

THE EFFECTS OF WATER QUALITY ON NURSERY PIG
PERFORMANCE AND HEALTH

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It truly does take a village, and

“Even statues have to start as a ball of clay.” - Dr. Lee Johnston

In all these things we are more than conquerors through him who loved us. For I am convinced that neither death nor life, neither angels nor demons, neither the present nor the future, nor any powers, neither height nor depth, nor anything else in all creation, will be able to separate us from the love of God that is in Jesus Christ our lord.

(Romans 8:37-39)

DEDICATION

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CHAPTER 1: INTRODUCTION

In the swine industry, water is known as one of the most essential components required to achieve optimal pig performance and health, along with feed and air. Unlike feed and air, water has received little attention of researchers throughout the years. Historically, water has been low in cost, widely available, and of abundant supply in most geographic areas causing it to be known as the “forgotten nutrient.” Furthermore, little is known about the impact of water quality on pig growth performance and health. Water is complex and composed of numerous chemical and biological characteristics (Patience, 2013). However, definitions for “water quality” vary throughout literature leading to an unclear understanding of what is considered “good” and “bad” for the pig. Suggestions for livestock water quality were adopted from human standards and published by the Canadian Council of Ministers of the Environment (CCME) in 1987 and have not been revisited since (CCME, 2008). Along with that, there is a lack of published research that discusses water quality and identifies how it affects specific classes of pigs’ growth performance and health. Consequently, resources to help pork producers understand water quality are severely limited. As a result, water quality has received minimal attention by producers.

Recently, pork producers have observed suboptimal performance of nursery pigs and suspect that this compromised performance could be related to poor water quality. Increased incidence of “fall-behind” pigs, an increase in the presence and severity of diarrhea, and pigs that seem to transition poorly to the nursery following weaning are all examples of producer concerns. Therefore, producers have become increasingly interested in understanding how water quality may be impacting growth performance and health of nursery pigs.

CHAPTER 2: LITERATURE REVIEW

Water as an Essential Nutrient

Roles of water in the pig's body

Water is an essential nutrient for pigs because it is involved in most all physiological processes within the body. Furthermore, water comprises the largest portion of a pig's body. For a pig weighing 1.50 kg, water constitutes 82% of empty body weight and at market weight of 127 kg, water represents 42% of empty body weight (Figure 2.1; Shields et al., 1983).

In the pig, water is found throughout the body in three primary pools: intracellular space (69% of total body water); interstitial space (22% of total body water); and the vascular space (9% of total body water; Rose, 1989; Mroz et al., 1995). Water is required for body growth, tissue maintenance, fetal development, and lactation. More specifically, water aids in thermoregulation, mineral homeostasis, excretion of metabolites and/or anti-nutritional substances, attainment of satiety, and behavioral purposes; however, the physiological mechanisms for all of these functions are not well understood (Mroz et al., 1995). Because water is such a large portion of the pigs' body, pigs must drink and obtain enough water to maintain proper functioning of organs and physiological processes for optimal growth performance and health.

Quantity of water required by pigs

Establishing the requirement for water intake can be more challenging than establishing requirements for other nutrients such as minerals, amino acids, or energy because numerous factors can influence a pig's demand for water. Water is needed for growth and body maintenance, and a wide array of other physiological processes such as: lactation, waste product elimination, thermoregulation, nutrient transport, hydrolysis of nutrients, lubrication and cushioning for joints and other organs, and respiratory gas exchange (Roubicek, 1969; Thacker, 2001). Typically, pigs are allowed full access to water at all times which allows them to satisfy

their water needs by voluntary intake. This is necessary to satisfy physiological, biochemical, and nutritional requirements while balancing the losses from secretory and excretory routes. True drinking water intake is dependent on factors such as: environmental temperature, age, disease status, stage of production, composition of the diet, and water quality (Mroz et al., 1995; Carson, 2000; Patience, 2013). Further, if pigs are allowed ad libitum access to water, they may use it to fulfill various behavioral needs (Patience, 2013). Drinking water to alleviate the effects of hunger is an example of pigs consuming water to satisfy a behavioral need. When feed allowances are reduced or limited, pigs (typically gestating sows) drink more water than normal during the afternoon hour to achieve abdominal fill (Yang et al., 1981; Brooks et al., 1989). Pigs obtain water through three main methods: drinking water, moisture contained in the diet, and metabolic water that is formed during metabolic processes within the body.

Interactions among metabolic processes that require water in pigs and the rates pigs obtain water creates a challenge to establish water intake requirements. Consequently, defining a specific requirement of water has not been determined due to the complexity of measuring these interactions. Further, determining water intake from drinking water is challenging because one cannot easily and accurately determine water wastage. Water disappearance can be easily monitored by measuring the amount of water that enters a barn. Losses from drinkers via leakage can be minimized to decrease water wastage. But, the amount of water loss incurred from the mouth of pigs during drinking events typically cannot be measured in commercial production conditions making estimation of actual water intake difficult (Brooks et al., 1984). So, typical “water intake” estimates are better defined as water disappearance estimates. Water intake is the actual amount of water the pig ingests, while water disappearance can vary greatly and accounts for both consumed and wasted water. Brooks et al. (1984) found that when weanling pigs were fed commercial starter diets, similar to what is used in commercial pig production today, average

daily feed intake (ADFI) was the most accurate way to estimate water intake from three to seven weeks of age using the following equation:

$$\text{Average Daily Water Intake, L} = 0.149 + (3.053 * \text{ADFI, kg})$$

However, this equation may overestimate water intake. Studies completed in the mid-1980s concluded that weanling pigs (three weeks of age) are expected to require and consume roughly 0.49 – 0.72 L per pig per day during the first week post weaning and by seven weeks of age (four weeks post weaning) require 1.46 – 2.60 L per pig per day (Brooks et al., 1984; Gill et al., 1986).

Suggested requirements for quantity of drinking water varies among different classes of swine and is calculated as a ratio of water to feed intake. Notably, these requirements ignore effects of body weight, thermoneutral environment, and diet composition (Mroz et al., 1995; Brumm et al., 2000). The ratio of water to feed intake is then used to estimate total amount of water required per pig per day. Water intake requirements increase as pigs grow and as they are expected to perform different productive tasks such as growth, pregnancy, and lactation (Table 2.1). These estimates are subject to other factors that influence daily water needs.

However, if pigs are in a thermoneutral environment, as body weight increases the ratio of water-to-feed disappearance decreases (Brumm et al., 2000; Patience, 2013). In a thermoneutral environment the standard estimation for the ratio of water-to-feed is 2.5 kg of water disappearance per 1 kg feed (2.5:1) in early growing pigs. In late finishing pigs this ratio is 2 kg water disappearance to 1 kg feed (2:1), which suggests heavier, older pigs require less water per kg of feed consumed than lighter, younger pigs. For example, an early growing pig that consumes 0.5 kg of feed, approximately 1.25 kg of water is required, whereas a pig in late finishing consuming 3 kg of feed, would require 6 kg of water. Therefore, pigs in late finishing require more water than early growing pigs, because they are consuming more feed (Patience, 2013).

To maintain proper balance of water within the pig's body, pigs lose water via four different routes: respiration via lungs, defecation via intestines, urination via kidneys, and evaporation from wetting the pigs' skin to allow for cooling and heat loss. Defecation and urination are responsible for the greatest amount of water loss and respiration is responsible for a small constant water loss in pigs (Roubicek, 1969). As environmental temperature rises, amount of water required by pigs also rises due to additional evaporative and respiratory losses experienced by pigs as temperatures exceed their thermoneutral zone (Gill, 1989; Patience et al., 2005). In growing pigs, excess dietary crude protein may slightly increase daily water intake. Similarly, positive correlations were established among average daily water intake and dietary intake of nitrogen and potassium (Shaw et al., 2006).

Factors affecting water intake and usage by pigs

Pigs maintain a constant amount of body water but some events throughout pigs' lives may briefly disturb this balance which affects water intake (Roubicek, 1969; Thacker, 2001). For example, thermal stress causes an increase in panting, and salivation which results in a greater amount of water loss (than when pigs are in their thermoneutral zone) that needs to be regenerated by additional water intake by the pig (Roubicek, 1969). Another example is the stress imposed on pigs at weaning. The weanling pig abruptly transitions from consuming about 800 mL of sow's milk daily to about 200 mL of water when weaned partially due to stresses involved in locating and learning new sources for nutrition (Mroz et al., 1995). However, the amount of water consumed by the pig after weaning varies during the first four days where it will consume a total of about 1 L of water. This significant decrease in consumption, compared to when the pig was consuming sow's milk, has a negative effect on the pig's ability to properly digest a solid diet. A slight interruption in growth and an increase in diarrhea are observed typically. Diarrhea, which contains an excess of water in feces, creates an imbalance of water within the pig's body (Stockill, 1990; Mroz et al., 1995). Scours (diarrhea) are commonly used as an indicator of

compromised health status in weanling pigs. Thus, a negative water balance is associated with the presence of scours and commonly occurs in young pigs, as the balance of water within the pig can be interrupted during times of health challenges (Brooks et al., 1984; Stockill, 1990; McLeese et al., 1991). Ultimately, water balance in the pig can be disrupted by any event or process that causes excretion of water to exceed intake of water, resulting in compromised performance and health of pigs.

Water quality

In North America, water used in swine production systems commonly comes from one of three sources: surface water, ground or well water, and municipal water supplies. More specifically, production systems in Minnesota rely mainly on ground and municipal water supplies. Water quality is defined currently based on the quantity of chemical components and bacteria within the water (Carson, 2000; Thacker, 2001; Nyachoti and Kiarie, 2010).

Suggested water quality standards for livestock

Providing pigs with high quality water is important to producers. However, the definition of “quality” differs throughout scientific literature. Many established standards for livestock water quality have been adopted from human standards, many of which may be irrelevant for swine (Patience, 2013). Pigs are able to adapt to a wide range of water qualities and this range may be broader than for humans (NRC, 2012; Patience, 2013). There has been a lack of recent research that clearly defines standards for water quality as related to modern pig production. Thus, recommended levels of analytes commonly referenced in the swine industry are extracted from standards set in 1987 by the Canadian Council of Ministers of the Environment (Table 2.2; CCME 2008; Olkowski, 2009). Additionally, the challenging nature of defining component recommendations for livestock combined with minimal research conducted has resulted in some components in water lacking an established recommended level in drinking water for livestock.

Further, beyond the 23 analytes shown in Table 2.2, there are other characteristics such as conductivity, sodium absorption ratio, hardness, and pH that could be analyzed in water. However, a recommendation level for a majority of these other characteristics has not been established. Since the establishment of the recommended levels in 1987, the swine industry has advanced and changed in the areas of: genetics, nutritional strategies, health and environmental management, infrastructure, and technology all of which have contributed to developing a faster growing, leaner, more prolific pig over time. Further, since analyte recommendation levels have not been visited since their establishment, the effects of water quality on this modern pig are not well understood. In recent years, the effects of some individual analytes have been studied in isolation on pig performance and health. However, it is unknown how analytes in water might interact with one another and further affect pig performance and health.

pH

The pH of water is a scale used to describe whether water is acidic or basic. A pH lower than 7 indicates acidic properties and a pH higher than 7 indicates basic properties. Water with a pH equal to 7 is deemed neutral and is representative of pure water. For pigs, pH is not a major concern, unless rapid changes in pH are observed within the water. However, if a producer is treating their water, a low pH of water combined with some water medications and antibacterial products can cause formation of precipitates in the distribution system, whereas high pH can decrease efficiency of chlorinating products (Thacker, 2001). For example, at a pH of 3.5, 4, and 4.5 a precipitate in the form of a film was present after five days when tetracycline, chlortetracycline, and oxytetracycline were added to the water, respectively (Dorr et al., 2009). De Busser et al. (2011) determined nursery pigs receiving water with pH = 4 had a lower coliform (bacteria) load in the intestine than pigs receiving water with pH = 8. Furthermore, in pigs receiving water with pH = 4, there was an observed reduction in total count of *Escherichia coli* at day 14 post-weaning in comparison to day 7 post-weaning.

Hardness

Water becomes “hard” when it contacts soluble, divalent, metallic cations, such as calcium (Ca^{2+}) and magnesium (Mg^{2+}). Water hardness is more likely to occur in ground water than surface water because ground water is drawn through geological formations that cause calcium and magnesium to be dissolved into the water. Water dissolves calcium when it contacts limestone, while it dissolves magnesium when it contacts dolomite or other geological formations that contain magnesium. There are many other divalent ions present in water that also contribute to the hardness of water; however, the levels are not large enough to contribute greatly to the hardness of water (MRWA, 1994). Water hardness is reported as milligram equivalents of calcium carbonate (CaCO_3). In pork production, the largest concern with hard water is accumulation of mineral deposits (scales) within the water system. These mineral deposits can disrupt flow of water to pigs and can cause problems in managing water lines (Nyachoti and Kiarie, 2010).

Total Dissolved Solids

Total dissolved solids (TDS) are the amount of total inorganic matter that is dissolved in water (Thacker, 2001). Waters with high levels of TDS commonly contain calcium, magnesium, and sodium in the bicarbonate, chloride, or sulfate forms (Thulin and Brumm, 1991; Carson, 2000). Thus, TDS is also referred to as salinity which is defined as the total concentration of salts in water after all of the carbonates are converted to oxides, all bromides and iodides to chloride, and all organic matter have been oxidized (Carson, 2000). The Task Force on Canadian Water Quality’s recommended level of TDS in water is 3,000 ppm (CCME, 2008). In Saskatchewan, 7.4% of wells analyzed to evaluate ground water sources on swine farms contained TDS concentrations exceeding 3,000 ppm (McLeese et al., 1991). Because TDS is high in waters of many geographical areas, a small amount of research has been conducted to understand the impact of TDS in water on performance and health of pigs (Carson, 2000). Much of this scientific

literature has resulted in inconsistent results regarding the effect of TDS on pig performance and health. Anderson and Stothers (1978) found that as concentrations of TDS increased to 8,000 ppm, pig performance was not affected as assessed by average daily gain and feed to gain ratio. However, increasing TDS concentration elicited increased water consumption and presence of diarrhea in nursery pigs. A later study in which nursery pigs were provided unmedicated feed and water containing 4,390 ppm TDS had depressed average daily gain and gain to feed ratio when compared to pigs receiving water with 217 ppm of TDS (McLeese et al., 1992). Further, during the first five days post-weaning, there was no difference in the amount of water consumed as the level of TDS increased. However, two additional replications of the same study concluded that as TDS increased, both the overall average daily gain and water intake were not different in nursery pigs, but were variable, numerically. McLeese et al. (1992) also evaluated the presence and severity of diarrhea and concluded that TDS concentration in water positively correlated with presence and severity of diarrhea in nursery pigs.

Anderson et al. (1994) evaluated the effect of water TDS content and consumption in growing-finishing pigs and found that pigs consuming water with 8,000 ppm TDS (with high sodium concentration) had increased urinary excretion and drank 30 to 55% more water than pigs consuming water with 6,350 ppm or less TDS. No differences in average weekly weight gain were observed attributable to TDS concentration of water. Similarly, in a subsequent study, urinary excretion and water intake were increased in pigs consuming water with 11,700 ppm TDS (with high sodium-sulfate concentration) compared with pigs receiving water with 7,000 ppm TDS or less. However, average weekly weight gain was decreased in growing-finishing pigs as the level of TDS increased to 11,700 ppm (Anderson et al., 1994).

Thus, literature suggests growing-finishing pigs are capable or tolerating levels of TDS that are higher than the 3,000 ppm recommendation therefore the recommendation is well established. However, information regarding the effects of high TDS concentration on weanling

and nursery pigs is somewhat inconclusive, but overall does not seem to impact growth performance greatly, though does increase diarrhea. Being that growth performance is not greatly impacted by TDS concentrations, it is probable the recommended level of 3,000 ppm is also ideal for weanling and nursery pigs.

Sulfates

Among all of the mineral components present in water, sulfates are the main cause of compromised water quality in many North American regions (NRC, 2012). McLeese et al. (1991) observed sulfate levels above 1,000 ppm in 25% of Canadian wells. The maximum recommended sulfate concentration in water of 1,000 ppm has not caused significant declines in pig growth performance (Anderson and Stothers, 1978; Paterson et al., 1979; Anderson et al., 1994; CCME, 2008; Nyachoti and Kiarie, 2010; NRC, 2012). When sulfate concentration reached 1,600 ppm, growth rate and gain to feed ratio of weanling pigs did not differ compared to pigs fed water containing less than 20 ppm of sulfates (Patience et al., 2004). In the same study, researchers found that sulfate concentration of water did not affect apparent digestibility of dry matter, gross energy, crude protein, acid detergent fiber (ADF), or neutral detergent fiber (NDF; Patience et al., 2004). Veenhuizen et al. (1992) studied effects of sulfate in drinking water < 2,000 ppm on post-weaning pigs and found no differences in gain of pigs provided with 54 ppm, 600 ppm, 1,200 ppm, or 1,800 ppm sulfates. Additionally, researchers observed increased water consumption as sulfate concentration increased (to 1,800 ppm) and the prevalence of diarrhea also increased as well. Because this study also evaluated pigs for presence of common post-weaning pathogens (*E. coli* and *Streptococcus*), diarrhea was determined to be related to quality of water as pathogen presence did not increase among treatments with increased prevalence of diarrhea (Veenhuizen et al., 1992). Similarly, when pigs weaned at four weeks of age received 3,000 ppm of sulfate through the water, no differences were observed in growth performance or feed intake. However, water disappearance increased by 30 to 50% which was accompanied by a significant increase in

diarrhea of pigs receiving 3,000 ppm of sulfates in the water (Paterson et al., 1979). Although increasing concentrations of sulfates in water may not affect growth performance of nursery pigs, there is an increased presence of diarrhea when sulfates exceed 7,000 ppm (Anderson et al., 1994). Further, sulfates are known to have laxative effects and inhibit water absorption in the gut, specifically in the small intestine (Robinson, 1970; Harvey and Read, 1973). This inhibition occurs due to the release of CCK (cholecystokinin) from mucosal cells in the duodenum, further causing small intestinal motor activity to increase the speed of passage (Harvey and Read, 1973). Cholecystokinin and pancreatic secretions are released simultaneously and decrease the reabsorption of water. The abundance of liquid is rapidly transported from the small intestine to the colon where the colon is unable to reabsorb the entirety of liquids, thus resulting in diarrhea (Harvey and Read, 1973). Therefore, presence of diarrhea suggests that sulfates are not well tolerated by the gut of pigs (Robinson, 1970; Harvey and Read, 1973; Anderson and Stothers, 1978; Anderson et al., 1994).

Nitrates and Nitrites

Nitrates and nitrites in water are derived mainly from leaching out of soil or from surface water runoff that has been exposed to animal manure, nitrogen fertilizers, decaying organic matter, or soils high in nitrogen-fixing organisms (Nyachoti and Kiarie, 2010). Generally, nitrate levels that are found in drinking water are well tolerated by pigs because they are less susceptible to nitrate poisoning than cattle, goats, and sheep (Sidhu et al., 2014). However, nitrates can be converted to nitrites in the large intestine. Nitrite is extremely toxic to monogastric animals if absorbed and pigs are very susceptible to nitrite poisoning (Sidhu et al., 2014). Nitrites greatly decrease oxygen-carrying capacity of blood because they facilitate conversion of hemoglobin to methemoglobin (Carson, 2000). Nitrites are known to be ten times more toxic than nitrates in monogastric animals (Thacker, 2001; Nyachoti and Kiarie, 2010; NRC, 2012). Therefore, the

established recommendations for nitrite and nitrate + nitrite according to the Canadian Water Quality Guidelines are 10 ppm and 100 ppm, respectively (Table 2.2; CCME, 2008).

Heavy Metals and Trace Minerals

Other heavy metals and trace minerals can be found in water such as: arsenic, cadmium, copper, lead, manganese, and nickel. Some of these have recommended maximum concentrations (e.g. arsenic, cadmium, copper, lead). Others are listed as “none” (iron and manganese) leaving speculation around what the maximum concentration livestock can tolerate, as iron and manganese are found in many waters. Maximum concentrations of heavy metals and trace minerals have been derived from human recommendations. Little research has been conducted with livestock to validate these maximum concentrations. However, established maximum concentrations of heavy metals and trace minerals provide no evidence of detrimental effects to pig performance and health, when studied singly (CCME, 2008; NRC, 2012). However, interactive effects of multiple heavy metals and trace minerals have not been studied.

Bacterial contamination

Bacterial contaminations are a major cause of compromised water quality for livestock. The standard for determining the sanitary state of water is typically determined by presence of bacteria (Thulin and Brumm, 1991). Coliforms and bacteria may cause significant infections in pigs if consumed. The term, “coliform,” is used to describe the presence of bacteria in water but does not necessarily indicate the presence of pathogens in the water (Thacker, 2001; Nyachoti and Kiarie, 2010). Further, coliforms are the largest, oldest indicator of bacterial and fecal presence in water which, historically, was a determinant of water quality (Cohen and Shuval, 1973). Coliforms are split into two sub-categories, total coliforms and fecal coliforms. Total coliforms live in the intestines of warm-blooded animals or are found naturally in soil, vegetation, and water. Total coliforms are often linked to disease outbreaks and generally are found in water

polluted by feces (Oshiro, 2002). The second classification of coliforms is fecal coliforms, which is often indicative of *E. coli* presence in the water. Fecal coliforms are found in feces and are a more accurate indicator of fecal contamination and potential pathogens (Oshiro, 2002). Coliform concentration in water for pigs should not exceed 5,000 CFU (colony forming units) per 1,000 mL (NRC, 2012). Coliforms in drinking water and their impact on pig performance and health is still poorly understood but has gained some research attention over time (Nyachoti and Kiarie, 2010). A study conducted by LeJeune et al. (2006) found that coliform and *E. coli* concentrations were lower in pigs that consumed lesser amounts of feed. However, these researchers could not determine definitely whether the high concentrations of coliforms and *E. coli* were caused by a poor balance of microorganisms in the gut, or if the high concentrations were causing the low feed intake (LeJeune et al., 2006).

Biofilms

Biofilms are best defined as complex structures consisting of various microcolonies of many organisms within a medium of extracellular organic polymers that adhere to solid, moist surfaces (Geldreich, 1996; Mah, 2012). Biofilms are often formed in surface water sources, and drinking water distribution systems (Kalmbach et al., 1997). Biofilms are formed when pioneering organisms enter the distribution system and become entrapped in slow-flow areas, dead-end sections, or line obstructions leading to further biofilm build up on areas such as pipe surfaces, water storage tanks, and valves. However, biofilm formation is not only determined by their attachment surface, but by the characteristics of the bacteria present and environmental factors (Van Houdt and Michiels, 2010). Environmental signals cause a transition from single-celled, planktonic cells to a multicellular population attached to a surface and encased in a polysaccharide matrix (Costerton et al., 1995). Biofilm matrices contain water channels, which makes up 40 to 60% of the total volume (Geldreich, 1996). Thus, the bacteria and microorganisms not only coexist, but also have access to nutrients in water passing through.

Biofilms play a role in development of antimicrobial and disinfectant resistance which is critical to human medicine (Mah, 2012). However, there is little understanding of how biofilms are directly important to the swine industry, but potential impacts have been considered. Because bacteria within biofilms are partially protected from antibacterial compounds, development of multi-factorial resistance to antibiotics in bacteria entrapped in biofilms is greater than free flowing bacteria in water (Singh et al., 2017). Increased occurrence of multi-factorial resistance to antibiotics could result in persistent disease in pigs as has been observed with humans. If biofilms are not monitored in drinking water distribution systems and growth is rapid, biofilms can potentially block delivery of water to drinkers, leaving pigs without water.

Managing biofilms can be accomplished by maintaining a dry environment, because biofilms cannot survive without water, however in a water delivery system this is impossible. Therefore, biofilms are best managed in wet environments by using different methods to achieve electrochemical oxidization (Olmedo et al., 2015; Roy et al., 2018). One way to achieve this is by applying oxidizing biocides to water, which either kill the microorganisms (biocidal effect) or inhibit growth of microorganisms (biostatic effect; Bajpai, 2015). Oxidizing biocides such as chlorine and chlorine dioxide attack microorganisms by causing an electron transfer reaction in the cell structure, which disrupts nutrient transport across the cell wall. This transport disruption irreversibly damages the cell (Bajpai, 2015; Sultana et al., 2016). Further, once biofilms are eliminated from the distribution system, routine sanitation and treatments can ultimately decrease the ability for biofilms to form in water distribution systems.

SUMMARY

Water is an essential nutrient for pigs because it is necessary for most all physiological and biological processes within the body. The quantity of water required by pigs increases with increasing body weight and with changes in physiological state (growth, reproduction, lactation, heat stress). Consumed water is important for the pig to maintain proper water balance of the

body. Furthermore, water intake requirements are often overestimated as it is difficult to distinguish water intake from water wastage by the pigs.

There has been little published research to understand water quality as related to pig performance and health. Most of the published research focuses on individual components or analytes contained within water rather than overall quality of water. Furthermore, the most recent guidelines for water quality fed to livestock date back nearly 30 years. There are no comprehensive data that provide support for these old recommendations.

Therefore, the purpose of Chapter 3 is to gain an understanding of pork producers' perception of water quality in Minnesota nursery barns. Further, we investigated the quality of water in a number of nursery barns throughout the state. Finally in Chapter 3, we compare qualities of water presently used in Minnesota nursery barns. Based on water quality standards from 30 years ago, waters of differing qualities perceived as either "good" or "poor" were selected to be fed to pigs in a performance experiment. Chapter 4 provides an investigation of the effects of varying water qualities on nursery pig performance and health.

Table 2.1. Estimated water requirements for different classes of pigs

Class	Liters/pig/day
Suckling pigs	0.46 ¹
Weanling pigs	1 – 4 ^{2,3,4}
Growing/finishing pigs	2 – 10 ^{4,5,6}
Gestating gilts/sows	11 – 21 ⁴
Lactating sows	17 – 27 ^{7,8,9}

¹Fraser et al., 1988.

²Gill et al., 1986.

³Brooks et al., 1984.

⁴Froese, 2003.

⁵Brumm et al., 2000.

⁶Li et al., 2005.

⁷Quiniou et al., 2000.

⁸Oliviero et al., 2009.

⁹Kruse et al., 2011.

Table 2.2. Water Quality Guidelines for Livestock

Item	Recommended maximum (ppm)
Arsenic	0.50
Cadmium	0.02
Calcium	1,000
Chromium	1.0
Cobalt	1.0
Copper	5.0
Fluoride	2.0
Iron	n.d. ^a
Lead	0.10
Manganese	n.d. ^a
Mercury	0.003
Molybdenum	0.5
Nickel	1
Nitrate + Nitrite	100
Nitrite	10
Selenium	0.05
Sulfate	1,000
Total dissolved solids	3,000
Uranium	0.2
Vanadium	0.1
Zinc	50
Fecal coliforms	< 1 CFU per liter
Total bacterial	< 10,000 CFU per liter

(CCME, 2008)

^aNot determined.

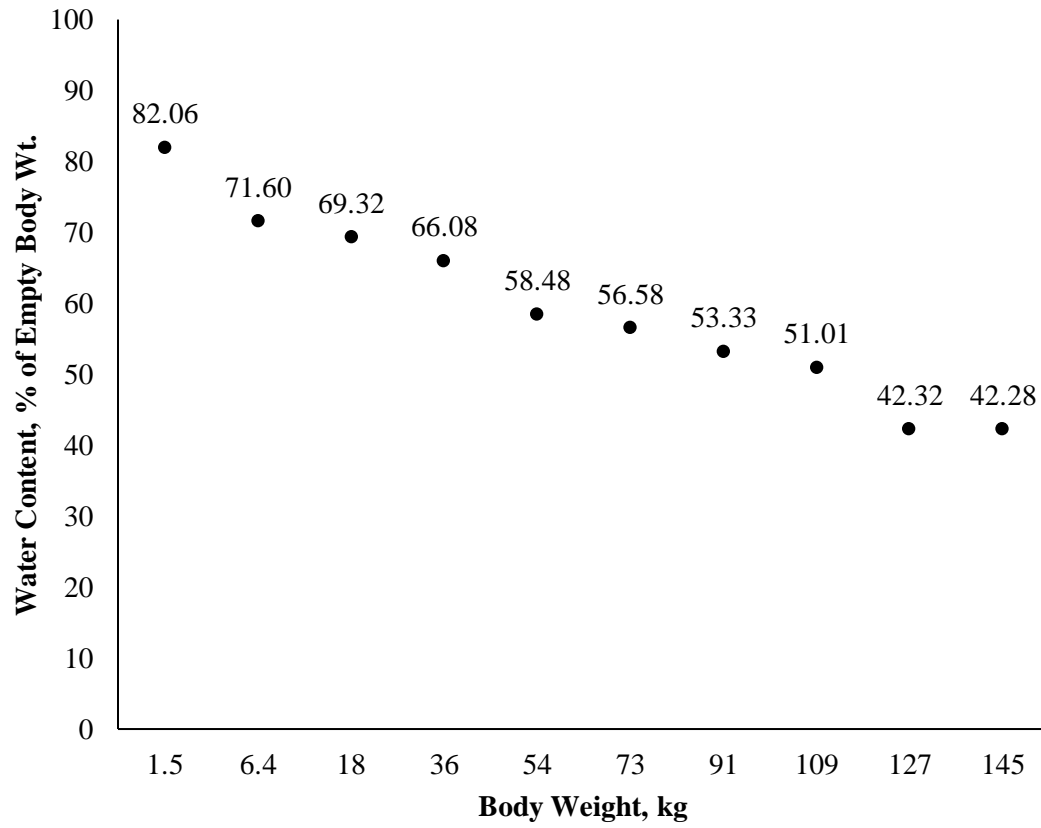


Figure 2.1. Water content of the pig's empty body weight (Shields et al., 1983)

CHAPTER 3

Survey of Minnesota Pork Producers for Their Perceptions of

Water Quality in Nursery Pig Barns

SYNOPSIS

There is no universal system or established guidelines to assist pork producers in evaluating the quality of water. This lack of clarity results in varying perceptions of water quality among producers. Definitions of water quality also vary based on different resources making it difficult to distinguish “good” water from “bad.” There are minimal literature and water quality suggestions available to aid in describing ideal drinking water for pigs. An initial survey was conducted to understand how Minnesota pig producers perceive the quality of water in their nursery barns. This survey revealed that both perceptions of and priorities for water quality vary among producers. Further, almost all respondents indicated that water quality is important to the pig and that poor water quality could have negative impacts on growth performance and health. However, only about 50% of respondents had analyzed their water to determine its quality in recent years. Initial survey responses were summarized and 15 barns with perceived “excellent” or “intolerable” water quality were chosen to be sampled and analyzed for 29 analytes. Water quality analyses revealed that water quality does differ widely among nursery barns in Minnesota. From abundant discussion around water quality of the 15 analyzed barns we selected two barns that were similar in that the analyte levels (Barns 6 and 12) were notably higher or closer to the recommended level than the third barn (Barn 14) selected. Given that the three barns varied greatly in a number of water quality analytes, we perceive that as analytes become close to (or exceed) the recommended levels, it is of poor quality. Because barns 6 and 12 had many analytes closer to recommended levels, we chose them as “poor” water quality. Barn 14 had the most

analytes that measured low or the lowest among barns analyzed therefore we chose it to represent “good” quality.

Key words: water quality, nursery, pig, producer perceptions

INTRODUCTION

Pigs need three things to survive: feed, water, and air. Thus, Minnesota pork producers value the availability of water in all barns. Water is particularly important in nursery barns. The weaning event results in many stressors being placed on the pig. These stressors include transition from a liquid diet supplied via milk from the sow to a solid diet; social stresses; and changes in drinking water. During this transition period, availability of water is particularly important to the vulnerable weanling pig. Recently, producers have been interested in helping improve this transition for the pig because they notice pigs falling behind or not starting on feed as easily as desired. Since producers in Minnesota typically have readily accessible water from wells and municipal supplies, effects of water on nursery pig performance have often been overlooked. However, quality of water has become increasingly a point of interest for some producers in their attempt to ease transitions of weanling pigs. A general understanding of what classifies water as “good” or “poor” can vary among producers and experienced industry professionals.

Therefore the objectives of this effort were: 1. to gain an understanding of producers’ perspectives on water quality in Minnesota nursery barns and identify “good” and “poor” water; 2. to physically sample and assess water quality in these nursery barns; and 3. to identify three sources of water that varied in quality to be used in a subsequent pig performance experiment.

MATERIALS AND METHODS

Part 1: Initial survey of producers

A survey, combining a mixture of approaches, was used to understand how pork producers in Minnesota perceive quality of water supplied to pigs in their nursery barns. The survey was approved by the University of Minnesota's Institutional Review Board under protocol #STUDY00006627. Purposive sampling was used to target pork producers in the state of Minnesota and is defined as choosing participants due to specific qualities or knowledge that will benefit the survey (Etikan et al., 2016). To include as many producers as possible, the survey was designed to give each participant a number of submission options and was distributed via online blogs, email lists, word of mouth, personal communications, and social media platforms between May 31 and July 3, 2019. Because of the diverse methods used to distribute the survey, we do not know how many producers received the survey. Pork producers were eligible to participate in the survey if they owned or operated a nursery barn located in Minnesota and had knowledge of the barn's water (and quality) as well as having basic production records for the barn. Once the survey was distributed, data were collected by various methods which included: Google Forms (Google, Mountain View, CA), Microsoft Excel file, PDF file, personal communication, or by completing the form and returning it via U.S. mail. Pork producers were also given the option to submit data for multiple nursery barns if they wished to do so.

Survey description

The survey consisted of 10 complete questions that were a combination of multiple choice and open-ended questions (Appendix). Data were collected that included the number of pigs owned, geographical location of the nursery barn, source of water supply, and the producer's perception of the water quality in their barn and if water quality impacted pig performance and health. Respondents also reported if the water or water supply lines were treated in any way and,

if so, what products were used. Producers were asked if the water quality in their barn(s) had been analyzed and if they would share results of these analyses. Finally, we asked if producers were willing to collaborate in future research efforts.

Part 2: Survey of water quality in 15 nursery barns throughout Minnesota

Based on responses of the initial survey, barns where producers indicated quality of water was perceived as “excellent” or “intolerable” were selected for a more in-depth analysis.

Producers who owned and operated those barns (n = 15) were contacted to determine their willingness to cooperate in the study. For consenting producers, barns were visited during July, 2019 while abiding by biosecurity measures required by each producer. Water samples were collected as close to the well head as possible without interrupting barn operations. Before collecting water samples at each barn, each producer was interviewed to further evaluate their perception of water quality within their barn. Three separate water samples were collected at each barn on one day by the same researcher using a precise and identical collection protocol.

Sampling protocols were adapted from industry water sampling protocols:

1. Before entering a barn or well house, ensure that all equipment is clean, disinfected, and compliant with the barn’s biosecurity practices.
2. Select a faucet for sample collection located BEFORE any sort of treatment system (i.e. water softener, heater, iron filtration unit, carbon filter). The selected faucet should be one that is used regularly and does not leak.
3. Disconnect anything attached to the faucet (i.e. hoses) and, using a gloved hand, remove aerators or screens from the faucet.
4. Sterilize the faucet (if it is metal) with a propane torch until it is heated throughout (about 1-2 minutes). If the faucet is not completely metal, or if methane gas might be present, use isopropyl alcohol to disinfect the opening of the faucet.
5. Run the faucet at medium to high flow rate for 5 minutes (minimum) to clean the entire

supply line. This ensures water is sampled from the source directly and not stagnant water present in the supply line. To ensure supply lines have been flushed adequately, use pH strips every 2 to 3 minutes to determine pH of water. Once the pH is consistent for three consecutive samples, water supply lines have been flushed adequately.

6. Decrease flow of water to a small steady stream (approximately the width of a pencil) and let run for 2 to 3 more minutes.
7. With a new pair of gloves on, uncap the sterile sample bottle and fill the bottle to the top without overflowing. (Hold the cap in a gloved hand being sure it does not come into contact with anything other than your gloved hand. Do not touch the inside of the bottle or cap, in the event that a bottle or cap is dropped/contaminated, repeat collection with new bottle and cap.) If needed to eliminate any head space, fill the cap of the bottle and invert the full lid onto the bottle. (Head space can cause separation or loss of components.)
8. Once the bottle is full, replace the cap on the bottle quickly and make sure that the lid is on the bottle tightly to reduce risk of spilling. Dry top area of bottle with paper towels and secure the top of the bottle with a small amount of packing tape.
 - a. Perform process from steps #7 and #8 three times in this order:
 1. Bottle 1 – Coliforms, use extra caution when collecting this sample to not cause contamination as this sample is collected in a sterile bottle.
 2. Bottle 2 – 500 mL used for measuring anions
 3. Bottle 3 – 500 mL used for heavy metals. Collect water first and add Nitric Acid vial that was provided by Midwest Labs (Omaha, NE).
9. Place bottles in Styrofoam™ cooler inside separate Ziploc™ bags, upright, surrounded with bagged wet ice cubes and/or gel ice packs.
10. Once bottles are in the cooler with ice, fill out all sample submission forms and ship to the lab so that samples arrive within 24 hours of the time of collection. Attach

information sheets to each bottle with rubber bands for ease of identification at the lab.

11. Wipe down torch and any other tools used with disinfecting wipes. Dispose of any trash and gloves.

A handwritten log was kept of each barn's address, ambient air temperature at the time of sample collection, weather conditions, any discoloration or odor of the water, sample collection location, water pH, sample ID, and any additional information pertaining to the well and barn based on the producer interview at the time of sample collections. Midwest Labs (Omaha, NE) supplied each of the collection bottles, labels, nitric acid, coolers, sample submission forms, and shipping containers and labels. The lab specified that water samples needed to be received within 24 hours of being collected. Consequently, water samples were only collected on Monday, Tuesday, or Wednesday during the week and were collected as late in the afternoon as possible with overnight shipping to the lab. Water samples needed to be 4 to 6°C upon arrival for proper analysis. For shipping, each bottle was placed in a separate Ziploc™ bag to ensure no cross contamination among bottles in the event of a leak or spill. Styrofoam™ coolers were packed with bags of ice cubes to keep sample temperatures from fluctuating.

Water samples were analyzed at Midwest Laboratories (Omaha, NE) for 29 different analytes:

1. Arsenic (method: USEPA 200.7; 1994)
2. Bicarbonate (as CaCO₃; method: SM 2320 B; 1999)
3. Boron (method: USEPA 200.7; 1994)
4. Cadmium (method: USEPA 200.7; 1994)
5. Calcium (method: USEPA 200.7; EPA 1994)
6. Carbonate (as CaCO₃; method: SM 2320 B; 1999)
7. Chloride (method: USEPA 300.0 Cl; 1993)
8. Chromium (method: USEPA 200.7; 1994)
9. Conductivity (method: SM 2510 B; 2017)

10. Copper (method: USEPA 200.7 Cu; 1994)
11. Fecal coliforms (method: SM 9222 D; 2017)
12. Fluoride (method: USEPA 300.0; 1993)
13. Hardness (method: calculation)
14. Iron (method: USEPA 200.7 Fe; 1994)
15. Lead (method: USEPA 200.7; 1994)
16. Magnesium (method: USEPA 200.7; 1994)
17. Manganese (method: USEPA 200.7; 1994)
18. Mercury (method: USEPA 245.1; 1994)
19. Nickel (method: USEPA 200.7; 1994)
20. Nitrate-N (method: USEPA 300.0; 1993)
21. Nitrite-N (method: SM 4500-NO₂-B; 2017)
22. pH (method: SM 4500 H+ B; 2017)
23. Phosphorus (method: USEPA 200.7; 1994)
24. Potassium (method: USEPA 200.7; 1994)
25. Sodium (method: USEPA 200.7; 1994)
26. Sodium Absorption Ratio (SAR; method: calculation)
27. Sulfate (method: USEPA 300.0 SO₄; 1993)
28. Total Dissolved Solids (TDS; method: calculation)
29. Zinc (method: USEPA 200.7; 1994)

Part 3: Selection of three water sources for pig experiment

As results were received from Midwest Laboratories, reports were reviewed and compiled into a spreadsheet that allowed side-by-side comparison of each barn/s analyses. Recommendation levels for water quality in livestock (CCME, 2008) were also included in the spreadsheet. Once all results were received, boundaries were set highlighting any result above the

recommended level established by the CCME. The number of analytes that were above the recommended level per barn were recorded and taken into consideration when selecting the three waters that would be used for the subsequent experiment. To further assist in selecting the three waters, industry experts in water quality were consulted and asked for their opinion of which analytes should be prioritized and why. Keeping the barn names and locations anonymous, industry experts were also given the spreadsheet and asked which barn(s) of the 15 farms had the “best” quality and which barn(s) had the “worst” quality water.

RESULTS AND DISCUSSION

Part 1: Initial survey of producers

Description of Respondents

Fifteen pork producers completed the initial survey and represented 48 different nursery barns throughout Minnesota. Of the respondents, five pork producers produced less than 1,000 nursery pigs, five producers raised between 1,000 and 5,000 nursery pigs, and five producers produced greater than 10,000 nursery pigs annually. A majority of the barns represented in the initial survey were located in central, southwest, and south-central areas of Minnesota where pig production is concentrated (Figure 3.1). Respondents indicated that eight nursery barns utilized a municipal water source, and 37 barns relied on well water. Two barns used a municipal water supply to start pigs in the nursery and then switched to well water a week or two post-weaning. One small nursery barn in northern Minnesota was supplied water from a dugout.

Producer Perception of Water Quality

Eleven of the fifteen pork producers indicated that they thought water quality could be having a negative impact on nursery pig growth performance and/or health. The remaining four producers did not think water quality had a negative impact on performance and/or health of their nursery pigs. Respondents were asked to rate water quality in each barn using a subjective scale

(excellent, good, average, marginal, intolerable) before any water or waterline treatment protocol. Nine out of 48 barns were rated as excellent and seven of these barns were supplied by municipal water sources. Four barns were thought to have “intolerable” quality water. Six barns were classified as good, 16 barns had average water quality, and finally 13 barns were identified to have marginal water quality.

Survey responses indicated that 21 of the 48 barns did not treat water or waterlines within the barn, and one respondent indicated they had treated water lines twice in the barn’s history to clean and sanitize them. In the remaining 26 barns, producers routinely used waterline treatments. Two barns were equipped with treatment systems that continuously treat all water coming into the barn. These producers stated that their water quality was improved from “average” to “excellent” and “intolerable” to “marginal”. One barn had a low-dose continuous bleach system in place which changed the perceived water quality from average to excellent. Another producer responded their barn contained a double water softener system and felt it improved water quality from intolerable to marginal. In 16 barns, either a hydrogen peroxide or citric acid-based products were used for some duration (not specified in the response) of the nursery phase to clean waterlines or improve water quality. Responses regarding the perceived changes in water quality in these barns were inconsistent among producers. In four barns, producers perceived some sort of water quality improvement, but in 12 barns producers observed no change in water quality regardless of the treatment in place.

The variability in responses of water quality and changes in quality from treatment systems may infer that perceptions of water quality differs among producers and may vary depending on efficacy of the treatment systems that producers have implemented. Additionally, some producers may prioritize a specific characteristic when they evaluate water quality, while other producers value a different characteristic. Producers may also assess their water quality based on subjective observations which can differ among producers as well. There is no universal

system for analyzing water quality in the barn that all producers are accustomed and trained to use. Further, there are limited definitions and guidelines established for assessing water quality and distinguishing between “good” and “poor” water thus producers’ opinions vary based on the source of their definition.

Producers analyzed water in 28 barns at some point in the barn’s history, but 20 barns have never had water quality analyzed. In fourteen of the 28 nursery barns where the water quality was analyzed, water samples were collected at the water drinker within pens. Water samples in 13 barns were collected at both the well head and end of the distribution system at the water drinker. In one barn, water was collected from the barn office sink. Finally, in all 28 barns with known water quality analyses, producers stated that water sampling occurred within the previous year.

Further, since water is one of the “big three” for pigs to thrive (along with feed and air), producers prioritize supplying clean water to all pigs. However, they have not emphasized water quality until recent years. Historically, producers have been more attentive to the quantity of water supplied to pigs.

Part 2: Survey of water quality in 15 nursery barns throughout Minnesota

Based on responses from the initial survey, a majority of barns where producers indicated a perception of either “excellent” or “intolerable” water quality along with a few others with “good” or “marginal” water quality were asked to participate in a more in-depth survey. The second survey included analysis of water collected from the well head. All of the selected barns were located in swine-dense areas of Minnesota (Figure 3.2). Distance of barns from the West Central Research and Outreach Center (WCROC) was considered because three barns would be selected for a subsequent experiment that required water to be transported to the WCROC (Morris, MN).

Part 3: Selection of three waters for pig experiment

Six barns had 10 or greater analytes that were above or outside of recommended levels for the specific analyte and the remaining five barns had 9 or less analytes outside of the recommendation (Table 3.1a and 3.1b). Barn 4, a barn supplied by a municipal water source, had the least number of analytes (6) that were outside of the recommended levels. Similarly, barn 2, supplied by the same municipal water source as barn 4 but in a different location had the next lowest number of analytes (7) outside of suggested ranges. The number of analytes outside of recommended ranges and the magnitude of that deviation were considered. For instance, barn 12 contained the highest level of sulfates (1,120 ppm) while no other barn exceeded the recommended level of 1,000 ppm. Further, no barn exceeded the recommended level of total dissolved solids (TDS; 3,000 ppm) but barns 12 and 15 measured the highest levels of TDS with 1,500 and 1,330 ppm, respectively. The recommended level of hardness in water for livestock is 0 ppm, but all barns exceeded this standard to some degree with barn 12 reaching a level of 1,410 ppm. In contrast, barn 14 had 7 analytes that were outside the recommended range, but water in this barn deviated the least from the recommendation. All of these observations were taken into consideration when selecting the final three waters that would be used in the pig experiment.

Barn 14 was deemed as “good” water quality and was selected to be used for the pig experiment based on advice and agreement from industry professionals and the research team. Barn 14 had the lowest deviation from the recommended levels for all analytes (Table 3.2). Additionally, barns 6 and 12 were chosen for the pig experiment as they were perceived to be of “poor” quality water because a number of analyte concentrations were much higher than the remaining sampled barns (Table 3.2). Examples of large differences among the three barns are observed in analytes such as: calcium, conductivity, hardness, iron, magnesium, potassium, sodium, sulfates, and total dissolved solids. The variation among barns suggests differences in water quality but only partially helps to distinguish which barns had “good” or “poor” quality

water. Another factor used to identify barn 14 as “good” and barns 6 and 12 as “poor” quality was the recommendation levels compared to each barn’s analyses and further compared to other barns. Exceeding the recommended levels is not ideal for water quality. Therefore, in the selection process, it was important to consider that barns 6 and 12 had the most analytes with concentrations that were elevated closer to the maximum recommended levels compared to barn 14. Additionally, nitrates, nitrites, and coliforms were not elevated in any barn meaning that the contamination of wells by manure and fertilizers was not compromising the water quality and indicated good well structure.

Swine industry professionals that work in the area of water quality were asked to provide insight into which barns they perceived had the “best” and “worst” quality of water. These consultations provided a different perspective in understanding quality of water. While opinions and rationale among industry professionals varied slightly of what water characteristics were most important in defining water quality, all agreed that water present in barn 14 was “good” quality water for a nursery pig.

CONCLUSION

Results of the initial survey indicated that when asked to assess water quality of nursery pig barns in Minnesota, defining water quality and priorities of water quality varied among producers. Survey responses indicated water for nursery barns in Minnesota is predominately sourced from wells. Producers responded water quality was of importance to their pigs and almost 75% of respondents felt that water quality could have a negative effect on nursery pig performance and/or health but only 58% of producers had analyzed the water in their barns. Perceptions of water quality and water quality changes resulting from treatment is highly variable among producers. Therefore, a survey alone is not adequate to assess water quality of nursery barns throughout the state. Based on laboratory analysis of water quality of some nursery barns in swine-dense areas of Minnesota, water quality differs from barn to barn but analytes rarely

exceed recommended levels. Because of this and the nature of the recommended levels, it is challenging to distinguish “good” water quality from “poor” water quality. However, experiences of swine water quality professionals and the little available published literature are the best guides thus far.

Further research should be considered to understand what analytes should be prioritized in determination of water quality for pigs. Also, a more definite and universal method for assessing water quality in-barn that can help producers distinguish water quality and compare it to other barns should be considered.

Table 3.1a. Received water quality analysis from Midwest Labs (Omaha, NE) of barns 1 - 8 selected from survey responses.

Analyte	Recommended Level ^a	Barn*							
		1	2	3	4	5	6	7	8
Source of water supply	-	Well	Municipal	Well	Municipal	Well	Well	Well	Well
Arsenic, ppm	0.5	< 0.10	< 0.10	< 1.0	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10
Bicarbonate (as CaCO ₃), ppm	-	376	42	322	274	365	375	421	416
Boron, ppm	5	0.3	0.18	0.28	0.06	0.23	0.24	0.35	0.37
Cadmium, mg/L	0.02	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002
Calcium, ppm	1000	180	93	167	91	84	214	164	165
Carbonate (as CaCO ₃)	-	< 1.0	1.3	< 1.0	< 1.0	1.6	< 1.0	1.1	< 1.0
Chloride, ppm	None	4	6	11	12	3	0	1	1
Chromium, mg/L	1	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Conductivity, mmhos/cm ^c	0.55 - 0.75	1.36	0.82	1.16	0.70	0.90	1.62	1.29	1.32
Copper, ppm	0.5 - 5.0	0.02	n.d. ^b	0.02	n.d. ^b	n.d. ^b	0.02	n.d. ^b	0.01
Fecal Coliforms, cfu/100mL ^d	None	< 2	< 2	< 2	< 2	n.d. ^b	< 2	< 2	< 2
Fluoride, mg/L	1 - 2	0.2	0.5	0.2	0.2	0.3	0.2	0.2	0.2
Hardness, mg Eq CaCO ₃ /L	None	707	391	616	367	390	909	646	656
Iron, ppm	None	3.23	0	0.34	0	0.93	5.22	3.47	3.68
Lead, mg/L	0.1	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Magnesium, ppm	None	63	39	48	34	44	91	58	59
Manganese, ppm	None	0.178	< 0.005	0.899	< 0.005	0.028	0.117	0.094	0.1
Mercury, mg/L	0.003	< 0.0004	< 0.0004	< 0.0004	< 0.0004	< 0.0004	< 0.0004	< 0.0004	< 0.0004
Nickel, mg/L	1	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Nitrate, ppm	23	n.d. ^b	0.5	2.3	5.6	n.d. ^b	n.d. ^b	n.d. ^b	n.d. ^b
Nitrite, mg/L	10	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
pH	7.4 - 8.8	7.24	8.52	7.13	7.34	7.68	7.38	7.46	7.29
Phosphorus, ppm	-	< 0.05	1.14	< 0.05	< 0.05	0.22	0.15	0.16	0.17
Potassium, ppm	None	7.39	4.79	7.48	2.81	3.67	6.33	8.32	8.91
SAR ^e	-	0.5	0.5	0.4	0.3	1	0.5	0.7	0.8
Sodium, ppm	None	33.3	24.5	25.7	11.6	45	37.4	42.8	46.1
Sulfate, ppm	1000	419	413	320	67	138	617	344	344
TDS, ppm ^f	3000	884	535	754	458	586	1,050	838	858
Zinc, ppm	50	0.04	0.02	0.02	< 0.01	0.01	< 0.01	< 0.01	0.02

^a (CCME, 2008)

^b n.d., Not detected

^c Conductivity- ability of water to pass an electrical current, increases with salinity

^d Fecal coliforms - bacteria caused by presence of sewage contamination

^e Sodium absorption ratio - ratio of sodium to calcium and magnesium

^f Total dissolved solids

* All values with a border exceed the recommended concentration level

Table 3.1b. Received water quality analysis from Midwest Labs (Omaha, NE) of 9 - 15 barns selected from survey responses.

Analyte	Recommended Level ^a	Barn*						
		9	10	11	12	13	14	15
Source of water supply	-	Well	Well	Well	Well	Well	Well	Well
Arsenic, ppm	0.5	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10
Bicarbonate (as CaCO ₃), ppm	-	391	386	425	397	462	270	279
Boron, ppm	5	0.18	0.21	0.12	0.25	0.22	0.13	1.16
Cadmium, mg/L	0.02	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002
Calcium, ppm	1000	100	161	96.4	284	100	59	146
Carbonate (as CaCO ₃)	-	< 1.0	< 1.0	1.3	< 1.0	< 1.0	< 1.0	< 1.0
Chloride, ppm	None	0	2	0	2	2	2	11
Chromium, mg/L	1	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Conductivity, mmhos/cm ^c	0.55 - 0.75	0.75	1.15	0.87	2.31	0.93	0.54	2.04
Copper, ppm	0.5 - 5.0	n.d. ^b	n.d. ^b	n.d. ^b	n.d. ^b	0.07	0.02	0.01
Fecal Coliforms, cfu/100mL ^d	None	< 2	< 2	< 2	< 2	< 2	< 2	< 2
Fluoride, mg/L	1 - 2	0.2	0.2	0.2	0.2	0.3	0.4	0.3
Hardness, mg Eq CaCO ₃ /L	None	387	638	451	1,410	457	235	529
Iron, ppm	None	1.48	2.52	1.38	5.43	1.83	1.33	0.52
Lead, mg/L	0.1	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Magnesium, ppm	None	33	57	51	171	50	21	40
Manganese, ppm	None	0.125	0.115	0.041	0.048	0.027	0.045	0.317
Mercury, mg/L	0.003	< 0.0004	< 0.0004	< 0.0004	< 0.0004	< 0.0004	< 0.0004	< 0.0004
Nickel, mg/L	1	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Nitrate, ppm	23	n.d. ^b	n.d. ^b	n.d. ^b	n.d. ^b	n.d. ^b	n.d. ^b	0.5
Nitrite, mg/L	10	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
pH	7.4 - 8.8	7.35	7.29	7.52	7.02	7.3	7.54	7.35
Phosphorus, ppm	-	0.1	0.1	0.36	0.12	0.24	0.1	< 0.05
Potassium, ppm	None	6.41	6.08	2.62	5.34	4.15	2.67	12.3
SAR ^e	-	0.4	0.6	0.6	0.7	0.7	0.8	4.3
Sodium, ppm	None	15.9	34.8	29.2	64	36.2	29.4	229
Sulfate, ppm	1000	32	312	34	1,120	47	2	784
TDS, ppm ^f	3000	486	748	567	1,500	604	348	1,330
Zinc, ppm	50	< 0.01	< 0.01	0.02	0.03	0.29	0.05	0.03

^a(CCME, 2008)

^b n.d., Not detected

^c Conductivity- ability of water to pass an electrical current, increases with salinity

^dFecal coliforms - bacteria caused by presence of sewage contamination.

^eSodium absorption ratio - ratio of sodium to calcium and magnesium

^fTotal dissolved solids

* All values with a border exceed the recommended concentration level

Table 3.2. Initial water quality analysis from Midwest Labs (Omaha, NE) of 3 selected barns for pig experiment

Analyte	Recommended Level ^a	Barn 6	Barn 12	Barn 14
Arsenic, ppm	0.5	< 0.10	< 0.10	< 0.10
Bicarbonate (as CaCO ₃), ppm	-	375	397	270
Boron, ppm	5	0.24	0.25	0.13
Cadmium, mg/L	0.02	< 0.002	< 0.002	< 0.002
Calcium, ppm	1000	214	284	58.7
Carbonate (as CaCO ₃)	-	< 1.0	< 1.0	< 1.0
Chloride, ppm	None	0	2	2
Chromium, mg/L	1	< 0.01	< 0.01	< 0.01
Conductivity, mmhos/cm ^b	0.55 - 0.75	1.62	2.31	0.536
Copper, ppm	0.5 - 5.0	0.02	n.d. ^d	0.02
Fecal coliforms, cfu/100mL ^c	None	< 2	< 2	< 2
Fluoride, mg/L	1 - 2	0.2	0.2	0.4
Hardness, mg Eq CaCO ₃ /L	None	909	1410	235
Iron, ppm	None	5.22	5.43	1.33
Lead, mg/L	0.1	< 0.05	< 0.05	< 0.05
Magnesium, ppm	None	90.9	171	21.4
Manganese, ppm	None	0.117	0.048	0.045
Mercury, mg/L	0.003	< 0.0004	< 0.0004	< 0.0004
Nickel, mg/L	1	< 0.01	< 0.01	< 0.01
Nitrate, ppm	23	n.d. ^d	n.d. ^d	n.d. ^d
Nitrite, mg/L	10	< 0.02	< 0.02	< 0.02
pH	7.4 - 8.8	7.38	7.02	7.5
Phosphorus, ppm	-	0.15	0.12	0.1
Potassium, ppm	None	6.33	5.34	2.67
SAR ^e	-	0.5	0.7	0.8
Sodium, ppm	None	37.4	64	29.4
Sulfate, ppm	1000	617	1120	2
TDS, ppm ^f	3000	1050	1500	348
Zinc, ppm	50	< 0.01	0.03	0.05

^a (CCME, 1987)

^b Conductivity – the ability of water to pass an electrical current, increases with salinity

^c Fecal coliforms – bacteria caused by presence of sewage contamination

^d n.d., Not detected

^e Sodium absorption ratio - ratio of sodium to calcium and magnesium

^f Total dissolved solids

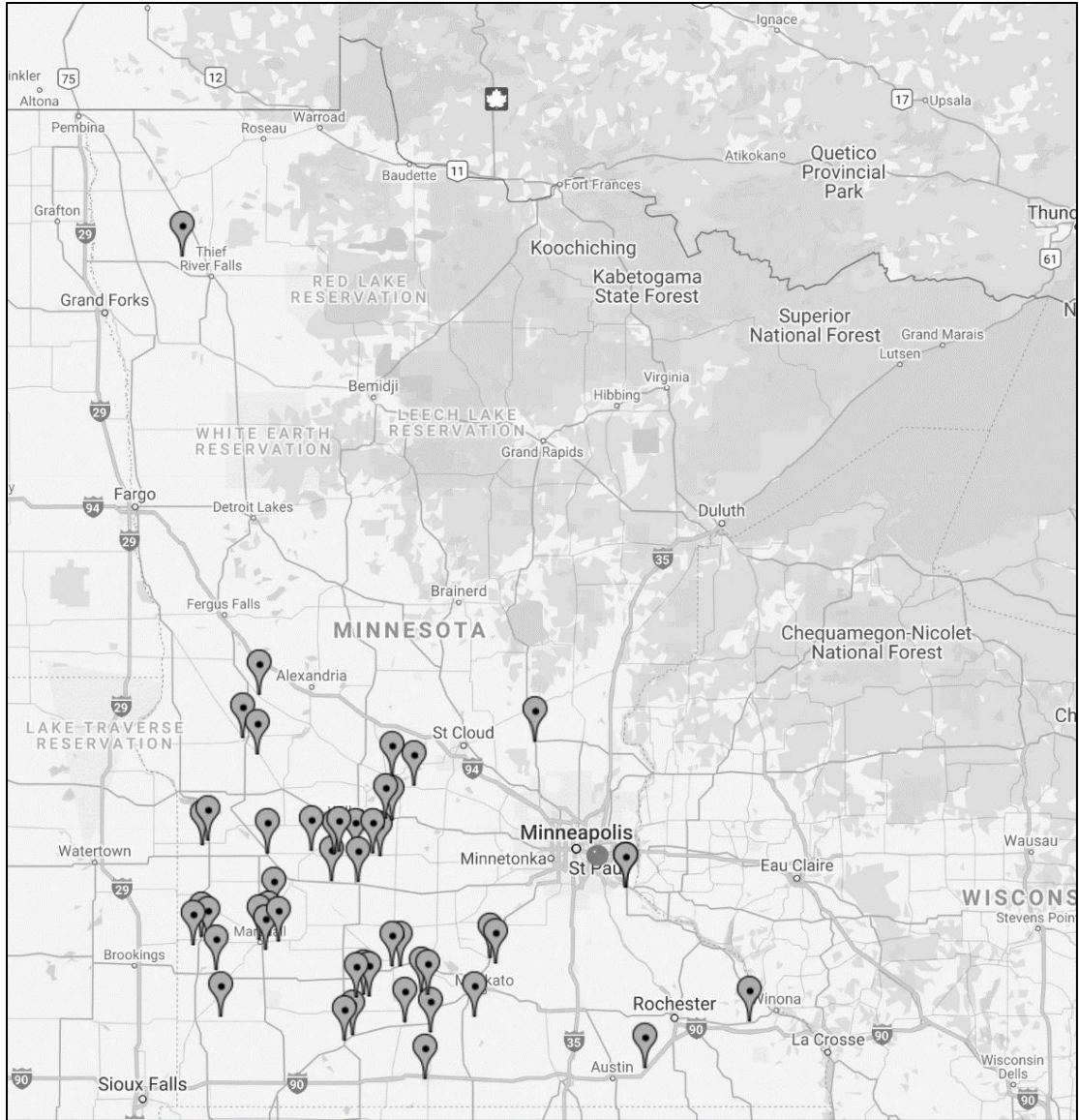


Figure 3.1. Approximate locations of 48 barns included in initial survey responses

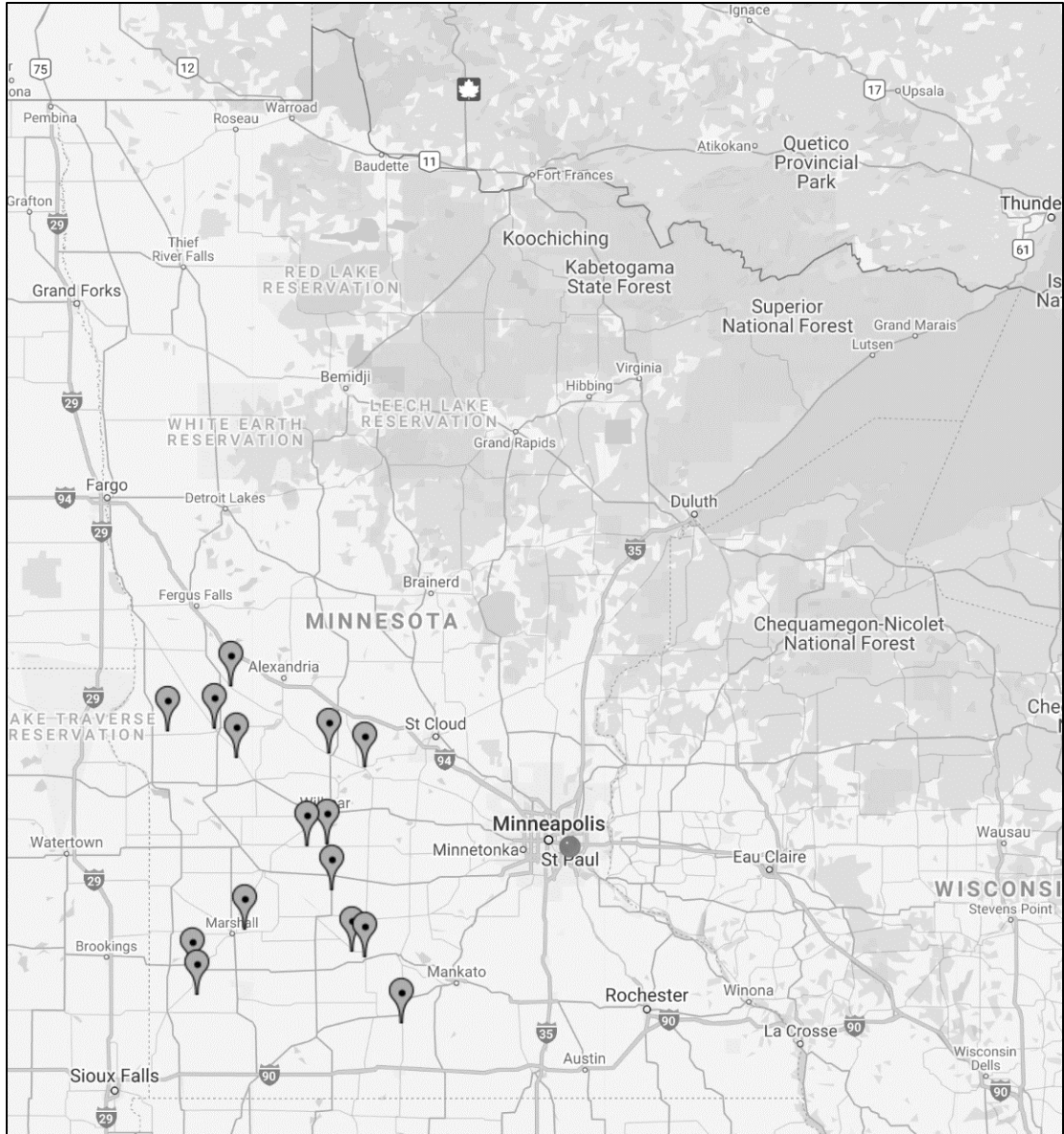


Figure 3.2. Approximate locations of 15 barns used for water quality analysis

APPENDIX



Research Survey

Project title: **Effects of water quality on nursery pig performance and health**
Project sponsors: University of Minnesota and Minnesota Pork Board

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Researchers at the University of Minnesota in collaboration with the Minnesota Pork Board would like to learn more about how quality of water supplied to nursery pigs might affect their health and performance. In the initial stage of this project, we hope to learn the range of water quality being offered to pigs in Minnesota. As a pork producer, you can help us with this project. We are asking you to complete the short survey below and return it to us as soon as possible, or before **June 21, 2019**. Responses and responder identity's will be held confidential to the research group. Any release of information to the public will be done in aggregate with no identification of individual farms. With this information, we will determine the range of water quality being fed to pigs and identify water sources that would be helpful in future studies. We are interested in learning about good quality water and poor quality water. Our ultimate goal is to determine the effects of water quality on health and performance of nursery pigs and to explore effective treatment options for water in swine nurseries.

We would greatly appreciate your time and efforts in assisting us to accomplish this project through your responses to the following questions. **Please complete one survey for each nursery barn that has a different water supply with this document or by using: <https://z.umn.edu/watersurvey2019>. Or to submit this survey for multiple barns please use: <https://z.umn.edu/WaterSurvey2019MassSubmit>.**

1. How many nursery pigs do you own/manage? (Check one)
 < 500 500 – 1,000 1,000 – 5,000 5,000 – 10,000 >10,000
2. Where is your nursery barn located (nearest town or zip code)?

3. What is the source for the water supply? (Check all that apply)
 Municipal Water Well Water Both
Other: _____
4. Do you believe that quality of water has a negative impact on nursery pig performance?
 Yes No
5. Rate the quality of water in your nursery barn before any type of treatments using the following scale:
 Excellent Good Average Marginal Intolerable



6. Do you treat your water and/or water lines in any way?

- Yes, water
 Yes, water lines
 Yes, both
 Neither

If yes, please state what is used:

7. How often do you treat your nursery barn water (if applicable)?

- Continuously
 Each turn
 Quarterly
 Yearly

Other (please specify):

8. Rate the quality of water in your nursery barn after any type of treatments using the following scale:

- Excellent
 Good
 Average
 Marginal
 Intolerable

9. Do you have any lab analyses of your water that you would be willing to share with us?

- Yes
 No

a. If yes, where was your sample collected?

- Well Head
 Drinker/Nipple
 Other (Please describe)

Other: _____

b. Approximately when was the last water sample collected and analyzed?

10. May we contact you to discuss potential use of your water in a future pig performance experiment?

- Yes
 No

If yes, please provide your preferred contact details:

Name: _____

Address: _____

Phone: _____

Email: _____

Thank you for your participation in this survey! If you have any additional questions or comments, please contact us.

To submit single barn information:
<https://z.umn.edu/watersurvey2019>

To submit information for multiple barns:
<https://z.umn.edu/WaterSurvey2019MassSubmit>

Or return this form to Brigit Lozinski at lozin012@umn.edu by **June 21st, 2019.**

CHAPTER 4

Effects of Water Quality on Nursery Pig Growth Performance and Health

SYNOPSIS

An experiment was conducted to determine effects of drinking waters of differing qualities on growth performance and health of nursery pigs. Weanling pigs (n = 450) were randomly assigned to one of three treatments consisting of varying water quality. Treatments were: 1) water perceived as poor quality (**water A**); 2) water perceived as poor quality (**water B**); and 3) water perceived as good quality (**water C**). Pigs received *ad libitum* access to their respective water sources for the duration of the study which began at the time of weaning (21 d of age) and ended 40-d later (61-d of age). Individual pig weights were recorded weekly along with feed intake on a pen basis. Occurrences of morbidity and mortality were recorded daily. Subjective fecal scores were assigned on a pen basis and blood samples were used to evaluate blood chemistry, cytokine concentrations, and phagocytic activity. A differential sugar absorption test was used to assess intestinal permeability. Fecal grab samples were used to establish diet digestibility and video cameras were used to assess if pigs had any aversions to the quality of water provided. The statistical model considered fixed effects of treatment, room, and their interaction with random effects of pen. A repeated measures analysis was conducted to determine the effect of water treatments over time. Water quality did not change while stored throughout the experiment. There were no differences among treatments in ADG (**A**, 0.46 kg; **B**, 0.46 kg; **C**, 0.47 kg) or ADFI (**A**, 0.68 kg; **B**, 0.69 kg; **C**, 0.71 kg). In health measures, there were no differences in blood chemistry, cytokine concentrations, gut permeability, or diet digestibility attributed to water quality treatments. Further, phagocytic activity of pigs fed different water sources also was not different for the percentages of monocytes (73.2 to 74.5%) and granulocytes (93.6 to 95.3%) ($P = 0.91$ and 0.45 , respectively). These results suggest that the gut health and

immune status of pigs consuming water sources of variable quality were similar and did not affect morbidity (9, 5, and 8 pigs, respectively) and mortality (0, 1, and 1 pigs, respectively). Pigs did not have an aversion to the waters provided, as total time spent at the drinker did not differ among treatments on d 1 ($P = 0.65$), 2 ($P = 0.82$), or d 3 ($P = 0.79$). Overall, these data indicate the sources of water with different qualities studied in this experiment did not impact growth performance or health of nursery pigs.

Key words: drinking behavior, growth performance, health, nursery pigs, water quality

INTRODUCTION

The role of water quality on pig health and growth performance is poorly understood in the swine industry, and more research has been conducted to determine optimal water availability than on quality of water for pigs. In recent years, some pork producers have questioned if the quality of water in their barns potentially contributes to observed increases in the percentage of pigs that lag behind their contemporaries post-weaning. Unfortunately, there has been little research published recently that has evaluated effects of water quality on nursery pig growth performance and health, while focusing on single factors associated with water quality rather than interactions among multiple factors of pigs. McLeese et al. (1992) studied effects of total dissolved solids (TDS) content in water on post-weaning pigs and found that as levels of TDS increased, there was a slight depression in nursery pig growth and an increase in the frequency and severity of diarrhea. Similarly, as concentrations of sulfates in water increased, presence and severity of diarrhea also increased with no differences in pig growth performance observed (Paterson et al., 1979; Anderson et al., 1994). Beyond these few studies, there has been little published literature on water quality and pig growth performance and very minimal information on water quality and health of nursery pigs.

Over time, pork production systems have advanced in areas such as: genetics, facilities, management, feeding strategies, and technology. Recommendations for water quality date back to the 1970s and 1980s and there is a lack of literature describing ideal concentrations of analytes in water for modern swine production which contributes to the lack of knowledge of water quality. Therefore, objectives of this study were to evaluate effects of varying drinking water quality on growth performance, diet nutrient digestibility, health, immunity, blood chemistry, and behavior of pigs post-weaning. We hypothesized that “poor” water quality would decrease growth performance and compromise health status of pigs compared with water perceived to be of “good” quality.

MATERIALS AND METHODS

This experiment was conducted in the research nursery barn at the West Central Research and Outreach Center (WCROC, University of Minnesota, Morris, MN). The experimental protocol was reviewed and approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC # 1907-37251A) and the study was conducted from September 11 to October 21, 2019.

Animals, Housing, and Treatments

Weanling pigs (n = 450; 19 ± 2 days of age) were sourced from a single, commercial sow farm (C16, Christensen Farms, Sleepy Eye, MN) that was negative for Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and *Mycoplasma hyopneumoniae*. Pigs were assigned randomly to one of three water quality treatments upon arrival at the WCROC nursery barn. Treatments were assigned randomly to pairs of adjacent pens.

Water treatments consisted of: 1) **Water A**, poor quality water containing 1,410 ppm hardness, 1,120 ppm sulfates, and 1,500 ppm total dissolved solids (TDS); 2) **Water B**, poor quality water containing 909 ppm hardness, 617 ppm sulfates, and 1,050 ppm total dissolved

solids; and 3) **Water C**, good quality water containing 235 ppm hardness, 2 ppm sulfates, and 348 ppm TDS (Table 4.1). Pigs remained on their assigned water quality treatment for 40-d and were provided with ad libitum access to water. All pigs received ad libitum access to the same high quality, commercial industry-relevant, four phase nursery feeding program across all water treatments (Table 4.2). The phase one diet was a proprietary pelleted diet provided by VitaPlus Corp. (Madison, WI) containing 21% crude protein, 5.4% crude fat, 0.70% calcium, 0.67% phosphorus, and 1.4% total lysine. Phase one also included a combination of tiamulin (Denagard®) and CTC (chlortetracycline) at 40 and 440 ppm in the final diet. Although pigs were sourced from a sow farm of high health, the genetic line of pigs is susceptible to infections of *Streptococcus suis* in the WCROC nursery, thus the phase 2 diet included Aureomycin 50® (chlortetracycline; Zoetis, Parsippany-Troy Hills, NJ) as a precaution.

Pigs were housed in pens of 10 pigs with floor space allowance of 0.3 m² per pig for the duration of the experiment. Two mirror-image identical rooms were used, each room contained 32 pens. Each pen was equipped with one stainless steel feeder with 4 feeding spaces and one water cup (Drink-o-Mat, Vittetoe Inc; Keota, IA) with fully slotted, plastic flooring over a deep manure pit. For this experiment, 45 pens (15 pens per treatment) were utilized (22 pens in Room 1 and 23 pens in Room 2). Each room possessed an independent ventilation controller (AP Expert Series 2; AGCO; Montreal, Canada) that controlled all heaters and ventilation fans. Daily high and low temperatures were recorded using Hobo temperature recorders (HOBO MX2203 Underwater Temp Recorder; ONSET; Bourne, MA) that were placed at pig level in the middle of each room.

Water Storage and Quality Management

For this experiment, water was transported to the WCROC nursery barn via insulated, milk tanker trucks (Nelson Trucking; Sauk Center, MN). Water was stored in separate water bladders (4.26 m long by 3.66 m wide) with the capacity to hold 9,464 L (Potable Pillow Bladder

Tank, Aire Industrial; Meridian, ID; Figures 4.1 – 4.3). Each water treatment was stored separately in its own individual bladder. Bladders were located on a level platform made of CDX-grade plywood (2.54 cm thick) on the northwest side of the barn. Shade cloth (90% SunBlocker™; FarmTek; Dyersville, IA) was used to create a lean-to shelter extending from the fascia of the nursery barn roof out past the end of the bladders. Each water bladder was connected to a series of valves that led to individual water pressure tanks located directly inside the nursery. Pressure tanks were set to maintain a flow of about 0.47 L/minute to drinker cups within the pen. Pressure tanks were connected to a new water distribution system (PVC pipe) that was attached to the ceiling of rooms housing pigs. Each water source was capable of being delivered to each pair of adjacent pens through manifolds mounted over water cups (Figure 4.4). Previously existing standpipes (stainless steel pipe connecting manifold to water cup) and water drinker cups in the nursery rooms were used during the study. However, both were de-scaled by removing the drinking nipple in the water cup and using pressurized water and a nylon power tube brush to flush the standpipe. Water drinker cups were cleaned with a sponge and warm, soapy water. New water drinking nipples were installed and calibrated to deliver a uniform flow rate throughout the nursery barn.

Water flow rates were recorded at the pen level weekly by collecting water from each water drinker cup for 30 seconds into a Ziploc® bag. Flows were measured at every pen on test, until weeks 5 and 6 where every other drinker was measured. Water flow rate was maintained at about 0.47 L/min for each drinker

Weekly water samples were collected from the same 3 water drinkers per treatment and pooled (n = 18 samples). Water samples were analyzed at Midwest Laboratories (Omaha, NE) for *E. coli* concentration (generic; method SM9223B), total coliform concentration (method SM9223B), and aerobic plate count (method SM9215B) to evaluate bacterial loads over time at the drinker cup (SM 9223 and 9215, 2017). To further monitor water quality during the

experiment, water samples were collected from pressure tanks 1-d after initial arrival of water, 1-d before the second delivery of water (to determine if water quality changed during the first portion of the experiment), and on the last day of the experiment (to determine if the water quality changed during the second portion of the experiment).

Ambient temperature near each water bladder was recorded every 10 min via HOBO temperature recorders (HOBO MX2203 Underwater Temp Recorder) for the duration of the experiment. One HOBO recorder was placed on the plywood platform in close proximity to, but not touching each water bladder near the middle of the bladder.

Pig Growth Performance and Health Measurements

Pigs were identified by individual ear tags and initial body weight and sex of each pig was recorded. All feed additions to feeders were weighed and recorded. Individual pig weights and pen feed disappearance were measured each week to determine average daily gain (ADG), average daily feed intake (ADFI) and the gain:feed ratio (G:F). Pigs were observed multiple times daily to identify any adverse health conditions. Any instances of morbidity were recorded and included: pig identification number, sex, pen number, date, clinical signs, if any treatment was administered, drug name, withdrawal period, and the outcome of the treatment procedure. Records of mortality were maintained and included: pig identification number, sex, bodyweight, pen number, date, and suspected cause of death. To assess any instances of diarrhea, fecal grab samples were collected from 2 randomly selected pigs in each pen daily during the first 7 d of the experiment. Fecal samples were pooled within pen for determination of fecal dry matter. Fecal grab samples were collected once daily in the morning, placed in Ziploc® bags, and stored by day at -20°C. To determine moisture content, samples were dried in a forced draft oven at 60°C and weighed twice daily until samples maintained a constant weight. Fecal scores were assigned to each pen during the first 7 d of the experiment and were recorded by the same researcher each

day for consistency. Fecal scores were assigned on a scale of 1 to 4 where 1 = firm feces and 4 = liquid consistency (Wellock et al., 2006).

Apparent total tract digestibility of nutrients

The phase 2 diet contained 0.5% titanium oxide to act as an indigestible marker for nutrient digestibility determination. This diet was introduced to all pigs on day 4 of the experiment. Pigs were allowed a 5-d adaptation period before fecal grab samples were collected. On d 10, 11, and 12 of the experiment (d 6, 7, and 8 of consuming the phase 2 diet) fecal grab samples were collected from 2 randomly selected pigs per pen in the morning, pooled in a Ziploc® bag (pen-basis), and stored at -20°C. To determine moisture content of feces, samples were dried in a forced draft oven at 60°C and weighed twice daily until samples maintained a constant weight. After drying, feces were ground through a 1-mm screen and samples were pooled on a pen-basis across all 3 collection days. Pooled samples were stored in Whirl-pak® bags until analysis.

Approximately 2 kg of the phase 2 diet were collected at mixing and on each fecal collection day and were stored at -20°C. A subsample of feed from each collection day was pooled and sent to the University of Missouri Agriculture Experiment Station Chemical Laboratories (Columbia, MO) for proximate analysis and determination of titanium concentration. Similarly, dried and ground fecal samples (12 to 15 g) were submitted for proximate analysis and determination of titanium concentrations. Standard procedures (AOAC International, 2006) were followed for the analysis of moisture (method 934.01), ash (method 942.05), crude protein (LECO; method 990.03), crude fat (method 920.39), and crude fiber (method 978.19). Diet and fecal samples were analyzed for titanium concentration according to procedures from Myers et al. (2004). Gross energy content of diet and fecal samples was determined using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL) using benzoic acid as the internal standard.

Apparent total tract apparent digestibility (ATTD) was calculated using the index method with the following equation:

$$\text{ATTD (\%)} = 100 - [100 \times ([\text{titanium in feed}] \times [\text{nutrient in feces}] / [\text{titanium in feces}] \times [\text{nutrient in feed}])] \text{ (Adeola, 2001).}$$

Gut Permeability Measurement

A differential sugar absorption test was conducted to assess gut barrier function. On d 12 of the experiment, feed was removed at about 0800 h from 8 randomly-selected pens per treatment (n = 24) for a 3-h fasting period and time of removal was recorded. Pigs had ad libitum access to water during the fast. Following the 3 hour fast, 1 pig was selected randomly from each fasted pen (8 pigs/treatment; n = 24) for blood collection via blind venipuncture. Blood was collected in a heparinized vacuum tube (5 mL; Vacutainer®; Becton, Dickinson and Company, Franklin Lakes, NJ) and a vacuum tube treated with no additive (5 mL; Vacutainer®; Becton, Dickinson and Company, Franklin Lakes, NJ). The time of blood collection was recorded. Blood samples were stored on ice following collection until further processing at the end of the procedure.

Immediately following initial blood collection, each pig was dosed orally with a water-based solution that contained 15 g of a sugar mixture (90% lactulose, 6% L-rhamnose, 1.2% 3-O-methylglucose, and 2.8% D-xylose).

The sugar mixture was prepared for the entire group immediately before the dosing procedure:

1. With a cylinder, measure 200 mL of distilled water and pour into flask that will be used for the sugar solution. Place a magnetic stirrer into flask and mark where the 200 mL volume of water and stirrer is.
2. Remove the water from the flask and discard.

3. Using clean weighing boats and weighing paper for each sugar, weigh the sugars; (to make 200 mL of solution) 180 g Lactulose, 12 g L-rhamnose, 2.4 g 3-O-methylglucose, and 5.6 g D-xylose.
4. Add each sugar to the flask and rinse weighing boat with distilled water to ensure all sugar is transferred to the flask.
5. Once all sugars are transferred to flask, place magnetic stirrer in flask and add distilled water to the marked volume established in Step 1.
6. Mix solution until all sugars have dissolved and solution is clear.

Each pig was dosed using a sterile, disposable plastic syringe (10 mL; with no needle) and was given 5 mL of the mixed sugar solution. The sugar solution was administered at a slow rate to ensure all of the mixture was ingested by the pig. The time of dosing was recorded for each pig. A sample of the sugar solution (5 mL) was retained for analysis and stored at -20°C. Pigs were allowed two hours from the time of dosing for sugars to be absorbed into the blood stream. Two hours after dosing, blood was again collected in a heparinized vacuum tube (5 mL; Vacutainer®; Becton, Dickinson and Company, Franklin Lakes, NJ) and a vacuum tube treated with no additive (5 mL; Vacutainer®; Becton, Dickinson and Company, Franklin Lakes, NJ), and the time of blood collection was recorded. Blood samples were placed on ice immediately after collection until further processing at the end of the procedure (about 4 hours after dosing).

All blood samples collected prior to and following dosing were centrifuged at 2,000 x g for 10 min at 4°C. Once centrifuged, plasma (1.0 mL) and serum (0.5 mL) were aliquoted from the heparinized and additive-free blood tubes, respectively, into new tubes, sealed, and stored at -80°C. Plasma samples were analyzed for concentrations of lactulose, L-rhamnose, xylose, and 3-O-methylglucose by liquid chromatography-mass spectrometry. The ratios of sugar in the plasma were calculated and normalized to the concentration of 3-O-methylglucose.

Immune Function Measurements

To assess immune status, three different analyses were performed including: a blood chemistry panel, a cytokine analysis, and a Phagotest™.

For blood chemistry and cytokine analyses, blood (5 mL) was collected in a heparinized vacuum tube (Vacutainer®; Becton, Dickinson and Company, Franklin Lakes, NJ) from 1 pig per pen (n = 45) via blind venipuncture on d 8 of the experiment. Immediately following blood collection, each sample was placed on ice. After all samples had been collected (about 2 hours), blood samples were centrifuged at 2,000 x g for 10 min at 4°C. Keeping samples as cool as possible, serum (0.5 mL to 1.0 mL) was aliquoted into 2 separate sample tubes and stored at -80°C until analysis.

One of the two aliquoted samples from each pen (n = 45) was analyzed at Marshfield Labs (Marshfield, WI) to determine blood chemistry (ANP2 Large Animal Profile). The other aliquoted sample from each pen (n = 45) was analyzed to determine cytokine concentrations (MILLIPLEX Porcine Cytokine Magnetic Bead Panel; Merck Millipore; Darmstadt, Germany).

To determine phagocytic activity of monocytes and granulocytes, a PHAGOTEST kit (ORPEGEN Pharma, Heidelberg, Germany) was used. On d 11, blood (1 mL) was collected in a heparinized vacuum tube (Vacutainer®; Becton, Dickinson and Company, Franklin Lakes, NJ) from 8 randomly selected pigs per treatment (1 pig per pen; n = 24) via blind venipuncture. Blood was kept whole and at room temperature (20 to 25°C) during transport to the lab for processing within 24 hours of collection. Whole blood was incubated with opsonized *Escherichia coli*-FITC (fluorescein isothiocyanate). Both monocytes and granulocytes were gated using forward-scatter plot versus side-scatter dot plots. Further, side-scatter versus *E. coli*-FITC dot plots were used to measure the phagocytic capacity of monocytes and granulocytes. The resulting percentage of *E. coli*-FITC-positive cells was indicative of phagocytic capacity (Hodkinson et al., 2006).

Pig Behavior Determinations

To investigate whether pigs displayed any aversion to their assigned water treatment, a behavioral assessment was conducted using video recordings. Digital cameras (TruVision High Definition TVI Bullet Camera TVB-4403, Interlogix, Costa Mesa, CA, USA) were used to capture video footage of 5 pens per treatment for 7 h per day (0900 h to 1600 h) over the first 3-d of the experiment.

Recordings were later transcribed using GeoVision video capture management software (Geo Vision Multicam Digital Surveillance System V8.2; USA Vision Systems Inc., Irvine, CA). Continuous observation was used to record the number of drinking bouts (number of times pigs visited the drinker) and duration of drinking bouts (amount of time spent at the drinker per pig per visit) for 6 continuous hours per day.

Statistical Analysis

Data were evaluated for the presence of outliers and normal distribution among treatments. Outliers were deemed as any value greater than or less than two standard deviations from the mean. Outliers were removed from the final analysis to ensure normal distribution of data among treatments. Experimental data were analyzed using the PROC GLIMMIX procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC). Pen was considered the experimental unit. The statistical model included fixed effects of treatment, room, and their interaction with random effects of pen. Treatment means were separated using the PDIFF option and the Tukey-Kramer adjustment for multiple comparisons. A similar model was used for a repeated measures analysis to evaluate changes over time.

To determine the influence of water treatment on categorical response variables (fecal score, number of treatment days, number of pigs treated, and number of dead pigs), a Chi-square

analysis was used. The significance level was declared at $P < 0.05$ and trends are described at $0.10 > P > 0.05$.

RESULTS AND DISCUSSION

Water Storage and Quality Management

The average water flow at the drinker cup in pens over the entire experiment did not differ among treatments (Figure 4.5). However during week 2, water source B had a lower flow rate than water source C but was not different than water source A. This aberration resulted in additional monitoring of water flow rates and an adjustment of pressure for water source B to improve consistency. The aberrant low flow rate returned to the desired level and was the same as water sources A and C for the rest of the experiment. Over time, there were no differences observed among treatments in flow rates of water at the drinker (Figure 4.5). Within each treatment, little to no changes occurred in analyte concentrations of water from the initial sample collected at the source nursery barns in July 2019 to completion of the experiment in Morris, MN in October 2019 (Tables 4.3, 4.4, and 4.5). Differences in water quality that were initially reported for each water, were the same differences that were analyzed at the end of the experiment. This observation implies that quality of well water does not change readily which concurs with work of Vinten and Dunn (2001) who reported well water did not change over the course of 10 years.

To assess bacterial loads in drinking water, water samples were collected from drinker cups in 3 pens/treatment and pooled. For both generic *E. coli* and total coliforms, the upper limit of detection was 2,400 MPN (most probable number) per 100 mL. Values in excess of this limit are reported as >2,400 so differences were unable to be determined (Table 4.6). Regarding aerobic plate counts, data fluctuated on a week to week basis among treatments. The fluctuation in values may be indicative of presence of fecal matter or other environmental contaminants in

water drinker cups. Water was collected directly from drinker cups rather than the drinker nipple because drinker cup design did not allow direct access to the water nipple. We assessed bacterial growth in the water after storage to determine if bacterial contamination increased during storage. We found no evidence of increased bacterial growth as storage time increased. One of the reasons water bladders were chosen over a standard water tank was the fact that there was practically no headspace in the bladder with minimal air which presumably would limit aerobic bacterial growth. In hindsight, the water samples for bacterial contamination should have been collected from the bladders themselves instead of the water drinker. Sampling from the bladder directly would have been more reflective of any bacterial growth during storage than sampling at drinkers.

Although water bladders were stored on a platform and underneath a shade cloth, the outdoor environment could have impacted water temperature and potentially caused water quality to change in the bladder. Therefore, temperatures around each water bladder were recorded and summarized into 12-h averages for both daytime (0700 h to 1900 h) and nighttime (1900 h to 0700 h). Among treatments, the ambient temperatures remained similar for both daytime (Figure 4.6) and nighttime (Figure 4.7). This measurement further served as a validation that all water bladders and waters experienced the same conditions and were handled similarly.

Pig Growth Performance

There were no differences in bodyweight of piglets among treatments at the initiation or conclusion of the study (Table 4.7) nor any differences in ADG, ADFI, or G:F (Figure 4.8). Furthermore, there were no differences among treatments in ADG (Figure 4.9), ADFI (Figure 4.10), or G:F (Figure 4.11) at any week throughout the experiment. Our hypothesis was that poor-quality water (A and B) would have a negative impact on pigs' growth performance, but this did not occur.

One potential reason water source did not affect pig growth performance may have been related to the diet that was fed. The phase 1 and phase 2 diets contained a number of specialty feeding ingredients, such as spray-dried bovine plasma, dried whey, specialty soy proteins, and fish meal that are common in the initial nursery phase diets. Specialty products increase the complexity and quality (and cost) of the diet by packing dense, easily digestible ingredients into the diet to accommodate the expected low feed intake during weaning and transition to the nursery. Further, use of complex, high quality diets aims to encourage feed consumption during the nursery phase and essentially set the pig up for success during the grow-finish period. Therefore, we wonder if use of a less complex, lower cost diet that does not contain as many specialty products would cause differences to occur in pigs consuming waters of different qualities.

At weaning, pigs are expected to quickly adapt to a new environment (Patience, 2013). Further, while the specific time point is unknown, it is known that pigs adapt to water quality within a few weeks (NRC, 2012; Patience, 2013). Adaptation to water differences is thought to occur quicker in pigs consuming lower levels of sulfates and TDS (Paterson et al., 1979; McLeese et al., 1992; Patience, 2013). Recall that waters A and B contained 1,120 and 617 ppm sulfates, respectively and 1,500 and 1,050 ppm TDS, respectively. Results from studies reported in literature would suggest that fecal scores were increased in pigs consuming water greater than or equal to 3,000 ppm sulfates. We did not observe differences in fecal scores, and this is in agreement with the 3,000 ppm limit observed in the literature (Paterson et al., 1979). Further, McLeese et al. (1992) found that growth performance was decreased in pigs consuming 4,390 ppm TDS in an unmedicated diet. In the same experiment, there was a tendency for pigs to grow faster when consuming water with a low TDS level and a medicated diet (McLeese et al., 1992). Therefore, because extreme levels of analytes with published impacts on pig performance were not reached in this experiment, we wonder if the pigs maybe adapted to the quality of water they

were given quickly which resulted in the absence of growth performance differences in this experiment.

Apparent Total Tract Diet Digestibility of Nutrients and Energy

Pigs consumed a similar amount of feed and has similar growth rates throughout the experiment, regardless of which water source they received. This includes the period of time (day 4 – 14) when TiO₂ was present in the diet. Fecal samples were collected during week 2 of the experiment. Digestibility of dry matter, crude protein, and gross energy of diets did not differ among treatments (Table 4.8). Furthermore, digestibility of lipid and fiber also did not differ among treatments. However, for both lipid and fiber there were a number of observations that resulted in negative digestibility values (17 and 6 negative values, respectively) that impacted the overall means for these two variables. These negative results may be indicative of large endogenous losses from metabolic functions occurring within the pig. Additionally, nursery diets fed in this experiment contained very low amounts of lipids and fiber, which limited our ability to determine digestibility using the indirect method (Zhang and Adeola, 2017). Lastly, ash digestibility of diets was different among treatments ($P = 0.016$). Pigs consuming water source C had greater ash digestibility compared with pigs consuming water sources A and B. Both ash and TDS are similar in their properties, composed mainly of inorganic salts. Water source C contained the lowest TDS concentration which may have supported a greater proportion of consumed ash to be digested compared to waters A and B. Except for ash digestibility, diet digestibility was not affected by the quality of water consumed by pigs.

Intestinal Permeability

To determine permeability and absorptive capacity of the intestine, an orally administered, non-metabolizable, sugar mixture was used to measure the ratios of specific sugars in the blood similar to the methods used in humans (Zuckerman et al., 2004; Wijtten et al., 2011).

A smaller ratio of d-xylose to l-rhamnose indicates a lower permeability and a healthier intestine (Zuckerman et al., 2004). In this experiment, there were no differences observed in the ratio of d-xylose to l-rhamnose among water source (Table 4.9). To evaluate intestinal absorptive capacity, the ratio of l-rhamnose to 3-O-methyl-glucose is compared among treatments, where a greater ratio is desired to indicate a larger absorptive capacity (Zuckerman et al., 2004). However, in this experiment, water qualities A, B, and C did not cause any differences in intestinal absorptive capacity of pigs. This result concurs with the assessment of intestinal permeability. Intestinal permeability is loss of gut epithelial wall integrity, resulting in unwanted material from the lumen entering the blood stream, also known as a “leaky gut” (McLeod et al., 2019). Whereas intestinal absorption is the movement of wanted nutrients from the small intestine into the blood supply by transport proteins (Kiela and Ghishan, 2017). The ratio of d-xylose to 3-O-methyl-glucose was not different among water qualities and suggests similar intestinal integrity. In humans, a higher ratio of d-xylose to 3-O-methyl-glucose is associated with the presence of illness or disease due to a decrease in d-xylose metabolism after absorption (Zuckerman et al., 2004).

Some observations (4 from d-xylose : l-rhamnose ratio, 6 from l-rhamnose : 3-O-methyl-glucose ratio, and 4 from d-xylose : 3-O-methyl-glucose ratio) were omitted from the data set because sugar measurements were below the minimum threshold of detection in blood. There are two possible causes for the low sugar concentration in blood. First, pigs may not have ingested the full dose of sugar mixture because they did not swallow all of the dose or they regurgitated a portion of the dose. Another explanation is that pigs were allowed too much time to absorb the mixture. In this study, pigs were allowed two hours whereas in other published studies both pigs and humans are only given one hour for sugar absorption prior to blood collection (Peled et al., 1991; Wijtten et al., 2011). With extended time for sugar absorption, we may have missed the timepoint when sugars peaked in the blood due to rapid clearance. By hour two, pigs may have been clearing the sugars from their bloodstream thus decreasing their presence.

Pig Morbidity and Mortality

Number of pigs treated and total number of injections administered were not different among water treatments (Table 4.10). Mortality for the entire experiment was 0.44% among all treatments with only 2 mortalities that were unrelated to quality of water. By comparison, pork industry average mortality during the nursery phase during 2019 was 3.06% (MetaFarms and Swine Management Services, 2020). Postmortem examinations by a veterinarian revealed that one pig fed water B died from *Streptococcus suis* and another pig fed water C died from an intestinal torsion. There were no differences in presence of scours among treatments during the first week of the study (Figure 4.12). However, subjective scour scores were not assigned on days 1 and 2 due to a lack of fecal matter present in the pens. Additionally, fecal scores and percentages of fecal dry matter were not different among treatments over the course of days 3 to 7 post-weaning (Figures 4.12 and 4.13).

Pigs used for this experiment were sourced from a commercial sow farm with high health status (negative for PRRSV and Mycoplasma). As a result, pigs received were also of high health upon arrival. Based on mortality and morbidity data, pigs maintained a high health status throughout the experiment.

Immune Function

Cytokine Analysis. Cytokines are key in assessing immune status of pigs due to their role in mediating and regulating immune and inflammatory responses. Thirteen different cytokines were analyzed from blood collected on day 8 of the experiment (Table 4.11). Within the Interleukin-1 (IL-1) family, water treatment had no effect on the proinflammatory cytokines, IL-1 α , IL-1 β , IL-18, or GM-CSF, or the anti-inflammatory cytokines of the IL-1 family; IL-1Ra (Interleukin-1 receptor antagonist). Further considering the other proinflammatory cytokines measured, water quality did not influence concentrations of TNF α , IFN γ , or IL-8. Additionally,

concentrations of anti-inflammatory cytokines, IL-4 and IL-10 also did not differ among treatments along with the intermediary IL-6. Finally, IL-2 and IL-12, whose inflammatory response depends on specific conditions within the body, also were not influenced by quality of water fed to pigs. The lack of difference in cytokine concentrations suggests that the innate and adaptive immune systems of pigs fed different water sources were similar among treatments.

Phagotest. Phagocytic capacity of pigs assigned to each water treatment was evaluated using the PHAGOTEST kit (ORPEGEN Pharma, Heidelberg, Germany). Phagocytic capacity is the overall percentage of cells that show phagocytosis. This measure evaluates the ability of immune cells to phagocytize a virus, particle, or infected cell. Opsonization is the process by which bacteria are able to easily be engulfed by phagocytes. Phagocytic capacity is then increased as opsonization is decreased. Therefore, when less opsonin-coated bacterial cells are present, there are more monocytes and granulocytes available to pursue phagocytosis (Magnusson and Greko, 1998; Hodkinson et al., 2006). Higher percentages of monocytes and granulocytes are desired, as their increased availability to participate in phagocytosis increases the pig's ability to fight infections.

There were no observed differences in the percentage of total monocytes that displayed phagocytosis among treatments (Table 4.12). Further, there was no influence of water quality on total percentage of granulocytes showing phagocytosis.

During stress, phagocyte distribution changes and an inverse relationship is observed between monocytes and granulocytes. Monocyte presence increases at the onset of stress and then quickly decreases to pre-stress baseline levels shortly after cessation of the stress. In contrast, granulocyte presence increases during stress and takes much longer than monocytes to return to pre-stress baseline levels (Dhabhar, 2002). We did not measure cortisol levels to quantify presence of stress in pigs fed water sources A, B, or C. So, we can not determine if stress levels of pigs fed different water could have influenced activity of monocytes and granulocytes.

Blood Chemistry

Blood chemistry was determined by analyzing blood for 22 different parameters. Water quality did not influence blood chemistry of pigs among treatments (Table 4.13). Furthermore, most parameters observed for each treatment were within optimal swine reference ranges (Table 4.13; Marshfield Labs, Marshfield, WI). However, for water A, both SDH (sorbitol dehydrogenase) and bilirubin were slightly above the specified reference ranges.

The SDH value for pigs fed water A was 24.6 U/L, where the range for SDH in swine is 4.2 to 24.3 U/L. Sorbitol dehydrogenase is an enzyme useful for indicating hepatic injuries that can result from the ingestion of toxic substances (i.e. blue-green algae, cyanamide, gossypol, and algae; Smith et al., 2013; Foreman, 2014). However, the SDH level of pigs drinking water source A was slightly out of the range and water characteristics did not change during the course of the experiment so we concluded that the elevated SDH level in pigs fed water source A was of no physiological significance.

Bilirubin of pigs fed water source A was significantly higher than for pigs fed water sources B and C ($P = 0.030$). One outlier was removed from the data set because it was more than two standard deviations from the mean. Bilirubin is a measure for improper liver function due to factors such as viral and bacterial infections, consumption of toxic chemicals, and poor nutrition (Hepatobiliary Disease, 1999; Karalyan et al., 2016). The elevation in blood bilirubin levels in pigs can also result from starvation or near starvation, suggesting low feed consumption of pigs post-weaning (Cornelius, 1980; Smith et al., 2013). However, feed intake for the first 7 days of the experiment for pigs fed water source A was 110 to 180 g per pig per day which was not different from the intake of pigs drinking water source B (110 to 180 g per pig per day) and water source C (130 to 170 g per pig per day). Feed intakes recorded in this study are consistent with industry published data suggesting that 150 to 200 g of feed intake per pig per day during the first

week post-weaning is common among commercial production conditions (Whittemore et al., 2001).

Collectively, blood chemistry measurements were not different among the varying water qualities. This observation suggests that on d-8 of the experiment, regardless of the water pigs were given, pigs' bodies were operating similarly.

Behavior

To assess whether pigs had an aversion to water that they were provided, video cameras were used to monitor pig behavior. Number of times the drinker was visited by pigs for more than one second, average time per visit, and total amount of time spent at the drinker per day was not influenced by quality of water pigs were given on d 1, 2, or 3 of the experiment. Similarly, over the course of d-1 to d-3 post-weaning, there were no observed differences among treatments in any of the three behavior measures evaluated (Figures 4.14, 4.15, and 4.16). This would suggest that pigs did not have any aversions to the water offered and were willing to drink the water they were given.

CONCLUSION

The three qualities of water compared in this controlled research setting did not yield differences in growth performance or health of nursery pigs. While we expected to see differences in some facet, we recognize the pig may be more tolerant of varying water quality than producers might expect. Further, as pigs are expected to adapt to the nursery environment rapidly post-weaning, they may adapt to water quality faster than expected especially combined with the presence of antibiotics in feed. The perceived poor-quality waters did not achieve the extreme levels of compounds that have depressed pig performance in literature reports. Therefore, the waters in this experiment may not have been of poor enough quality to negatively affect pig performance. It is possible that pigs of high health are less likely to respond negatively to poor

water quality due to the robustness of their immune systems. Lastly, observing growth performance differences is often challenging in studies with a small population, so more pigs or an identical replicate could be beneficial in seeing differences among waters. This experiment provides a foundation that future water quality research can be built upon. Specific research priorities should include: understanding characteristics of water, water management, and recommendations for water characteristics that support optimal pig performance and health.

Table 4.1. Initial composition of water provided to nursery pigs

Analyte	Water Source		
	Barn A	Barn B	Barn C
Arsenic, ppm	< 0.10	< 0.10	< 0.10
Bicarbonate (as CaCO ₃), ppm	397	375	270
Boron, ppm	0.25	0.24	0.13
Cadmium, ppm	< 0.002	< 0.002	< 0.002
Calcium, ppm	284	214	58.7
Carbonate (as CaCO ₃), ppm	< 1.0	< 1.0	< 1.0
Chloride, ppm	2	0	2
Chromium, ppm	< 0.01	< 0.01	< 0.01
Conductivity, mmhos/cm	2.31	1.62	0.536
Copper, ppm	n.d.	0.02	0.02
Fecal coliforms, cfu/100mL	< 2	< 2	< 2
Fluoride, ppm	0.2	0.2	0.4
Hardness, mg EQ CaCO ₃ /L	1410	909	235
Iron, ppm	5.43	5.22	1.33
Lead, ppm	< 0.05	< 0.05	< 0.05
Magnesium, ppm	171	90.9	21.4
Manganese, ppm	0.048	0.117	0.045
Mercury, ppm	< 0.0004	< 0.0004	< 0.0004
Nickel, ppm	< 0.01	< 0.01	< 0.01
Nitrate, ppm	n.d. ^a	n.d. ^a	n.d. ^a
Nitrite (NO ₂), ppm	< 0.02	< 0.02	< 0.02
pH	8	8	7.5
Phosphorus, ppm	0.12	0.15	0.1
Potassium, ppm	5.34	6.33	2.67
SAR ^b	0.7	0.5	0.8
Sodium, ppm	64	37.4	29.4
Sulfate, ppm	1120	617	2
TDS, ppm ^c	1500	1050	348
Zinc, ppm	0.03	< 0.01	0.05

^a n.d., Not detected

^b SAR; Sodium absorption ratio

^c TDS; Total dissolved solids

Table 4.2. Ingredient and nutrient composition of nursery diets (as-fed basis)

Ingredient, %	Phase 2 ^a	Phase 3 ^b	Phase 4 ^c
Corn	47.25	54.00	64.84
Soybean meal	12.50	25.00	30.50
Titanium dioxide pre-blend ^d	4.00	-	-
Soy oil	1.25	1.00	1.00
Aureomycin 50® ^e	0.10		
Dical 21%	0.51	0.69	0.91
Calcium carbonate	0.47	0.49	0.90
Salt, White	0.46	0.40	0.58
L-Lysine 98.5%	0.39	0.47	0.48
Zinc oxide 72%	0.32	0.32	-
Vitamin trace mineral premix	0.25	0.27	0.17
Specialty proteins ^f	30.37	16.09	-
Other ^g	2.24	1.28	0.64
Calculated nutrient composition:			
ME, kcal/kg	3,372.21	3,367.25	3,364.72
Crude protein, %	21.96	21.69	21.29
Crude fat, %	4.26	4.05	3.92
Calcium, %	0.68	0.71	0.63
Phosphorus, %	0.66	0.63	0.55
SID Lys, % ^h	1.42	1.39	1.27
SID Trp, % ^h	0.26	0.26	0.23
SID Met + Cys, % ^h	0.80	0.81	0.72
SID Thr, % ^h	0.91	0.86	0.77

^a All ingredients minus corn, soybean meal, soy oil, and pre-blend are provided by Nursery Base 700 (Team Nutrition, Inc., Cyrus, MN)

^b All ingredients minus corn, soybean meal, and soy oil provided by TNI 400 Nursery Base (Team Nutrition, Inc., Cyrus, MN)

^c All ingredients minus corn, soybean meal, and soy oil provided by TNI 25-80 NG Premix (Team Nutrition, Inc., Cyrus, MN)

^d Composed of 46.5% Soybean Meal (87.5%) and titanium dioxide (12.5%)

^e Aureomycin 50® (Zoetis, Parsippany-Troy Hills, NJ) added to Phase 2 diet to control *Streptococcus suis*

^f Specialty proteins (mix of specialty animal and plant proteins)

^g Other (mixture of carbohydrate sources, synthetic amino acids, flavors, preservatives, and yeast products)

^h SID is standard ileal digestible

Table 4.3. Analysis of water source A from beginning to end of experiment

Analyte	Water A			
	Initial ^a	Arrival ^b	End of first delivery ^c	Final ^d
Arsenic, ppm	< 0.10	< 0.10	< 0.10	< 0.10
Bicarbonate (as CaCO ₃), ppm	397	398	387	398
Boron, ppm	0.25	0.25	0.25	0.21
Cadmium, ppm	< 0.002	< 0.002	< 0.002	< 0.002
Calcium, ppm	284	259	275	264
Carbonate (as CaCO ₃), ppm	< 1.0	1.6	1.3	< 1.0
Chloride, ppm	2	1	1	2
Chromium, ppm	< 0.01	< 0.01	< 0.01	< 0.01
Conductivity, mmhos/cm	2.31	2.33	2.28	2.16
Copper, ppm	n.d. ^e	n.d. ^e	n.d. ^e	n.d. ^e
Fecal coliforms, cfu/100mL	< 2	< 2	< 10	< 4
Fluoride, ppm	0.20	0.20	0.20	0.20
Hardness, mg CaCO ₃ /L	1,410	1,260	1,370	1,330
Iron, ppm	5.43	1.52	0.66	1.20
Lead, ppm	< 0.05	< 0.05	< 0.05	< 0.05
Magnesium, ppm	171	149	165	163
Manganese, ppm	0.048	0.046	0.044	0.048
Mercury, ppm	< 0.0004	< 0.0004	< 0.0004	< 0.0004
Nickel, ppm	< 0.01	< 0.01	< 0.01	< 0.01
Nitrate, ppm	n.d. ^e	n.d. ^e	n.d. ^e	n.d. ^e
Nitrite (NO ₂), ppm	< 0.02	< 0.02	< 0.02	< 0.02
pH	8.00	7.62	7.38	7.32
Phosphorus, ppm	0.12	< 0.05	< 0.05	< 0.05
Potassium, ppm	5.34	5.40	5.63	5.49
SAR	0.70	0.70	0.80	0.80
Sodium, ppm	64	60.7	65.6	63.4
Sulfate, ppm	1,120	1,100	1,130	1,130
TDS, ppm	1,500	1,510	1,480	1,400
Zinc, ppm	0.03	0.05	0.03	0.02

^a Sample collected 7/22/2019 at the source barn for water A

^b Sample collected 9/12/2019 at WCROC

^c Sample collected 10/7/2019 at WCROC

^d Sample collected 10/23/2019 at WCROC

^e n.d. Not Detected

Table 4.4. Analysis of water source B from beginning to end of experiment

Analyte	Water B			
	Initial ^a	Arrival ^b	End of first delivery ^c	Final ^d
Arsenic, ppm	< 0.10	< 0.10	< 0.10	< 0.10
Bicarbonate (as CaCO ₃), ppm	375	388	389	410
Boron, ppm	0.24	0.22	0.23	0.2
Cadmium, ppm	< 0.002	< 0.002	< 0.002	< 0.002
Calcium, ppm	214	214	223	220
Carbonate (as CaCO ₃), ppm	< 1.0	1.2	1.1	< 1.0
Chloride, ppm	n.d. ^e	n.d. ^e	n.d. ^e	n.d. ^e
Chromium, ppm	< 0.01	< 0.01	< 0.01	< 0.01
Conductivity, mmhos/cm	1.62	1.67	1.64	1.56
Copper, ppm	0.02	n.d. ^e	n.d. ^e	n.d. ^e
Fecal coliforms, cfu/100mL	< 2	4	< 10	< 10
Fluoride, ppm	0.20	0.20	0.20	0.20
Hardness, mg CaCO ₃ /L	909	897	931	918
Iron, ppm	5.22	0.67	7.94	8.51
Lead, ppm	< 0.05	< 0.05	< 0.05	< 0.05
Magnesium, ppm	91	88	88.5	90
Manganese, ppm	0.117	0.108	0.111	0.111
Mercury, ppm	< 0.0004	< 0.0004	< 0.0004	< 0.0004
Nickel, ppm	< 0.01	< 0.01	< 0.01	< 0.01
Nitrate, ppm	n.d. ^e	n.d. ^e	n.d. ^e	n.d. ^e
Nitrite (NO ₂), ppm	< 0.02	< 0.02	< 0.02	< 0.02
pH	8.00	7.52	7.33	7.24
Phosphorus, ppm	0.15	< 0.05	0.05	< 0.05
Potassium, ppm	6.33	6.03	6.17	6.05
SAR	0.50	0.50	0.50	0.50
Sodium, ppm	37.4	36.9	37.3	36.2
Sulfate, ppm	617	613	613	632
TDS, ppm	1,050	1,080	1,070	1,010
Zinc, ppm	< 0.01	0.01	0.02	< 0.01

^a Sample collected 7/16/2019 at the source barn for water B

^b Sample collected 9/12/2019 at WCROC

^c Sample collected 10/7/2019 at WCROC

^d Sample collected 10/23/2019 at WCROC

^e n.d. Not Detected

Table 4.5. Analysis of water source C from beginning to end of experiment

Analyte	Water C			
	Initial ^a	Arrival ^b	End of first delivery ^c	Final ^d
Arsenic, ppm	< 0.10	< 0.10	-	< 0.10
Bicarbonate (as CaCO ₃), ppm	270	279	-	280
Boron, ppm	0.13	0.14	-	0.12
Cadmium, ppm	< 0.002	< 0.002	-	< 0.002
Calcium, ppm	58.7	54.7	-	57.2
Carbonate (as CaCO ₃), ppm	< 1.0	1.2	-	< 1.0
Chloride, ppm	2	2	-	2
Chromium, ppm	< 0.01	< 0.01	-	< 0.01
Conductivity, mmhos/cm	0.54	0.54	-	0.50
Copper, ppm	0.02	n.d. ^e	-	0.02
Fecal coliforms, cfu/100mL	< 2	< 2	-	8
Fluoride, ppm	0.40	0.40	-	0.40
Hardness, mg EQ CaCO ₃ /L	235	218	-	230
Iron, ppm	1.33	0.51	-	8.15
Lead, ppm	< 0.05	< 0.05	-	< 0.05
Magnesium, ppm	21	20	-	21
Manganese, ppm	0.045	0.046	-	0.062
Mercury, ppm	< 0.0004	< 0.0004	-	< 0.0004
Nickel, ppm	< 0.01	< 0.01	-	< 0.01
Nitrate, ppm	n.d. ^e	n.d. ^e	-	n.d. ^e
Nitrite (NO ₂), ppm	< 0.02	< 0.02	-	< 0.02
pH	7.50	7.67	-	7.42
Phosphorus, ppm	0.10	0.28	-	0.28
Potassium, ppm	2.67	8.27	-	2.41
SAR	0.80	0.80	-	0.80
Sodium, ppm	29.4	28.1	-	29.2
Sulfate, ppm	2	3	-	n.d. ^e
TDS, ppm	348	352	-	325
Zinc, ppm	0.05	< 0.01	-	0.02

^a Sample collected 7/22/2019 at the source barn for water C

^b Sample collected 9/12/2019 at WCROC

^c Sample was not collected due to a logistical error

^d Sample collected 10/23/2019 at WCROC

^e n.d. Not Detected

Table 4.6. Weekly bacterial counts of water from drinkers in WCROC nursery barn

Item	Water A ^a	Water B ^b	Water C ^c
Generic <i>E. coli</i> , MPN/100mL			
Initial ^d	<1	8	1
Wk 1	>2,400	>2,400	>2,400
Wk 2	>2,400	687	687
Wk 3	>2,400	126	>2,400
Wk 4	142	>2,400	866
Wk 5	>2,400	>2,400	>2,400
Wk 6	>2,400	345	>2,400
Total Coliforms, MPN/100mL			
Initial ^d	>2,400	2,420	26
Wk 1	>2,400	>2,400	>2,400
Wk 2	>2,400	>2,400	>2,400
Wk 3	>2,400	>2,400	>2,400
Wk 4	>2,400	>2,400	>2,400
Wk 5	>2,400	>2,400	>2,400
Wk 6	>2,400	>2,400	>2,400
Aerobic Plate Count, cfu/mL			
Initial ^d	139,000	115,000	1,194,000
Wk 1	774,500	1,017,000	653,000
Wk 2	745,000	640,000	720,000
Wk 3	644,000	625,500	7,600,000
Wk 4	3,345,000	2,200,000	1,670,000
Wk 5	655,000	1,750,000	3,100,000
Wk 6	760,000	1,140,000	2,100,000

^a Water source A samples pooled from pens: 6, 34, 57

^b Water source B samples pooled from pens: 3, 24, 42

^c Water source C samples pooled from pens: 11, 39, 62

^d Initial sample was collected on the day pigs arrived (9/11/19) prior to pig placement

Table 4.7. Effect of differing water qualities on initial and final BW of nursery pigs

Item	Treatment			SE	<i>P</i> value
	Water A	Water B	Water C		
No. of pens	15	15	15	-	-
No. of pigs	150	150	150	-	-
Initial BW, kg	6.18	6.20	6.34	0.150	0.691
Final BW, kg	24.60	24.55	25.37	0.440	0.357

Table 4.8. Effects of water quality on apparent nutrient digestibility in diets fed to nursery pigs (d 10, 11, and 12 of experiment)

Item, %	Treatment			SE	<i>P</i> -value
	Water A	Water B	Water C		
No. of observations	15	15	15	-	-
Dry matter	79.05	78.00	78.30	0.41	0.195
Crude protein	71.72	69.84	70.05	1.07	0.170
Crude fiber	26.04	16.34	16.17	3.34	0.995
Ash	56.57 ^a	56.55 ^a	59.02 ^b	0.66	0.016
Ether extract	5.62	2.40	6.60	4.69	0.804
Gross energy	76.45	75.12	75.24	0.50	0.125

^{ab} Means within a row with different superscripts differ ($P < 0.05$).

Table 4.9. Effect of water quality on intestinal integrity of nursery pigs

Ratio	Treatment			SE	<i>P</i> -value
	Water A	Water B	Water C		
No. of observations	8	8	8	-	-
Xylose/Rhamnose	2.97	1.99	2.39	0.457	0.337
Rhamnose/3-O-Methyl-Glucose	1.39	2.03	1.36	0.237	0.143
Xylose/3-O-Methyl-Glucose	3.67	3.64	3.03	0.557	0.655

Table 4.10. Effect of water quality on morbidity and mortality of nursery pigs

Item	Treatment			P-value
	Water A	Water B	Water C	
Total pigs, no.	150	150	150	-
Pigs treated, no.	9	5	8	0.472
Injections administered, no. ^a	20	19	18	0.606
Mortality, no.	0	1	1	-

^a Injections administered are injections of antibiotics for pigs that exhibited compromised health.

Table 4.11. Effect of differing water qualities on plasma cytokine concentrations of nursery pigs (d 8 of experiment)

Item	Treatment			SE	P-value
	Water A	Water B	Water C		
No. of observations	24	24	24	-	-
GM-CSF, pg/mL ^a	1,605	1,748	1,566	-	1.000
IFN γ , pg/mL ^b	89,783	88,097	120,401	4,867	0.960
IL-1 α , pg/mL ^c	700	682	693	24	0.860
IL-1 β , pg/mL ^d	3,758	3,544	3,587	145	0.538
IL-1ra, pg/mL ^e	7,201	7,059	6,383	329	0.223
IL-2, pg/mL	11,234	10,497	10,648	419	0.431
IL-4, pg/mL	118,032	115,685	120,401	8,464	0.931
IL-6, pg/mL	4,049	3,684	3,884	215	0.480
IL-8, pg/mL	523	535	445	31	0.101
IL-10, pg/mL	20,102	19,415	19,776	3,049	0.899
IL-12, pg/mL	3,246	3,353	3,442	117	0.845
IL-18, pg/mL	32,194	31,394	32,215	1,318	0.891
TNF, pg/mL ^f	1,248	1,261	1,050	89	0.195

^a Granulocyte-macrophage colony-stimulating factor

^b Interferon-gamma

^c Interleukin-1 alpha

^d Interleukin-1 beta

^e Interleukin-1 receptor antagonist

^f Tumor necrosis factor

Table 4.12. Effect of water quality on percentage (%) of total monocytes and granulocytes displaying phagocytosis in nursery pigs (d 11 of the experiment)

Item	Treatment			SE	P-value
	Water A	Water B	Water C		
No. of observations	8	8	8	-	-
Total Monocytes	74.28	73.20	74.49	2.296	0.913
Total Granulocytes	95.27	93.56	93.79	0.967	0.451

Table 4.13. Effect of differing water qualities on blood chemistry of nursery pigs (d 8 of experiment)

Item	Treatment				SE	P-value	Ref. Ranges ^a
	Water A	Water B	Water C				
No. of observations	24	24	24	-	-	-	
Glucose, mg/dL	103.2	103.9	105.8	2.93	0.80	57 – 113	
AST, U/L ^b	40.3	38	36.7	2.91	0.68	14 – 61	
SDH, U/L ^c	24.6	23.4	23	1.07	0.55	4.2 – 24.3	
Bilirubin, mg/dL	0.35 ^x	0.19 ^{xy}	0.16 ^y	0.05	0.03	0.0 – 0.4	
Cholesterol, mg/dL	78	73	66.5	4.58	0.21	53 – 103	
Total Protein, g/dL	4.2	4.4	4.2	0.19	0.79	4.0 – 8.4	
Albumin, g/dL	3.1	3.1	3	0.09	0.54	2.0 – 4.4	
Urea N, mg/dL	8.1	7.9	8.2	0.96	0.97	5 – 24	
Creatinine, mg/dL	1.1	1	1	0.04	0.88	0.5 – 0.6	
Phosphorus, mg/dL	8.5	8.5	8.4	0.18	0.90	5.3 – 11.1	
Calcium, mg/dL	10.6	10.5	10	0.4	0.46	8.8 – 11.2	
Potassium, mmol/dL	5.78	5.87	6.05	0.15	0.48	123 – 144	
Sodium, mmol/dL	142	143	143	0.94	0.50	3.1 – 6.7	
Chloride, mmol/dL	104	106	106	0.84	0.42	84 – 106	
CK, U/L ^d	240	382	332	70.05	0.35	129 – 1,409	
Gamma-CT, U/L	25.98	23.22	24.25	2.13	0.65	23 – 62	
Anion Gap, mmol/L	19	19	18	0.82	0.44	10 – 27	
Globulin, g/dL	1.4	1.3	1.3	0.05	0.20	1.6 – 4.9	
A/G Ratio	2.3	2.4	2.4	0.12	0.60	-	
Urea-Creatinine Ratio	7.5	7.6	7.8	0.78	0.96	-	
Sodium-Potassium Ratio	24.7	24.5	23.6	0.55	0.31	-	
Bicarbonate, mmol/dL	23	24	26	1.00	0.09	22 - 36	

^a Reference ranges of Marshfield Labs. Marshfield, WI.

^b AST; aspartate aminotransferase

^c SDH; sorbitol dehydrogenase

^d CK; creatine kinase

^{xy} Means within a row with different superscripts differ ($P < 0.05$).



Figure 4.1. Photo of filled water bladders under a lean-to shelter (North side)



Figure 4.2. Photo of full (foreground) and empty water bladders (West side)



Figure 4.3. Photo of full water bladders and pump connections (East side)



Figure 4.4. Photo of water system and manifold from ceiling to standpipe

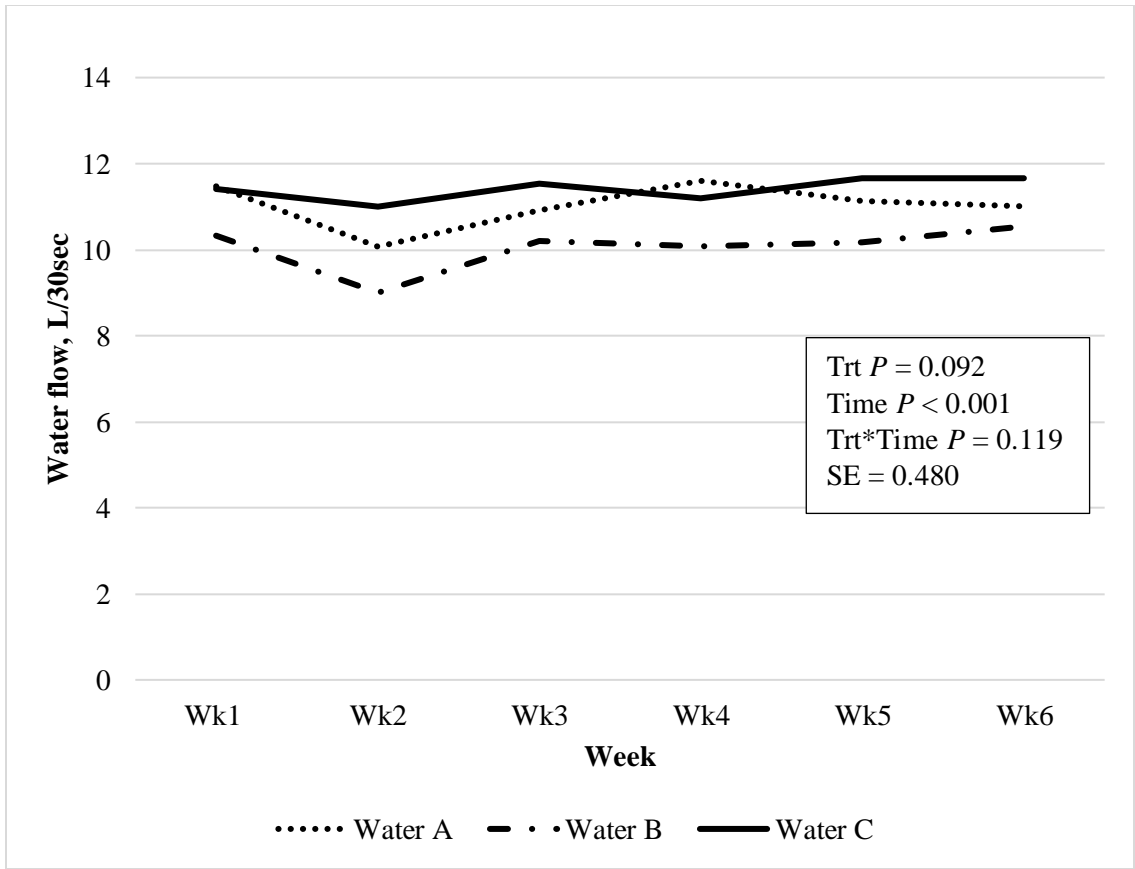


Figure 4.5. Average water flow at each drinker over the 6 wk period in pens with different qualities of water.

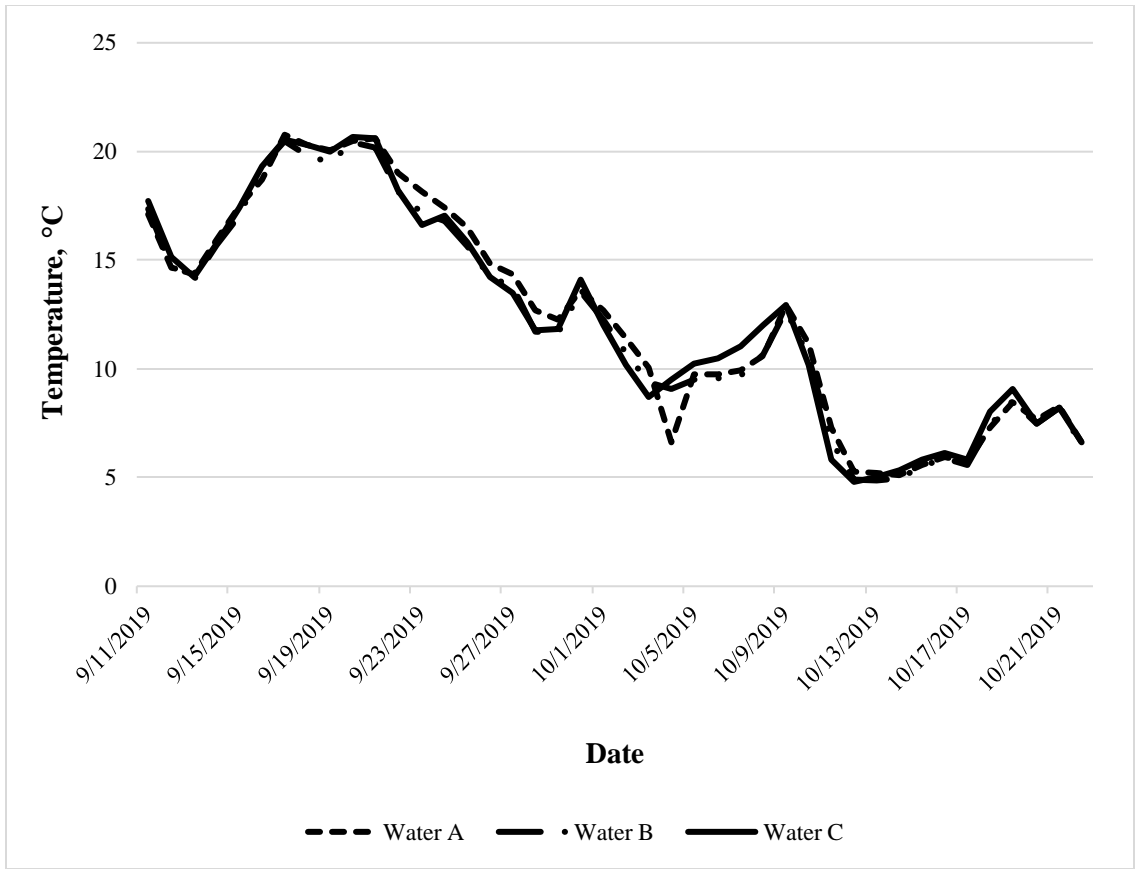


Figure 4.6. Average ambient daytime temperature of water bladders. Data summarized into 12-h intervals from 0700 h to 1900 h

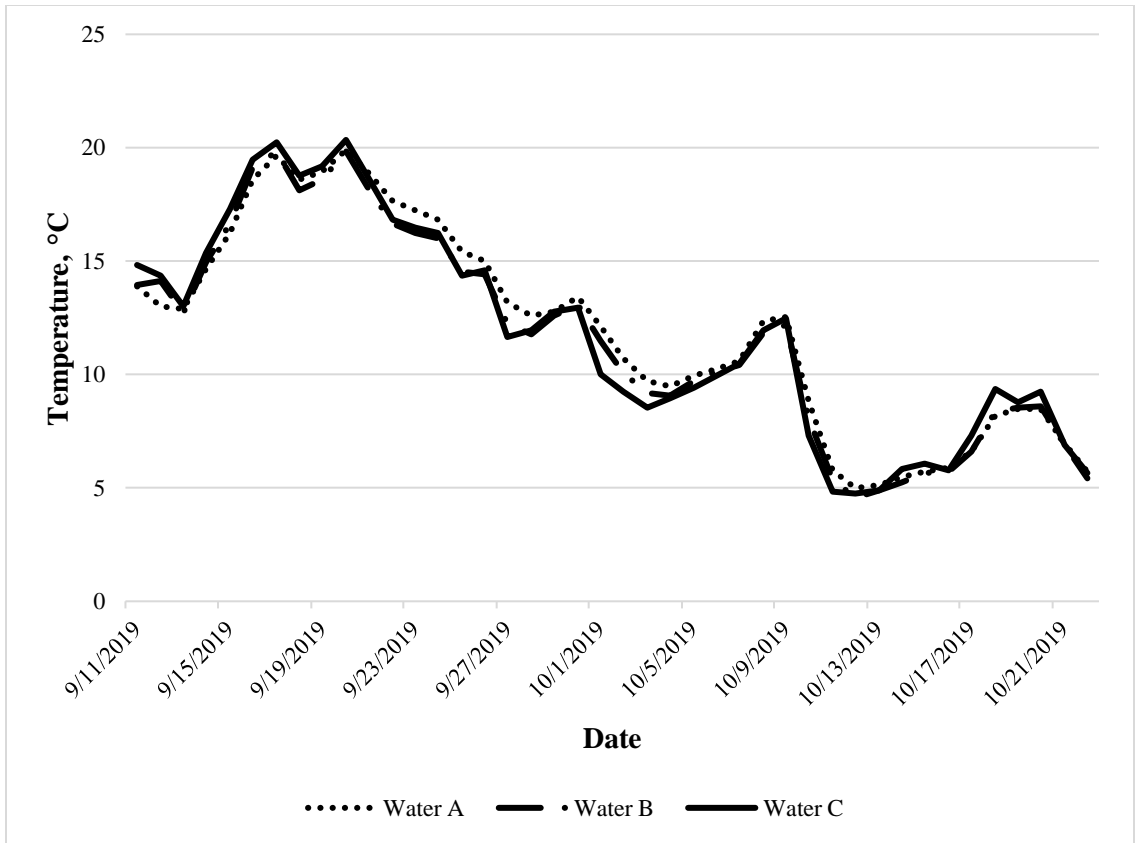


Figure 4.7. Average ambient nighttime temperature of water bladders. Data summarized into 12-h intervals from 1900 h to 0700 h

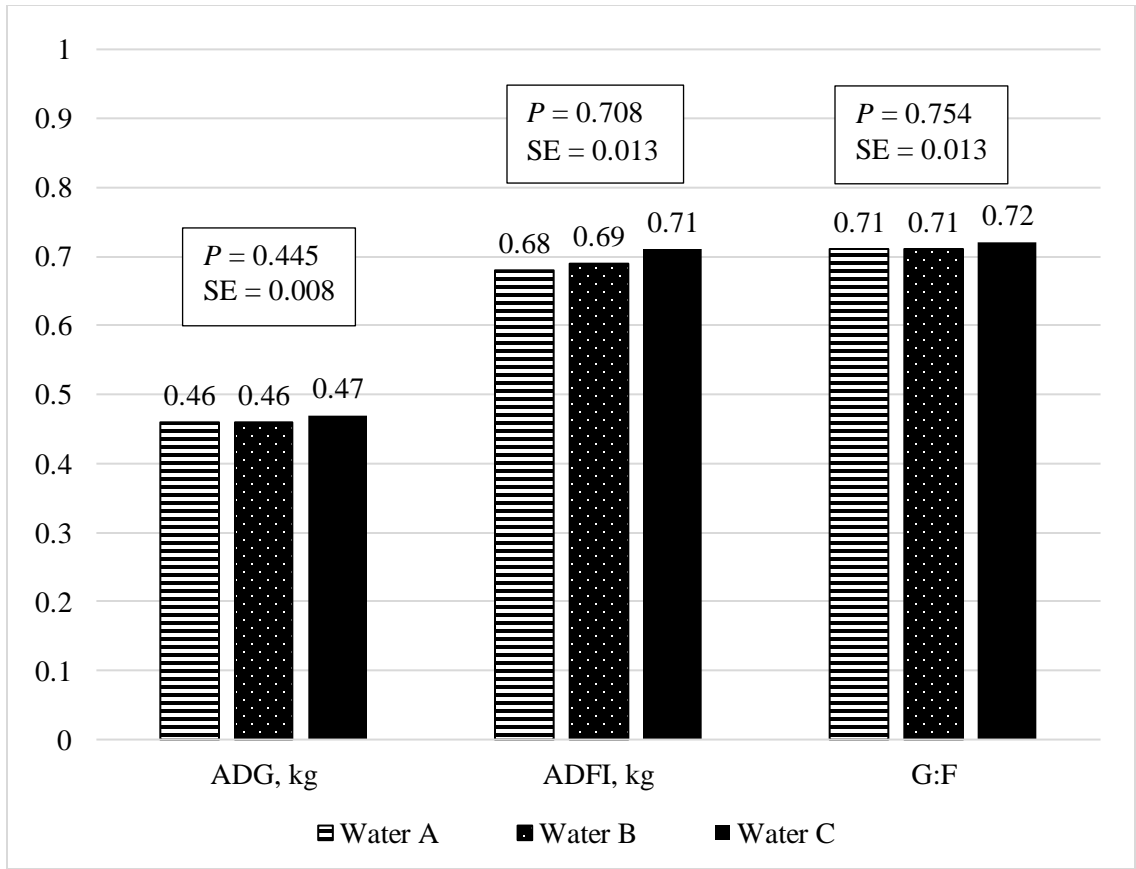


Figure 4.8. Overall growth performance of nursery pigs fed differing waters

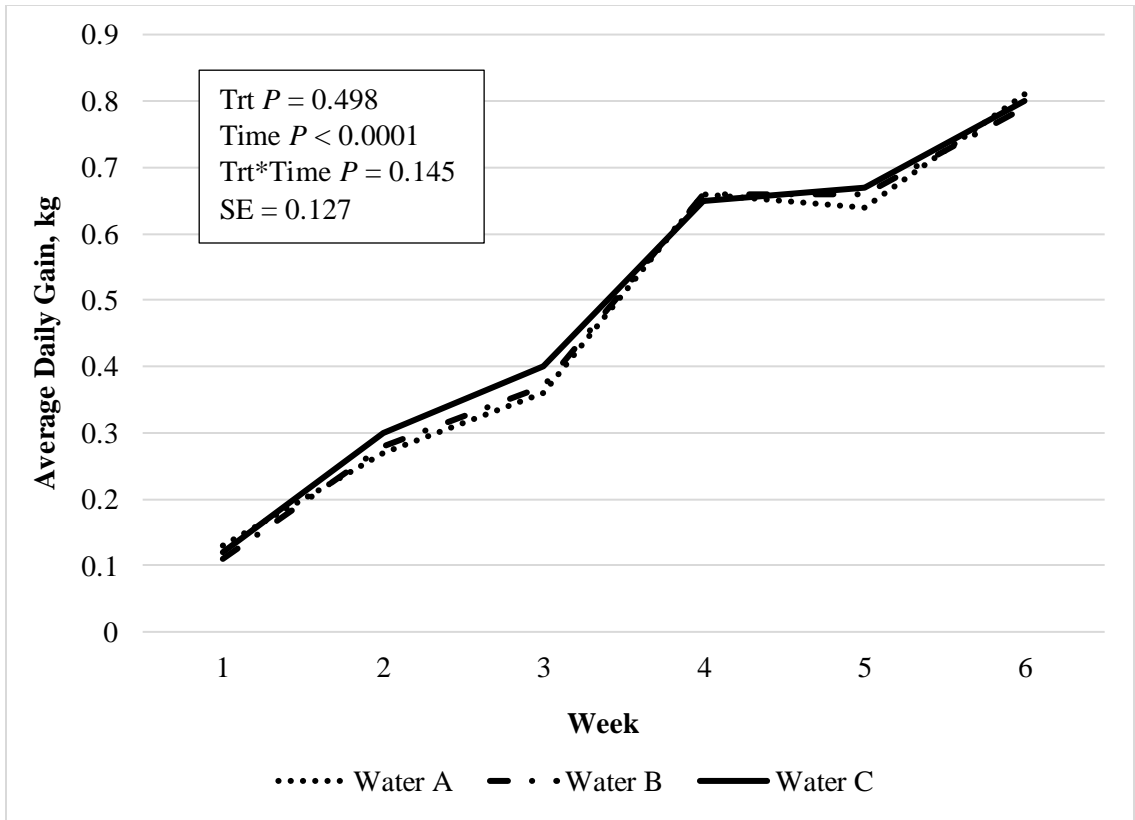


Figure 4.9. Effect of water quality on average daily gain of nursery pigs over time

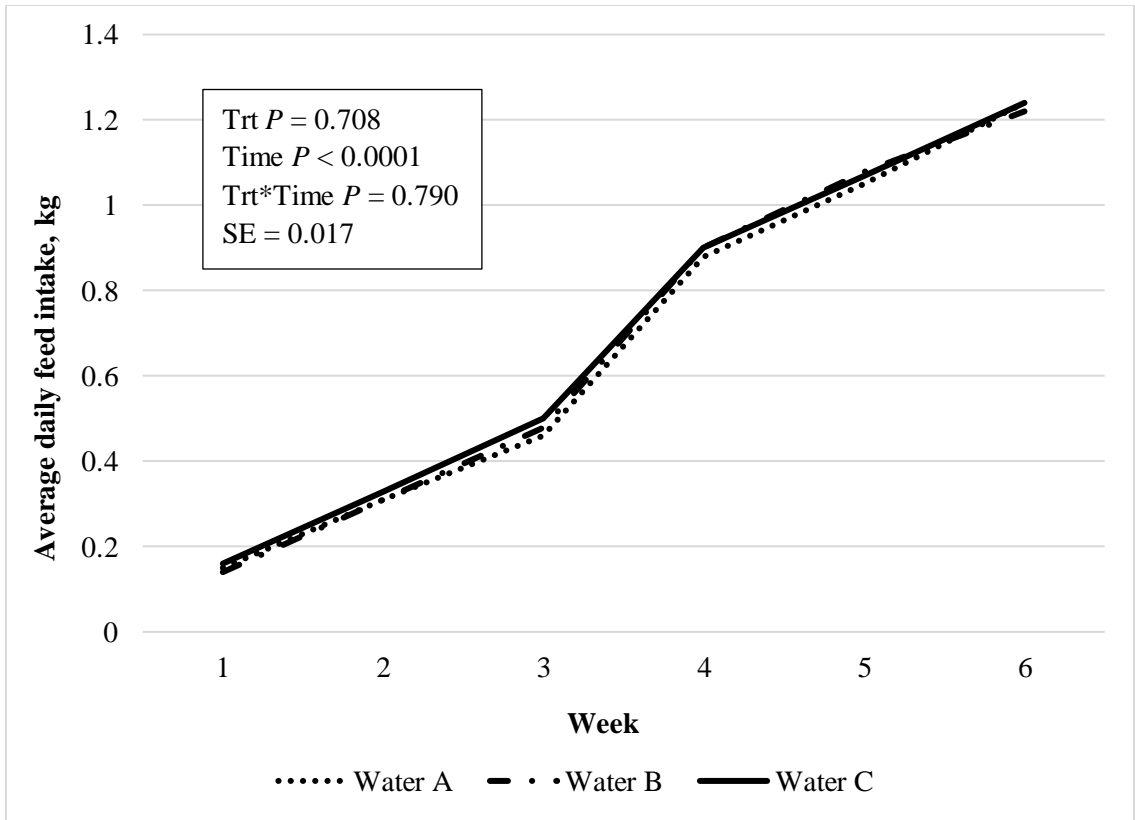


Figure 4.10. Effect of water quality on average daily feed intake of nursery pigs over time

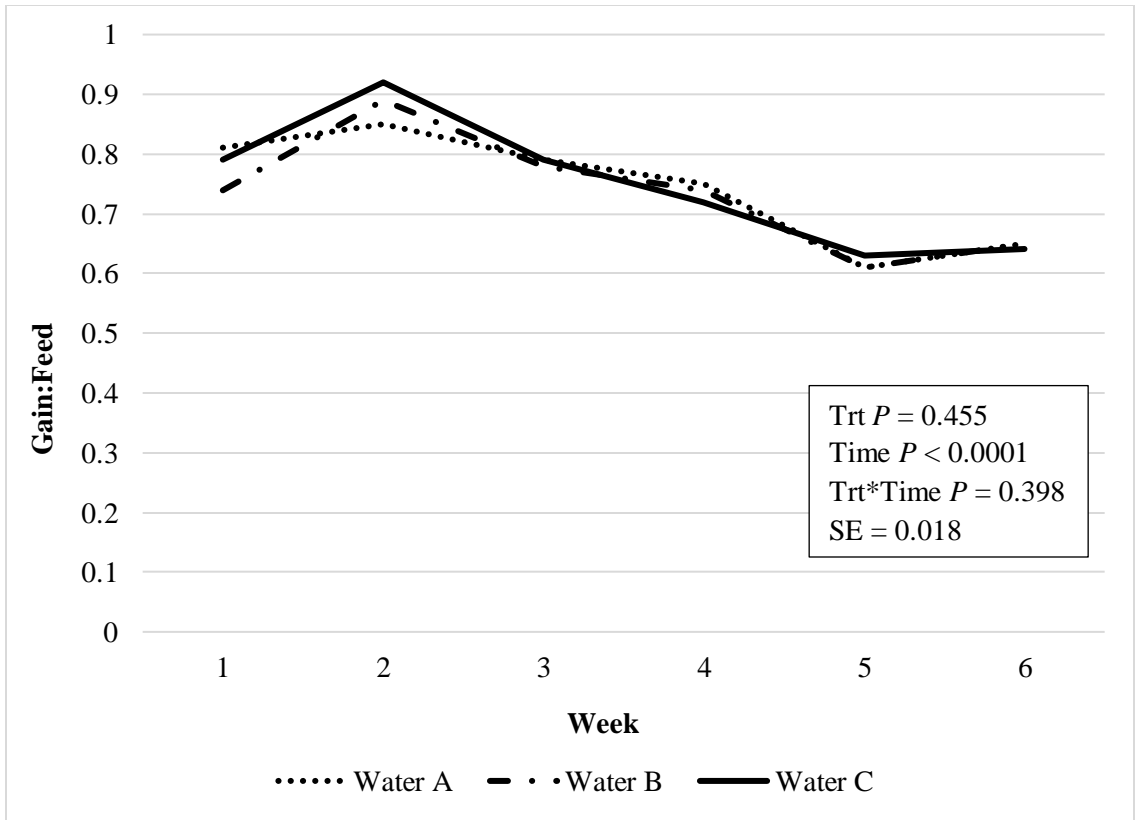


Figure 4.11. Effect of water quality on gain efficiency of nursery pigs over time

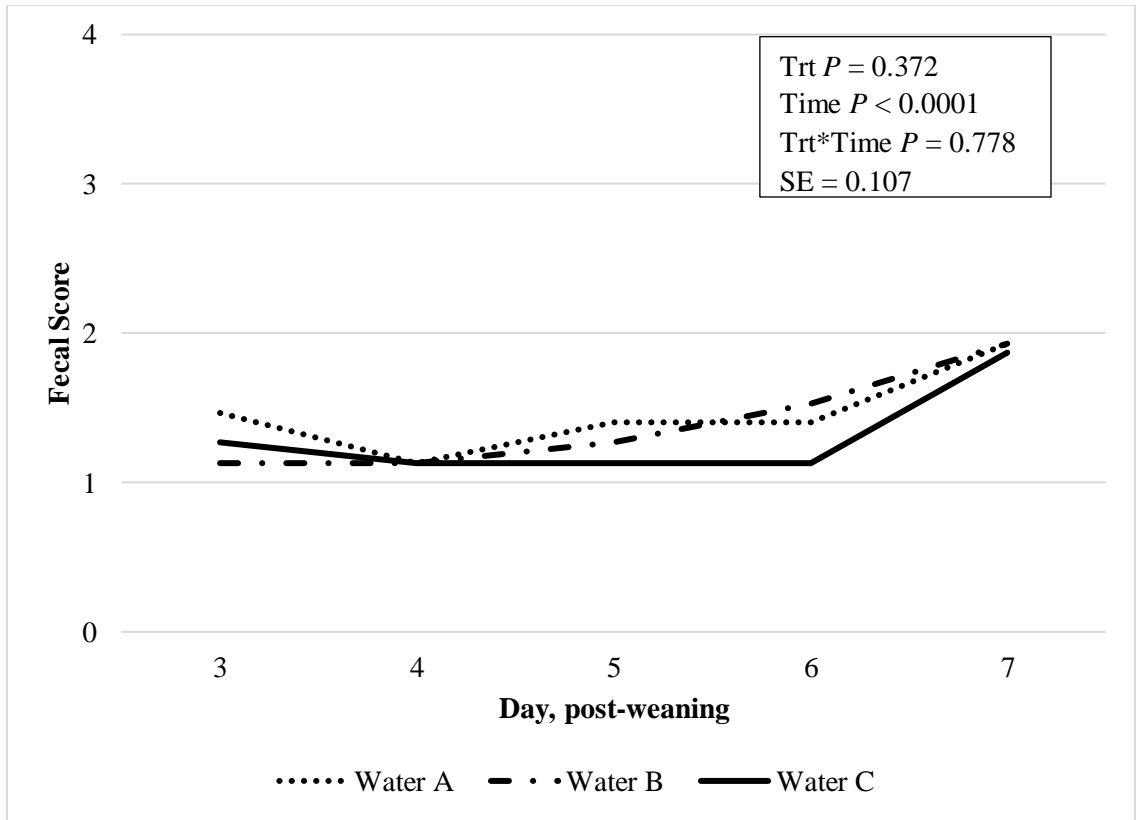


Figure 4.12. Effect of water quality on average fecal score of nursery pigs over time (d 3 to d 7 post-weaning)

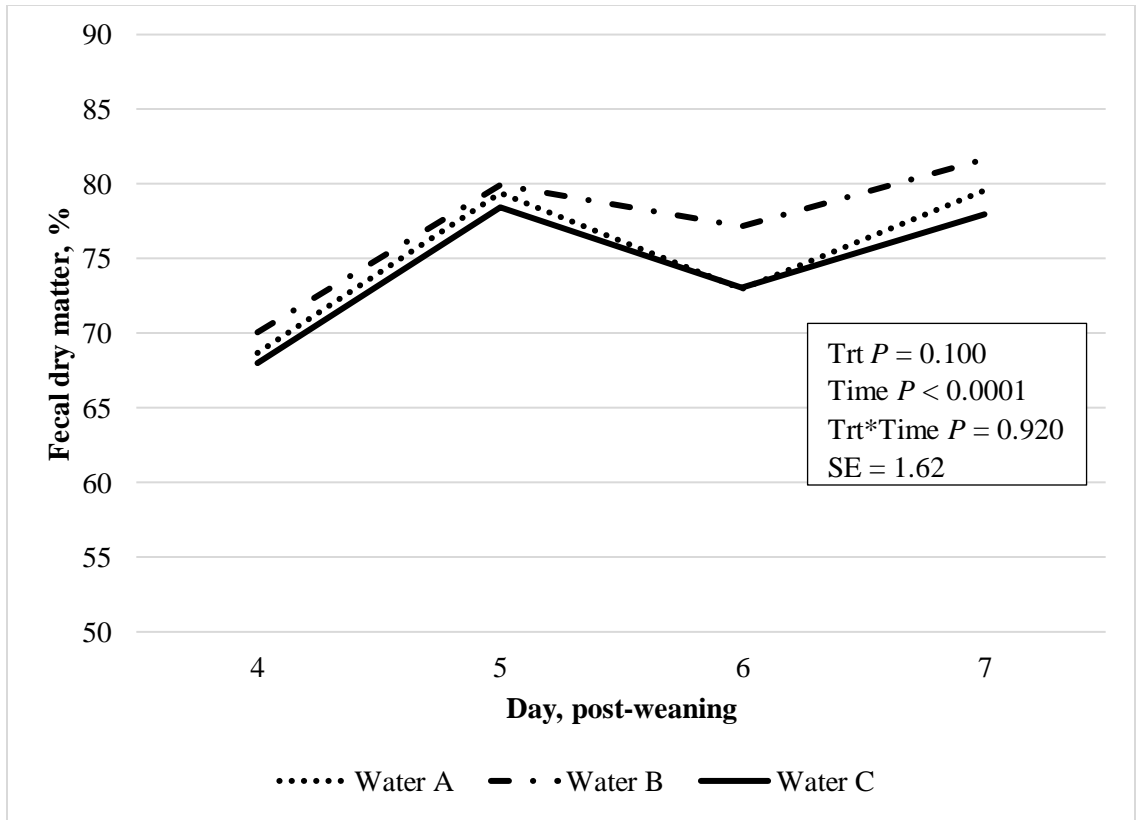


Figure 4.13. Effect of water quality on average fecal dry matter (%) of nursery pigs over time (d 4 to 7 post-weaning)

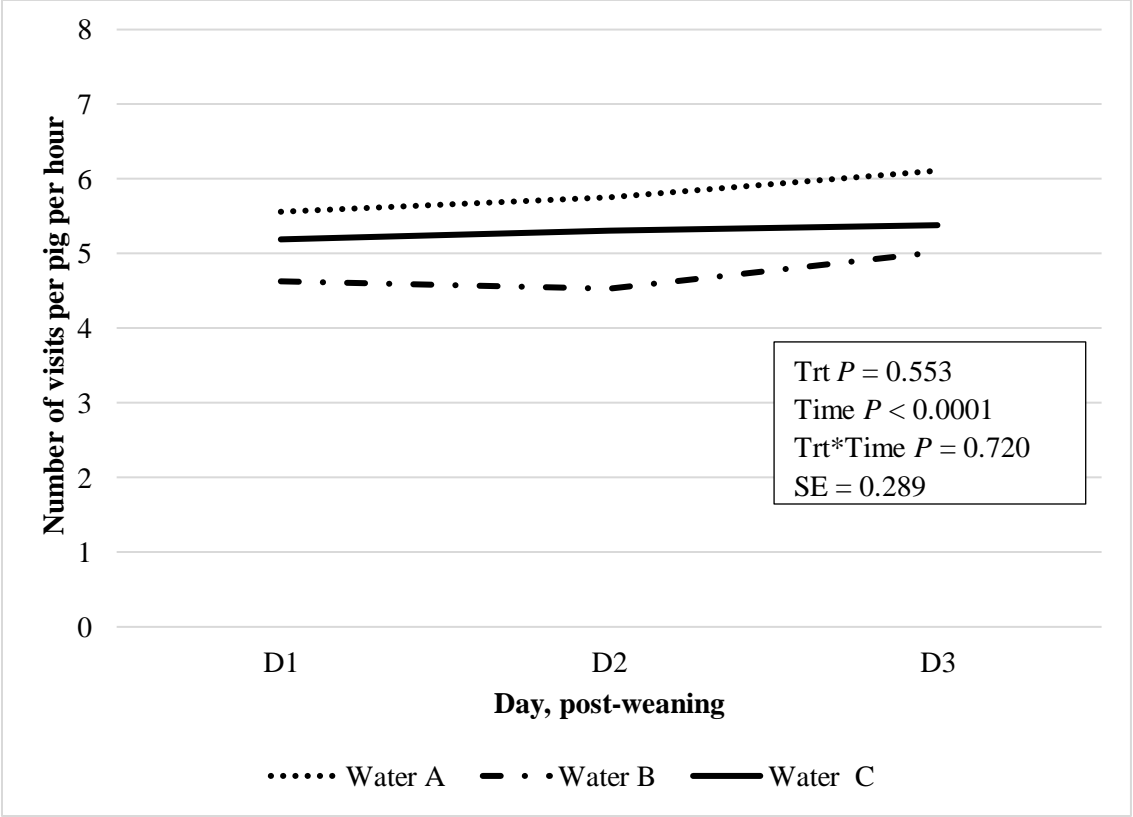


Figure 4.14. Effect of water quality on the average number of visits to the drinker per pig per hour (d 1 to 3 post-weaning)

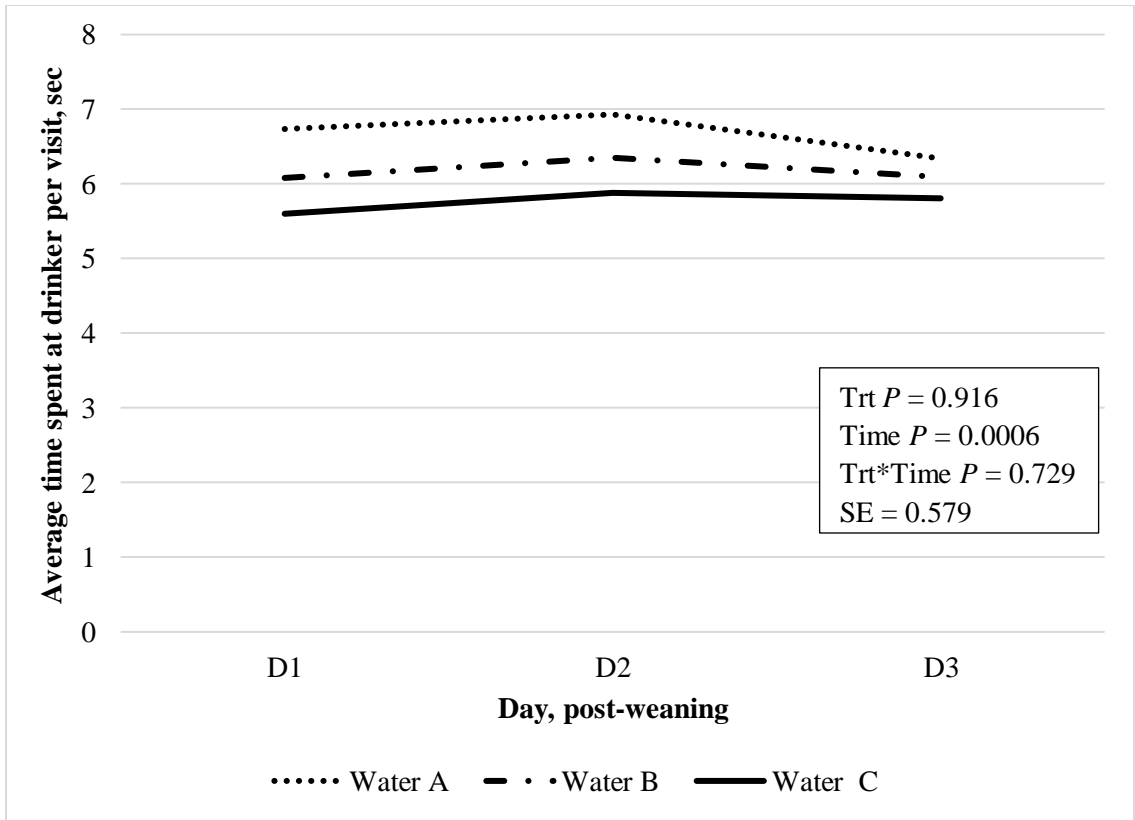


Figure 4.15. Effect of water quality on the average time spent at the drinker per visit (d 1 to 3 post-weaning)

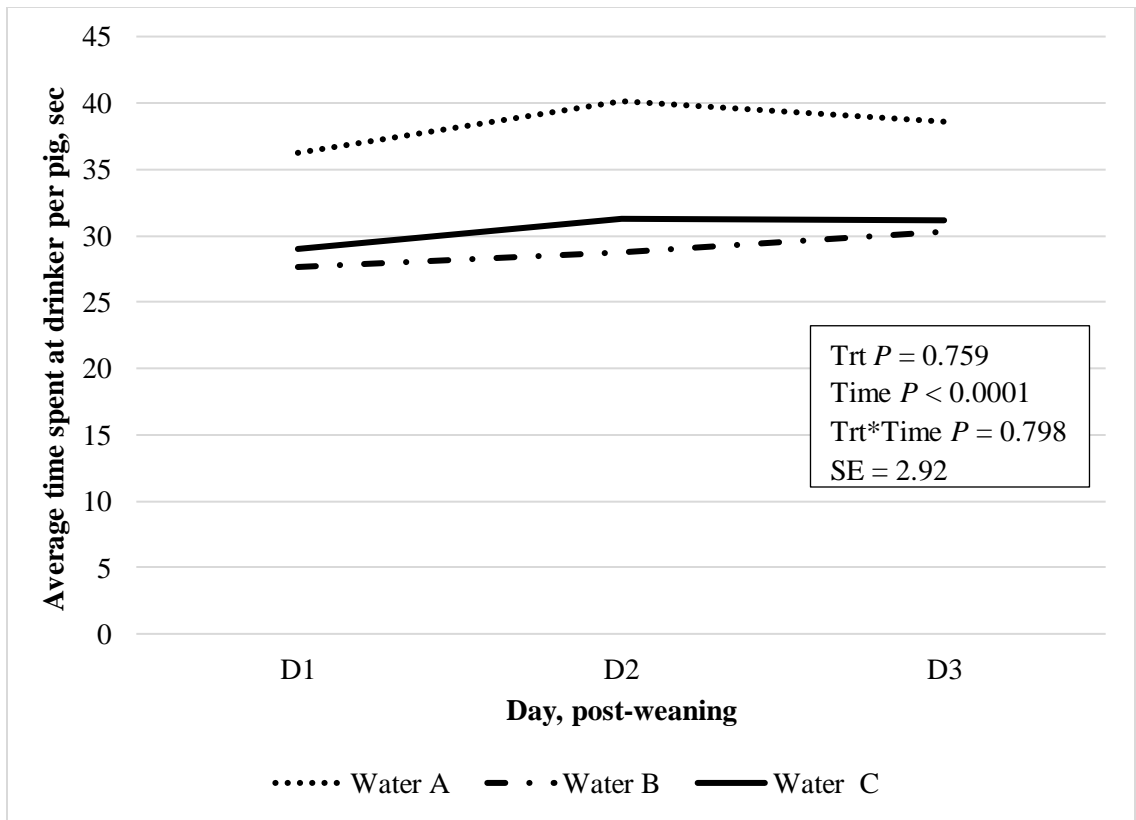


Figure 4.16. Effect of water quality on the average time spent at the drinker per pig (d 1 to 3 post-weaning)

CHAPTER 5: OVERALL SUMMARY

Water makes up the largest portion of a pig's body and is an essential nutrient for most all physiological processes within the body. Because water is so critical to the pig, it is valuable to understand the pig's requirements for water. Pigs can obtain water through three methods: mainly drinking water and small amounts from moisture in the diet and metabolic water. However, water needs of the pig are typically satisfied by voluntary consumption of water. Due to the difficulty associated with determining true water wastage, defining water requirements for pigs is challenging. Consequently, "water intake" estimates are better explained as water disappearance which is more easily able to be measured in commercial production. Estimated water requirements are calculated as a ratio of water to feed intake but fail to include a number of factors that can alter water intake leading to overestimation. As the pig grows and our expectations for different productive tasks changes, requirements for water intake also change.

Maintaining proper water balance within the pig's body is also crucial for health and growth reasons, thus pigs must also excrete water. Main water losses come from urination and defecation while a small constant loss occurs from respiration. A number of factors can disrupt the balance of water in pigs including social and thermal stresses. The weaning event specifically causes a large disruption in the pig's water balance which decreases water intake as they transition from a liquid diet to a solid diet. Additionally, water intake is generally low during the first week post-weaning, which can result in compromised performance and health.

Ground (well) water and municipal water supplies make up a majority of water sources used in Minnesota swine production. Providing pigs with high quality water is important to producers but the term "quality" is relatively misunderstood. Published livestock water quality guidelines were adapted from human standards about 40 years ago and may not be applicable to pigs because pigs can adapt to a wide range of water quality. There is a lack of published research in understanding optimal recommended levels of many characteristics found in water. Available

water research with swine has focused primarily on individual analytes in isolation, but not water quality holistically and the impact it may have on growth performance and health. This lack of research causes challenges in defining what should be considered as “good” and “poor” water for the pig.

Results obtained from the survey in Chapter 3 suggest that water quality perceptions and priorities vary among swine producers in Minnesota. The survey also indicated that producers believe water quality is important and can have a negative impact on growth performance and health of their pigs but only half of the producers had analyzed the water quality to determine analyte concentrations. Furthermore, analyses of water quality from some survey respondents revealed that quality of water varies in nursery barns throughout Minnesota. However, distinguishing “good” water from “poor” water for a pig experiment was challenging. Finally, two barns with similar water quality were selected and considered to have “poor” quality due to high concentrations of many analytes that came close to exceeding recommended levels. Another barn was selected and considered to have “good” quality water due to a large number of analytes having very low concentrations.

Results obtained from the pig experiment in Chapter 4 suggest that when comparing the specific qualities of water sources selected for this experiment, there were no effects on growth performance or health of nursery pigs. Additionally, no differences were observed throughout the study in mortality and occurrences of morbidity or diarrhea. Behavior of pigs also was not different, suggesting that pigs did not have an aversion to the quality of water they were provided. Moreover, diet digestibility, gut permeability, and immune functions of pigs did not differ across all treatments.

The presented survey and experiment had a few limitations. Firstly, survey responses came from a relatively concentrated area in Minnesota and the number of barns chosen for the water quality analysis was limited to 15. A broader geography and more water quality analyses

may have resulted in the discovery of water with higher analyte concentrations leading us to find more extreme water sources to be used in the pig experiment. A limitation of the pig experiment included utilizing a controlled research setting to analyze water quality's impact on the nursery pig. We recognize that in the field, there are many variables among nursery barns that can impact water quality and pig performance and health, however our aim was to truly assess water quality itself. Although trained individuals were used to carry out the experiment and analyses, logistical, lab, and human error in diet digestibility and intestinal permeability resulted in a number of missing data. Increasing the number of replicates for all measurements of the experiment would reduce error and allow researchers to observe greater differences.

With these limitations, our results were still able to explore methods of comparing water quality sources in pigs and ultimately provide a comparison among different qualities of water found in Minnesota. Within the range of water quality traits studied in this project, quality of water did not influence nursery pig growth performance or health.

CHAPTER 6: LITERATURE CITED

- Adeola, O. 2001. Digestion and balance techniques in pigs. In: Swine Nutrition, 2nd Edition. A. J. Lewis and L. L. Souther, ed. CRC Press. P 903-916.
- Anderson, D. M., and S.C. Stothers 1978. Effects of saline water high in sulfates, chlorides and nitrates on the performance of young weanling pigs. *J. Anim. Sci.* 47:900-907.
- Anderson, J. S., D. M. Anderson, and J. M. Murphy. 1994. The effect of water quality on nutrient availability for grower/finisher pigs. *Can. J. Anim. Sci.* 74:141-148.
- AOAC International. 2006. Official Methods of Analysis of AOAC International. 18th ed. AOAC Int., Arlington, VA.
- Bajpai, P. 2015. The control of microbiological problems. In: Pulp and Paper Industry: Microbiological Issues in Papermaking. Elsevier, Waltham, MA. 103-195. doi: 10.1016/B978-0-12-803409-5.00008-2
- Brooks, P. H., S. J. Russell, and J. L. Carpenter. 1984. Water intake of weaned piglets from three to seven weeks old. *Vet. Rec.* 20:513-515. doi: 10.1136/vr.115.20.513
- Brooks, P. H., J. L. Carpenter, J. Barber, and B. P. Gill. 1989. Production and welfare problems relating to the supply of water to growing-finishing pigs. *UK Pig Vet. J.* 23:51-66.
- Brumm, M. C., J.M. Dahlquist, and J. M. Heemstra. 2000. Impact of feeders and drinker devices on pig performance, water use, and manure volume. *Swine Health Prod.* 8:51-57.
- Canadian Council of Ministers of the Environment (CCME). 2008. Summary – Guidelines for livestock drinking water quality. In: Canadian. Environ. Qual. Guide. Table 4.10.
- Carson, T. L. 2000. Current knowledge of water quality and safety for livestock. *Veterinary Clinics: Food Anim. Prac.* 16:455-464.
- Cohen, J., and H. I. Shuval. 1973. Coliforms, fecal coliforms, and fecal streptococci as indicators of water pollution. *Water, Air, and Soil Pollution* 2:85–95. doi: 10.1007/bf00572392
- Cornelius, C. E. 1980. Liver Function. In: Clinical biochemistry of domestic animals. 3rd ed. Academic Press, New York, NY. 201–250.
- Costerton, J. W., Z. Lewandowski, D. E. Caldwell, D. R. Korber, and H. M. Lappin-Scott. 1995. Microbial biofilms. *Annu. Rev. Microbiol.* 49:711-745.
- De Busser, E. V., J. Dewulf, L.D. Zutter, F. Haesebrouck, J. Callens, T. Meyns, W. Maes, and D. Maes. 2011. Effect of administration of organic acids in drinking water on faecal shedding of *E. Coli*, performance parameters and health in nursery pigs. *Vet. J.* 2:184-188. doi:10.1016/j.tvjl.2010.04.006.
- Dhabhar, F. S. 2002. Stress-induced augmentation of immune function—The role of stress hormones, leukocyte trafficking, and cytokines. *Brain, Behavior, and Immunity* 16:785–798. doi: 10.1016/S0889-1591(02)00036-3

- Dorr, P. M., D. Madsen, S. Wayne, A. Sheidt, and G. W. Almond. 2009. Impact of pH modifiers and drug exposure on the solubility of pharmaceutical products commonly administered through water delivery systems. *J. Swine Health Prod.* 4:217-222.
- Etikan, I., S. A. Musa, and R. S. Alkassin. 2016. Comparison of convenience sampling and purposive sampling. *Amer. J. Theoretical and Applied Stat.* 5:1-4.
doi: 10.11648/j.ajtas.20160501.11
- Foreman, J. H. 2014. Overview of Hepatic Disease in Large Animals - Digestive System. Merck Veterinary Manual. Available from: <https://www.merckvetmanual.com/digestive-system/hepatic-disease-in-large-animals/overview-of-hepatic-disease-in-large-animals>.
- Fraser, D., W. B. Peeters, P. Phillips, and B. Thompson. 1988. Use of water by piglets in the first days after birth. *Can. J. Anim. Sci.* 68:603-610.
- Froese, C., 2003. Water usage and manure production rates in today's pig industry. *Proc. Manitoba Swine Sem.* 14:218-223.
- Geldreich, E. E. 1996. Biofilms in water distribution systems. In: *Microbial Quality of Water Supply in Distribution Systems*. CRC Press LLC, Boca Raton, FL. 159-215.
- Gill, B. P., P. H. Brooks, and J. L. Carpenter. 1986. The water intake of weaned pigs from three to six weeks of age. *Anim. Prod.* 42:470 (Abstract).
- Gill, B. P. 1989. Water use by pigs managed under various conditions of housing, feeding, and nutrition. PhD Diss. University of Plymouth Polytechnic. Plymouth, England.
- Harvey, R. F., and A. E. Read. 1973. Saline purgatives act by releasing cholecystokinin. *The Lancet* 302:185-187. doi: 10.1016/S0140-6736(73)93011-0
- Hepatobiliary Disease. 1999. In: *Diet and health: implications for reducing chronic disease risk*. Net Library, Inc., Boulder, CO. p. 633-636.
- Hodkinson, C. F., J. M. O'Connor, H. D. Alexander, I. Bradbury, M. P. Bonham, B. M. Hannigan, W. S. Gilmore, J. J. Strain, and J. M. W. Wallace. 2006. Whole blood analysis of phagocytosis, apoptosis, cytokine production, and leukocyte subsets in healthy older men and women: The ZENITH Study. *J. Gerontol.* 61:907-917.
doi:10.1093/gerona/61.9.907
- Kalmach, S., W. Manz, and U. Szewzyk. 1997. Dynamics of biofilm formation in drinking water: phylogenetic affiliation and metabolic potential of single cells assessed by formazan reduction and in situ hybridization. *FEMS Microb. Ecol.* 22:265-279. doi: 10.1111/j.1574-6941.1997.tb00379.x
- Karalyan, Z., H. Zakaryan, E. Arakelova, V. Aivazyan, M. Tatoyan, A. Kotsinyan, R. Izmailyan, and E. Karalova. 2016. Evidence of hemolysis in pigs infected with highly virulent African swine fever virus. *Vet. World* 9:1413-1419. doi:10.14202/vetworld.2016.1413-1419

- Katouzian, F., T. Sblattero, T. Not, A. Tommasini, E. Giusto, D. Meiaccio, M. Stebel, R. Marzari, A. Fasano, and A. Ventura. 2005. Dual sugar gut-permeability testing on blood drop in animal models. *J. Clin. Chim. Acta.* 352:191-197. doi: 10.1016/j.cccn.2004.09.023
- Kiela, P. R., and F. K. Ghishan. 2017. Physiology of intestinal absorption and secretion. *Best Pract. Res. Clin. Gastroenterol.* 30:145-159. doi: 10.1016/j.bpg.2016.02.007
- Kruse, S., I. Traulsen, and J. Krieter. 2011. Analysis of water, feed intake and performance of lactating sows. *Livest. Sci.* 135:177-183. doi:10.1016/j.livsci.2010.07.002
- LeJeune, J. T., M. D. Kauffman, M. D. Amstutz, and L. A. Ward. 2006. Limited effects of a commercial direct-fed microbial on weaning pig performance and gastrointestinal microbiology. *J. Swine Health Prod.* 14:247–252.
- Li, Y. Z., L. Chenard, S. P. Lemay, and H. W. Gonyou. 2005. Water intake and wastage at nipple drinkers by growing-finishing pigs. *J. Anim. Sci.* 83:1413-1422. doi: 10.2527/2005.8361413x
- Magnusson, U., and C. Greko. 1998. Assessment of phagocytic capacity and opsonic activity in blood and mammary secretions during lactation in sows. *J. Vet. Med.* 45:353–361.
- Mah, T. F. 2012. Biofilm-specific antibiotic resistance. *Fut. Microbiol.* 9:1061-1072. doi: 10.2217/fmb.12.76
- McLeese, J. M., J. F. Patience, G. I. Christison, and M. S. Wolynetz. 1991. Evaluation of the quality of ground water supplies used on Saskatchewan swine farms. *Can. J. Anim. Sci.* 71:191-203.
- McLeese, J. M., M. L. Tremblay, J. F. Patience, and G. I. Christison. 1992. Water intake patterns in the weanling pig: effect of water quality, antibiotics and probiotics. *J. Anim. Prod.* 54:135–142. doi: 10.1017/s0003356100020651
- McLeod, K. H., J. L. Richards, Y. A. Yap, and E. Marino. 2019. Dietary short chain fatty acids: how the gut microbiota fight against autoimmune and inflammatory diseases. In: R. R. Watson and V. R. Preedy, editors. *Arthritis and autoinflammatory diseases: second edition.* Elsevier, Waltham, MA. 139-159. doi: 10.1016/B978-0-12-813820-5.00007-6
- MetaFarms, and Swine Management Services, LLC. 2020. Production Analysis Summary for U.S. Pork Industry: 2017 – 2019. 2019:1-15.
- Minnesota Rural Water Association (MRWA). 1994. Chapter 15: Hardness. In: *Minnesota water works operations manual.* Minnesota Rural Water Association. Elbow Lake, MN. 15.1-15.4.
- Mroz, Z., A. W. Jongbloed, N. P. Lenis, and K. Vreman. 1995. Water in pig nutrition: Physiology, allowances and environmental implications. *Nutr. Res. Rev.* 8:137-164.
- Myers, W. D., P. A. Ludden, V. Nayigihugu, and B. W. Hess. 2004. Technical Note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. *J. Anim. Sci.* 82:179–183. doi: 10.2527/2004.821179x

- NRC. 2012. Nutrient requirements of swine. 11th ed. Natl. Acad. Press, Washington, DC.
- Nyachoti, M., and E. Kiarie. 2010. Water in swine production: A review of its significance and conservation strategies. Manitoba Swine Seminar. 217-232.
- Oliviero, C., T. Kokkonen, M. Heinonen, S. Sankari, and O. Peltoniemi. 2009. Feeding sows with high fiber diet around farrowing and early lactation: Impact on intestinal activity, energy balance, related parameters and litter performance. *J. Res. Vet. Sci.* 86:314-319. doi: 10.1016/j.rvsc.2008.07.007.
- Olkowski, S. A. 2009. Water requirements for swine. In: *Livestock water quality: a field guide for cattle, horses, poultry, and swine.* Canadian Council of Ministers of the Environment, Winnipeg. 30-34.
- Olmedo, G. M., M. Grillo-Puertas, L. Cerioni, V. A. Rapisarda, and S. I. Volentini. 2015. Removal of pathogenic bacterial biofilms by combinations of oxidizing compounds. *Can. J. Microbiol.* 61:351-356. doi: 10.1139/cjm-2014-0747
- Paterson, D., R. C. Wahlstrom, G. W. Libal, G. W., and O. E. Olson. 1979. Effects of sulfate in water on swine reproduction and young pig performance. *South Dakota Swine Field Day Proceedings and Research Reports, 1979.* 5.
- Patience, J. 2013. Water in Swine Nutrition. In: L. I. Chiba, editor, *Sustainable Swine Nutrition.* Wiley-Blackwell, Ames, IA. 3-22.
- Patience, J., A. Beaulieu, and D. Gillis. 2004. The impact of ground water high in sulfates on the growth performance, nutrient utilization, and tissue mineral levels of pigs housed under commercial conditions. *J. Swine Health Prod.* 12:228-236.
- Patience, J., J. F. Umboh, R. K. Chaplin, and C. M. Nyachoti. 2005. Nutritional and physiological responses of growing pigs exposed to a diurnal pattern of heat stress. *J. Liv. Prod. Sci.* 96:205–214. doi: 10.1013/j.livprodsci.2005.01.012
- Peled, Y., O. Doron, H. Laufer, Y. Bujanover, and T. Gilat. 1991. D-Xylose absorption test. Urine or blood? *Dig. Dis. Sci.* 36: 188–192.
- Quiniou, N., D. Renaudeau, S. Dubois, and J. Noblet. 2000. Influence of high ambient temperatures on food intake and feeding behavior of multiparous lactating sows. *J. Anim. Sci.* 70:471-479. doi:10.1017/S1357729800051821
- Robinson, J. W. L. 1970. Comparative aspects of the response of the intestine to its ionic environment. *Comp. Biochem. Physiol.* 34:641–655. doi: 10.1016/0010-406X(70)90290-2
- Rose, B. D. 1989. Physiology of Body Fluids. In: Health Professions Division, editor, *Clinical physiology of acid base and electrolyte disorders.* 3rd ed. McGraw-Hill, New York, NY. p. 4-24.
- Roubicek, C. B. 1969. Water Metabolism. In: E. S. E. Hafez and I. A. Dyer, editors, *Animal Growth and Nutrition.* Lea & Febiger, Philadelphia, PA. p. 353-373.

- Roy, R., M. Tiwari, G. Donelli, and V. Tiwari. 2018. Strategies for combating bacterial biofilms: a focus on anti-biofilm agents and their mechanisms of action. *Virulence* 9:522-554. doi: 10.1080/21505594.2017.1313372
- Shaw, M. I., A. D. Beaulieu, and J. F. Patience. 2006. Effect of diet composition on water consumption in growing pigs. *J. Anim. Sci.* 84:3123–3132. doi: 10.2527/jas.2005-690
- Shields, R. G., Jr, D. C. Mahan, and P. L. Graham. 1983. Changes in swine body composition from birth to 145 kg. *J. Anim. Sci* 57:43–54.
- Sidhu, P., V. Mahajan, S. Verma, Ashuma, and M. Gupta. 2014. Toxicological and pathological review of concurrent occurrence of nitrite toxicity and swine fever in pigs. *Toxicol. Int.* 21:186-190. doi: 10.4103/0971-6580.139806
- Singh, S., S. K. Singh, I. Chowdhury, and R. Singh. 2017. Understanding the mechanism of bacterial biofilms resistance to antimicrobial agents. *Microbiol. J.* 11:53-62. doi: 10.2174/1874285801711010053
- Smith, G. S., G. L. Walter, and R. M. Walker. 2013. Clinical pathology in non-clinical toxicology testing. In *Haschek and Rousseaux's Handbook of Toxicologic Pathology*; Academic Press: Cambridge, MA, USA, 2013. 565–594 doi:10.1016/B978-0-12-415759-0.00018-2
- Standard Methods. 2320 B, Titration method. 1999. Standard methods for the examination of water and wastewater. 20th ed. American Public Health Association. Washington, D.C.
- Standard Methods. 2510 B, Conductivity. 2017. Standard methods for the examination of water and wastewater. 23rd ed. American Public Health Association. Washington, D.C. doi: 10.2105/SMWW.2882.027
- Standard Methods. 4500-H+, pH value. 2017. Standard methods for the examination of water and wastewater. 23rd ed. American Public Health Association. Washington, D.C. doi:10.2104/SMWW.2822.082
- Standard Methods. 4500-NO₂, Nitrogen (nitrite). 2017. Standard methods for the examination of water and wastewater. 23rd ed. American Public Health Association. Washington, D.C. doi: 10.2105/SMWW.2882.088
- Standard Methods. 9215 Heterotrophic Plate Count. 2017. Standard methods for the examination of water and wastewater. 23rd ed. American Public Health Association, Washington, D.C. doi: 10.2105/SMWW.2882.188
- Standard Methods. 9222 D, Membrane filter technique members of the coliform group. 2017. Standard methods for the examination of water and wastewater. 23rd ed. American Public Health Association, Washington, D.C. doi: 10.2105/SMWW.2882.193
- Standard Methods. 9223 Enzyme substrate coliform test. 2017. Standard methods for the examination of water and wastewater. 23rd. ed. American Public Health Association, Washington D.C. doi: 10.2105/SMWW/2882.194
- Stockill, P. 1990. Water: Why it should not be the neglected nutrient for pigs. *Feed Int.* 11:10-18.

- Sultana, S. T., J. T. Babauta, and H. Beyenal. 2016. Electrochemical biofilm control: a review. *Biofouling*. 31:745-758. doi: 10.1080/08927014.2015.1105222
- Thacker, P. A. 2001. Water in swine nutrition. In: *Swine Nutrition*, 2nd ed. CRC Press LLC, Boca Raton, Florida. p. 381-398.
- Thulin, A. J., and M. C. Brumm. 1991. Water: The forgotten nutrient. In: E. R. Miller, D. E. Ullrey, and A. J. Lewis, Editors, *Swine nutrition*. Butterworth-Heinemann, Stoneham, MA. p. 315-324.
- USEPA. 1994. U.S. Method 200.7: Determination of metals and trace elements in water and wastes by inductively coupled plasma-atomic emission spectrometry. Revision 4.4. doi: 10.1016/b978-0-8155-1398-8.50010-0
- USEPA. 1994. U.S. Method 245.1: Determination of mercury in water by cold vapor atomic absorption spectrometry. Revision 3.0.
- USEPA. 1993. U.S. Method 300.0: Determination of inorganic anions by ion chromatography. Revision 2.1.
- Van Houdt, R., and C. W. Michiels. 2010. Biofilm formation and the food industry, a focus on the bacterial outer surface. *J. Appl. Microbiol.* 109:1117-1131. doi: 10.1111/j.1365-2672.2010.04756.x
- Veenhuizen, M. F., G. C. Shurson, and E. M. Kohler. 1992. Effect of concentration and source of sulfate on nursery pig performance and health. *J. Am. Vet. Med. Assoc.* 201:1203-1208.
- Vinten, A., and S. Dunn. 2001. Assessing the effects of land use on temporal changes in well water quality in a designated nitrate vulnerable zone. *Sci. Total. Envir.* 265:253-268. doi: 10.1016/S0048-9697(00)00662-8
- Wellock, I. J., P. D. Fortomaris, J. G. M. Houdijk, and I. Kyriazakis. 2006. The effect of dietary protein supply on the performance and risk of post-weaning enteric disorders in newly weaned pigs. *J. Anim. Sci.* 82:327-335. doi:10.1079/ASC200643
- Whittemore, C. T., and D. M. Green. 2001. Growth of the young weaned pig. In: *The weaner pig: nutrition and management*. CABI Publishing, Wallingford, UK. 1-17. doi: 10.1079/9780851995328.000
- Wijtten, P. J. A., J. V. D. Meulen, and M. W. A. Verstegen. 2011. Intestinal barrier function and absorption in pigs after weaning: a review. *Brit. J. Nutr.* 105:967-981. doi: 10.1017/S0007114510005660
- Yang, T. S., B. Howard, and W. V. Macfarlane. 1981. Effects of food on drinking behavior of growing pigs. *App. Anim. Ethol.* 7:259-270.
- Zhang, F. and Adeola O. 2017. Techniques for evaluating digestibility of energy, amino acids, phosphorus, and calcium in feed ingredients for pigs. *J. Anim. Nutr.* 3:344-252. doi: 10.1016/j.aninu.2017.06.008

Zuckerman, M. J., I. S. Menzies, H. Ho, G. G. Gregory, N. A. Casner, R. S. Crane, and J. A. Hernandez. 2004. Assessment of intestinal permeability and absorption in cirrhotic patients with ascites using combined sugar probes. *Dig. Dis. Sci.* 49:621–626. doi: 10.1023/B:DDAS.0000026307.56909.21