Heat dissipation mechanisms associated with heat stress in cattle involve a combination of radiation, convection, conduction, and evaporation (Mader *et al*. 2003). In order for thermal equilibrium to be maintained, radiation exchange is necessary. Radiant energy from the sun is reflected by clouds and the ground surface, as well as received as diffuse radiation from the sun due to atmospheric scattering. This exchange of radiant energy is also affected by the animal's orientation to the sun, as well as its reflectivity and absorption characteristics. As the ambient temperature approaches or exceeds body temperature, the animal must either escape, or increase its active cooling by evaporating water from the respiratory tract or by sweating (Lee 1967). Cattle's hair coat presents a range of insulation values, ranging from fine and glossy to thick and woolly; this coat will affect heat exchange by convection and sweat evaporation (Blackshaw & Blackshaw 1994).

Cattle in feedlots, fed a high-energy diet and unable to escape their environment, are at risk of heat stress. The degree of heat stress will depend on several factors, including external heat load, metabolic heat increment, and the efficiency of heat loss mechanisms. The reduction of solar radiation, which is a significant source of heat for cattle, by shading may be beneficial in maintaining feed supply and growth, as well as extending the time during which they are able to survive (Blackshaw & Blackshaw 1994).

Compared to other animals, cattle cannot dissipate their heat load very effectively (Klein 2013). Cattle undergo heat stress when the temperature is above 27 ℃, resulting in increased maintenance requirements. Beef cattle compensate for increased body temperature by homeostatic mechanisms such as sweating, panting, urination, and

behavioral alterations such as reduced activity, increased water intake, and reduced feed intake (Magrin *et al*. 2017). Feedlot cattle are more susceptible to heat stress than cattle on pasture. Cattle on pasture can seek shade, water, and air movement to cool themselves while feedlot cattle cannot. Additionally, radiant heat from dirt or a concrete floor in a feedlot can increase heat stress. Brahman cattle has genetically increased heat tolerance capabilities, productivity, and growth performance under heat stress, compared to other beef cattle breeds (Blackshaw & Blackshaw 1994).

Moreover, water consumption is the fastest way to reduce core body temperature. A 1000 kg animal needs about 5.7 liters gallons of water per hour (Pfost *et al.*, 2007). Cattle need three inches of linear water space per head during summer (Pfost *et al*., 2007). Before extreme heat events, extra water tanks should be introduced to encourage cattle to consume adequate water. The water supply should be able to deliver 1.1% of the body weight of the cattle per hour. Providing shade and water availability are efficient attempts to overcome heat stress in feedlots (Blackshaw & Blackshaw 1994). Although providing shade and utilizing sprinkler systems are useful for preventing heat stress, building this infrastructure is expensive. The current production systems of feedlots in U.S. use several other alternatives to overcome heat stress such removing excess manure. Livestock manure releases CH_4 and N_2O gas (EPA, 1999). Environment conditions, handling systems, and duration of waste management affect N_2O emissions from manure storage. Steinfeld *et al*. (2006) reported that N_2O emissions from stored manure are equivalent to 10 million tons N per year. Modification of production and management systems may include diversifying livestock and crops, integrating livestock systems with forestry and crop production, and altering the timing and rotation of livestock (Kurukulasuriya and Rosenthal 2003).

Increasing drought and heat stress tolerance through diversification of livestock and crops may increase livestock production when animals are exposed to high temperatures and precipitation stresses (Batima *et al.* 2008). Rotation of livestock and crop production through different pastures could reduce soil erosion and improve moisture and nutrient retention (Kurukulasuriya and Rosenthal 2003). Crop rotations and changing timing of management operations such as grazing, planting, spraying, irrigating could also be adapted to changes in growing season duration, heat waves, and precipitation variability (Batima *et al*. 2008).

The THI threshold temperature for beef cattle is set at 30°C with relative humidity below 80% and 27°C with relative humidity above 80% (SCAHAW 2001). Cattle begin to experience heat stress when temperatures fall outside the thermal neutral zone, resulting in increased maintenance requirements and decreased appetite (Ahmed *et al*. 2015). When cattle overheat, the amount of panting is increased which decreases the time for ruminating and eating (Brody 1945). The actual heat load of the animal is increased after ingesting feed as metabolic activity increases to digest the feed (Collins *et al*. 2018). This heat load comes to a peak 4 to 6 hours after feeding. If cattle are fed in the morning, heat production peaks in the middle of the day when environmental temperature is also often highest. Under heat stress, cattle also experience decreased growth rates, milk production and reproductive performance which result in economic losses (Beede and Collier 1986).

Influencing water intake through excretion

A significant amount of water can be lost through excretion in the form of urine, feces, lungs, and skin. Several factors such as water intake, ambient temperature and physical activity determine the amount of water excreted from the kidneys as urine. If an animal is

not water restricted, urinary excretion rate can usually be reduced without weakening the ability of the kidneys to excrete body wastes (Sands & Layton 2009). Urinary loss accounts for the majority of water loss and acts as a tool to dispose of toxic metabolic products (Hristov *et al.* 2019). Urine production is controlled by vasopressin, also called antidiuretic hormone (ADH). Vassopressin is secreted by the pituitary gland and controls water reabsorption from renal tubules and ducts. Cattle will reduce urine volume and increase renal water conservation during water restriction (Weeth *et al*. 1967). The water requirement of cattle increases when the diet is high in diuretic components such as salt and protein (NRC 2001).

Fecal output is determined by the composition of the ration. More water is consumed when cattle are fed less moisture feeds like green chop, silage, or growing pasture whereas low moisture feeds such as winter pasture and hay result in high water consumption. Additionally, high energy diets produce more metabolic water and contribute more water to feces (NRC 2001). Compared to non-ruminants, ruminants require more water to carry the ingesta through the gastrointestinal tract because of the higher fiber diets. Cattle feces contain 75-85% water and sheep and goat feces contain 60-65% water. Sheep and goats can reabsorb water in the lower gut and excrete dry fecal pellets which is one mechanism to conserve water (NRC 1981). When heifers had access to ad libitum water, they lost on average 6.8 kg more water per day in feces than in urine (Weeth *et al.* 1967). According to Thornton and Yates (1968), the reduction in water content within feces was more pronounced than the reduction in urine water content. Consequently, this suggests that fecal water output exhibits a greater degree of variability in response to diet changes compared

to water output in urine. The implication drawn is that diet has a more substantial influence on the output of fecal matter than it does on urine output.

Water loss from the respiratory tract is associated with relative humidity and respiration rate. When relative humidity decreases, respiratory water loss increases. Respiratory water loss is increased when respiration rate increases. High temperatures also increase respiration rate. According to Roubicek (1969), cattle may lose 23 ml/m^2/h at 27°C and up to 50 ml/m^2/h under severe heat stress. Evaporation of water from the skin is the major route of heat loss in cattle during high temperatures (Robertshaw *et al*. 1966). The threshold skin temperature for sweat production is about 25°C in cattle (McDowell *et al.* 1954).

Water restriction effects on digestibility

Feed and water consumption varies among diets and water consumption is significantly reduced when cattle are water restricted and given a high roughage diet (Bond *et al.* 1976). According to Bond *et al.* (1976), water restriction causes a reduction in feed intake. When water intake of beef cattle is restricted, digestibility of a ration is increased (Balch *et al.* 1953). As a result of restricted water intake, animals' reticulo-rumens adjust to maintain a water-to-DM ratio similiar to those whose water intake is unrestricted (Balch *et al*. 1953). Saliva production increases when water is absent, which creates favorable conditions for fermentation. Thus, rumen conditions are more favorable for rumen fiber breakdown when water intake is restricted (Balch *et al*. 1953). In support of this finding, water restriction increased cellulose degradation in the rumen ventral sac (Thornton and Yates 1968).

The restriction of water intake has been found to reduce ruminant feed intake (Bianca 1966). Ruminants secrete much more saliva during eating than monogastric animals and

they can buffer osmotic changes in the rumen derived from digesta as they have a large fluid reserve in the rumen (Bailey 1961). During water deprivation, lactating cows gradually reduced feed intake and did not compensate for the weight loss resulting from dehydration by increasing feed intake during the subsequent rehydration period. Several mechanisms may contribute to the limitation of the dehydration-induced energy deficit in ruminants; 1) forage-based diets may be more digestible during dehydration and 2) resting metabolic rate may decrease during dehydration. These adaptive mechanisms have mostly been observed in nonlactating, mostly small and desert-adapted ruminants (Brosh *et al.* 1986).

During an experiment conducted by Burgos *et al*. (2001) ad libitum water intake was compared with 25% and 50% water restriction over an 8-day period. Milk and blood urea concentrations, plasma sodium concentrations, and hematocrit levels were increased by 50% water restriction. Water restriction did not affect energy balance, but the nitrogen balance became negative because nitrogen excretion via urine and milk was higher compared to intake. Energy balance did not change significantly with 50% water restriction since the decrease in excretion rates paralleled the decrease in intake rates. After rehydration, the body weight of both restriction groups immediately increased above the baseline level and feed intake immediately returned to the baseline level. The ability of the rumen to retain water, while suppressing feed intake and enhancing digestibility, allows ruminants to survive periods of limited water availability. According to Burgos *et al.* (2001), dehydration is prevented by this homeostatic mechanism.

Effects of feed and water restriction on carcass characteristics

Several research teams have investigated the effects of restricted feeding on carcass characteristics of feedlot steers (Sainz *et al*. 1995; Rossi *et al.* 2001). According to Bryant *et al.* (1999), a restricted feed intake of 5 to 15% below ad libitum intake resulted in decreased quality grade of the animal during the finishing stage. The effect of programmed feeding methods which restrict feeding in the growth period and then increase feed in the finishing stage to maximize compensatory gain was usually not significantly associated with quality grade (Bryant *et al.* 1999). Results from the study done by Jones *et al.* (1985) show that pork color is slightly darker and carcass weight is decreased when swine are denied access to feed and water for 48 hours prior to harvest. The effect of water restriction on carcass quality in beef cattle has not yet been explored.

GLOBAL CLIMATE CHANGES

Globally, climate change is a major concern for current livestock systems. Climate change is defined by the United Nations Framework Convention on Climate Change (UNFCCC) as changes induced by long-term direct and indirect activities that are more than natural fluctuations (UNFCCC 1992). The primary cause of global climate change is greenhouse gas emissions (GHG) that cause the atmosphere to warm (IPCC 2013). A 14.5% share of global GHG emissions is attributed to livestock (Gerber *et al*. 2013). Global mean surface temperatures are projected to rise by about 3.7°C (between 2.6°C and 4.8°C) by 2100 (IPCC 2013). Climate change is influenced by livestock through land use change, feed production, animal production, manure, and processing and transportation. According to Cassandro (2020), the livestock sector is directly and indirectly responsible for GHG through animal physiology, animal housing, manure treatment and storage, chemical

fertilizer, and land application. The direct sources of emissions from animals are enteric fermentation, respiration, and excretion (Jungbluth *et al*. 2001). Indirect emissions from animal sources include farm operations, manure applications, livestock product processing, transportation, and land use allocation for livestock production such as deforestation, desertification, carbon released from cultivated soils (Mosier *et al*. 1998). As a result of livestock production, indirect emissions contribute more to the release of carbon into the atmosphere than direct emissions (Grossi *et al.* 2019).

Increases in livestock production may lead to natural resource bases as livestock use a large amount of natural resources (Thornton and Herrero 2010). Thirty percent of the planet's ice-free surface area is occupied by livestock, and 8% of the fresh water supply is consumed by livestock (Steinfeld *et al*. 2006). Worldwide, 20% of the energy required by cattle to reach market weight is derived from cereal crops, while the rest comes from rangeland, pasture, and other roughage sources. Livestock, especially ruminants, occupy 80% of anthropogenic land use and consume about 35% of agricultural crops (Foley *et al*. 2011). They compete with crop production for human consumption and with alternative land uses, such as the production of bioenergy crops and the conservation of nature (Smith *et al*. 2010). The difference in meat consumption between developed and developing countries is enormous, with developed countries consuming far more animal products per capita than developing countries. Overusing resources and the subsequent wastage of the end product result in increased greenhouse gas emissions in developed countries (Steinfeld *et al*. 2006).

The primary livestock GHG emissions are carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O). These gases are emitted by feed production and manure, which affects climate change. The GHG emissions from livestock are largely accounted for by beef and

dairy cattle, which contribute 65% of the total while pork, poultry, buffaloes, and small ruminants contribute about 7 to 10% (Gerber *et al.* 2013). The enteric fermentation produced by ruminant livestock such as cattle, sheep, and goats emit globally between 87 and 94 Teragram (Tg; 1 Tg=1 million tons) of methane annually (IPCC 2013). Mixed crop-livestock systems account for 64% of global enteric fermentation methane emissions where grazing systems account for 35%, and industrial 1% (Steinfeld *et al*. 2006). Globally, livestock contribute 44% of anthropogenic CH_4, 53% of anthropogenic N_2O and 5% of anthropogenic CO_2 emissions. The IPCC (2006) reported that the warming potential of CH_4 is 25 carbon dioxide equivalents (CO_2-eq), while N_2O is 298 CO_2-eq. The UNFCCC (2014) also reported that CH_4 emissions have a warming potential of 21 CO_2-eq, and N_2O is 310 CO_2-eq over a 100-year time horizon. It is estimated that livestock unit output of CH_4 ranges between 194 and 390 grams per day (1 Livestock Unit (LU) equals 500 kg) and primary determinants of CH_4 emissions are animal weight, gross energy intake and milk production. The highest CH_4 emissions occur during feeding and rumination (Jungbluth *et al.* 2001). Emission levels are mainly influenced by animal weight, diet, and milk yield. Furthermore, management and environment may affect GHG emissions from cattle. Amon *et al.* (1998) found that deep litter systems with straw seem to have higher N_2O emissions compared to tied stalls with solid and liquid manure systems. It was found that N_2O emissions increased at high temperatures (Cheng *et al*. 2020). Hahne *et al*. (1999) found that when the air exchange rate is lower in autumn and winter, higher levels of CH_4 are emitted. Oxygen availability over the emitting surfaces might also increase CH_4 production (Hahne *et al.* 1999).

Effect of climate on cattle performance

Climate change can adversely affect livestock by changing the quality and quantity of feed, milk production, disease prevalence, pregnancy rate, and biodiversity (Nardone *et al*. 2010). Impacts of climate change are being driven by higher temperatures and atmospheric CO_2 concentrations, as well as precipitation variation (Henry *et al*. 2012; Thornton *et al*. 2009). Changes in temperature, CO_2 and precipitation affect forage quantity and quality. Temperature and precipitation variations have a strong influence on livestock diseases (Rojas-Downing *et al*. 2017).

Increasing temperature also is associated with changes milk and meat production. Dairy cows have a significant impact on their organic and inorganic milk composition due to climate change (Mariani *et al*. 1998). Summer *et al*. (2019) showed the negative effects of heat stress on both milk protein and casein content. Dairy cattle are typically more sensitive to heat stress due to their higher metabolic rate and body heat production than beef cattle (Summer *et al*. 2019). Beef cattle tend to be less stressed by heat than dairy cattle, as beef cattle tend to have a higher average temperature-humidity index threshold (St-Pierre *et al*. 2003). Additionally, beef cattle compensate for increased body temperature by using homeostatic mechanisms (panting, sweating, and urination) and making behavioral changes such as reduced activity, increased water intake, and reduced feed intake. These changes lead to reduced growth performance.

Effect of climate on water intake

The livestock sector, which uses water for animal consumption, feed crops, and product manufacturing, will be affected by water availability issues (Thornton *et al*. 2009). The

livestock sector accounts for about 8% of global human water use and an increase in temperature may increase animal water consumption by a factor of two to three (Nardone *et al*. 2010). Climate conditions determine how much water livestock consume, with a higher requirement under hot conditions (Ward and McKague 2019), putting an increased strain on water supplies, especially in areas already experiencing shortages. For example, the intake of drinking water by poultry can rise by 50% if warm air temperatures exceed 30°C. Moreover, warmer temperatures result in more glacier depletion, disrupting historic surface water flow patterns. Higher temperatures and extreme events like floods and droughts are likely to reduce water quality for animal consumption by increasing the concentration of pathogens, sediments, salts, nutrients, or pollutants in water (Godde *et al*. 2021).

Climate change is also expected to reduce the quality of raw water (Jiménez Cisneros *et al*. 2014). In turn, animals consume less water, eat less and are less healthy. A warmer climate, sea level rise, or heavy rainfall can lead to poor local water quality (Sharma *et al*. 2016). Global warming is causing global mean sea level to rise (Meehl *et al*. 2005), leading to more saltwater introduced into coastal freshwater. Saltwater adds to chemical and biological contaminants and high concentrations of heavy metal that may hinder livestock production (Nardone *et al*. 2010). As a result of increased contaminants and heavy metals, cardiovascular, excretory, skeletal, nervous, and respiratory systems may be affected (Nardone *et al*. 2010). Animal fertility, metabolism, and digestion could also be negatively affected by water salination. The tolerance of animals to different levels of total dissolved solids (including inorganic salts) in water vary considerably among various species of

animals, with poultry, buffaloes, and dairy cattle (2,000–2,500 ppm) having lower tolerance levels than beef, sheep and pigs (4,000 ppm) (Godde *et al*. 2021).

Based on the Livestock Weather Safety Index (LWSI; LCI 1970), benchmark levels of heat stress have been established using the THI. The THI is used to estimate the level of heat stress in cattle (Tom 1959). This index is adapted to report ambient temperature and humidity that cause heat stress in cattle (Dikmen & Hansen 2009). The THI values are only a rough measure of the effect of heat stress on production. Heat stress is affected by other variables, including wind speed and solar radiation (West 2003; Mader *et al*. 2006). However, the LWSI uses the THI equation containing only temperature and humidity as reported by Thom (1959) and NOAA (1976).

HYPOTHALAMIC INTEGRATION OF BODY FLUID REGULATION

Thirst is an important component of control mechanisms that ensure body fluid homeostasis and is essential for survival. Both intracellular and extracellular compartments are depleted when the body loses water. Sodium chloride loss combined with water loss results in a proportionately greater reduction of extracellular fluid than if water alone is lost (Bhave & Neilson 2011). As a result of compartment depletion, a variety of compensatory responses are triggered such as vasopressin secretion, stimulation of the renin-angiotensin aldosterone system, sympathetic activation, and reduced renal solute and water excretion that have the effect of minimizing changes in body fluid volume and composition. These mechanisms do not restore body fluids to the original state. Therefore, thirst provides the motivation to drink which replenishes fluid losses. Increases of 1-2% in the effective osmotic pressure of plasma result in the stimulation of thirst in mammals. In mammals, thirst is stimulated when the plasma osmolality (usually in the range of 280-295

Osm/kg H_2O) is increased because of increasing the concentration of solutes such as NaCl that do not readily pass across cell membranes (Zerbe & Robertson 1983). The main stimulant is peptide angiotensin II (ANG II), which is secreted by the kidneys in response to sodium deficiency. Additionally, reduced concentrations of Na+ in the adrenal arterial blood or increased concentrations of K+ directly stimulate secretion, as do stress-released ACTH (Denton *et al*. 1986).

Water moves out of cells by osmosis through a transmembrane osmotic gradient, resulting in cellular dehydration. Permeating solutes do not result in cellular dehydration in the latter case, and it is thought that osmoreceptors in the brain respond to cellular dehydration to generate neural mechanisms that cause thirst (Fitzsimons 1979). Studies have shown that systemically administered renin or ANG II generates water intake in hydrated rats. As with osmotically stimulated drinking, ANG-II induced thirst relies on the lamina terminalis (SFO, MnPO, and OVLT) for sensing circulating peptides (particularly SFO), as well as for integrating peripherally derived information into the central nervous system (Johnson *et al*. 1996). Bengt Andersson, who stimulated goats' thirst by electrical or chemical means in the early 1950s, showed that the hypothalamus plays a role in thirst generation by producing vasopressin which plays essential roles in the control of the body’s osmotic balance and blood pressure regulation (Andersson 1953).

ANATOMY OF HYPOTHALAMUS AND PITUITARY GLAND

Hypothalamus

The hypothalamus is located at the base of the brain, around the third ventricle, and extends from a plane immediately anterior to the optic chiasma to immediately posterior to the

mamillary bodies. Anteriorly, its borders are roughly the optic tract, pes pedunculi, the internal capsule, globus pallidus, and ansa peduncularis at different anteroposterior planes, where it does not extend above the anterior commissure. The hypothalamus is a small mass of brain tissue whose nuclei are extremely well defined (Clark 1938). Supraoptic nuclei are almost entirely composed of large nerve cells, while paraventricular nuclei are mainly composed of large nerve cells but also has smaller nerve cells (Hope 1975). The large nerve cells are the most notable as they produce vasopressin and oxytocin, as well as a cystine-rich binding protein, neurophysin (Watkins 1975). Vasopressin plays essential roles in the control of the body's osmotic balance, blood pressure regulation, sodium homeostasis, and kidney functioning and indirectly affect thirst. Therefore, it is important to have a thorough knowledge of anatomy and physiology of hypothalamus when studying thirst responses.

The hypothalamus has symmetric walls and each of the two symmetric walls of the hypothalamus are divided into four surfaces. These surfaces are 1) a lateral surface contiguous with the thalamus, subthalamus and internal capsule, 2) a medial surface extending to the wall of the third ventricle, 3) a superior surface corresponding to the hypothalamic sulcus that separates the hypothalamus from the central mass of the thalamus, and 4) an inferior surface that is contiguous with the floor of the third ventricle. An external protuberance of the hypothalamic floor is called the *tuber cinereum*, and its central part forms a funnel-shaped process called the infundibulum or median eminence. The infundibulum is contiguous with the infundibular stem of the posterior pituitary gland, and the pars tuberalis of the anterior pituitary forms the pituitary stalk. Two additional symmetric eminences include 1) the lateral eminences, corresponding to the most lateral portion of the hypothalamic wall and 2) the postinfundibular eminence, as well as the

symmetric mammillary bodies, which complete the macroscopic morphology of the hypothalamic floor.

The vertebrate hypothalamus contains a number of nuclear groups and fiber tracts. The hypothalamus can be divided into three regions: the lateral, medial, and periventricular regions. In the human hypothalamus, an anatomical boundary divides the hypothalamus into medial and lateral subdivisions. This boundary is created by the anterior column of the fornix, also known as anterior pillars, and fornicolumns extends face-caudally through the hypothalamus to the mammillary bodies and the mammilo-thalamic tract, which extends from the mammillary bodies upward to the thalamus (Swanson 1987).

A 5 - 6 cell layer thick nuclear group contained within the medial subdivision is the periventricular subdivision which surrounds the third ventricle that is readily identifiable in rodents using standard vital stains. The periventricular subdivision has less clearly defined anatomical boundaries in humans. Both medial and periventricular subdivisions contain a high density of neuronal cell bodies arranged into nuclear groups. These subdivisions are important for the regulation of the anterior and posterior pituitary glands. The medial subdivision also contains nuclear groups that relay binary information received from the neuronal cortex, limbic system, and autonomic sensory centers in the brain stem to initiate certain homeostatic behaviors, including thirst, hunger, thermoregulation, the sleep-wake cycle, and reproductive behavior (Swanson 1987). The lateral hypothalamus constitutes the largest portion of the hypothalamus by volume. Compared to the medial hypothalamus, it contains fewer neurons and fewer nuclei intercalated within the massive forebrain bundle (MFB) are present (Monroe 1967).

Pituitary Gland

The pituitary gland is located in the middle cranial fossa (sella turcica) and consists of the anterior lobe (adenohypophysis) and the posterior lobe (neurohypophysis). The anterior lobe contains three subdivisions including the *pars distalis, pars intermedia* and *pars tuberalis*. The *pars distalis* makes up the bulk of the anterior pituitary and is responsible for the secretion of anterior pituitary hormones into the peripheral circulation. The *pars intermedia* lies between the *pars distalis* and the posterior pituitary (Ciocca *et al*. 1984). The *pars tuberalis* surrounds the infundibular stem (Harris 1948). The posterior pituitary and hypothalamus are anatomically connected via a rich nervous pathway of which the supraoptico-hypophysial tract is the most important. After the pituitary gland matures, communication between the hypothalamus and *pars distalis* is dependent on the hypophysial portal system, a vascular link connecting the hypothalamus to the pituitary gland (Cohen & Radovick 2002).

Venous drainage refers to veins that drain into the right atrium without passing through two vascular beds (Nakazawa *et al*. 1978). This advanced physiological structure of blood supply from the hypothalamus to pituitary gland helps carry the hypothalamic hormones to other organs in the body. In both the anterior and the posterior lobes, the venous drainage originates from the adjacent venous sinuses. However, blood supply to the pituitary gland is systemic from the internal carotid artery. The *pars distalis* and infundibular process have independent vascular fields, whereas the *pars intermedia* is relatively avascular. A portal system of blood vessels connects the *pars distalis* to the median eminence. Between the *pars tuberalis* and median eminence, twigs of the internal carotid arteries supply a rich vascular plexus. As a result of this plexus, " vascular tufts " or " sinusoidal loops " protrude

into the region coextensive with the *pars tuberalis*. These loops form the wide trunks of the portal vessels, which descend to the pars distalis, where they drain into the sinusoids of this part of the gland (Harris 1948).

Circumventricular organs

Circumventricular organs (CVO) are structures in the brain characterized by their extensive and highly permeable capillaries which are different from the rest of the brain where there exists a blood–brain barrier (BBB) at the capillary level. The CVO contains a capillary plexus and, except for the subcommissural organ, possesses a fenestrated endothelium which places the structures outside of the BBB. As a result of this feature, brain-derived products can be secreted into the peripheral circulation and circumventricular organs can be targeted for blood-borne information that can be transmitted to the brain (Johnson & Gross 1993). Circumventricular organs include the organum vasculosum of the lamina terminalis (OVLT), subfornical organ, choroid plexus, pineal gland, subcommissural organ and area postrema (Figure 1). In this review, only the median eminence, lamina terminalis and subfornical will be discussed in more detail considering their role in thirst response and water regulation.

Median eminence (ME)

The median eminence is a circumventricular organ which is also one of the most important regions of the hypothalamus and is involved in pituitary gland regulation as well. The ME is in the basal hypothalamus ventral to the third ventricle and adjacent to the arcuate nucleus. According to Weindl (1973), the hypophysiotropic hormones congregate in this area before they are transported to the pituitary gland.

Organum vasculosum of the lamina terminalis (OVLT)

The organum vasculosum of the lamina terminalis, another important circumventricular organ, is located midline of the lamina terminalis (Figure 1). The lamina terminalis, a thin lamina, consists of the median portion of the wall of the forebrain which stretches from the interventricular foramen (Foramen of Monro) to the recess at the base of the optic stalk (optic nerve). The OVLT contains the vascular organ of the lamina terminalis, which regulates osmotic concentration. The ventral surface of OVLT is directly in contact with the prechiasmatic cistern which is formed as the interpeduncular cistern extends forward across the optic chiasm and onto the upper surface of the corpus callosum. The dorsal surface of OVLT protrudes into the third ventricle cavity positioned to be bathed by cerebrospinal fluid (CSF) in both ventricular and cisternal spaces (Weindl & Joynt 1972). Weindl & Joynt (1972) also mentioned a variety of cell types present in the OVLT such as specialized neurons, ciliated ependyma, and glial cell tanycytes.

In the OVLT, small, fenestrated capillaries derived from the preoptic arteries are present that form a dense network of small vessels in the pia mater lining the lamina terminalis and loop up towards the ventricular lumen (Yamaguchi *et al*. 1993). These vessels surround interstitial spaces filled with cellular processes and secretory nerve endings that contain a variety of neurotransmitters, including atrial natriuretic peptide, vasopressin, somatostatin, and gonadotropin-releasing hormone (GnRH). Neurons in the OVLT project to the preoptic nucleus, subfornical organ, arcuate nucleus, supraoptic nucleus, medial thalamus, cingulate, temporal, and insular cortices. Therefore, the OVLT is an ideal location to receive blood-borne information and then transmit this information to specific regions of the brain. Furthermore, the OVLT is involved in fluid regulation and osmoregulation

through osmoreceptor cells with Transient Receptor Potential Vanilloid1 (TRPV) 1 protein and responds to angiotensin II and relaxin levels in the blood (Ciura & Bourque 2006). The transient receptor potential channel (TRP channel) is an evolutionary conserved membrane protein (Samanta *et al*. 2018)**.**

Subfornical Organ (SFO)

The subfornical organ is located at the point where the lamina terminalis joins the tela choroidea of the third ventricle (Dellmann 1985; Figure 1). A tela choroidea is a region of the meningeal pia mater that forms a choroid plexus within each of the four brain ventricles after attaching to the underlying ependyma. The SFO's "core" is thought to contain major hormone receptor fields and fiber terminals of its afferent neuronal innervation, especially the median preoptic nucleus. The SFO's "perimeter" refers to where SFO axons exit from the hypothalamus and project directly to the preoptic nucleus, OVLT, supraoptic nucleus, paraventricular nucleus, and lateral hypothalamus. The SFO has a crucial role in coordinating fluid balance with blood pressure and drinking behavior, particularly during hemorrhage and hypovolemia (Tanaka *et al*. 1993). Through the rich vasculature of the SFO, circulating angiotensin II stimulates intrinsic neurons through angiotensin type 1 receptors. The SFO has direct projections to the paraventricular nucleus, supraoptic nucleus and accessory magnocellular cell groups of the hypothalamus. The paraventricular nucleus (PVN, PVA, or PVH) is located in the hypothalamus, is anatomically adjacent to the third ventricle, and many of its neurons project to the posterior pituitary. These projecting neurons secrete oxytocin and a smaller amount of vasopressin, but they also release corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH). The supraoptic nucleus (SON) consists of magnocellular neurosecretory cells in the

hypothalamus of mammals which is located at the base of the brain, adjacent to the optic chiasm. These neurons produce oxytocin and a smaller amount of vasopressin as well (Bhumbra *et al.* 2004). The SFO neurons activate the posterior pituitary to release vasopressin (Ferguson 1992). Several neural sites associated with fluid and blood pressure balance and vasopressin release can be affected by SFO neurons. Additionally, paraventricular nucleus neurons may stimulate sympathetic nerve endings to release vasoactive peptides like vasoactive intestinal polypeptide (VIP) (Sakai *et al*. 2007). Peptides such as obestatin, somatostatin, and thyrotropin-releasing hormone are present in the SFO, which may play a role in the coordination of liquid with solid ingestive behavior and sleep (Hajdu *et al*. 2003). Leptin receptors are found in the SFO, and their deletion abolishes the leptin-driven increase in sympathetic outflow to the kidney which leads to increased fluid retention and vasoconstriction as well as activation of the renin-angiotensin system. As a result of connections with the preoptic nucleus and OVLT, the SFO also controls thirst and drinking locomotion (Gross 1985).

THE ROLE OF VASOPRESSIN IN REGULATING THE WATER BALANCE

Vasopressin synthesis and processing

The neurohypophyseal antidiuretic hormone vasopressin (VP) is one of the major regulators of water balance (Ball 2007). Vasopressin is synthesized and secreted by chromaffin cells either located in the medulla or scattered throughout the cortex, with more concentration in the zona glomerulosa (Gallo & Guillon 1998). Most of the VP is produced in large secretory neurons found in the paraventricular nucleus and the supraoptic nucleus of the hypothalamus. The nerve terminals of these magnocellular neurons terminate on capillaries of the inferior hypophyseal artery in the posterior pituitary. The VP gene is

located on chromosome 20 in humans (Mohr *et al*. 1988). It is made up of three exons encoding a 145 amino-acid modular polypeptide consisting of an amino-terminal signal peptide, the VP peptide sequence, VP-specific mid-molecule peptide neurophysin-II (NPII), and the carboxyl-terminal peptide involved in post-translational glycosylation. Synthesis of VP precursor (VP-NPII pre-prohormone) undergoes significant posttranslational processing. The carboxyl-terminal domain is glycosylated, and the precursor packaged into vesicles of the regulated secretory pathway in which it undergoes cleavage as these vesicles migrate within magnocellular neurons toward nerve terminals in the posterior pituitary (Ball 2007).

As a result of this sequential process, mature hormones are stored in secretory granules in the posterior pituitary after posttranslational processing (Russell *et al*. 1980). Its short circulating half-life is only five to fifteen minutes (Lauson 1974). The VP protein binds to specific receptors on platelets, even though it circulates unbound to plasma proteins. Degradation of VP occurs by several means, including circulating and endothelial enzymes (Bichet *et al*. 1987). The action of VP is mediated by bonding to one of three G-protein-coupled receptors (V-Rs) found on the plasma membrane of target cells: V1-V3. The V-Rs are encoded by different genes, and they differ in their tissue distribution, signal transduction, and function.

The principal physiological action of VP is the regulation of water resorption by the kidney. In the renal interstitium, distal nephrons pass through a high-osmolar environment enroute to the collecting duct. The distal nephron has selective VP-dependent water channels (AQPs) in the cellular membranes that allow water to flow from the collecting duct lumen into the renal interstitium and excrete concentrated urine. Among the 11 different

mammalian AQPs, seven (AQP1—4, AQP6--8) are found in the kidney. Some AQPs have a significant amount of functional redundancy. The isotonic movement of fluid is facilitated by the expression of AQP1 in both the apical and basolateral membranes of the proximal tubule and descending loop of Henle (Verkman 2002). A defect in renal water conservation occurs when AQP1 is mutated (Schnermann 2000). The AQP2 channel is expressed on the surface of collecting duct cells and is responsible for VP-dependent water transport from the lumen of the nephron into the collecting duct cells. In collecting duct cells, activation of V2-R increases AQP2 gene expression and accelerates AQP2 trafficking to luminal membranes, where AQP2 monomers are assembled into functional homo-tetrameric water channels. Therefore, the expression AQP2 is VP-dependent.

PHYSIOLOGICAL REGULATION OF THIRST IN CATTLE

Drinking behavior of cattle

Cattle are often kept close to water; water is often freely available in intensive production systems such as feedlots, dairying, and small grazing enterprises. However, water is not always freely available in extensive grazing systems (ARC 1980). Cattle tend to concentrate their grazing around water points (Low *et al*. 1978). When water is provided only at drinking facilities for dairy cows, access to water coincides with milking times (Cowan *et al*., 1978). Low *et al*. (1978) suggested that the distance cattle graze from water influences their drinking behavior. According to Castle *et al*. (1950), cattle in small paddocks (<15 ha) drink multiple times per day and lactating dairy cows (*Bos taurus*) in temperate climates drink 2–4 times per day. In cool climates, growing *Bos taurus* beef cattle drink on average 4–7 times per day (Coimbra *et al*. 2010). Drinking frequency of cattle may affect water intake, feed intake and performance of cattle. Total deprivation of

water for 72 h has been observed to reduce feed intake and live weight gain in beef cattle (Ahmed and El Hadi 1996). Limiting the amount of water ingested by animals for 2 hours, though not completely depriving them of it, reduces both feed intake by cattle as well as live weight gain and milk yield by dairy cows (Balch *et al*. 1953).

Restricting access to water reduced water intake in beef cattle (Williams *et al*. 2017). Even though cattle with restricted access to water drink more at every opportunity than cattle with more frequent access, the amount of water consumed was not sufficient to offset this restriction over time (Payne 1965). Schmidt *et al*. (1980) showed that a control group that was allowed to drink twice a day consumed approximately 15 kg of water each time and approximately 35 kg of water was consumed by the group of cattle that was allowed to drink only once every second day. At each drinking opportunity, the treatment group consumed more than twice as much as the control group, but over time consumed 43% less water.

Thirst response in cattle

Animals are motivated to drink fluids because of thirst, which is a subjective experience. Thirst is a result of deficits in either intracellular or extracellular fluid volumes, and the thirst response is important for maintaining body fluid homeostasis, which ultimately determines survival. Afferent signals from intrathoracic baroreceptors via the hindbrain could produce a thirst response by integrating signals arising from osmotic and hormonal influences on the lamina terminalis. (McKinley & Johnson 2004). As reductions in fluid content in various compartments, hypertonicity of extracellular fluid, or rises in circulating dipsogenic hormone concentrations occur, a thirst response arises.

Normally, when the body loses water, it is taken from both the extracellular and intracellular compartments, but it is not necessarily lost equally from both compartments (Thomas *et al*. 1998). NaCl is the major solute of extracellular fluid. Water loss alone does not deplete the extracellular fluid as much as NaCl and water loss combined (Schrier 2011). Vomiting or diarrhea may result in fluid loss from the alimentary tract, and if this fluid loss is in the form of an isotonic fluid, then the extracellular fluid will be drained entirely. Additionally, adding hypertonic fluid to the extracellular compartment will increase extracellular osmolarity, water is osmosed out of the cells into the extracellular space (McKinley & Johnson 2004). The NaCl remains mostly in the extracellular compartment, and fluid diffuses from the cells into the extracellular space to achieve osmotic equilibrium. As a result, the extracellular volume increases (greater than the added fluid volume), the intracellular volume decreases, and the osmolarity rises in both compartments (Guyton & Hall 2000).

When either the intra- or extracellular compartment is depleted, a range of compensatory responses such as vasopressin secretion, stimulation of the renin-angiotensin-aldosterone system, sympathetic activation of the nervous system, and reduced renal solute and water excretion are activated (McKinley & Johnson 2004). The effect of these responses is to minimize changes in body fluid volume and composition, but these changes do not restore body fluids to the original state. In order for fluid restoration to occur, fluid losses should be replenished. Hence, thirst, which provides the driver for drinking, plays a critical role in maintaining the composition and volume of body fluids by the coordinated action of numerous physiological factors (Anderson 1977).

Osmosis results in the movement of water out of cells via a transmembrane osmotic gradient. An increase of 12% in the effective osmotic pressure of plasma stimulates thirst in mammals (Stricker 1966). The increase in plasma osmolality that takes place in experimentation by increasing the concentration of solutes that do not readily cross cell membranes, such as NaCl or sucrose, induced thirst in both human subjects and mice (McKinley & Johnson 2004). Infusion of concentrated solutes such as urea and D-glucose, which more readily pass through nerve cell membranes, are relatively ineffective at stimulating thirst. In humans and other mammals, increasing the concentration of NaCl or sucrose - molecules that cannot readily cross cell membranes - increased plasma osmolality, which in turn stimulated thirst (McKinley *et al*., 1978). The permeating solutes do not result in dehydration of cells in this case, and it is thought that brain cells called osmoreceptors (which were first associated with vasopressin production) initiate neural mechanisms that result in thirst (Zerbe & Robertson 1983).

A study by Andersson (1978) found that electrical or chemical stimulation of the hypothalamus stimulated water consumption in goats. The injection of hypertonic saline into the hypothalamus near the fornix induced drinking, but the solutions injected were grossly hypertonic, which made it difficult to conclude physiologically relevant osmoreceptors for thirst were present in this region. Andersson suggested that rostral tissue in the anterior wall of the third ventricle was more likely to be the site of sensors mediating osmotic thirst and proposed a role for ambient Na + concentrations in this region of the third ventricle in triggering osmotic thirst in rats.

Neural mechanisms of water regulation

There is sufficient evidence to suggest that the anterior wall of the third ventricle plays a crucial role in thirst mechanisms because tissue ablation in anteroventral third ventricle walls (AV3V region) of goats, and rats caused temporary or permanent adipsia (Andersson 1978). Destruction of this area by narrow, medially placed radiofrequency lesions had drastic effects on the water balance of goats, including apparent adipsia, absent or greatly impaired ADH and hypernatremia (Andersson *et al*. 1975). Despite severe dehydration and hypernatremia, those with such lesions have a complete and persistent lack of thirst. It was noted that there was no apparent ADH release in animals with these lesions in response to hypertonic NaCl infusion or intracarotid infusion with angiotensin II. As an acute effect of such lesions, an uncompensated, temporary diuresis was observed, which rapidly induced hypernatremia and hypovolemia. Loss of dipsogenic response to osmotic and angiotensin (ANG) stimuli was evident in those animals with lesions that recovered spontaneous water drinking. According to McKinley *et al*. (1978), the cerebral osmoreceptors regulating thirst and vasopressin secretion are located, at least in part, in brain regions lacking a blood-brain barrier in sheep. McKinley *et al*. (2003) confirmed that during the embryonic stage, osmoreceptors reside within both the subfornical organ (SFO) as well as the organum vasculosum of the lamina terminalis (OVLT). Both the SFO and OVLT are circumventricular organs that lack blood-brain barriers and are located inside the anterior wall of the third ventricle (the lamina terminalis). In rats, the dorsal part of the OVLT and the peripheral SFO are osmosensitive.

Moreover, the preoptic nucleus of the median ventricular atria (AV3V), which is found in the lamina terminalis between the two circumventricular structures, is also strongly

affected by osmotic stimulation. Johnson *et al*. (1996) have shown that both osmotic and hormonal signals are relayed to the median preoptic nucleus (MnPO) by neural inputs from the SFO and possibly the OVLT, which may be responsible for producing thirst in rats. Osmoregulatory drinking may also be inhibited pharmacologically by intracerebral injection of ANG antagonists, indicating a central angiotensinergic pathway in mammals. The angiotensinergic synapse would likely be located in the MnPO, which is rich in ANG type 1 receptors but not amenable to circulating ANG II (Johnson *et al.* 1996). The MnPO receives neural signals from the SFO and OVLT and integrates these signals with hindbrain visceral input. Absence of both the SFO and OVLT, while leaving a significant part of the MnPO intact, reduced but did not eliminate osmotically induced drinking (McKinley *et al*. 1999). Accordingly, neurons in the MnPO are likely to be osmoreceptive or receive osmotically related inputs from other parts of the brain, such as the dessertma (AP) or hepatic portal system.

The lamina terminalis is a region of the brain where stimulated changes in blood circulation, such as hypertonicity or hormones (e.g., AN II, relaxin) that have a dipsogenic effect, are processed. The lamina terminalis may send efferent neural pathways to other brain regions (including the cerebral cortex) to create thirst. Three areas of the hypothalamus receive neural input from the lamina terminalis and may be involved in the generation of thirst: the lateral hypothalamus, hypothalamic paraventricular nucleus, and periaqueductal gray.

The brain renin-angiotensin system

Fitzsimons (1979) found that renin and its effector peptide, ANG II, were highly effective as dipsogenic stimuli in the rat. In sated rats, systemically administered renin or ANG II

induced water intake. As is true for osmotically stimulated drinking, ANG-induced thirst required the lamina terminalis structures (i.e., SFO, MnPO, and OVLT) to sense circulating peptides (especially the SFO), as well as to process and integrate data from peripheral sources (Johnson 1996). An ANG injection directly into the brain mimics the action of ANG II at one or more periventricular brain sites. The brain renin-angiotensin system with all the components of the metabolic cascade as well as receptors are synthesized *de novo* in the brain (McKinley & Johnson 2004). Johnson & Thunhorst (1997) mentioned that if ANG II is a neurotransmitter, then circulating ANG II acts as a hormone on forebrain circumventricular organs (SFO, OVLT) and activates angiotensinergic pathways.

Vlasenko & Kotov (2003) have shown that the effector peptides of the renin-angiotensin system (RAS) initiate thirst. Furthermore, angiotensins facilitate aggressive and comfort behaviors, induce feeding motivation, and stimulate orientational-investigative responses, in addition to drinking behavior (Brown & Holtzman 1981). Several types of receptors such as AT1-4 are associated with angiotensins and mediate their functions. The AT1 receptors are responsible for dipsogenic activity (DGA) (Ardaillou 1999). Angiotensin II (A-II) and angiotensin III (A-III) play a crucial role in in the main pathway for the metabolic conversion of the α2-globulin angiotensinogen, while [desAsp1]-A-I is involved in one of the alternative pathways for the metabolism of this peptide. These peptides have different affinity for AT1 receptors, such that they have different "dipsogenic potentials" in terms of their dose-dependent initiation of drinking behavior (Vlasenko & Kotov 2007).

LITERATURE CITED

Agricultural Research Council (1980) The Nutrient Requirements of Ruminant Livestock. Technical Review by an Agricultural Research Council Working Party. Commonwealth Agricultural Bureaux, Slough, England.

Ahmed, A., Tiwari, R. P., Mishra, G. K., Jena, B., Dar, M. A., & Bhat, A. A. (2015). Effect of environmental heat stress on reproduction performance of dairy cows-a review. *International Journal of Livestock Research*, 5(4), 10-18.

Ahmed, M. M., & El Hadi, H. M. (1996). Water metabolism and dehydration in two types of cattle given poor and good quality roughages. *Journal of Arid Environments*, 34(2), 225-233.

Alparslan, Ö. S., & Demirbaş, N. (2020). Red meat and processed red meat consumption behaviour of healthcare professionals: do they participate in the World Health Organization's view of red meat carcasses and red meat carcinogens?. *Public Health Nutrition*, 23(2), 214-220.

Amon, B., Kryvoruchko, V., Amon, T., & Zechmeister-Boltenstern, S. (2006). Methane, nitrous oxide and ammonia emissions during storage and after application of dairy cattle slurry and influence of slurry treatment. *Agriculture, Ecosystems & Environment*, 112(2-3), 153-162.

Anderson, B. (1977). Regulation of body fluids. *Annual Review of Physiology*, 39(1), 185-200.

Anderson, V. L., Ilse, B., Dhuyvetter, J., Stoltenow, C., Burr, D., Schroeder, T., & Ingebretson, T. (2011). *Winter Management of the Beef Cow Herd*. NDSU Extension Service.

Andersson, B. (1953). The Effect of Injections of Hypertonic NaCl-Solutions into Different Parts of the Hypothalamus of Goats. *Acta Physiologica Scandinavica*, 28(2-3), 188-201.

Andersson, B. (1978). Regulation of water intake. *Physiological Reviews*, 58(3), 582-582.

Andersson, B., & Olsson, K. (1973). On central control of body fluid homeostasis. *Conditional Reflex: A Pavlovian Journal of Research & Therapy*, 8(3), 147-159.

Andersson, B., Leksell, L. G., & Lishajko, F. (1975). Perturbations in fluid balance induced by medially placed forebrain lesions. *Brain research*, 99(2), 261-275

Ardaillou, R. (1999). Angiotensin II receptors. *Journal of the American Society of Nephrology*, 10, S30-9.

Arias, R. A., & Mader, T. L. (2010). Determination of potential risk of heat stress of cattle in four locations of Central and Southern Chile. *Archivos de Medicina Veterinaria*, 42(1), 33-39.

Arias, R. A., & Mader, T. L. (2011). Environmental factors affecting daily water intake on cattle finished in feedlots. *Journal of Animal Science*, 89(1), 245-251.

Babinszky, L., Halas, V., & Verstegen, M. W. (2011). Impacts of climate change on animal production and quality of animal food products. *Climate change socioeconomic effects. Rijeka: InTech*, 165-190.

Bailey, C. B. (1961). Saliva secretion and its relation to feeding in cattle: 3. The rate of secretion of mixed saliva in the cow during eating, with an estimate of the magnitude of the total daily secretion of mixed saliva. *British Journal of Nutrition*, 15(3), 443-451.

Balch, C. C., Balch, D. A., Johnson, V. W., & Turner, J. (1953). Factors affecting the utilization of food by dairy cows: The effect of limited water intake on the digestibility and rate of passage of hay. *British Journal of Nutrition*, 7(3), 212-224.

Ball, S. G. (2007). Vasopressin and disorders of water balance: the physiology and pathophysiology of vasopressin. *Annals of Clinical Biochemistry*, 44(5), 417-431.

Batima, P., Bold, B., Sainkhuu, T., & Bavuu, M. (2008). Adapting to drought, zud and climate change in Mongolia's rangelands. *Climate Change and Adaptation*, 197-210.

Beckett, J. L., & Oltjen, J. W. (1993). Estimation of the water requirement for beef production in the United States. *Journal of Animal Science*, 71(4), 818-826.

Beede, D. K. (2005). Assessment of water quality and nutrition for dairy cattle. In *Proc. Mid-south Ruminant Nutrition Conference*, pp. 1-19.

Beede, D. K., & Collier, R. J. (1986). Potential nutritional strategies for intensively managed cattle during thermal stress. *Journal of Animal Science*, 62(2), 543-554.

Bentley, J. (2019). *U.S. Per Capita Availability of Red Meat, Poultry, and Seafood on the Rise*. Retrieved from Economic Research Service U.S. Department of Agriculture:

https://www.ers.usda.gov/amber-waves/2019/december/us-per-capita-availability-ofred-meat-poultry-and-seafood-on-the-rise/. Accessed 08 November 2021.

Bhave, G., & Neilson, E. G. (2011). Volume depletion versus dehydration: how understanding the difference can guide therapy. *American Journal of Kidney Diseases*, 58(2), 302-309.

Bhumbra, G. S., Inyushkin, A. N., & Dyball, R. E. J. (2004). Assessment of spike activity in the supraoptic nucleus. *Journal of Neuroendocrinology*, 16(4), 390-397.

Bianca, W. (1966). Heat tolerance in dehydrated steers. *The Journal of Agricultural Science*, 66(1), 57-60.

Bichet, D. G., Arthus, M. F., Barjon, J. N., Lonergan, M., & Kortas, C. (1987). Human platelet fraction arginine-vasopressin. Potential physiological role. *The Journal of Clinical Investigation*, 79(3), 881-887.

Blackshaw, J. K., & Blackshaw, A. W. (1994). Heat stress in cattle and the effect of shade on production and behaviour: a review. *Australian Journal of Experimental Agriculture*, 34(2), 285-295.

Brosh, A., Aharoni, Y., Degen, A. A., Wright, D., & Young, B. A. (1998). Effects of solar radiation, dietary energy, and time of feeding on thermoregulatory responses and energy balance in cattle in a hot environment. *Journal of Animal Science*, 76(10), 2671-2677.

Brosh, A., Shkolnik, A., & Choshniak, I. (1986). Metabolic effects of infrequent drinking and low-quality feed on Bedouin goats. *Ecology*, 67(4), 1086-1090.

Brown, D. R., & Holtzman, S. G. (1981). Suppression of drinking by naloxone in the rat: a further characterization. *European Journal of Pharmacology*, 69(3), 331-340.

Bryant, T. C., Rivera, J. D., Galyean, M. L., Duff, G. C., Hallford, D. M., & Montgomery, T. H. (1999). Effects of dietary level of ruminally protected choline on performance and carcass characteristics of finishing beef steers and on growth and serum metabolites in lambs. *Journal of Animal Science*, 77(11), 2893-2903.

Burgos, M. S., Senn, M., Sutter, F., Kreuzer, M., & Langhans, W. (2001). Effect of water restriction on feeding and metabolism in dairy cows. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 280(2), R418-R427.

Carson, T. L. (2000). Current knowledge of water quality and safety for livestock. *Veterinary Clinics of North America: Food Animal Practice*, 16(3), 455-464.

Cassandro, M. (2020). Animal breeding and climate change, mitigation and adaptation. *Journal of Animal Breeding and Genetics*, 137(2), 121-122.

Castle, M. E., Foot, A. S., & Halley, R. J. (1950). 423. Some observations on the behaviour of dairy cattle with particular reference to grazing. *Journal of Dairy Research*, 17(3), 215-230.

Chan, E. L. P., & Swaminathan, R. (1994). The effect of high protein and high salt intake for 4 months on calcium and hydroxyproline excretion in normal and oophorectomized rats. *The Journal of Laboratory and Clinical Medicine*, 124(1), 37-41.

Cheng, J., Xu, L., Jiang, M., Jiang, J., & Xu, Y. (2020). Warming increases nitrous oxide emission from the littoral zone of Lake Poyang, China. *Sustainability*, 12(14), 5674.

Ciocca, D. R., Puy, L. A., & Stati, A. O. (1984). Constitution and behavior of follicular structures in the human anterior pituitary gland. *The American Journal of Pathology*, 115(2), 165.

Ciura, S., & Bourque, C. W. (2006). Transient receptor potential vanilloid 1 is required for intrinsic osmoreception in organum vasculosum lamina terminalis neurons and for normal thirst responses to systemic hyperosmolality. *Journal of Neuroscience*, 26(35), 9069-9075.

Cohen, L. E., & Radovick, S. (2002). Molecular basis of combined pituitary hormone deficiencies. *Endocrine Reviews*, 23(4), 431-442.

Coimbra, P. A. D., Machado Filho, L. C. P., Nunes, P. A., Hötzel, M. J., De Oliveira, A. G. L., & Cecato, U. (2010). Effect of water trough type on the drinking behaviour of pasture-based beef heifers. *Animal*, 4(1), 116-121.

Collins, T., Hampton, J. O., & Barnes, A. L. (2018). A systematic review of heat load in Australian livestock transported by sea. *Animals*, 8(10), 164.

Cowan, R. T., Shackel, D., & Davison, T. M. (1978). Water intakes, milk yield and grazing behaviour of Friesian cows with restricted access to water in a tropical upland environment. *Australian Journal of Experimental Agriculture*, 18(91), 190-195.

Daniel, P. M. (1976). Anatomy of the hypothalamus and pituitary gland. *Journal of Clinical Pathology. Supplement (Ass. Clin. Path.)*, 7, 1.

Daniel, P. M., & Prichard, M. M. (1975). Studies of the hypothalamus and the pituitary gland. *European Journal of Endocrinology*, 80(4), S1-S205.

De Gasparo, M., Catt, K. J., Inagami, T., Wright, J. W., & Unger, T. H. (2000). International union of pharmacology. XXIII. The angiotensin II receptors. *Pharmacological Reviews*, 52(3), 415-472.

Dellmann, H. D. (1985). Fine structural organization of the subfornical organ. A concise review. *Brain Research Bulletin*, 15(1), 71-78.

Denton, D. A., McKinley, M. J., Osborne, P., Tarjan, E., & Weisinger, R. S. (1986). The phylogeny and ontogeny of thirst. In *The Physiology of Thirst and Sodium Appetite* (pp. 1-12). Springer, Boston, MA.

Denton, D., Shade, R., Zamarippa, F., Egan, G., Blair-West, J., McKinley, M., & Fox, P. (1999). Neuroimaging of genesis and satiation of thirst and an interoceptor-driven theory of origins of primary consciousness. *Proceedings of the National Academy of Sciences*, 96(9), 5304-5309.

Dikmen, S. E. R. D. A. L., & Hansen, P. J. (2009). Is the temperature-humidity index the best indicator of heat stress in lactating dairy cows in a subtropical environment?. *Journal of Dairy Science*, 92(1), 109-116.

Dutton, A., & Dyball, R. E. J. (1979). Phasic firing enhances vasopressin release from the rat neurohypophysis. *The Journal of Physiology*, 290(2), 433-440.

EPA (U.S. Environmental Protection Agency), 1999. "*Livestock manure management*." https://www3.epa.gov/npdes/pubs/cafo_manure_guidance.pdf. Accessed 08 November 2021.

EU SCAHAW (2001). EU Scientific Committee on Animal Health and Animal Welfare Report on the Welfare of Cattle Kept for Beef Production. *European Commission, Brussels, Belgium*.

Felton, C. A., & DeVries, T. J. (2010). Effect of water addition to a total mixed ration on feed temperature, feed intake, sorting behavior, and milk production of dairy cows. *Journal of Dairy Science*, 93(6), 2651-2660.

Ferguson, A. V. (1992). Neurophysiological analysis of mechanisms for subfornical organ and area postrema involvement in autonomic control. *Progress in Brain Research*, 91, 413-421.

Fitzgerald, K. T. (1979). The structure and function of the pars tuberalis of the vertebrate adenohypophysis. *General and Comparative Endocrinology*, 37(3), 383-399.

Fitzsimons, J. T. (1979). Nonregulatory drinking and renal function. *Behavioral and Brain Sciences*, 2(1), 105-106.

Fitzsimons, J.T. (1979). The Physiology of Thirst and Sodium Appetite. Cambridge University Press, Cambridge, UK.

Foley, J. A., Ramankutty, N., Brauman, K. A., Cassidy, E. S., Gerber, J. S., Johnston, M., & Zaks, D. P. (2011). Solutions for a cultivated planet. *Nature*, 478(7369), 337-342.

Gallo-Payet, N., & Guillon, G. (1998). Regulation of adrenocortical function by vasopressin. *Hormone and Metabolic Research*, 30(06/07), 360-367.

Gaughan, J. B., Davis, M. S., & Mader T. L. (2004). Wetting and the physiological responses of grain-fed cattle in a heated environment. *Australian Journal of Agriculture Research*, 55, 253–260.

Gerbens-Leenes, P. W., Mekonnen, M. M., & Hoekstra, A. Y. (2013). The water footprint of poultry, pork and beef: A comparative study in different countries and production systems. *Water Resources and Industry*, 1, 25-36.

Gerber, P. J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., & Tempio, G. (2013). *Tackling climate change through livestock: a global assessment of emissions and mitigation opportunities*. Food and Agriculture Organization of the United Nations (FAO), Rome.

Godde, C. M., Mason-D'Croz, D., Mayberry, D. E., Thornton, P. K., & Herrero, M. (2021). Impacts of climate change on the livestock food supply chain, a review of the evidence. *Global Food Security*, 28, 100488.

Gross, P. M. (1985). The subfornical organ as a model of neurohumoral integration. *Brain Research Bulletin*, 15(1), 65-70.

Grossi, G., Goglio, P., Vitali, A., & Williams, A. G. (2019). Livestock and climate change: impact of livestock on climate and mitigation strategies. *Animal Frontiers*, 9(1), 69-76.

Guyton, A. C., & Hall, J. E. (2000). The body fluid compartments: extracellular and intracellular fluids; interstitial fluid and edema. *Textbook of medical physiology*, 9, 306-8.

Hajdu, I., Szentirmai, E., Obal Jr, F., & Krueger, J. M. (2003). Different brain structures mediate drinking and sleep suppression elicited by the somatostatin analog, octreotide, in rats. *Brain Research*, 994(1), 115-123.

Harris, G. W. (1948). Hypothalamus and pituitary gland. *British Medical Journal*, 1(4546), 339.

Hoffman, M. P., & Self, H. L. (1972). Factors affecting water consumption by feedlot cattle. *Journal of Animal Science*, 35(4), 871-876.

Hristov, A. N., Bannink, A., Crompton, L. A., Huhtanen, P., Kreuzer, M., McGee, M., & Yu, Z. (2019). Invited review: nitrogen in ruminant nutrition: a review of measurement techniques. *Journal of Dairy Science*, 102(7), 5811-5852.

IFAD (International Fund for Agricultural Development), (2010). "Livestock and climate change." https://www.uncclearn.org/wp-content/uploads/library/ifad81.pdf. Accessed 08 November 2021.

Iwaki, M., Iwane, A. H., Shimokawa, T., Cooke, R., & Yanagida, T. (2009). Brownian search-and-catch mechanism for myosin-VI steps. *Nature Chemical Biology*, 5(6), 403-405.

Jéquier, E., & Constant, F. (2010). Water as an essential nutrient: the physiological basis of hydration. *European Journal of Clinical Nutrition*, 64(2), 115-123.

Johnson, A. K., & Gross, P. M. (1993). Sensory circumventricular organs and brain homeostatic pathways. *The FASEB Journal*, 7(8), 678-686.

Johnson, A. K., & Thunhorst, R. L. (1997). The neuroendocrinology of thirst and salt appetite: visceral sensory signals and mechanisms of central integration. *Frontiers in Neuroendocrinology*, 18(3), 292-353.

Johnson, A. K., Cunningham, J. T., & Thunhorst, R. L. (1996). Integrative role of the lamina terminalis in the regulation of cardiovascular and body fluid homeostasis. *Clinical and Experimental Pharmacology and Physiology*, 23(2), 183-191.

Jones, S. D. M., Rompala, R. E., & Haworth, C. R. (1985). Effects of fasting and water restriction on carcass shrink and pork quality. *Canadian Journal of Animal Science*, 65(3), 613-618.

Jungbluth, T., Hartung, E., & Brose, G. (2001). Greenhouse gas emissions from animal houses and manure stores. *Nutrient Cycling in Agroecosystems*, 60(1), 133-145.

Klein, B. G. (2013). Cunningham's Textbook of Veterinary Physiology-E-Book. Elsevier Health Sciences, Amsterdam, Netherlands.

Kreith, M., & Davis, C. A. (1991). *Water inputs in California food production*. Water Education Foundation, Sacramento, CA.

Kurukulasuriya, P., Rosenthal, S., 2003. Climate change and agriculture: a review of impacts and adaptations. Climate Change Series Paper No. 91, World Bank, Washington DC.

Lauson, H. D. (1974). Metabolism of the neurohypophysial hormones. *Handbook of Physiology*, 4, 287-393.

Lee, D. H. (1967). Principles of homeothermic adaptation. *In Biometeorology. Proceedings of Annual Biological Colloquium, 28th*, ed. W. P. Lowry, 113-30. Oregon State University Press, Corvallis, Oregon.

Lorenzo, I., Serra-Prat, M., & Yébenes, J. C. (2019). The role of water homeostasis in muscle function and frailty: a review. *Nutrients*, 11(8), 1857.

Low, W. A., Hodder, R. M., & Abel, D. E. (1978). Watering behaviour of British breed cattle in central Australia. In: K.M. W. Howes (eds), Studies of the Australian Arid Zone. 3. Water in Rangelands. CSIRO Australia, Melbourne, VIC, pp. 165–177.

Lukas, J. M., Reneau, J. K., & Linn, J. G. (2008). Water intake and dry matter intake changes as a feeding management tool and indicator of health and estrus status in dairy cows. *Journal of Dairy Science*, 91(9), 3385-3394.

Mader, T. L. (2003). Environmental stress in confined beef cattle. *Journal of Animal Science*, 81(14), E110-E119.

Mader, T. L., Dahlquist, J. M., Hahn, G. L., & Gaughan, J. B. (1999). Shade and wind barrier effects on summertime feedlot cattle performance. *Journal of Animal Science*, 77(8), 2065-2072.

Mader, T. L., Davis, M. S., & Brown-Brandl, T. (2006). Environmental factors influencing heat stress in feedlot cattle. *Journal of Animal Science*, 84(3), 712-719.

Mader, T. L., Davis, M. S., & Gaughan, J. B. (2007). Effect of sprinkling on feedlot microclimate and cattle behavior. *International Journal of Biometeorology*, 51(6), 541-551.

Mader, T. L., Johnson, L. J., & Gaughan, J. B. (2010). A comprehensive index for assessing environmental stress in animals. *Journal of Animal Science*, 88(6), 2153-2165.

Magrin, L., Brscic, M., Lora, I., Rumor, C., Tondello, L., Cozzi, G., & Gottardo, F. (2017). Effect of a ceiling fan ventilation system on finishing young bulls' health, behaviour and growth performance. *Animal*, 11(6), 1084-1092.

Mathews Jr, K. H., & Johnson, R. J. (2013). Alternative beef production systems: issues and implications. *US Department of Agriculture, Economic Research Service, LDPM-218-01*.

Mayer, A. (1900). Variations de la tension osmotique du sang chez les animaux prives de liquides. *Comptes Rendus des Seances de la Societe de Biologie*, 52, 153-155.

McDowell, R. E., Lee, D. H., & Fohrman, M. H. (1954). The measurement of water evaporation from limited areas of a normal body surface. *Journal of Animal Science*, 13(2), 405-416.

McKinley, M. J., & Johnson, A. K. (2004). The physiological regulation of thirst and fluid intake. *Physiology*, 19(1), 1-6.

McKinley, M. J., Denton, D. A., & Weisinger, R. S. (1978). Sensors for antidiuresis and thirst—osmoreceptors or CSF sodium detectors? *Brain Research*, 141(1), 89-103.

McKinley, M. J., Denton, D. A., Leksell, L. G., Mouw, D. R., Scoggins, B. A., Smith, M. H., ... & Wright, R. D. (1982). Osmoregulatory thirst in sheep is disrupted by ablation of the anterior wall of the optic recess. *Brain Research*, 236(1), 210-215.

McKinley, M. J., Mathai, M. L., Pennington, G., Rundgren, M., & Vivas, L. (1999). Effect of individual or combined ablation of the nuclear groups of the lamina terminalis on water drinking in sheep. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 276(3), R673-R683.

McKinley, M. J., McAllen, R. M., Davern, P. J., Giles, M. E., Penschow, J., Sunn, N., & Oldfield, B. (2003). The sensory circumventricular organs of the mammalian brain: subfornical organ, OVLT and area postrema. *Advances in Anatomy, Embryology and Cell Biology* 172, 1-127.

Meehl, G. A., Washington, W. M., Collins, W. D., Arblaster, J. M., Hu, A., Buja, L. E., & Teng, H. (2005). How much more global warming and sea level rise?. *Science*, 307(5716), 1769-1772.

Mekonnen, M. M., & Hoekstra, A. Y. (2012). A global assessment of the water footprint of farm animal products. *Ecosystems*, 15(3), 401-415.

Miglierina, M. M., Bonadeo, N., Ornstein, A. M., Becú-Villalobos, D., & Lacau-Mengido, I. M. (2018). In situ provision of drinking water to grazing dairy cows improves milk production. *New Zealand Veterinary Journal*, 66(1), 37-40.

Mohr, E., Schmitz, E., & Richter, D. (1988). A single rat genomic DNA fragment encodes both the oxytocin and vasopressin genes separated by 11 kilobases and oriented in opposite transcriptional directions. *Biochimie*, 70(5), 649-654.

Monroe, B. G. (1967). A comparative study of the ultrastructure of the median eminence, infundibular stem and neural lobe of the hypophysis of the rat. *Zeitschrift für Zellforschung und Mikroskopische Anatomie*, 76(3), 405-432.

Mosier, A., Wassmann, R., Verchot, L., King, J., & Palm, C. (2004). Methane and nitrogen oxide fluxes in tropical agricultural soils: sources, sinks and mechanisms. *Environment, Development and Sustainability*, 6(1), 11-49.

Murphy, M. R. (1992). Water metabolism of dairy cattle. *Journal of Dairy Science*, 75(1), 326-333.

Murray, D. M. (1966). A comparison of cutaneous evaporation rates in cattle exposed to heat in a climate laboratory and in the field. *The Journal of Agricultural Science*, 66(2), 175-179.

Nakazawa, H. K., Roberts, D. L., & Klocke, F. J. (1978). Quantitation of anterior descending vs. circumflex venous drainage in the canine great cardiac vein and coronary sinus. *American Journal of Physiology-Heart and Circulatory Physiology*, 234(2), H163-H166.

Nardone A, Ronchi B, Lacetera N, Ranieri M.S, & Bernabucci U. (2010). Effects of climate change on animal production and sustainability of livestock systems. *Livestock Science*, 130, 57–69.

National Agricultural Statistics Service (2012). "Cattle and Calves Inventory: 2012 and 2007." United States Department of Agriculture.

National Research Council. (1981). Effect of environment on nutrient requirements of domestic animals. National Academies Press, Washington (DC).

Network, W. F. (2017). Water footprint of crop and animal products: a comparison. *https://waterfootprint.org/en/water-footprint/product-water-footprint/water-footprint-crop-and-animal-products/.* Accessed 11 August 2021.

Østergaard, S., & Gröhn, Y. T. (2000). Concentrate feeding, dry-matter intake, and metabolic disorders in Danish dairy cows. *Livestock Production Science*, 65(1-2), 107-118.

Parker, D. B., & Brown, M. S. (2003). Water consumption for livestock and poultry production. *Encyclopedia of Water Science; Howell, Basata, Ed.; Marcel Dekker Inc.; New York, NY, USA*, 588-591.

Payne, W. J. A., & Hutchison, H. G. (1963). Water metabolism of cattle in East Africa I. The problem and the experimental procedure. *The Journal of Agricultural Science*, 61(2), 255-266.

Petersen, M. K., Muscha, J. M., Mulliniks, J. T., & Roberts, A. J. (2016). Water temperature impacts water consumption by range cattle in winter. *Journal of Animal Science*, 94(10), 4297-4306.

Pfost, D. L., Gerrish, J. R., Davis, M., & Kennedy, M. (2007). Pumps and Watering Systems for Managed Beef Grazing. *http://hdl.handle.net/10355/10036*. Accessed 25 August 2021.

Provenza, F. D., Kronberg, S. L., & Gregorini, P. (2019). Is grassfed meat and dairy better for human and environmental health? *Frontiers in Nutrition*, 6, 26.

Reynolds, C., Crompton, L., & Mills, J. (2010). Livestock and climate change impacts in the developing world. *Outlook on Agriculture*, 39(4), 245-248.

Robbins, J. (1998). *Diet for a New America*. Stillpoint Press, Walpole, NH.

Robertshaw, D., & Whittow, G. C. (1966). The effect of hyperthermia and localized heating of the anterior hypothalamus on the sympatho-adrenal system of the ox (*Bos taurus*). *The Journal of Physiology*, 187(2), 351-360.

Rojas-Downing, M. M., Nejadhashemi, A. P., Harrigan, T., & Woznicki, S. A. (2017). Climate change and livestock: Impacts, adaptation, and mitigation. *Climate Risk Management*, 16, 145-163.

Rossi, J. E., Loerch, S. C., Keller, H. L., & Willett, L. B. (2001). Effects of dietary crude protein concentration during periods of feed restriction on performance, carcass characteristics, and skeletal muscle protein turnover in feedlot steers. *Journal of Animal Science*, 79(12), 3148-3157.

Roubicek, C. B. (1969). Water metabolism. *Animal Growth and Nutrition.* Lea and Febiger, Philadelphia, USA, 353-373.

Russell, J. T., Brownstein, M. J., & Gainer, H. (1980). Biosynthesis of vasopressin, oxytocin, and neurophysins: isolation and characterization of two common precursors (propressophysin and prooxyphysin). *Endocrinology*, 107(6), 1880-1891.

Rutkowska, J., Sadowska, E. T., Cichoń, M., & Bauchinger, U. (2016). Increased fat catabolism sustains water balance during fasting in zebra finches. *Journal of Experimental Biology*, 219(17), 2623-2628.

Sainz, R. D., De la Torre, F., & Oltjen, J. W. (1995). Compensatory growth and carcass quality in growth-restricted and refed beef steers. *Journal of Animal Science*, 73(10), 2971-2979.

Sakai, K., Agassandian, K., Morimoto, S., Sinnayah, P., Cassell, M. D., Davisson, R. L., & Sigmund, C. D. (2007). Local production of angiotensin II in the subfornical organ causes elevated drinking. *The Journal of Clinical Investigation*, 117(4), 1088-1095.

Samanta, A., Hughes, T. E., & Moiseenkova-Bell, V. Y. (2018). Transient receptor potential (TRP) channels. *Membrane Protein Complexes: Structure and Function*, 141-165.

Sands, J. M., & Layton, H. E. (2009). The physiology of urinary concentration: an update. *Seminars in Nephrology*, 29(3), 178-195.

Sapouckey, S. A., Deng, G., Sigmund, C. D., & Grobe, J. L. (2017). Potential mechanisms of hypothalamic renin-angiotensin system activation by leptin and DOCA-salt for the control of resting metabolism. *Physiological Genomics*, 49(12), 722-732.

SAS. 1985. SAS User's Guide: Statistics. SAS Inst. Inc., Cary, NC. Schnermann, J. (2000). NaCl transport deficiencies–hemodynamics to the rescue. *Pflügers*

Schrier, R. W. (2011). Diagnostic value of urinary sodium, chloride, urea, and flow. *Journal of the American Society of Nephrology*, 22(9), 1610-1613.

Sexson, J. L., Wagner, J. J., Engle, T. E., & Eickhoff, J. (2012). Predicting water intake by yearling feedlot steers. *Journal of Animal Science*, 90(6), 1920-1928.

Shirahata, S., Hamasaki, T., & Teruya, K. (2012). Advanced research on the health benefit of reduced water. *Trends in Food Science & Technology*, 23(2), 124-131.

Sileshi, Z., Tegegne, A., & Tsadik, G. T. (2003). Water resources for livestock in Ethiopia: implications for research and development. *Integrated water and land management research and capacity building priorities for Ethiopia*, 66.

Smith, J., Pearce, B. D., & Wolfe, M. S. (2010). Reconciling productivity with protection of the environment: Is temperate agroforestry the answer?. *Renewable Agriculture and Food Systems*, 28(1), 80-92.

Solomon, S., Manning, M., Marquis, M., & Qin, D. (2007). *Climate change 2007-the physical science basis: Working group I contribution to the fourth assessment report of the IPCC* (Vol. 4). Cambridge University Press. Cambridge, UK.

Steinfeld, H., Gerber, P., Wassenaar, T. D., Castel, V., Rosales, M., Rosales, M., & de Haan, C. (2006). *Livestock's long shadow: environmental issues and options*. Food & Agriculture Organization, Rome, Italy

Stocker, T. F., Qin, D., Plattner, G. K., Alexander, L. V., Allen, S. K., Bindoff, N. L., & Xie, S. P. (2013). Technical summary. In *Climate change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of*

the Intergovernmental Panel on Climate Change (pp. 33-115). Cambridge University Press.

St-Pierre, N. R., Cobanov, B., & Schnitkey, G. (2003). Economic losses from heat stress by US livestock industries. *Journal of Dairy Science*, 86, E52-E77.

Stricker, E. M. (1966). Extracellular fluid volume and thirst. *American Journal of Physiology-Legacy Content*, 211(1), 232-238.

Summer, A., Lora, I., Formaggioni, P., & Gottardo, F. (2019). Impact of heat stress on milk and meat production. *Animal Frontiers*, 9(1), 39-46.

Swanson L.W. 1987 The hypothalamus, *in* Bjorklund, A., Hokfelt, T., Swanson, L.W. (eds), Handbook of Chemical Neuroanatomy, vol 5, Integrated Systems of the CNS, part 1, Elsevier Science Publisher, Amsterdam, Netherlands. pp 1-124.

Tanaka, J., Nojima, K., Yamamuro, Y., Saito, H., & Nomura, M. (1993). Responses of subfornical organ neurons projecting to the hyphotalamic paraventricular nucleus to hemorrhage. *Brain Research*, 608(1), 141-144.

Thom, E. C. (1959). The discomfort index. *Weatherwise*, *12*(2), 57-61.

Thomas, B. J., Ward, L. C., & Cornish, B. H. (1998). Bioimpedance spectrometry in the determination of body water compartments: accuracy and clinical significance. *Applied Radiation and Isotopes*, 49(5-6), 447-455.

Thornton, P. K., & Herrero, M. (2010). Potential for reduced methane and carbon dioxide emissions from livestock and pasture management in the tropics. *Proceedings of the National Academy of Sciences*, 107(46), 19667-19672.

Thornton, R. F., & Yates, N. G. (1968). Some effects of water restriction on apparent digestibility and water excretion of cattle. *Australian Journal of Agricultural Research*, 19, 665-672.

UNFCCC (United Nations Framework Convention on Climate Change), 2008. Challenges and opportunities for mitigation in the agricultural sector: technical paper. http://unfccc.int/resource/docs/2008/tp/08.pdf. Accessed 28 September 2021.

United States Department of Agriculture (2021). *"Sector at a Glance. Cattle & Beef."* Economic Research Service, https://www.ers.usda.gov/topics/animal-products/cattle-beef/sector-at-a-glance/. Accessed 05 August 2021

United States Department of Agriculture. (2016b). *"Irrigation & Water Use: Background."* Economic Research Service, http://www.ers.usda.gov/topics/farm-practicesmanagement/irrigation-water-use/background/#definitions. Accessed 12 July 2021.

Verkman, A. S. (2002). Physiological importance of aquaporin water channels. *Annals of Medicine*, 34(3), 192-200.

Visschers, V. H. (2018). Public perception of uncertainties within climate change science. *Risk Analysis*, 38(1), 43-55.

Vlasenko, R. I., & Kotov, A. V. (2003). Comparative analysis of motivation-activated effects of angiotensins in rats. *Zhurnal vysshei nervnoi deiatelnosti imeni IP Pavlova*, 53(1), 113-116.

Vlasenko, R. Y., & Kotov, A. V. (2007). Effector peptides of the renin-angiotensin system in the central mechanisms of acquired and innate behavior in thirst in rats. *Neuroscience and Behavioral Physiology*, 37(3), 263-270.

Weeth, H. J., Sawhney, D. S., & Lesperance, A. L. (1967). Changes in body fluids, excreta and kidney function of cattle deprived of water. *Journal of Animal Science*, 26(2), 418-423.

Weindl A. (1973). Neuroendocrine aspects of circumventricular organs, in Ganong W, Martini L (eds): Frontiers in Neuroendocrinology, Oxford University Press, pp 3-32.

Weindl, A., & Joynt, R. J. (1972). Ultrastructure of the ventricular walls: three-dimensional study of regional specialization. *Archives of Neurology*, 26(5), 420-427.

West, J. W. (2003). Effects of heat-stress on production in dairy cattle. *Journal of Dairy Science*, 86(6), 2131-2144.

WHO Edition, F. (2011). Guidelines for drinking-water quality. *WHO Chronicle*, 38(4), 104-108.

Williams, L. R., Jackson, E. L., Bishop-Hurley, G. J., & Swain, D. L. (2017). Drinking frequency effects on the performance of cattle: A systematic review. *Journal of Animal Physiology and Animal Nutrition*, 101(6), 1076-1092.

Winchester, C. F., & Morris, M. J. (1956). Water intake rates of cattle. *Journal of Animal Science*, 15(3), 722-740.

Wright, C. L. (2007). Management of water quality for beef cattle. *Veterinary Clinics of North America: Food Animal Practice*, 23(1), 91-103.

Xuereb, G. P., Prichard, M. M., & Daniel, P. M. (1954a). The arterial supply and venous drainage of the human hypophysis cerebri. *Quarterly Journal of Experimental Physiology and Cognate Medical Sciences: Translation and Integration*, 39(3), 199-217.

Yamaguchi, K., Morimoto, A., & Murakami, N. (1993). Organum vasculosum laminae terminalis (OVLT) in rabbit and rat: topographic studies. *Journal of Comparative Neurology*, 330(3), 352-362.

Zerbe, R. L., & Robertson, G. L. (1983). Osmoregulation of thirst and vasopressin secretion in human subjects: effect of various solutes. *American Journal of Physiology-Endocrinology and Metabolism*, 244(6), E607-E614.

CHAPTER II: IDENTIFICATION OF GENES EXPRESSED IN ANTERIOR PITUITARY ASSOCIATED WITH WATER RESTRICTION IN CATTLE

ABSTRACT

Water is vital for the growth, metabolism, lactation, and reproduction of beef cattle. However, there is a lack of recent research on water requirements for beef cattle despite changes in their characteristics. The Nutrient Requirements of Beef Cattle relies on outdated literature from the 1950's or earlier to define water requirements, highlighting the need for updated information. Climate change exacerbates the need for reassessing water requirements, as weather conditions affect animal performance and productivity. Changes in feed resources due to climate change impact livestock productivity, rangeland carrying capacity, and disease distribution. Water scarcity caused by droughts and elevated temperatures intensifies pressure on water resources. Research is necessary to update water requirement knowledge, considering current beef cattle characteristics and the challenges of climate change. This would contribute to sustainable livestock management and the well-being and productivity of beef cattle in evolving environmental conditions. RNA-seq analysis was performed on the anterior pituitary of beef cattle subjected to water restriction (control, n=3 and water restriction, n = 3). The analysis revealed that out of 14,280 expressed genes, 40 genes exhibited significant differential expression between the control and water-restricted animals. These genes showed a ≥2-fold change in expression and were statistically significant at a false discovery rate of 0.1%. Further analysis using GOseq/KEGG pathway analysis identified one KEGG pathway and six Curated Reactomes pathways associated with G protein-coupled receptor incorporated hormone secretion and transport that were significantly associated with the differentially expressed genes.

Notably, all these pathways were related to arginine vasopressin (AVP), a major regulator of water balance. The RNA-seq analysis suggested that the major changes in gene expression observed in the anterior pituitary of water-restricted cattle were related to the release of AVP into the circulation and its transportation to the kidney, where AVP binds to the vasopressin type 2 receptor. This finding indicates the crucial role of AVP in regulating plasma osmolarity in response to water restriction. This study provides insights into the molecular mechanisms involved in the anterior pituitary's response to water restriction in beef cattle. The differential gene expression patterns, particularly those related to AVP and its associated pathways, highlight the importance of AVP in regulating plasma osmolarity and maintaining water balance during water restriction.

Keywords: RNA-seq, Arginine Vasopressin, Bovine, anterior pituitary

INTRODUCTION

Since the U.S. beef cattle have grown in size, the amount of water required for the cattle population likely has increased, even though no recent estimates are available. Water is one of the major nutrients required for growth, metabolism, lactation, and reproduction (Jéquier & Constant 2010). Water is important for regulating temperature, nutrient hydrolysis, cellular metabolism, excretion of wastes, mineral homeostasis, lubrication of the joints, cushioning of the nervous system, and eyesight (NASEM 2016). Growth and performance of the animal depend on an adequate supply of water (NRC 1981). Water requirements of cattle are influenced by breed, size, stage of production, milk production, ration composition, and environmental factors, such as temperature, heat stress, and water quality (Mader *et al.* 2006; Winchester and Morris 1956). In the United States, approximately 760 billion liters of water are consumed annually by beef cattle alone, according to a study conducted nearly two decades ago (Beckett and Oltjen 1993). Since 1950, water requirements in livestock have not received much attention from the research community. For example, the most recent edition of the Nutrient Requirements of Beef Cattle (8th edition) relies on literature from the 1950's or earlier to define beef cattle water requirements. However, since the 1950's dramatic changes in body size, genetics, and productivity of beef cattle have occurred.

Global surface temperatures have increased over the past 20 years and this climate change is leading to increased climate variability, drought conditions, and desertification (IPCC 2014). Livestock can be affected by climate directly and indirectly. Weather conditions such as air temperature, humidity, and wind speed affect animal performance, including growth, milk production, wool production, and reproduction (Houghton, 2001). Changes

in feed resources are one of the most visible effects of climate change on livestock production. Though indirect, feed resources have a significant impact on livestock productivity, rangeland carrying capacity, ecosystems' buffering power and sustainability, as well as livestock diseases and parasite distribution (Thornton *et al*. 2007). As a result of severe droughts and elevated temperatures, water resources have become scarce in some regions. These factors are placing increased pressure on water resources, emphasizing the importance of evaluating livestock water requirement (Falkenmark and Widstrand 1992). Understanding water requirements of cattle will also help improve animal production (NRC 1996). Balch *et al*. (1953) has shown that limiting water intake of cattle increased dry matter digestion. Burgos *et al*. (2001) examined the effect of chronic water restriction over an eight-day period and concluded that body weight and intake declined at the onset of the restriction period but stabilized after day three. These results indicate that cattle can acclimate to limited intake of water and stabilize performance under restricted conditions. However, we understand little about the biology of this response to water restriction.

Nutrigenomics is concerned with the interaction between the (epi) genome and nutrition and involves affecting the balance between health and disease by altering the expression, structure of an individual's genetic makeup, or both (Kaput & Rodriguez 2004). By this definition, it is understood that dietary nutrients influence gene expression directly or indirectly, thereby affecting protein expression, metabolism, and signaling in cells, and, consequently, tissues, organs, and entire organisms (Rubhana & Cravioto, 2009). Therefore, nutritional molecules are no longer just energy sources and "building blocks" for cells but are also signals detected by cellular sensors that stimulate cellular changes; therefore, they are bioactive as well. In addition to specific bioactive elements, other

components of the diet can have nutrigenomic effects; for example, limiting the amount of dietary energy may have such an effect (Abete *et al*. 2012; Müller & Kersten 2003).

In most livestock research, nutrigenomics studies have focused on interactions between genomics and dry matter component of the diet and how they impact the physiology of livestock. In recent years, much effort has been expended on nutrigenomics in cattle (Ladeira *et al.* 2016; Bionaz *et al*. 2015). Published nutrigenomic research has mainly focused on changes in gene expression resulting from diet restriction, but few have focused on changes in gene expression resulting from water restriction. For example, Bionaz *et al.* (2015) described how dietary components such as feed and energy intake, fatty acids, and amino acids can affect gene expression with the focus of the way nutrients act as signaling molecules through their interactions with transcription factors (specifically, ligand-dependent nuclear receptors, such as PPARs) to affect gene expression. They concluded that, although the field of nutrigenomics is young, fine-tuning cow metabolism and ration formulation to improve performance, milk quality, and animal welfare was possible. According to Müller and Kersten (2003), nutrients can directly affect the transcriptional regulatory factors in monogastrics. These transcriptional regulatory factors include ligand-dependent nuclear receptors (LdNR), including peroxisome proliferator-activated receptors (PPAR), liver X receptors (LXR), and hepatic nuclear factor 4 (HNF4), which are activated by macronutrients. Neither of these studies investigated how water restriction changes the transcriptome of tissues.

Water restriction has been studied in mice. Cai *et al*. (2006) removed water access for 48 h to increase endogenous levels of circulating vasopressin. Furthermore, they performed cDNA analysis of renal medullary gene expression with the aim of identifying new gene

targets of potential vasopressin action. They identified several mRNAs whose expression was either significantly increased or decreased in the renal medulla following water restriction. To our knowledge, transcriptome and genomic variation have not been studied in response to water restriction in cattle. Due to the importance of water in livestock diets, this gap in our knowledge of livestock nutrigenomics is significant. Our objective was to identify genes expressed in the hypothalamus and anterior pituitary gland associated with water restriction in beef calves.

MATERIAL AND METHODS

Animal and Sample Collection

All animal experimentation was approved by the South Dakota State University Institutional Animal Care and Use Committee (No. 19-009E). Calves were housed at the Cow-Calf Education and Research Facility (CCERF), South Dakota State University, Brookings, South Dakota. The animals were purchased from a commercial livestock sale barn in Sioux Falls, South Dakota. Calves were housed in the same pen at the South Dakota State University CCERF for 67 days prior to the experiment. The calves were part of a larger sample of calves used for an observational study on the effect of weather on feed and water intake in beef calves.

Bos taurus cattle (n = 6) from this contemporary group were randomly allocated into two treatment groups (control, n=3 and water restriction, n = 3). Water intakes were collected using an Insentec automated system (Insentec, The Hague, Netherlands). The waterers were 1-m wide, 0.75-m high, and 0.84-m deep and had a capacity of 40 kg of water. Calves were fitted with an electronic identification tag. When a calf approaches a waterer, a transponder identifies the electronic tag, records the calf identification, and a barrier preventing access to the waterer lowers. After the calf leaves the waterer, the barrier closes, and the difference between beginning and end water weights and duration of time at the waterer is recorded. The difference in weight was assumed to be water intake. The waterer refills once the water level reaches a predefined weight after the calf leaves the waterer. Calves had ad libitum access to feed (Table 2.1). The average water intake of sampled calves was within the range of 30 to 65 kilograms (kg) per day prior to water restriction. Water was withheld from calves in the water restriction group at 2000 hours on the night

before slaughter. The control calves continued to access water ad libitum. The average water consumption for all calves in the same contemporary group as the experimental calves on the day of water restriction was 35.68 kg. All calves were slaughtered the next day between 800 am and 1300 pm. Thus, calves in the water restriction group were restricted from consuming fluid water for at least 12 hours before slaughter. The control calves were allowed to consume water freely after 2000 hours and consumed liquid water overnight, as confirmed from records collected by the Insentec automated system. The animals were harvested at the Meat Science Lab, South Dakota State University, Brookings, South Dakota in the morning of 10/20/2020. Immediately following exsanguinations and head removal, the top part of the skull and brain was removed using a reciprocating saw with a 12-inch blade. The cut was made through the cerebrum close to the thickest part of the brain. The bottom part of the brain was then removed, and the hypothalamus was identified by the pituitary stalk. Hypothalamus containing the supraoptic and paraventricular nuclei and anterior pituitary were collected, each divided into two pieces roughly equal in size, and snap frozen in liquid nitrogen. The samples were stored at -80°C prior to RNA extraction.

RNA Extraction

Total RNA was isolated from both hypothalamus and pituitary samples using RNeasy Lipid tissue mini kit (Qiagen, BD, Germany). Ten mg of tissue were homogenized in QIAzol Lysis Reagent using a PowerLyzer 24 bead beater (MO BIO, Carlsbad, CA USA). After addition of chloroform, the homogenate was separated into aqueous and organic phases by centrifugation. The upper, aqueous phase, where RNA was collected, was removed and ethanol was added to the aqueous phase. The sample was then applied to the

RNeasy spin column, where total RNA (up to 100 µg) binds to the membrane and phenol and other contaminants are removed. High-quality RNA was then eluted in 50 µl of RNase-free water. Concentration and purity of isolated RNA samples were determined using a Nanodrop ND- 2000 spectrophotometer. The quality of RNA was evaluated using RNA gel electrophoresis.

Library Preparation and Sequencing

Total RNA samples on dry ice were submitted to the Genomic Sequencing Facility, South Dakota State University, Brookings, South Dakota, USA for library construction and sequencing. As a result of technical issues that occurred at the Genomic Facility, only four out of the original six samples were successfully processed and analyzed. The RNA-Seq libraries were prepared with Illumina® TruSeq Stranded Total RNA Library PrepKit (Illumina, San Diego, CA) as per the manufacturer's instructions. For the initial step of library preparation, polyadenylated RNA was isolated from total RNA with poly (T) oligonucleotides attached to magnetic beads and then fragmented into small pieces using divalent metal cations under elevated temperatures. First strand cDNA synthesis was performed using reverse transcriptase and random primers. Cleaved RNA fragments were copied into first strand cDNA in the presence of Actinomycin D to prevent spurious DNA-dependent synthesis while allowing RNA-dependent synthesis improving strand specificity. Double-stranded cDNA then was synthesized by using DNA Polymerase I in the presence of RNase H. Strand specificity was achieved by replacing dTTP with dUTP. The cDNA was then purified using AMPure XP beads and modified by end-repair, poly(A) addition, and adaptor ligation. The fragment size 200 bp of the double-stranded cDNA was

selected using AMPure XP beads. Finally, fragments were enriched by polymerase chain reaction (PCR).

RNA-Seq

The library products were sequenced with Illumina Hiseq2000, obtaining single end 75 bp reads. The libraries were labelled as Control (C) and Treatment (T). Quality assessment and adapter trimming was completed using Trim Galore! Quality and adapter trimmer of reads (Galaxy Version 0.6.3) to obtain high-quality reads. Low-quality base calls were trimmed off from the 3' end of the reads of Illumina standard adapters ('AGATCGGAAGAGC') by default before adapter removal which efficiently removes poor quality portions of the reads with Phredscore of less than 20. The Concatenate datasets tail-to-head (cat) (Galaxy Version 0.1.0) was used to concatenate reads before mapping the reads using HISAT2 (Galaxy Version 2.1.0+galaxy5).

Bioinformatics analysis

Trim Galore (Galaxy Version 0.6.3) was used to perform quality and adapter trimming. It removes two additional bases that contain a cytosine which was artificially introduced in the end-repair step during the library preparation. Concatenate datasets tail-to-head (cat) (Galaxy Version 0.1.0) was used to link trimmed reads together. The linked trimmed reads were aligned to the Bovine reference genome (Bos_taurus.ARS-UCD1.2) using HISAT2 (Galaxy Version 2.2.1+galaxy0) to identify transcripts. The cDNA sequencing reads were aligned against the virtual transcriptome build. Then the reads were mapped to the reference genome to extract transcript sequences and resulting alignments were stored in ‘BAM’ files.

Feature Counts (Galaxy Version 1.6.4+galaxy1) was used to count the number of sequences reads for a transcript per sample after mapping. The expression level for each gene was determined by the number of reads mapped to the specific gene and by the total number of mapped reads in the sample. The variable read per kilobase of exon per million mapped reads (RPKM) method was used to determine the expression levels of various genes and compare them between samples. The DESeq program (Galaxy Version 2.11.40.6+galaxy1) was used to assess differential expression features from count tables between treatment and control samples. Fold-changes (FC) between the control and treatment were calculated. The P-value was adjusted to control the false discovery rate (FDR) and differentially expressed genes (DEGs) were defined as those with an $|FC| > 2$ and FDR <5%.

Gene ontology (GO) and pathway enrichment analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the differentially expressed genes were completed by iDEP (integrated Differential Expression and Pathway analysis). iDEP is a bioinformatics resource consisting of an integrated biological knowledgebase and analytic tools that systematically extract enriched biological and functional meaning to a given list of gene with q-value (FDR adjusted P-value) < 0.05. All the GO terms are significantly enriched among DEGs, compared with the background genome, and DEGs were categorized as biological processes, cellular components, and molecular functions. The KEGG pathways are manually drawn diagrams displaying networks of biochemical reactions and molecular interactions in various cellular processes.

RESULTS

Pre-process and EDA Pre-process

iDEP was used with the *Bos taurus* genome assembly ARS-UCD1.2 (GCA_000003205.6) to analyze gene expression changes following water restriction. Using iDEP, a total of 15,484 genes were identified and analyzed in relation to their differential expressions. This means that the expression levels of these genes were compared between the control group (non-water-restricted) and the water-restricted group.

A minimum threshold of 0.5 counts per million reads mapped (CPM) was used to filter the gene expression data. This threshold ensures that only genes with a minimum level of expression are included in the analysis, excluding genes with very low expression levels that might be less biologically relevant. To gain further insights into the functional relationships and interactions among the differentially expressed genes, STRINGdb (version 93) was utilized. By utilizing STRINGdb, you were able to explore the potential interactions and associations among the identified genes to uncover functional modules or networks that may be relevant to water restriction and its effects.

Total read counts per library of two controls and two water restricted groups were 36 897 254, 61 330 330, 46 176 621 and 66 588 925, respectively (Figure 2.1.A). The treatment group had a higher total number of reads compared to the control group. In this study, the average count suggests that a substantial amount of sequencing data was generated, allowing for a reasonably robust analysis of gene expression. Furthermore, the observation that the treatment group had a higher total number of reads compared to the control group indicates that more sequencing reads were obtained for the samples subjected to water restriction. This difference in sequencing depth between the treatment and control groups

may have implications for the statistical analysis and the ability to detect differentially expressed genes accurately. A higher number of reads in the treatment group could potentially increase the statistical power to identify gene expression changes associated with water restriction. It allows for a more comprehensive examination of the molecular responses to water restriction and enhances the chances of capturing subtle but biologically significant alterations in gene expression patterns.

Figures 2.1.B-C depict the distribution of the transformed data where the count data was transformed by using EdgeR: log2(CPM+c). The density plot estimates the count data expression distribution of treatment and control groups. The density plot is negatively skewed that suggests that there might be a subset of genes with relatively low expression levels in the dataset. In the context of water restriction in beef cattle, the negative skewness of the density plot may imply that water restriction has an impact on the expression of certain genes, leading to reduced expression levels. This suggests that the restriction of water intake may trigger regulatory mechanisms or biological responses that result in decreased gene expression in some genes. The presence of lower expression levels in a subset of genes may be indicative of specific molecular pathways and biological processes affected by water restriction in beef cattle. Further exploration of these genes and associated pathways can provide insights into the mechanisms underlying the response to water restriction and the adaptation of beef cattle to limited water availability.

Figure 2.1.D with an R-value of 0.858 indicates a strong positive correlation between the two groups and there is a strong linear association between the two groups being compared. The scatter plot shows that as the values of one group increase, the values of the other group also tend to increase, following a relatively linear pattern. The strong positive

correlation implies that there may be a consistent relationship between the water-restricted beef cattle group and the control beef cattle group in terms of the variable being measured or compared. The similarity in the trends between the two groups suggests that water restriction has influenced the measured variable in a comparable manner for both groups of cattle.

k-Means

In Table 2.3, upregulated genes in cluster D are strongly enriched in several pathways, including Class A/1 Rhodopsin-like receptors (FDR $P < 2.66 \times 10^{-4}$), GPCR ligand binding (FDR $P < 2.66 \times 10^{-4}$), peptide ligand-binding receptors (FDR $P < 5.88 \times 10^{-4}$), GPCR downstream signaling (FDR $P < 5.88 \times 10^{-4}$), signaling by GPCR (FDR $P < 5.90 \times 10^{-4}$), and G alpha S signaling events (FDR $P < 9.19 \times 10^{-4}$). These pathways are significantly associated with upregulated genes in cluster D, indicating their potential involvement in response to water restriction. On the other hand, cluster A shows higher upregulation in the control group compared to the water-restricted group. In this cluster, the control group is overrepresented with genes involved in hormone ligand-binding receptors (FDR $P < 4.58 \times 10^{-8}$) and peptide hormone metabolism (FDR $P < 2.34 \times 10^{-7}$) pathways. Notably, AVP is not directly implicated in these enriched pathways in cluster A.

These findings suggest that water restriction may lead to specific changes in gene expression patterns associated with different pathways. The upregulated genes in cluster D, enriched in GPCR-related pathways, may play a role in the response to water restriction, while the upregulated genes in cluster A, associated with hormone ligand-binding receptors and peptide hormone metabolism, were down regulated in response to water restriction. Further analysis and experimental validation would be needed to fully understand the

functional significance of these pathways and their relationship to AVP and water regulation.

In the KEGG pathways analysis, cluster A genes in the water-restricted group were significantly enriched in the neuroactive ligand-receptor interaction pathway with a high level of significance (FDR $P < 1.22 \times 10^{-12}$; Table 2.4). Water restriction may affect the expression of genes involved in this pathway, which is associated with the interaction between neuroactive ligands (such as neurotransmitters and hormones) and their corresponding receptors. Interestingly, the neuroactive ligand-receptor interaction pathway is also enriched in cluster D, but with a lower level of significance (FDR $P < 1.33 \times 10^{-5}$). In cluster D, AVP is strongly enriched, indicating its potential role in this pathway. The AVP is known to be involved in neuroactive signaling and can act as a neuroactive ligand, binding to specific receptors.

The differential enrichment of the neuroactive ligand-receptor interaction pathway in both cluster A and cluster D suggests that water restriction may influence expression of genes associated with neuroactive signaling and receptor interactions. While cluster A may involve a broader range of genes related to this pathway, cluster D, with the strong enrichment of AVP, may have a more specific focus on AVP-mediated neuroactive interactions. These findings highlight the potential involvement of neuroactive signaling pathways, including the role of AVP, in response to water restriction and provide valuable insights into the molecular mechanisms underlying the physiological adaptation to water restriction. Further investigation and functional studies are necessary to fully understand the specific contributions of these pathways and their relevance to water homeostasis regulation.

Principal Component Analysis (PCA)

In Figure 2.2, the PCA plot of differentially expressed genes (DEGs) for the treatment (water-restricted) and control groups is presented. The plot visualizes variation among samples based on their gene expression profiles. Each point represents a sample, and their positions in the plot reflect similarities or differences in gene expression patterns. Samples displaying similar expression profiles tend to group together, forming clusters. In this case, two control samples and one treatment sample have been grouped closely, while the remaining treatment sample exhibits notable variability compared to the other three samples. This dissimilarity is evident when examining the first component of a plot that explains a large portion (82%) of the overall variance in gene expression data.

Differentially expressed genes (DEGs)

The analysis of differentially expressed genes (DEGs) identified a total of 56 upregulated genes and 1 downregulated gene in response to water restriction. These DEGs were selected based on a threshold of false discovery rate (FDR) < 0.1 and a fold change greater than 2. The FDR is a statistical measure that helps control for multiple hypothesis testing and provides an estimation of the proportion of false positives among the significant results (Benjamini & Hochberg 1995).

Figure 2.3 displays the heatmap of genes that were consistently up-regulated or down-regulated between control and treatment group, respectively in response to water deprivation. resulting from Differentially Expressed Genes analysis. This heatmap shows discerning patterns and relationships among multiple variables. By visually representing data through color-coded cells, it allows for the intuitive interpretation of gene expression

dynamics. The hue of each cell conveys both the intensity and direction of gene expression. Cells with a darker shade indicate heightened expression strength, while the color spectrum indicates the nature of the expression change. The ability to visually distinguish between strong and weak gene expressions, as well as the direction of change, empowers researchers to discern important trends at a glance. This aids in generating hypotheses, guiding further investigations, and making informed decisions in fields such as molecular biology, genetics, and medical research.

As show in Figure 2.4, all Curated Reactome enriched pathways with the differentially expressed genes (DEGs) for control and water restricted groups in response to water restriction are related to the arginine vasopressin (AVP) gene (Table 2.2). This finding suggests that water restriction may have a significant impact on expression of genes associated with AVP and its associated pathways, highlighting the potential role of AVP in the response to water deprivation. The terms listed in Table 2.5 indicate the biological processes or pathways that are overrepresented among upregulated genes associated with AVP. The enriched terms include Class A/1 Rhodopsin-like receptors, peptide ligand-binding receptors, G-protein coupled receptor (GPCR) ligand binding, GPCR downstream signaling, and signaling by GPCR. These findings suggest that water restriction induced changes in expression of genes related to receptor signaling pathways, particularly those involving GPCRs.

The volcano plot (Figure 2.5 A) and the MA plot (Figure 2.5.B), provide visual representations of the transcriptomic response to water restriction. The volcano plot depicts fold change (log2) of gene expression on the x-axis and statistical significance (represented as -log10 FDR p-value) on the y-axis. In the volcano plot, the upregulated genes are located

on the right side of the plot with positive fold change values, indicating a higher expression level in the water-restricted group compared to the control group. The downregulated genes are positioned on the left side with a negative fold change value, indicating lower expression in the water-restricted group. The MA plot, on the other hand, displays the log-intensity ratios (M-values) on the y-axis, which represent the log2 fold change of gene expression, and the log-intensity averages (A-values) on the x-axis, which represent average expression levels. This plot visualizes the distribution and patterns of gene expression changes between the two groups.

Pathway analysis

The gene ontology (GO) analysis provides insights into functional categories and biological processes that are enriched among differentially expressed genes (DEGs) in response to water restriction. In the present study, several GO terms were found to be related and showed significant enrichment. Among the enriched GO terms, functional categories related to GPCR signaling, such as GPCR downstream signaling and GPCR ligand binding are included. These terms indicate involvement of G protein-coupled receptors (GPCRs) and their downstream signaling pathways in response to water restriction. The GPCRs are known to play crucial roles in cellular signaling and are involved in a wide range of physiological processes. Other enriched GO terms include extracellular matrix organization, which suggests that water restriction may impact regulation and remodeling of the extracellular matrix. This process is important for maintaining tissue structure and function.

In terms of biological processes, water restriction increased gene expression related to neuron development, neurogenesis, and response to external stimulus. These processes

indicate a potential neuronal response and adaptation to the water restriction condition. At the cellular component level, upregulated genes were associated with the extracellular region, extracellular space, distal axon, and axon. Water restriction may therefore affect cellular components involved in intercellular communication, neuronal function, and connectivity.

No significant enrichment was found for the molecular component GO terms, indicating that changes in gene expression may not be specifically associated with molecular functions in this context. In addition to the GO analysis, the KEGG pathway analysis revealed that neuroactive ligand-receptor interaction was the only significantly enriched pathway. The interaction between neuroactive ligands and their receptors may play a role in response to water restriction. Overall, these findings highlight the molecular processes and pathways that are influenced by water restriction and provide insights into the biological mechanisms underlying the response to this condition.

The pathway analysis using the Generally Applicable Gene Set Enrichment (GAGE) method further supports and reinforces the findings from the enrichment analysis based on differentially expressed genes (DEGs) and gene ontology (GO) terms. The GAGE analysis considers fold-change values of all genes and identifies coherently altered pathways. The coherently altered pathways are multiple interconnected biological pathways that have been modified in a synchronized manner, potentially due to a common factor, such as a disease, treatment, or environmental stimulus. The treatment group was compared to the control group using the curated reactome (http://curator.reactome.org) gene sets for the analysis. The results from GAGE analysis agree with enrichment analysis based on DEGs and GO terms. The pathways that showed enrichment, such as extracellular matrix

organization, GPCR downstream signaling, GPCR ligand binding, and signaling by GPCR, were consistent with previous findings. The hierarchical clustering tree, which measures the distance between terms based on the percentage of overlapped genes, provides a visual representation of relationships and similarities among the enriched pathways. This clustering analysis identifies clusters based on similarity of their gene composition (Figure 2.6). At the top of the tree, the root node representing the main category or overarching pathway, which GPCR in response to water restriction response. The network of GO terms (Figure 2.7) further illustrates connections and associations between enriched pathways. This network-based visualization provides a comprehensive view of the relationships and functional associations among the pathways affected by water restriction.

DISCUSSION

Summary

The RNA-seq analysis conducted on the anterior pituitary of beef cattle subjected to water restriction provides valuable insights into the molecular mechanisms involved in regulating water balance. The study identified a subset of genes that were differentially expressed between control and water-restricted animals, with 57 genes showing statistically significant differential expression at a false discovery rate of 0.1% and a fold change of ≥2. The subsequent GOseq/KEGG pathway analysis revealed an intriguing association between the differentially expressed genes and G protein-coupled receptor incorporated hormone secretion and transport. Notably, these pathways were all related to arginine vasopressin (AVP), a critical regulator of water balance. This finding suggests that the changes observed in gene expression in the anterior pituitary were specifically involved in the release of AVP into circulation and its transportation to the kidney, where it binds to the vasopressin type 2 receptor.

These results highlight the pivotal role of AVP in responding to water restriction and maintaining plasma osmolarity. AVP, also known as antidiuretic hormone (ADH), plays a crucial role in regulating water reabsorption in the kidney. By increasing water reabsorption, AVP helps conserve water and prevent excessive loss of fluids during periods of limited water availability. The identification of AVP-related pathways as the major pathways affected by water restriction underscores the importance of AVP in mediating the physiological response to changes in water balance. This study provides valuable insights into the molecular mechanisms underlying the regulation of plasma osmolarity in response to water restriction in beef cattle. Further research in this area could deepen our

understanding of water balance regulation and potentially lead to the development of novel strategies to improve water management and animal welfare in agricultural settings.

Principal Component Analysis

Based on the PCA plot of differentially expressed genes (DEGs), the observed distinction in the gene expression profile of the particular samples may indicate that water restriction has had a significant effect on its gene expression patterns compared to the control samples. This finding suggests that water restriction may induce distinct molecular responses within the affected individual, potentially influencing various biological processes and pathways related to water balance and homeostasis. The presence of this distinct sample highlights the importance of considering individual variation in response to water restriction. It suggests that while some animals may exhibit similar gene expression patterns under water-restricted conditions, others may demonstrate unique responses. Factors such as genetic variation, physiological differences, and environmental interactions may contribute to this individual variability.

Understanding the specific genes and pathways associated with this distinct gene expression profile in the water-restricted sample can provide insights into the molecular mechanisms underlying the response to water restriction in beef cattle. Further analysis and characterization of these differentially expressed genes could elucidate the biological pathways affected by water restriction and potentially uncover novel regulatory mechanisms involved in maintaining water balance and coping with limited water availability. It is important to note that this finding is based on the analysis of gene expression data and does not directly indicate the physiological or functional consequences

of the observed gene expression differences. Additional investigations, such as functional validation studies, are necessary to determine the implications of these gene expression variations on the animal's response to water restriction, its overall health, and productivity. The presence of a distinct gene expression profile in one of the water-restricted samples highlights the heterogeneity of individual responses to water restriction in beef cattle. Further exploration of the differentially expressed genes and associated pathways can contribute to a more comprehensive understanding of the molecular mechanisms underlying the effects of water restriction on livestock and potentially inform management practices aimed at optimizing water use and animal welfare under limited water availability conditions.

Our study focused on the differential gene expression in the bovine anterior pituitary gland under conditions of water restriction using RNA-seq. The main finding is that water deprivation has a positive effect on neuroactive ligand-receptor interaction, particularly involving AVP and GPCR receptors. Genes associated with ion transportation and neurotransmitter activity in the presence of GPCRs were also found to be differentially expressed following water restriction. The upregulation of AVP is particularly relevant as this protein is known to play a role in regulating both ion balance and water reabsorption in the body. Other studies have shown that molecular knockdown of AVP expression can impact ionocyte-related genes and reduce H+ secretion, although levels of Na+ and Ca^{2}+ remain unaffected. Furthermore, an osmoregulatory system in the neurohypophysis, involving pituicytes, taurine, and neuronal glycine receptors, controls calcium influx and neurohormone release from nerve terminals. These findings highlight the intricate molecular mechanisms involved in water regulation and osmoregulation in the body.

Overall, our study provides insights into the gene expression changes that occur in the anterior pituitary gland during water restriction, shedding light on the molecular processes associated with water regulation and signaling through neuroactive ligand-receptor interactions.

Role of Paired box gene 8

The *Pax-8* gene plays a crucial role in the development of renal and thyroid organs (Bouchard *et al*. 2002). *Pax-8* is involved in regulating the formation and differentiation of these organs during embryogenesis. *Pax-8* is often co-expressed with another member of its gene family, *Pax-2*, and their expression patterns help determine the lineage of nephric cells. In the adult kidney, *Pax-8* has been detected in the renal epithelial cells of renal tubules and Bowman's capsule, indicating its ongoing role in renal function and maintenance (Tong *et al.* 2009).

Previous studies have demonstrated that the expression of *Pax-2* and *Pax-8* can be influenced by factors such as high sodium chloride (NaCl) concentration. Cai *et al*. (2005) showed that increased NaCl concentration in cultured inner medullary cells led to an increase in mRNA levels of *Pax-2* and *Pax-8.* Furthermore, *in vivo* studies have indicated that the expression of *Pax-8* mRNA is significantly increased following water restriction. This finding suggests that water deprivation may contribute to the upregulation of *Pax-8* expression in the kidney. In this study, we observed a significant increase (2.63-fold) in *Pax-8* expression following water restriction in cattle. This finding aligns with previous research and suggests that water deprivation can influence the expression of *Pax-8,* potentially impacting renal function and adaptation to osmotic challenges (Cai *et al*. 2005).

The upregulation of *Pax-8* in response to water restriction highlights its involvement in renal adaptation and suggests its role in maintaining water homeostasis in cattle.

Proprotein Convertase Subtilisin/Kexin Type 2

The increase in *Proprotein Convertase Subtilisin/Kexin Type 2 (PCSK2)* mRNA abundance (FDR $P < 2.59 \times 10^{-6}$) following water restriction aligns with previous research findings in dehydrated rats and supports the role of *PCSK2* in the regulation of water homeostasis (Greenwood *et al*. 2014). The *PCSK2* gene belongs to the subtilisin-like proprotein convertase family, which is involved in the processing of protein and peptide precursors in secretory pathways. During intracellular transport, *PCSK2* undergoes autocatalytic processing and is activated. This protein interacts with neuroendocrine secretory proteins outside the endoplasmic reticulum (ER) and then enters secretory granules where *PCSK2* is further activated. This enzyme is responsible for proteolytic activation of polypeptide hormones and neuropeptide precursors.

The activation of pituitary hormones involved in regulating water homeostasis may be influenced by *PCSK2*, suggesting that *PCSK2* plays a role in processing and activation of these hormones in response to water deprivation. In terms of functional partners, Synaptotagmin-4 (SYT4) and Synaptotagmin-11 (SYT11) are predicted to interact with *PCSK2*. These proteins are involved in Ca^{2+}-dependent exocytosis of secretory vesicles, acting as Ca^{2+} sensors in vesicular trafficking and exocytosis processes. Their interaction with *PCSK2* may contribute to the regulated release of processed hormones. Regarding other members of the proprotein convertase family, PC1/3 and PC2 have been identified in non-neoplastic pituitary glands, particularly in corticotrophs, gonadotrophs, and thyrotrophs. In pituitary adenomas, adrenocorticotropic hormone (ACTH) has shown a

high prevalence of PC1/3 and PC2. This correlation suggests the involvement of *PCSK2* and related proprotein convertases in the processing and activation of pituitary hormones, including those related to water regulation. In general, the increased abundance of *PCSK2* mRNA following water restriction suggests its role in activation of pituitary hormones involved in water homeostasis. The potential interaction with Synaptotagmin proteins and association with other proprotein convertases further highlights the complex regulatory mechanisms involved in hormone processing and secretion in response to water deprivation.

Pro-opiomelanocortin (POMC) hormone precursor

In pituitary corticotrope tumor (AtT20) cells, pro-opiomelanocortin (POMC) hormone precursor is highly expressed and undergoes proteolytic cleavage to generate several biologically active peptides. These cells are derived from anterior pituitary corticotroph cells and serve as a well-characterized secretory cell line (Cawley *et al*. 2016). During dehydration, approximately 15-20% of rat corticotroph cells in the anterior lobe of the pituitary synthesize POMC. Proprotein convertase 1/3 (PC1/3), primarily found in neural and endocrine tissues, is responsible for processing neuropeptides and peptide hormones, including AVP, POMC, proinsulin, and proglucagon (Dong *et al*. 1997). The PC1/3 protein was highly expressed in pituitary corticotroph cells and plays a crucial role in coordinating the processing of POMC to produce adrenocorticotropic hormone (ACTH), which is an essential component of the stress response mediated by the hypothalamic-pituitary-adrenal (HPA) axis (Day *et al*. 1992; Zhou *et al*. 1993).

In response to stress, corticotropin-releasing hormone (CRH) is released from the axon terminals of parvocellular neurons in the paraventricular nucleus (PVN) of the

hypothalamus. This release stimulates ACTH release from the anterior pituitary, which in turn leads to the release of corticosterone from the adrenal cortex (Swanson *et al*. 1993). Dehydration initially results in an increase in plasma corticosterone concentrations (Windle *et al*. 1993). However, studies have shown a dissociation between responses of pituitary and adrenal glands to acute stress, indicating a decrease in adrenal sensitivity to ACTH in salt-loaded rats (Grinevich *et al*. 2001). Dehydration and salt loading have been demonstrated to affect plasma ACTH secretion (Grinevich *et al*. 2001; Amaya *et al*. 2001). Interestingly, although expression of POMC mRNA in the pituitary remains unchanged during dehydration (Aguilera *et al*., 1993), reduced PC1/3 expression, as shown by Greenwood *et al*. (2020), is responsible for attenuated ACTH secretion. This result suggests that decreased expression of PC1/3 in the anterior pituitary can alter the availability of ACTH and subsequently impact stress response in dehydrated animals (Pan *et al*. 2005). Studies have also demonstrated that exogenously expressed POMC can be fully processed into ACTH-related peptides in rat pituitary GH3 cells, which only express PC2 (Friedman *et al*. 1996). However, in the absence of PC1/3, ACTH is not detected in the pituitary. This finding indicates the importance of PC1/3 in the processing and availability of ACTH. Overall, the expression and activity of PC1/3 in pituitary corticotroph cells are crucial for the proper processing of POMC and the subsequent release of ACTH. Changes in PC1/3 expression during dehydration can affect the availability of ACTH and impact the stress response mediated by the hypothalamic–pituitary–adrenal (HPA) axis.

Biological pathways

Integration of the Anterior Pituitary, AVP, and Kidney

The hypothalamic-neurohypophysial system (HNS) plays a vital role in maintaining osmotic stability and responding to osmotic challenges in mammals (Antunes-Rodrigues *et al*. 2004). The HNS consists of magnocellular neurons (MCNs) located in the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of the hypothalamus (Brownstein *et al*., 1980). These MCNs extend their axons through the internal zone of the median eminence to terminate in the pituitary gland, where they release neuropeptides into the bloodstream. One of the key neuropeptides produced and secreted by the HNS is arginine vasopressin (AVP), also known as antidiuretic hormone. AVP is synthesized as a prepropeptide precursor in the cell bodies of PVN and SON MCNs (Brownstein *et al*. 1980). During anterograde axonal transport, the precursor is processed, and biologically active AVP is stored in the posterior pituitary gland terminals. Changes in MCN electrical activity, triggered by hyperosmolality, stimulate the mobilization and secretion of AVP into circulation (Bourque *et al*. 1994).

The regulation of AVP secretion involves intrinsic osmoreceptor mechanisms within MCNs, as well as inputs from circumventricular osmoreceptive neurons that detect increases in plasma osmolality (Bourque 1999). These osmoreceptive neurons project to the MCNs and provide glutamate receptor-mediated excitatory inputs, shaping the firing activity of magnocellular neuroendocrine cells for hormone secretion (Onaka & Yagi 2001). Once released, AVP travels through the bloodstream to specific receptor targets located in the kidney. In the kidney, AVP acts to decrease the urinary excretion of water by increasing the permeability of the collecting ducts. Increased permeability allows for

the reabsorption of water back into the circulation, leading to water conservation and concentrated urine production (Valenti *et al*. 2005). Therefore, the HNS and its production of AVP play a crucial role in the regulation of water balance and responding to osmotic challenges, ensuring the maintenance of osmotic stability in the body.

The hypothalamic-neurohypophysial system exhibits function-related plasticity in response to hyperosmotic cues, particularly dehydration (Hatton 1997). This plasticity involves reversible changes in various aspects of the system, including morphology, electrical properties, biosynthesis, and secretory activity (Tasker & Boudaba 2002; Wakerley *et al*. 1978). The plasticity of the HNS is a complex and dynamic process that is governed by a combination of intrinsic properties of the magnocellular neurons (MCNs), interactions among MCNs, interactions with glial cells, and extrinsic synaptic inputs. These factors contribute to the remodeling of the HNS in response to dehydration, allowing for enhanced hormone production and delivery (Tasker & Boudaba 2002). While it is possible to identify the processes through which changes in gene expression contribute to neuronal plasticity in the HNS, the specific molecular mechanisms underlying these changes are still largely unknown. Further research is needed to unravel the intricate molecular pathways and signaling mechanisms that drive the plasticity of the HNS in response to osmotic challenges. Understanding the molecular basis of HNS plasticity would provide valuable insights into the adaptive responses of the neuroendocrine system to maintain osmotic stability and regulate water balance in the body.

Maintaining body water homeostasis is crucial, and water homeostasis involves balancing renal and nonrenal water losses with water intake (Weitzman 1980). The thirst, which is primarily triggered by increased osmolality of body fluids, is sensed by anteroventral

hypothalamic osmoreceptors. Additionally, hypovolemia (low blood volume) can also influence thirst through arterial baroreceptors and the renin-angiotensin system. Water deprivation leads to increased plasma sodium concentration, urine osmolality, and sodium excretion, while simultaneously decreasing plasma volume and urine production (Goumi *et al.* 1993). Various proteins, such as the EnAC complex and Na+/K+ ATP enzymes, are involved in facilitating sodium absorption in renal tubules. Hormones like renin, angiotensin, aldosterone, and vasopressin play important roles in regulating sodium absorption (Geerling & Loewy 2008).

Activation of the Arginine Vasopressin (AVP) Pathway

The AVP (antidiuretic hormone) is responsible for regulating renal water loss. It is synthesized in the supraoptic and paraventricular nuclei of the hypothalamus and transported via axons to the posterior pituitary gland. When neurosecretory neurons depolarize, AVP and its carrier protein (neurophysin) are released into the bloodstream through exocytosis of granules. During dehydration, the synthesis of AVP increases to meet the additional demand for this hormone. The increased demand for AVP synthesis and secretion during dehydration requires changes in cellular components necessary for processing and secretion. These changes in cell components enable the proper synthesis, packaging, and release of AVP into the bloodstream to regulate water balance and maintain body water homeostasis.

In our RNA-seq study, we found that the AVP gene was the most significantly differentially expressed gene, showing up-regulation in water-restricted animals. The AVP gene was featured in two KEGG pathways, namely the neuroactive ligand-receptor interaction pathway and the calcium signaling pathway, with both pathways showing a fold

change of at least 2 (FDR $P < 3.2 \times 10^{-3}$ and FDR $P < 4.9 \times 10^{-2}$, respectively). Additionally, AVP was involved in six out of eight curated reactome pathways, including Class A/1 rhodopsin-like receptors, peptide ligand-binding receptors, GPCR ligand binding, Transport of organic anions, GPCR downstream signaling, and signaling by GPCR. All these pathways showed significant associations with AVP (FDR $P < 1.10 \times 10^{-4}$, 1.10×10^{-4}, 1.20×10^{-4}, 1.38×10^{-4}, 2.04×10^{-4}, 3.23×10^{-3}, 7.71×10^{-3}, and 7.73×10^{-3}, respectively). These findings highlight the central role of AVP in mediating neuroactive ligand-receptor interactions and calcium signaling pathways. A G protein-coupled receptor ligand, AVP is involved in various signaling cascades and downstream processes. The pathways associated with AVP provide insights into the molecular mechanisms underlying AVP's and the thirst response effects on hormone secretion, receptor binding, organic anion transport, and GPCR-mediated signaling.

In our RNA-seq study, one KEGG pathway and eight curated reactomes pathways showed significant differential expression of genes (≥2-fold change) related to G protein-coupled receptor associated hormone secretion and transport (Figure 2.4). These pathways are associated with release of AVP into circulation and its transportation to the kidney, where AVP binds to the vasopressin type 2 receptor and leads to the expression of aquaporin 2 channels. The findings suggest that during water deprivation in cattle, major changes in gene expression in the anterior pituitary gland are involved in regulating plasma osmolarity. These gene expression changes are associated with the secretion of AVP, its binding to receptors in the kidney, and subsequent expression of aquaporin 2 channels. Aquaporin 2 channels play a crucial role in water reabsorption in the kidney, facilitating the concentration of urine and reducing water loss. These results highlight the molecular

mechanisms involved in regulation of water balance and osmotic stability in response to water restriction, particularly focusing on the role of AVP and its downstream signaling pathways. By understanding gene expression changes associated with these processes, further insights can be gained into the molecular mechanisms underlying water homeostasis in mammals, specifically in cattle.

The significant up-regulation of AVP in water-restricted animals suggests its crucial role in response to osmotic challenges and the regulation of water balance. The involvement of AVP in multiple pathways further emphasizes its importance in coordinating physiological responses related to GPCR signaling and hormone secretion. The statistical significance of the differentially expressed AVP gene and its associations with pathways are based on the provided FDR-adjusted P-values. The AVP gene encodes a preproprotein that undergoes proteolytic processing to produce multiple peptides, including neurophysin 2, copeptin, and arginine vasopressin. Arginine vasopressin is a neuropeptide hormone belonging to the vasopressin/oxytocin family. The AVP is synthesized in the supraoptic nucleus and paraventricular nucleus of the hypothalamus and functions as a posterior pituitary hormone (Dutertre *et al*. 2008). After synthesis, AVP is transported axonally to the nerve endings in the neurohypophysis (posterior pituitary) and is either stored or secreted into the bloodstream along with its carrier protein, neurophysin 2 (Haley & Flynn, 2006). During its transport to the posterior pituitary, the precursor form of AVP is believed to be activated (Mitra 2021).

In the renal medulla, AVP interacts with G protein-coupled receptors expressed in the nephrons and microvasculature, specifically in the vasa recta (Bankir 2001). The primary function of AVP in the kidney is to stimulate osmotically driven water reabsorption,

leading to increased urine concentration, both in the cortex and medulla. Studies by Cai *et al*. (2006) demonstrated that water restriction in mice resulted in significant increases in urine osmolarity and the abundance of aquaporin 2 (AQP2) mRNA and protein after 48 hours. While the role of vasopressin in regulating water absorption is well-established, its influence on renal sodium absorption is less clear. Recent research suggests that AVP may have both antinatriuretic and natriuretic effects. Activation of the vasopressin receptor 1A (V1aR) is associated with natriuresis, whereas vasopressin receptor 2 (V2R) activation contributes to antinatriuresis (Bankir *et al*. 2005). The precise mechanisms by which AVP regulates sodium absorption in the kidney are still being elucidated. In summary, AVP, derived from the AVP gene, plays a vital role in regulating water balance and urine concentration. This protein functions as a neuropeptide hormone synthesized in the hypothalamus and subsequently transported to the posterior pituitary. In the kidney, AVP interacts with specific receptors, contributing to water reabsorption and influencing sodium absorption, although the exact mechanisms involved are still under investigation.

In our study, neuroactive ligand-receptor interaction KEGG pathway is up regulated (Table 2.6). Specifically, angiotensinogen (AGT) was found to be upregulated in this pathway. Angiotensinogen is a gene that encodes a protein known as pre-angiotensinogen or angiotensinogen precursor. This protein is primarily expressed in the liver and undergoes cleavage by the enzyme renin in response to low blood pressure. The cleaved product, angiotensin I, is further processed by the angiotensin-converting enzyme (ACE) to generate angiotensin II, which is a physiologically active molecule. Angiotensin II plays a crucial role in maintaining body fluid and electrolyte balance, as well as regulating blood pressure. The upregulation of AGT is likely a response to the depletion of water in both intracellular

and extracellular compartments (Hayashi *et al*. 2007). The depletion of fluid compartments triggers various compensatory responses within the body. These responses include the secretion of vasopressin (also known as antidiuretic hormone), activation of the renin-angiotensin-aldosterone system, stimulation of the sympathetic neuron system, and reduced excretion of solutes and water by the kidneys. These compensatory mechanisms aim to minimize changes in body fluid volume and composition. In general, upregulation of AGT and the associated activation of the renin-angiotensin-aldosterone system and other compensatory responses reflect the body's effort to maintain fluid and electrolyte balance in response to water depletion. Thirst in mammals is typically stimulated by an increase in plasma osmolality, which refers to the concentration of solutes in the blood (usually in the range of 280-295 Osm/kg H_2O). This increase in osmolality is primarily caused by the accumulation of solutes such as NaCl that do not readily pass across cell membranes (Zerbe & Robertson 1983). The main stimulant for thirst is peptide angiotensin II (ANG II), which is secreted by the kidneys in response to sodium deficiency (Mendelsohn *et al*. 1988).

Angiotensin II has multiple effects on the body. Angiotensin II increases blood pressure stimulates the release of aldosterone (a hormone that promotes sodium reabsorption) and vasopressin (also known as antidiuretic hormone) and increases water intake. It also has effects on anterior pituitary hormones, which are hormones released by the pituitary gland located at the base of the brain. Angiotensin II receptors are found on lactotropes (cells that produce prolactin) and corticotropes (cells that produce adrenocorticotropic hormone) in rats, as well as on thyrotropes (cells that produce thyroid-stimulating hormone) and other secretory cells (Ganong 1993). In addition to angiotensin II produced locally by the pituitary renin-angiotensin system, circulating angiotensin II can reach these receptors.

Furthermore, there are indirect effects produced by the effects of brain angiotensin II on the secretion of hypophyseotropic hormones, which are hormones that regulate the release of pituitary hormones (Deschepper 1991).

Signaling by G protein-coupled receptor

Signaling by GPCR (G protein-coupled receptor) was identified as the second most upregulated pathway associated with AVP in our study. The GPCRs share a common membrane-spanning helical architecture connected by intracellular and extracellular loops. The structural stability of GPCRs is maintained by disulfide bonds formed by two cysteine residues. The GPCRs have a broad range of ligand recognition capabilities, including light, odors, small molecules, hormones, and neurotransmitters (Jacob *et al.* 2006). Upon ligand binding, GPCRs function as guanine nucleotide exchange factors, promoting the exchange of GDP for GTP on associated heterotrimeric G proteins. This activation of G proteins leads to the initiation of downstream signaling cascades. The physiological functions of GPCRs are diverse and encompass various biological processes. They are involved in visual perception, smell, regulation of behavior, autonomic nervous system functions, as well as immune system modulation and inflammation regulation (Bockaert & Pin 1999). Given their broad ligand recognition and signaling capabilities, GPCRs play crucial roles in mediating cellular responses to environmental cues and maintaining overall physiological homeostasis.

In our study, we observed that pro-opiomelanocortin (POMC), which is a component of the KEGG Class A/1 rhodopsin-like receptors pathway, was upregulated in water-restricted cattle. The upregulation of POMC suggests its involvement in the regulatory response to

water restriction. POMC is a precursor protein that is processed into several biologically active peptides through proteolytic cleavage. These peptides include adrenocorticotropic hormone (ACTH), melanocyte-stimulating hormones (MSHs), and endorphins, among others. The processing of POMC occurs in specialized secretory cells. AtT20 cells are a well-characterized cell line derived from the corticotroph cells of the anterior pituitary. These cells express high levels of POMC and are commonly used in research to study the synthesis and secretion of POMC-derived peptides, particularly ACTH. The upregulation of POMC in water-restricted cattle suggests its involvement in the adaptive response to water deprivation, possibly through the production and release of ACTH and other POMC-derived peptides. These peptides play crucial roles in various physiological processes, including stress response, immune regulation, and melanin synthesis.

In our study, the TACR3 gene, which encodes the neuromedin-K receptor (NK3R), was upregulated in the pituitary gland following water restriction in cattle. The TACR3 gene is a member of the KEGG Class A/1 rhodopsin-like receptors pathway and is involved in the signaling of neuromedin-K, a tachykinin neuropeptide. The neuromedin-K receptor (NK3R) is a G-protein-coupled receptor that is heavily expressed in magnocellular neurons of the supraoptic (SON) and paraventricular nucleus (PVN) of the hypothalamus. Activation of NK3R leads to the activation of phosphatidylinositol-calcium second messengers through G proteins. In the hypothalamus, NK3R expressed by magnocellular neurons plays a role in the release of vasopressin (AVP) and oxytocin. Stimulation of NK3R increases calcium conductance, which leads to the induction of c-Fos expression in magnocellular neurons and the systemic release of AVP and oxytocin. Under conditions of hyperosmotic or hypotensive challenges, the release of neuromedin-K (NKB) occurs

locally in the hypothalamus, as NK3R is internalized within organelles. This release of NKB and activation of NK3R play a role in modulating the activity of vasopressin-producing (VP) magnocellular neurons. Immunohistochemical studies have shown that NK3R immunoreactivity can be detected in magnocellular VP neurons following the cytoplasmic sequestration of NK3R. This suggests that NK3R activation and NKB signaling are involved in the regulation of vasopressin release. The TACR3, which encodes NK3R, is not limited to the hypothalamus but is widely expressed throughout the brain. This expression pattern suggests that NK3R may have additional roles and functions beyond its involvement in vasopressin and oxytocin release in the pituitary gland.

Study limitations

Despite the valuable insights gained from our research findings, it is essential to recognize and address the limitations inherent in our study. These limitations highlight areas where further research and improvements can enhance the validity and generalizability of our results. Water was not withheld for longer than 12 hours for ethical reasons in the study. Ethical considerations play a crucial role in research involving animals. The welfare and well-being of the animals involved should always be a primary concern. In the case of water restriction studies, prolonged deprivation of water can potentially lead to dehydration, discomfort, and adverse health effects in the animals. It is important to ensure that the duration of water restriction does not cause undue distress or harm to the animals involved. By limiting the water restriction period to a maximum of 12 hours, we aimed to strike a balance between obtaining valuable scientific insights and maintaining the welfare

of the animals. This decision reflects a commitment to ethical research practices that prioritize animal welfare and minimize potential negative impacts on the subjects.

Understanding whether water restriction alone is sufficient to trigger a thirst response in these animals, given their ability to store water in their rumen, is also important. Animals with a large rumen, such as ruminants like cows, sheep, and deer, have evolved unique adaptations to their digestive systems. The rumen is a specialized stomach compartment where microbial fermentation takes place, aiding in the breakdown of complex plant materials. This fermentation produces volatile fatty acids and other byproducts, which can contribute to the animal's water needs. When considering whether water restriction is sufficient to cause a thirst response in such animals, several factors come into play. Animals with large rumens can store a significant amount of water in their rumen due to the fermentation process. This stored water can provide a buffer against short-term water shortages. The fermentation process in the rumen generates water as a byproduct. This metabolic water can contribute to the animal's hydration needs to some extent. Animals have varying water requirements based on factors like their size, activity level, diet, and environmental conditions. While they might have water stored in the rumen, their overall hydration needs may still necessitate additional water intake. Moreover, thirst is regulated by osmoregulation, which involves maintaining the balance of electrolytes and fluid levels in the body. Even if animals have access to stored water in the rumen, their bodies may still detect changes in osmolarity, triggering a thirst response. Therefore, it's important to consider the specific species, their physiological adaptations, and their environmental conditions when assessing the impact of water restriction on thirst response in these animals. Although we have no direct evidence that a thirst response was stimulated, several

genes (e.g., *Arginine Vasopressin*) involved with this thirst response were differentially abundant between the treatment and control group.

Another limitation of this study is only the anterior pituitary tissue was examined. This limitation suggests that the findings and interpretations of the study may be restricted to the molecular changes occurring specifically in the anterior pituitary gland in response to water restriction. By focusing solely on the anterior pituitary tissue, the study may have overlooked potential gene expression changes occurring in the posterior pituitary or other relevant tissues involved in water balance regulation. To obtain a more comprehensive understanding of the molecular response to water restriction, it would be beneficial to investigate gene expression patterns in additional tissues or organs involved in water regulation, such as the hypothalamus, kidney, or other relevant organs. Examining a broader range of tissues could provide a more complete picture of the systemic changes occurring in response to water restriction and enable a more comprehensive analysis of the nutrigenomic response in livestock.

Considering the limitations associated with studying a single tissue, future research could incorporate multiple tissue types and conduct a comprehensive analysis of gene expression changes across different organs involved in water balance regulation. This approach would enhance the understanding of the molecular mechanisms underlying the response to water restriction and provide a more comprehensive assessment of the nutrigenomic effects in cattle.

The observations that we made in our study could be caused by both water deprivation and a change in salt balance in animals. Both water deprivation and alterations in salt balance

can have significant effects on an animal's physiology and behavior. Both water deprivation and changes in salt balance can lead to increased thirst in animals. Dehydration resulting from water deprivation directly triggers a thirst response as the body attempts to restore its fluid balance. Similarly, an imbalance in salt levels (such as excessive salt intake) can lead to increased fluid retention and increased thirst as the body tries to dilute the excess salt. Both water deprivation and shifts in salt balance can result in electrolyte imbalances. Water deprivation can lead to decreased levels of important electrolytes like sodium, potassium, and chloride due to reduced intake and excretion. Changes in salt balance can disrupt the normal distribution of electrolytes in the body. Water deprivation can strain the kidneys as they work to conserve water and maintain blood volume. Changes in salt balance can affect the filtration and reabsorption processes in the kidneys, potentially leading to kidney dysfunction. While both factors can contribute to dehydration, water deprivation is a direct cause of dehydration due to insufficient fluid intake. Changes in salt balance may indirectly contribute to dehydration if they lead to increased fluid loss through urine or other means.

CONCLUSION

Our study on the gene expression of bovine thirst response in response to water restriction has provided insights into the specific molecular mechanisms and pathways involved in regulating water balance and osmoregulation. Transcriptomic analysis of the anterior pituitary gland and in water restricted cattle has revealed changes in gene expression patterns compared to control animals. These changes indicate the activation or suppression of specific genes in response to water restriction. Our study identifies pathways associated with AVP, the hormone involved in water conservation and osmoregulation. Changes in AVP-related genes suggest their importance in responding to water restriction and the coordination the physiological responses to limited water availability. However, genes related to metabolism, stress response, and immune function have shown differential expressions in response to water restriction, indicating potential interconnectedness between these systems. These findings contribute to a deeper understanding of the complex regulatory networks involved in maintaining water balance in cattle and provide potential targets for further investigation and management strategies to optimize water utilization and animal welfare.

LITERATURE CITED

Abete, I., Navas-Carretero, S., Marti, A., & Martinez, J. A. (2012). Nutrigenetics and nutrigenomics of caloric restriction. *Progress in Molecular Biology and Translational Science,* 108, 323-346.

Aguilera, G., Lightman, S., & Kiss, A. (1993). Regulation of the hypothalamic-pituitary-adrenal axis during water deprivation. *Endocrinology*, 132(1), 241-248.

Alfie, M. E., Alim, S., Mehta, D., Shesely, E. G., & Carretero, O. A. (1999). An enhanced effect of arginine vasopressin in bradykinin B2 receptor null mutant mice. *Hypertension*, 33(6), 1436-1440.

Amaya, F., Tanaka, M., Hayashi, S., Tanaka, Y., & Ibata, Y. (2001). Hypothalamo-pituitary-adrenal axis sensitization after chronic salt loading. *Neuroendocrinology*, 73(3), 185-193.

Antunes-Rodrigues, J., De Castro, M., Elias, L. L., Valenca, M. M., & McCann, S. M. (2004). Neuroendocrine control of body fluid metabolism. *Physiological Reviews*, 84(1), 169-208.

Balch, C. C., Balch, D. A., Johnson, V. W., & Turner, J. (1953). Factors affecting the utilization of food by dairy cows. *British Journal of Nutrition*, 7(03), 212-224.

Bankir, L. (2001). Antidiuretic action of vasopressin: quantitative aspects and interaction between V1a and V2 receptor-mediated effects. *Cardiovascular Research*, 51(3), 372-390.

Bankir, L., Bichet, D. G., & Morgenthaler, N. G. (2017). Vasopressin: physiology, assessment and osmosensation. *Journal of Internal Medicine*, 282(4), 284-297.

Bankir, L., Fernandes, S., Bardoux, P., Bouby, N., & Bichet, D. G. (2005). Vasopressin-V2 receptor stimulation reduces sodium excretion in healthy humans. *Journal of the American Society of Nephrology*, 16(7), 1920-1928.

Bealer, S. L., & Flynn, F. W. (2003). Central neurokinin 3 receptors increase systemic oxytocin release: interaction with norepinephrine. *Experimental Neurology*, 184(2), 1027-1033.

Beckett, J. L., & Oltjen, J. W. (1993). Estimation of the water requirement for beef production in the United States. *Journal of Animal Science*, 71(4), 818-826.

Beede, D. K., & Collier, R. J. (1986). Potential nutritional strategies for intensively managed cattle during thermal stress. *Journal of Animal Science*, 62(2), 543-554.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal statistical society: series B (Methodological)*, 57(1), 289-300.

Bionaz, M., Osorio, J., & Loor, J. J. (2015). TRIENNIAL LACTATION SYMPOSIUM: Nutrigenomics in dairy cows: Nutrients, transcription factors, and techniques. *Journal of Animal Science*, 93(12), 5531-5553.

Bockaert, J., & Pin, J. P. (1999). Molecular tinkering of G protein-coupled receptors: an evolutionary success. *The EMBO Journal*, 18(7), 1723-1729.

Bouchard, M., Souabni, A., Mandler, M., Neubüser, A., & Busslinger, M. (2002). Nephric lineage specification by Pax2 and Pax8. *Genes & Development*, 16(22), 2958-2970.

Bourque, C. W. (1999). Osmoregulation of vasopressin neurons: A synergy of intrinsic and synaptic processes. *Progress in Brain Research*, 119, 59-76.

Bourque, C. W., Oliet, S. H., & Richard, D. (1994). Osmoreceptors, osmoreception, and osmoregulation. *Frontiers in Neuroendocrinology*, 15(3), 231-274.

Brauner-Osborne, H., Wellendorph, P., & Jensen, A. A. (2007). Structure, pharmacology and therapeutic prospects of family C G-protein coupled receptors. *Current Drug Targets*, 8(1), 169-184.

Brownstein, M. J., Russell, J. T., & Gainer, H. (1980). Synthesis, transport, and release of posterior pituitary hormones. *Science*, 207(4429), 373-378.

Burgos, M. S., Senn, M., Sutter, F., Kreuzer, M., & Langhans, W. (2001). Effect of water restriction on feeding and metabolism in dairy cows. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 280(2), R418-R427.

Cai, Q., Dmitrieva, N. I., Ferraris, J. D., Brooks, H. L., van Balkom, B. W., & Burg, M. (2005). Pax2 expression occurs in renal medullary epithelial cells in vivo and in cell culture, is osmoregulated, and promotes osmotic tolerance. *Proceedings of the National Academy of Sciences*, 102(2), 503-508.

Cai, Q., Keck, M., McReynolds, M. R., Klein, J. D., Greer, K., Sharma, K., & Brooks, H. L. (2006). Effects of water restriction on gene expression in mouse renal medulla: identification of 3βHSD4 as a collecting duct protein. *American Journal of Physiology-Renal Physiology*, 291(1), F218-F224.

Cai, Q., Keck, M., McReynolds, M. R., Klein, J. D., Greer, K., Sharma, K., & Brooks, H. L. (2006). Effects of water restriction on gene expression in mouse renal medulla: identification of 3βHSD4 as a collecting duct protein. *American Journal of Physiology-Renal Physiology*, 291(1), F218-F224.

Cawley, N. X., Li, Z., & Loh, Y. P. (2016). Biosynthesis, trafficking and secretion of pro-opiomelanocortin-derived peptides. *Journal of Molecular Endocrinology*, 56(4), T77.

Cottrell, G. T., & Ferguson, A. V. (2004). Sensory circumventricular organs: central roles in integrated autonomic regulation. *Regulatory Peptides*, 117(1), 11-23.

Day, R., Schafer, M. K., Watson, S. J., Chretien, M., & Seidah, N. G. (1992). Distribution and regulation of the prohormone convertases PC1 and PC2 in the rat pituitary. *Molecular Endocrinology*, 6(3), 485-497.

Deschepper, C. F. (1991). The renin-angiotensin system in the pituitary gland. *Trends in Endocrinology & Metabolism*, 2(3), 104-107.

Dessauer, C. W., Tesmer, J. J., Sprang, S. R., & Gilman, A. G. (1998). Identification of a Giα binding site on type V adenylyl cyclase. *Journal of Biological Chemistry*, 273(40), 25831-25839.

Ding, Y. Q., Lü, B. Z., Guan, Z. L., Wang, D. S., Xu, J. Q., & Li, J. H. (1999). Neurokinin B receptor (NK3)-containing neurons in the paraventricular and supraoptic nuclei of the rat hypothalamus synthesize vasopressin and express Fos following intravenous injection of hypertonic saline. *Neuroscience*, 91(3), 1077-1085.

Dong, W., Seidel, B., Marcinkiewicz, M., Chretien, M., Seidah, N. G., & Day, R. (1997). Cellular localization of the prohormone convertases in the hypothalamic paraventricular and supraoptic nuclei: selective regulation of PC1 in corticotrophin-releasing hormone parvocellular neurons mediated by glucocorticoids. *Journal of Neuroscience*, 17(2), 563-575.

Dutertre, S., Croker, D., Daly, N. L., Andersson, A., Muttenthaler, M., Lumsden, N. G., & Lewis, R. J. (2008). Conopressin-T from Conus tulipa reveals an antagonist switch in vasopressin-like peptides. *Journal of Biological Chemistry*, 283(11), 7100-7108.

Falkenmark, M., & Widstrand, C. (1992). Population and water resources: a delicate balance. *Population Bulletin*, 47(3), 1-36.

Friedman, T. C., Cool, D. R., Jayasvasti, V., Louie, D., & Loh, Y. P. (1996). Processing of pro-opiomelanocortin in GH3 cells: inhibition by prohormone convertase 2 (PC2) antisense mRNA. *Molecular and Cellular Endocrinology*, 116(1), 89-96.

Ganong, W. F. (1993). Blood, pituitary, and brain renin-angiotensin systems and regulation of secretion of anterior pituitary gland. *Frontiers in neuroendocrinology*, 14(3), 233-249.

Ge, X. (2021). iDEP Web Application for RNA-Seq Data Analysis. In: Picardi, E. (eds) RNA Bioinformatics. *Methods in Molecular Biology*, vol 2284. Humana, New York, NY.

Geerling, J. C., & Loewy, A. D. (2008). Central regulation of sodium appetite. *Experimental Physiology*, 93(2), 177-209.

Goumi, M. B., Riad, F., Giry, J., De La Farge, F., Safwate, A., Davicco, M. J., & Barlet, J. P. (1993). Hormonal control of water and sodium in plasma and urine of camels during dehydration and rehydration. *General and Comparative Endocrinology*, 89(3), 378-386.

Greenwood, M. P., Greenwood, M., Romanova, E. V., Mecawi, A. S., Paterson, A., Sarenac, O., & Murphy, D. (2018). The effects of aging on biosynthetic processes

in the rat hypothalamic osmoregulatory neuroendocrine system. *Neurobiology of Aging*, 65, 178-191.

Greenwood, M., Bordieri, L., Greenwood, M. P., Melo, M. R., Colombari, D. S., Colombari, E., & Murphy, D. (2014). Transcription factor CREB3L1 regulates vasopressin gene expression in the rat hypothalamus. *Journal of Neuroscience*, 34(11), 3810-3820.

Greenwood, M., Paterson, A., Rahman, P. A., Gillard, B. T., Langley, S., Iwasaki, Y., & Greenwood, M. P. (2020). Transcription factor Creb3l1 regulates the synthesis of prohormone convertase enzyme PC1/3 in endocrine cells. *Journal of Neuroendocrinology*, 32(4), e12851.

Grinevich, V., Ma, X. M., Verbalis, J., & Aguilera, G. (2001). Hypothalamic Pituitary Adrenal Axis and Hypothalamic–Neurohypophyseal Responsiveness in Water-Deprived Rats. *Experimental Neurology*, 171(2), 329-341.

Gurevich, V. V., & Gurevich, E. V. (2008). GPCR monomers and oligomers: it takes all kinds. *Trends in Neurosciences*, 31(2), 74-81.

Haley, G. E., & Flynn, F. W. (2006). Agonist and hypertonic saline-induced trafficking of the NK3-receptors on vasopressin neurons within the paraventricular nucleus of the hypothalamus. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 290(5), R1242-R1250.

Harmar, A. J. (2001). Family-B G-protein-coupled receptors. *Genome Biology*, 2(12), 1-10.

Hatton, G. I. (1997). Function-related plasticity in hypothalamus. *Annual Review of Neuroscience*, 20(1), 375-397.

Hatton, G. I., & Walters, J. K. (1973). Induced multiple nucleoli, nucleolar margination, and cell size changes in supraoptic neurons during dehydration and rehydration in the rat. *Brain Research*, 59, 137-154.

Hayashi, Y., Edwards, N. A., Proescholdt, M. A., Oldfield, E. H., & Merrill, M. J. (2007). Regulation and function of aquaporin-1 in glioma cells. *Neoplasia*, 9(9), 777-787.

Houghton, J. T., Ding, Y. D. J. G., Griggs, D. J., Noguer, M., van der Linden, P. J., Dai, X., & Johnson, C. A. (Eds.). (2001). *Climate change 2001: the scientific basis: contribution of Working Group I to the third assessment report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK.

Howe, H. E., Somponpun, S. J., & Sladek, C. D. (2004). Role of neurokinin 3 receptors in supraoptic vasopressin and oxytocin neurons. *Journal of Neuroscience*, 24(45), 10103-10110.

Hussy, N., Bres, V., Rochette, M., Duvoid, A., Alonso, G., Dayanithi, G., & Moos, F. C. (2001). Osmoregulation of vasopressin secretion via activation of neurohypophysial nerve terminals glycine receptors by glial taurine. *Journal of Neuroscience*, 21(18), 7110-7116.

IPCC. (2014). *Climate change 2014 synthesis report. Contribution of working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [R. K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151pp.

Jacoby, E., Bouhelal, R., Gerspacher, M., & Seuwen, K. (2006). The 7 TM G-protein-coupled receptor target family. *ChemMedChem: Chemistry Enabling Drug Discovery*, 1(8), 760-782.

Jastrebski, S. F., Lamont, S. J., & Schmidt, C. J. (2017). Chicken hepatic response to chronic heat stress using integrated transcriptome and metabolome analysis. *PLoS One*, 12(7), e0181900.

Jensen, D., Zhang, Z., & Flynn, F. W. (2008). Trafficking of tachykinin neurokinin 3 receptor to nuclei of neurons in the paraventricular nucleus of the hypothalamus following osmotic challenge. *Neuroscience*, 155(1), 308-316.

Jéquier, E., & Constant, F. (2010). Water as an essential nutrient: the physiological basis of hydration. *European Journal of Clinical Nutrition*, 64(2), 115-123.

Joost, P., & Methner, A. (2002). Phylogenetic analysis of 277 human G-protein-coupled receptors as a tool for the prediction of orphan receptor ligands. *Genome Biology*, 3, 1-16.

Kaput, J., & Rodriguez, R. L. (2004). Nutritional genomics: the next frontier in the postgenomic era. *Physiological Genomics*, 16(2), 166-177.

Kristiansen, K. (2004). Molecular mechanisms of ligand binding, signaling, and regulation within the superfamily of G-protein-coupled receptors: molecular modeling and mutagenesis approaches to receptor structure and function. *Pharmacology & Therapeutics*, 103(1), 21-80

Ladeira, M. M., Schoonmaker, J. P., Gionbelli, M. P., Dias, J. C., Gionbelli, T. R., Carvalho, J. R. R., & Teixeira, P. D. (2016). Nutrigenomics and beef quality: A review about lipogenesis. *International Journal of Molecular Sciences*, 17(6), 918.

Lin, X. W., Blum, I. D., & Storch, K. F. (2015). Clocks within the master gland: hypophyseal rhythms and their physiological significance. *Journal of Biological Rhythms*, 30(4), 263-276.

Mader, T. L., Davis, M. S., & Brown-Brandl, T. (2006). Environmental factors influencing heat stress in feedlot cattle. *Journal of Animal Science*, 84(3), 712-719.

Matteri, R. L., Carroll, J. A., & Dyer, C. J. (2000). Neuroendocrine responses to stress. In The biology of animal stress: basic principles and Implications for animal welfare. (pp. 43-76). Wallingford UK: CABI publishing.

Mendelsohn, F. A., Allen, A. M., Clevers, J., Denton, D. A., Tarjan, E., & McKinley, M. J. (1988). Localization of angiotensin II receptor binding in rabbit brain by in vitro autoradiography. *Journal of Comparative Neurology*, 270(3), 372-384.

Mitra, A. K. (2021). Oxytocin and vasopressin: the social networking buttons of the body. *AIMS Molecular Science*, 8(1), 32-50.

Müller, M., & Kersten, S. (2003). Nutrigenomics: goals and strategies. *Nature Reviews Genetics*, 4(4), 315-322.

National Academies of Sciences, Engineering, and Medicine (2016). Nutrient requirements of beef cattle. 8th rev. ed., Natl. Acad. Press, Washington, DC, USA.

NRC (1981). *Nutrient requirement of small ruminants*. 1st Ed. Natl. Acad. Press, Washington, DC, USA.

Oldham, W. M., & Hamm, H. E. (2008). Heterotrimeric G protein activation by G-protein-coupled receptors. *Nature Reviews Molecular Cell Biology*, 9(1), 60-71.

Onaka, T., & Yagi, K. (2001). Involvement of N-methyl-D-aspartic acid receptor activation in oxytocin and vasopressin release after osmotic stimuli in rats. *Journal of Neuroendocrinology*, 13(2), 166-174.

Pan, H., Nanno, D., Che, F. Y., Zhu, X., Salton, S. R., Steiner, D. F., & Devi, L. A. (2005). Neuropeptide processing profile in mice lacking prohormone convertase 1. *Biochemistry*, 44(12), 4939-4948.

Peter, J., Burbach, H., Adan, R. A., Lolait, S. J., van Leeuwen, F. W., Mezey, E., & Barberis, C. (1995). Molecular neurobiology and pharmacology of the vasopressin/oxytocin receptor family. *Cellular and Molecular Neurobiology*, 15, 573-595.

Rubhana, R., & Cravioto, A. (2009). Nutrition, immunology, and genetics: future perspectives. *Nutrition Reviews*, 67(suppl_2), S227-S236.

Scanlon, B. R., Ruddell, B. L., Reed, P. M., Hook, R. I., Zheng, C., Tidwell, V. C., & Siebert, S. (2017). The food-energy-water nexus: Transforming science for society. *Water Resources Research*, 53(5), 3550-3556.

Smrcka, A. V. (2008). G protein βγ subunits: central mediators of G protein-coupled receptor signaling. *Cellular and Molecular Life Sciences*, 65, 2191-2214.

Soundararajan, M., Willard, F. S., Kimple, A. J., Turnbull, A. P., Ball, L. J., Schoch, G. A., & Siderovski, D. P. (2008). Structural diversity in the RGS domain and its interaction with heterotrimeric G protein α-subunits. *Proceedings of the National Academy of Sciences*, 105(17), 6457-6462.

Stachenfeld, N. S. (2014). Hormonal changes during menopause and the impact on fluid regulation. *Reproductive Sciences*, 21(5), 555-561.

Swanson, L. W., Sawchenko, P. E., Rivier, J., & Vale, W. W. (1983). Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. *Neuroendocrinology*, 36(3), 165-186.

Takumi, I., Steiner, D. F., Sanno, N., Teramoto, A., & Osamura, R. Y. (1998). Localization of prohormone convertases 1/3 and 2 in the human pituitary gland and pituitary adenomas: analysis by immunohistochemistry, immunoelectron microscopy, and laser scanning microscopy. *Modern pathology: an official journal of the United States and Canadian Academy of Pathology, Inc*, 11(3), 232-238.

Tasker, J. G., Di, S., & Boudaba, C. (2002). Functional synaptic plasticity in hypothalamic magnocellular neurons. *Progress in Brain Research*, 139, 113-119.

Tesmer, J. J., Sunahara, R. K., Gilman, A. G., & Sprang, S. R. (1997). Crystal structure of the catalytic domains of adenylyl cyclase in a complex with Gsα· GTPγS. *Science*, 278(5345), 1907-1916.

Thornton, P. K., Herrero, M. T., Freeman, H. A., Okeyo Mwai, A., Rege, J. E. O., Jones, P. G., & McDermott, J. J. (2007). Vulnerability, climate change and livestock opportunities and challenges for the poor. *Journal of Semi-Arid Tropical Agricultural Research*, 4 (1), 1-23.

Tong, G. X., Yu, W. M., Beaubier, N. T., Weeden, E. M., Hamele-Bena, D., Mansukhani, M. M., & O'toole, K. M. (2009). Expression of PAX8 in normal and neoplastic renal tissues: an immunohistochemical study. *Modern Pathology*, 22(9), 1218-1227.

Tong, S. K., Lee, H. L., Lee, Y. C., Wu, L. C., Tsou, Y. L., Lu, S. W., & Chou, M. Y. (2020). Arginine Vasopressin Modulates Ion and Acid/Base Balance by Regulating Cell Numbers of Sodium Chloride Cotransporter and H+-ATPase Rich Ionocytes. *International Journal of Molecular Sciences*, 21(11), 3957.

Torres, V. E., Bankir, L., & Grantham, J. J. (2009). A case for water in the treatment of polycystic kidney disease. *Clinical Journal of the American Society of Nephrology*, 4(6), 1140-1150.

Valenti, G., Procino, G., Tamma, G., Carmosino, M., & Svelto, M. (2005). Minireview: aquaporin 2 trafficking. *Endocrinology*, 146(12), 5063-5070.

Wakerley, J. B., Poulain, D. A., & Brown, D. (1978). Comparison of firing patterns in oxytocin-and vasopressin-releasing neurones during progressive dehydration. *Brain Research*, 148(2), 425-440.

Weitzman, R. E. (1980). The Clinical Physiology of Water Metabolism. *The Western Journal of Medicine*, 132(1), 16.

Winchester, C. F., & Morris, M. J. (1956). Water intake rates of cattle. *Journal of Animal Science*, 15(3), 722-740.

Windle, R. J., Forsling, M. L., Smith, C. P., & Balment, R. J. (1993). Patterns of neurohypophysial hormone release during dehydration in the rat. *Journal of Endocrinology*, 137(2), 311-319.

Zerbe, R. L., & Robertson, G. L. (1983). Osmoregulation of thirst and vasopressin secretion in human subjects: effect of various solutes. *American Journal of Physiology-Endocrinology and Metabolism*, 244(6), E607-E614.

Zhou, A. N., Bloomquist, B. T., & Mains, R. E. (1993). The prohormone convertases PC1 and PC2 mediate distinct endoproteolytic cleavages in a strict temporal order during proopiomelanocortin biosynthetic processing. *Journal of Biological Chemistry*, 268(3), 1763-1769.

Zingg, H. H. (1996). Vasopressin and oxytocin receptors. *Bailliere's Clinical Endocrinology and Metabolism*, 10(1), 75-96.

FIGURES

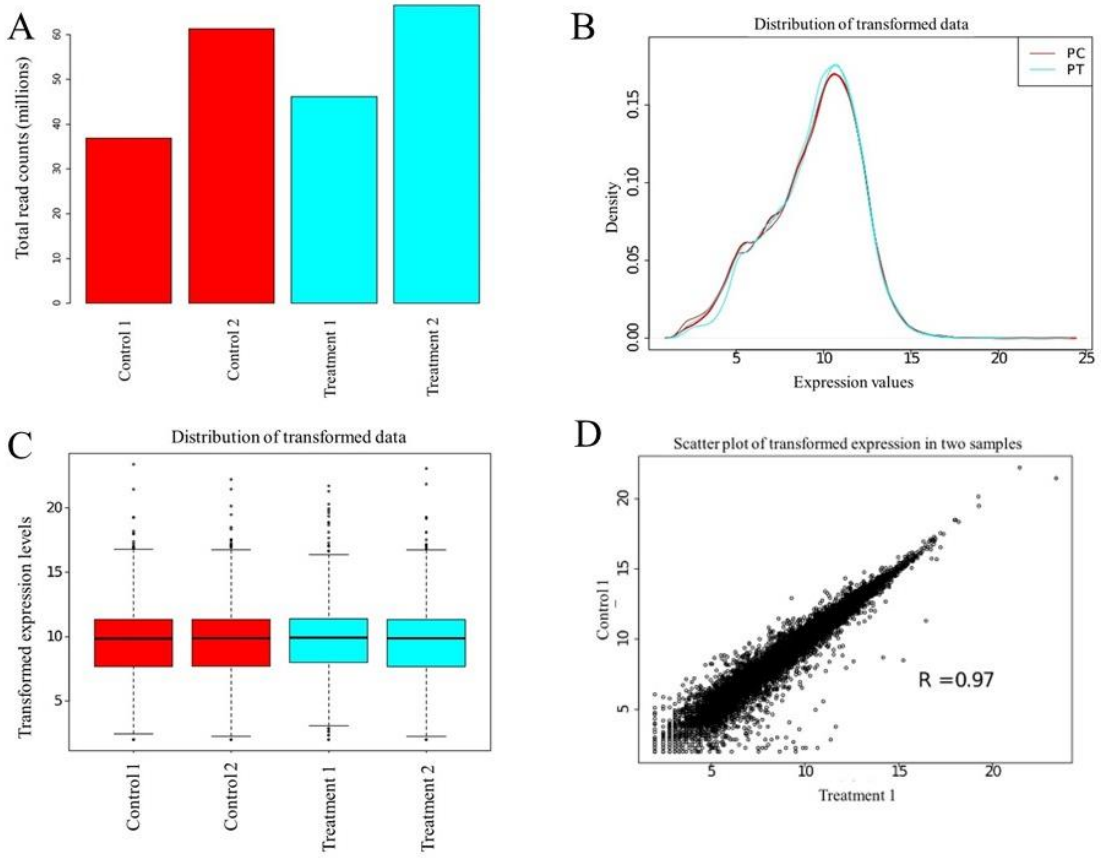

Figure 2. 1 Diagnostic plots for read-count data. (A) Total read counts per library of control and water restricted groups. (B) Distribution of log_2 transformed transcript counts per million reads. (C) Boxplot of log_2 transformed transcript counts per million reads. (D) Scatter plot of log_2 transformed transcript counts per million reads for control and water restricted groups.

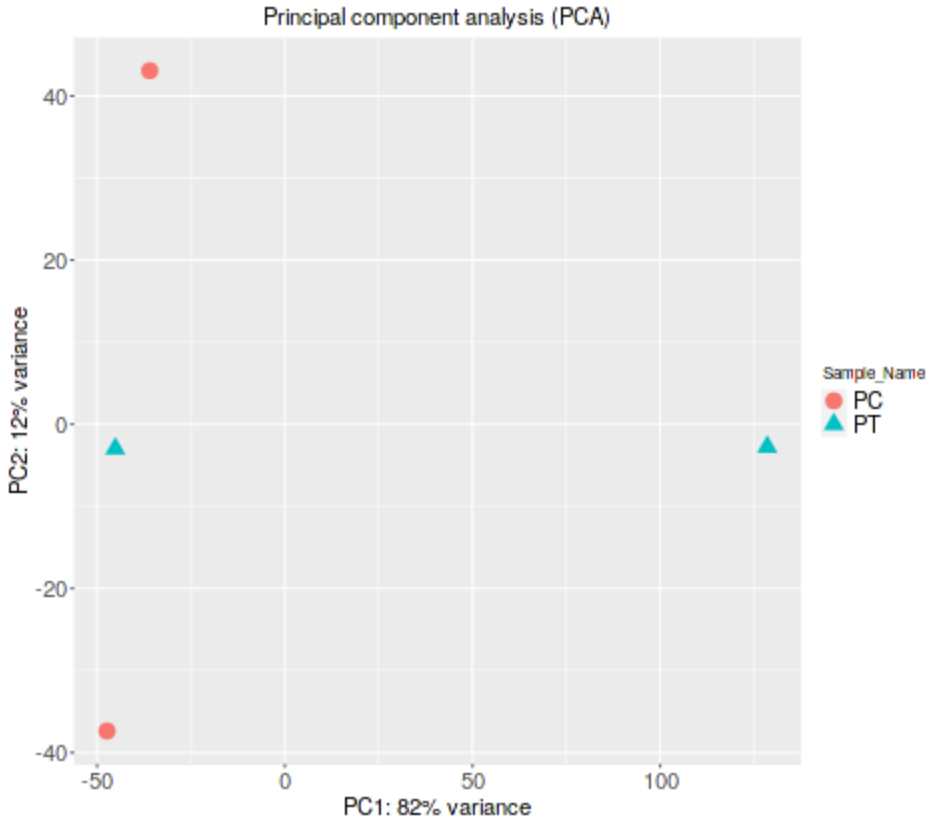

Figure 2. 2 Graphical presentations of the principal component analysis (PCA). The PCA was based on the normalized expression values using DESeq2. The red dots indicate the group had ad-libitum water (PC1 and PC2), whereas blue triangles represent the water restricted group (PT1 and PT2).

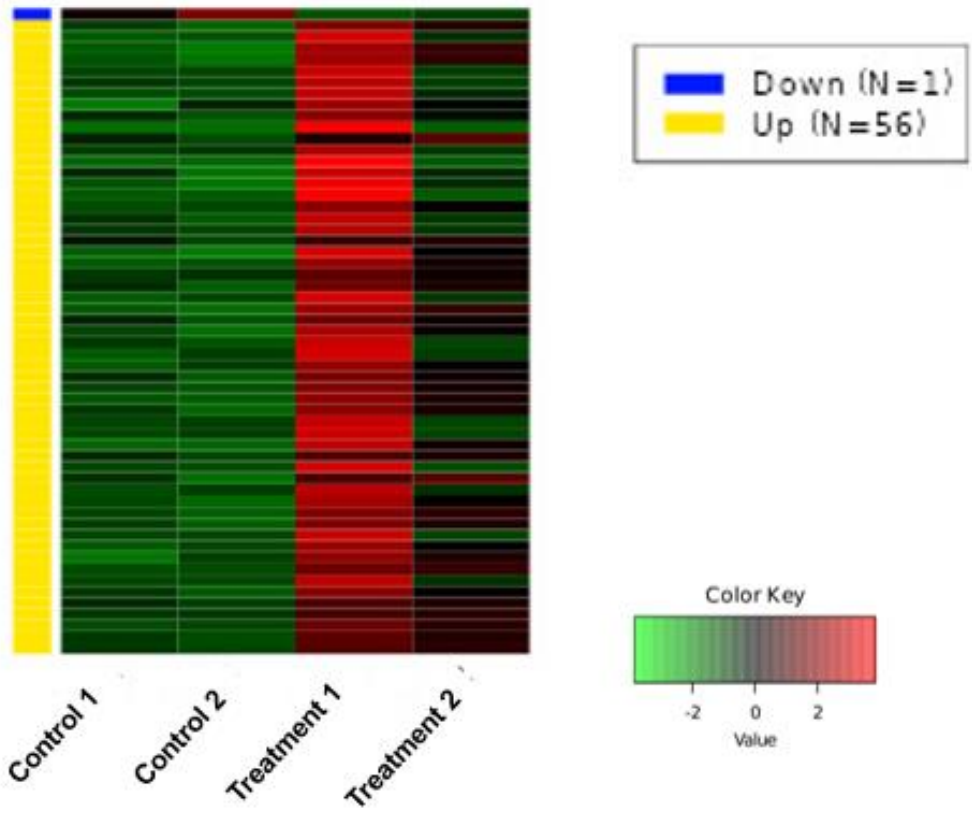

Figure 2. 3 Heatmap of genes that were up-or down-regulated (FDR $P < 0.05$) between control and treatment group (PC and PT), respectively in response to water deprivation.

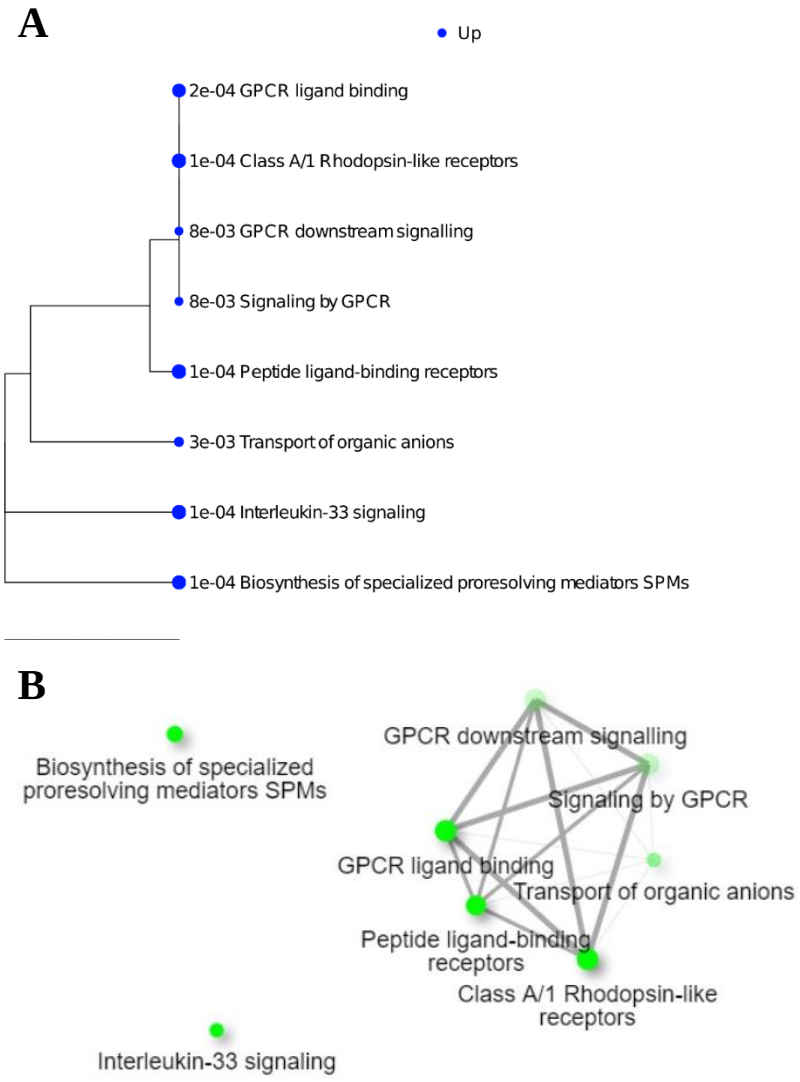

Figure 2. 4 A) Enriched Curated Reactome pathways for differentially expressed genes between treatment and control groups B) Network visualization of Enriched Curated Reactome pathways associated with *Arginine Vasopressin*.

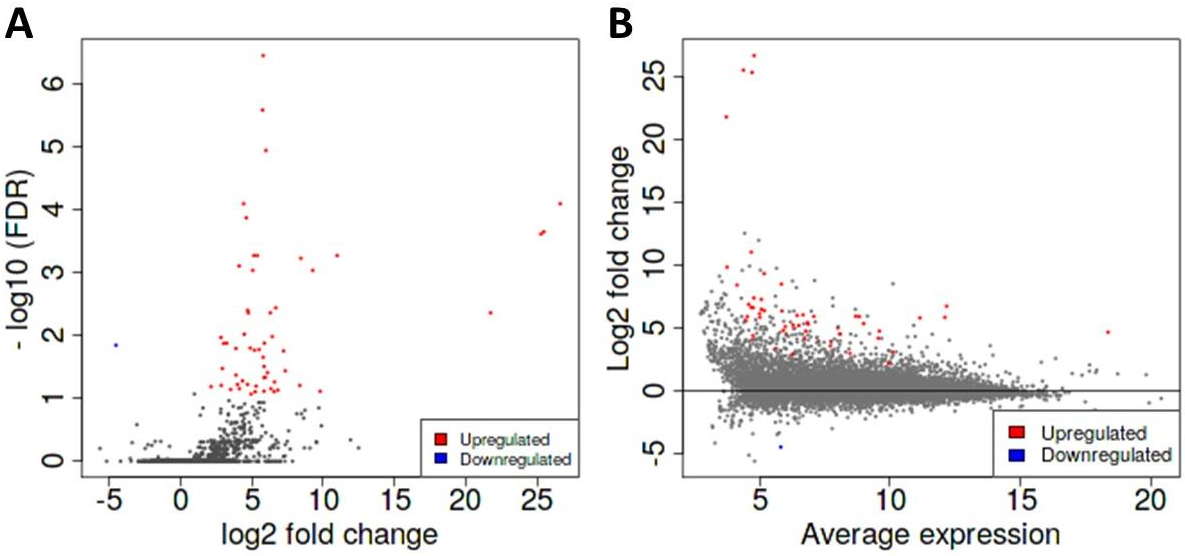

Figure 2. 5 Summary plots for differential expression analysis using DESeq2. (A) Volcano plot, and (B) MA plot.

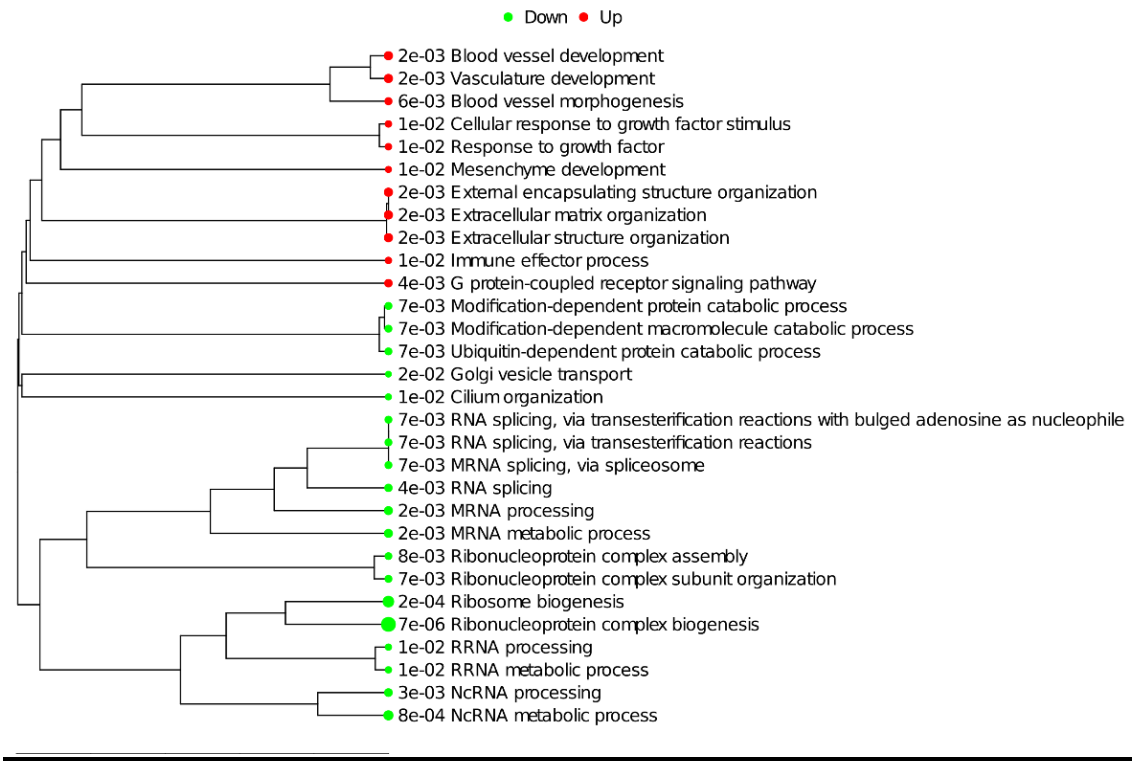

Figure 2. 6 Enriched significant gene ontology (GO) biological pathways from pathway analysis where upregulated genes are enriched in extracellular structure organization, blood vessel development and G protein-couple receptor signaling pathway.

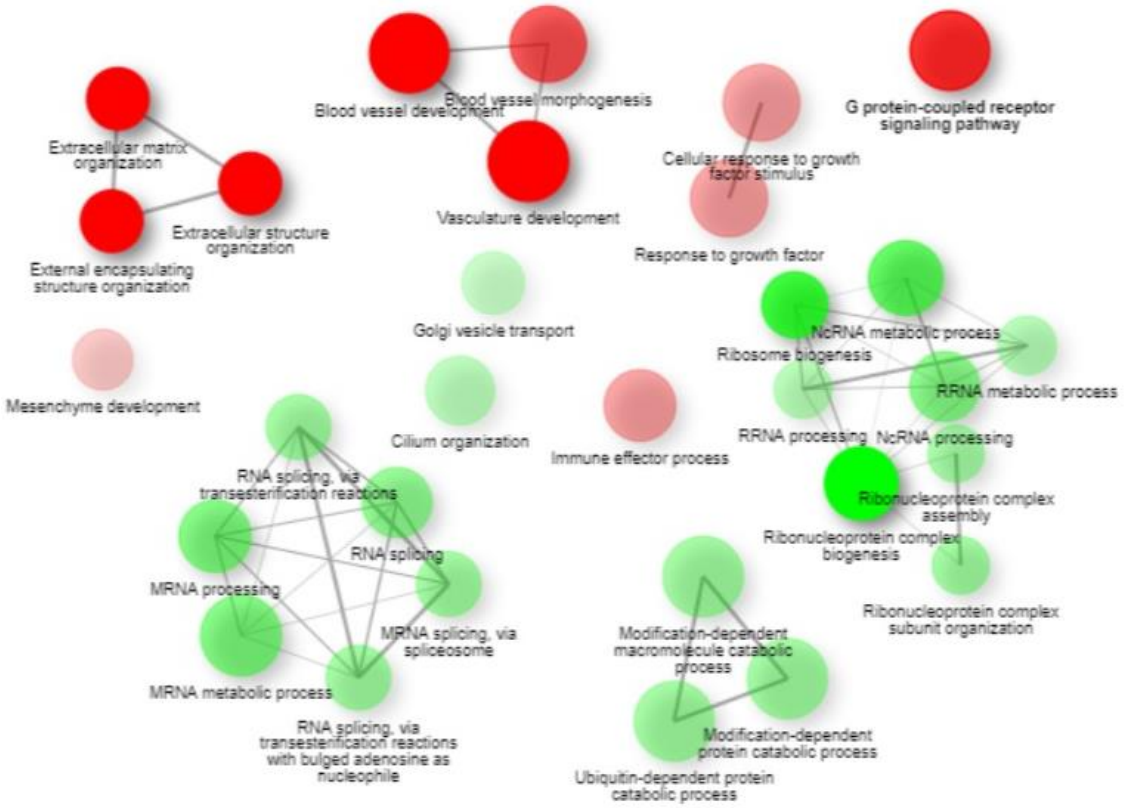

Figure 2. 7 Network of enriched significant gene ontology (GO) biological pathways from pathway analysis where upregulated genes are enriched in extracellular structure organization, blood vessel development and G protein-couple receptor signaling pathway.

TABLES

Table 2. 1. Predicted composition of feed

Ingredient	% of Diet (DM basis)
Corn Grain, Cracked	53.50
Distillers Grains, Dehydrated - Light	20.00
Brome Hay, Mature	5.00
Corn Silage, 50% Grain	15.00
DAKOTA LS 512.3R	6.50

Table 2. 2. Enriched Curated Reactome pathways among DEGs for control and water restricted groups.

Direction	Adj. P-value	Number of genes	Pathways
Upregulated	1.1×10^{-04}	6	Class A/1 Rhodopsin-like receptors
	1.1×10^{-04}	5	Peptide ligand-binding receptors
	1.2×10^{-04}	2	Interleukin-33 signaling
	1.4×10^{-04}	3	Biosynthesis of specialized proresolving mediators SPMs
	2.0×10^{-04}	6	GPCR ligand binding
	3.2×10^{-03}	2	Transport of organic anions
	7.7×10^{-03}	6	GPCR downstream signaling
	7.7×10^{-03}	6	Signaling by GPCR

Table 2. 3. Enriched pathways for each cluster in curated reactome pathways in DEGs for the comparison between treatment and control.

Cluster	Adj. P-value	Number of genes	Pathways
A	4.58×10^{-08}	4	Hormone ligand-binding receptors
A	4.58×10^{-08}	4	Glycoprotein hormones
A	4.58×10^{-08}	4	Peptide hormone biosynthesis
A	2.34×10^{-07}	5	Peptide hormone metabolism
A	2.32×10^{-04}	2	Mineralocorticoid biosynthesis
A	3.86×10^{-04}	2	TFAP2 AP-2 family regulates transcription of growth factors and their receptors
A	5.50×10^{-04}	2	Androgen biosynthesis
A	7.45×10^{-04}	4	G alpha s signalling events
A	7.45×10^{-04}	2	Prolactin receptor signaling
A	7.45×10^{-04}	7	Signaling by GPCR
A	7.45×10^{-04}	7	GPCR downstream signaling
A	8.15×10^{-04}	5	GPCR ligand binding
A	9.81×10^{-04}	2	Reactions specific to the complex N-glycan synthesis pathway

A	9.81×10^{-04}	2	Growth hormone receptor signaling
A	2.32×10^{-03}	4	Class A/1 Rhodopsin-like receptors
D	2.66×10^{-04}	7	Class A/1 Rhodopsin-like receptors
D	2.66×10^{-04}	8	GPCR ligand binding
D	5.88×10^{-04}	5	Peptide ligand-binding receptors
D	5.88×10^{-04}	10	GPCR downstream signaling
D	5.90×10^{-04}	10	Signaling by GPCR
D	9.19×10^{-04}	5	G alpha s signaling events

Number of clusters were chosen using the elbow method, therefore adding another cluster does not substantially reduce the within groups sum of squares

Table 2. 4. Enriched pathways for each cluster in KEGG pathways.

Cluster	Adj. P-value	Number of genes	Pathways
A	1.22×10^{-12}	13	Neuroactive ligand-receptor interaction
A	2.74×10^{-05}	5	Salivary secretion
A	1.98×10^{-04}	6	CAMP signaling pathway
A	7.39×10^{-04}	4	GnRH signaling pathway
A	9.39×10^{-04}	3	Ovarian steroidogenesis
A	9.39×10^{-04}	3	Autoimmune thyroid disease
A	1.19×10^{-03}	4	Growth hormone synthesis, secretion, and action
A	2.40×10^{-03}	3	GnRH secretion
A	2.59×10^{-03}	3	Prolactin signaling pathway
A	2.59×10^{-03}	3	Protein digestion and absorption
A	6.83×10^{-03}	2	Nicotine addiction
D	1.33×10^{-05}	10	Neuroactive ligand-receptor interaction

Table 2. 5. Differentially expressed genes between control and water restricted groups in response to water restriction.

Gene Name	log2 Fold Change	FDR P-value	Symbol
ENSBTAG00000038461	26.6	8.01×10^{-5}	Unidentified
Retina And Anterior Neural Fold	25.5	2.24×10^{-4}	RAX
Orthodenticle Homeobox 2	25.3	2.43×10^{-4}	OTX2
NK2 Homeobox 4	21.8	4.36×10^{-4}	NKX2-4
ENSBTAG00000055016	11	5.36×10^{-4}	Unidentified
Interleukin 1 Receptor Like 1	9.8	7.73×10^{-2}	IL1RL1
Arginine Vasopressin	9.3	9.21×10^{-4}	AVP
Solute Carrier Family Member 1C1	8.4	5.91×10^{-4}	SLCO1C1
FEZ Family Zinc Finger 1	8.4	6.24×10^{-2}	FEZF1
Thyroid Stimulating Hormone Receptor	7.3	3.65×10^{-2}	TSHR
Sodium Voltage-Gated Channel Alpha Subunit 5	7.2	1.76×10^{-2}	SCN5A
TUB Like Protein 1	6.8	7.42×10^{-2}	TULP1
Serpin Family E Member 2	6.7	3.63×10^{-3}	SERPINE2
Tryptase Beta 2	6.6	5.49×10^{-2}	TPSB2
Zona Pellucida Glycoprotein 2	6.6	7.81×10^{-2}	ZP2
Ventral Anterior Homeobox 1	6.4	1.04×10^{-2}	VAX1
Keratocan	6.3	7.04×10^{-2}	KERA

Cut Like Homeobox 2	6.3	4.36×10^{-3}	CUX2
ENSBTAG00000047529	6.1	3.90×10^{-2}E-02	Unidentified
Paired Box 7	6	1.1×10^{-5}	PAX7
ADAMTS Like 5	6	4.65×10^{-2}	ADAMTSL5
Endothelin Receptor Type B	5.9	1.33×10^{-2}	EDNRB
Peptidase Inhibitor 16	5.9	4.69×10^{-2}	PI16
Podocan	5.9	3.11×10^{-2}	PODN
C-Type Lectin Domain Family 3 Member A	5.9	7.70×10^{-2}	CLEC3A
ENSBTAG00000022829	5.8	3.51×10^{-7}	Unidentified
Prepronociceptin	5.8	2.22×10^{-2}	PNOC
proprotein convertase subtilisin/kexin type 2	5.8	2.59×10^{-6}	PCSK2
15-Hydroxyprostaglandin Dehydrogenase	5.5	1.67×10^{-2}-	HPGD
ENSBTAG00000051483	5.4	5.36×10^{-4}	Unidentified
Arachidonate 15-Lipoxygenase	5.3	6.39×10^{-2}	ALOX15
Fibroblast Growth Factor 7	5.2	7.86×10^{-2}	FGF7
ENSBTAG00000012692	5.2	1.71×10^{-2}	Unidentified
Cadherin 4	5.2	5.36×10^{-4}	CDH4
ENSBTAG00000051439	5.1	9.21×10^{-4}	Unidentified
Frizzled Related Protein	4.9	8.56×10^{-2}	FRZB

Endothelial Cell Specific Molecule 1	4.9	1.58×10^{-2}	ESM1
Tyrosine Hydroxylase	4.8	4.36×10^{-3}	TH
Ras Protein Specific Guanine Nucleotide Releasing Factor 1	4.7	3.96×10^{-3}	RASGRF1
NADH-ubiquinone oxidoreductase chain 6	4.7	6.02×10^{-2}	ND6
Proopiomelanocortin	4.6	1.34×10^{-4}	POMC
Myosin Heavy Chain 11	4.5	9.56×10^{-3}	MYH11
ENSBTAG00000052397	4.4	8.01×10^{-5}	Unidentified
TAFA Chemokine Like Family Member 3	4.3	5.27×10^{-2}	TAFA3
Netrin G2	4.1	7.04×10^{-2}	NTNG2
Tachykinin Receptor 3	4.1	7.82×10^{-4}	TACR3
ENSBTAG00000023648	4	6.02×10^{-2}	Unidentified
Potassium Voltage-Gated Channel Interacting Protein 3	3.9	1.61×10^{-2}	KCNIP3
CaM Kinase Like Vesicle Associated	3.9	4.26×10^{-2}	CAMKV
Interleukin 33	3.5	7.22×10^{-2}	IL33
Carbamoyl-Phosphate Synthase 1	3.2	1.33×10^{-2}	CPS1
Collagen Type XI Alpha 2 Chain	3	1.34×10^{-2}	COL11A2
Growth Associated Protein 43	2.9	3.36×10^{-2}	GAP43
Glutamate Metabotropic Receptor 8	2.9	6.24×10^{-2}	GRM8
G Protein-Coupled Receptor 6	2.8	1.07×10^{-2}	GPR6

ENSBTAG00000050723	2.1	6.52×10^{-2}	Unidentified
Chromosome 16 Open Reading Frame 89	-4.5	1.43×10^{-2}	C25H16orf89

FDR: False discovery rate

Positive value indicates greater abundance in the water restricted group and negative values indicate greater abundance in control group

Table 2. 6. Summary of KEGG pathways associated with ≥2-fold SDE genes.

Direction	GAGE analysis: PT vs PC	Number of Genes	Adj.Pval
Down	Ribosome	127	8.4×10^{-4}
	Protein processing in endoplasmic reticulum	157	2.8×10^{-3}
	Spliceosome	121	9.6×10^{-2}
Up	Neuroactive ligand-receptor interaction	195	3.2×10^{-3}
	Calcium signaling pathway	189	4.9×10^{-2}

Pathway maps for KEGG pathways associated with ≥2-fold SDE genes (FDR0.1%)