THE EFFECTS OF FEEDING SEAWEED EXTRACT IN THE DIET OF SWINE ON GUT

HEALTH, PERFORMANCE, CARCASS CHARACTERISTICS, AND PORK QUALITY

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Title

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The Supervisory Committee certifies that this ***disquisition*** complies with
North Dakota State University's regulations and meets the accepted
standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Consumers are concerned about antibiotic and ractopamine usage; therefore, alternatives need to be found. Objectives of this study were to investigate the effects of using seaweed as an alternative feed supplement and comparing performance, carcass, pork quality, and immune traits in pigs fed seaweed, control, and ractopamine diets. Pigs were allocated to one of three treatments (CON, SWE, RAC) at weaning (n = 40/treatment). Pigs were weighed every two weeks. Carcass characteristics, pork quality, and immune data were collected post-mortem. No differences were found between treatments for feed intake, growth, or feed efficiency. Pigs on RAC treatment had greater hot carcass weight and dressing percentage ($P < 0.05$). Chops from RAC pigs were lighter ($P = 0.05$), less red ($P < 0.05$), and tougher ($P = 0.08$). There were no differences between treatments for FABP2 gene expression, cell proliferation percentages, or crypt depths. Therefore, no negative effects of feeding seaweed to pigs were found in this study.

ACKNOWLEDGEMENTS

DEDICATION

This thesis is dedicated to my parents, Daniel and Icema Somers; my brothers, Paul and Oneil

Somers; and my sister, Ann- Marie Somers, and her daughter, Alaina Burrell. It is also to my

close friends. Without each of you, it would not have been possible to finish this Masters.

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LIST OF ABBREVIATIONS

ADFI ....average daily feed intake

ADG..average daily gain

β-AA ....beta-adrenergic agonists

β-AR..beta-adrenergic receptor

CON ....control treatment

DFD..dark, firm, and dry

FDA..Food and Drug Administration

G:F ....gain to feed ratio

GI ....gastrointestinal tract

HCW ....hot carcass weight

pH..potential hydrogen

PSE...pale, soft, and exudative

RAC ....ractopamine hydrochloride

SWE ....seaweed extract

US ....United States

USDA..United States Department of Agriculture

VFD..Veterinary Feed Directive

WBSF..Warner-Bratzler shear force

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

Introduction

Pork is the number one protein source consumed worldwide (USMEF 2015b; Garbossa et al., 2013). In the US, pork ranks third behind beef and chicken consumption (Davis and Lin, 2005). However, with an increase in the global consumption of pork, especially in major export markets such as China, Korea, and Japan, the US pork industry will have an opportunity to increase exports to meet the growing demand for meat. Furthermore, real per capita expenditures, a measure for pork demand, shows that there is an increase of 13.5 % for pork since 2012 (Meyer, 2015), indicating an increasing demand for pork. Additionally, increased pork consumption will be competing with other food products and an increasing population for land space. Based on statistical reports from the Department of Economic and Social Affairs of the United Nations, the world's population is expected to increase from the current 7 billion to approximately 9 billion by 2050 (Garbossa et al., 2013). Due to rapid population growth, the US has lost and continues to lose land to urban development (Becker, 2002), meaning that the US will have to produce more pork on less land to meet the demands of a growing global population. This trend maintained a steady increase during the period 1982-1997 which demonstrates that, with an increase of 17 % in the population, 47 % of land was converted into urban areas (Becker, 2002). With a decrease in rural land space, pigs will be raised in closer connection with other pigs, resulting in increased biosecurity concerns particularly with the spread of diseases. While medication is required to treat sick animals, consumers are concerned with antibiotic resistance and the impression of over-use of antibiotics in the livestock industry. As a result, alternatives to antibiotics to promote good health are a necessity.

Feed is one of the single most important factors in pork production because it accounts for approximately 60 to 70 % of the total cost of production (Lawrence et al., 2010, Patience et al., 2015). This has influenced producers to think of innovative ways in which they may improve feed efficiency due to its close relationship with feed costs (Patience et al., 2015). To improve feed efficiency, multiple strategies have been explored and implemented in the past. Lou and Gonyou (1997) found that adjustments in feeder dimensions to accommodate pigs with different weights and feeding behaviors minimize wastage and improve feed efficiency. Patience and others (2015) also found that changes in environmental management practices such as housing pigs indoor to reduce cold and heat stress increase feed efficiency. Advances in genetics and utilization of feeding material with higher protein and fiber content have shown favorable results regarding feed efficiency (FAO, 2004). Feed additives such as antibiotics and beta-adrenergic agonists (**β-AA**) have also been used to improve growth and feed efficiency. For example, ractopamine hydrochloride (**RAC**), which is a β-AA, has been used in the swine industry because it has been demonstrated to improve average daily gain (**ADG**) and gain-to-feed ratio (**G:F**) (Dunshea et al., 1993; Barker et al., 2002; Carr et al., 2005; Potter et al., 2009; Scramlin et al., 2010; Garbossa et al., 2013; Hinson et al., 2014).

Although feed additives have been commonly used in the pork industry to promote growth, concerns have arisen due to their use. The use of antibiotics as a growth promotant leads to concerns regarding antibiotic-resistant bacteria. As a result, the United States Food and Drug Administration (**FDA**) has implemented stricter guidelines on those antibiotics they deem "medically important" (FDA, 2013). Additionally, starting January 1, 2017, the FDA implemented a Veterinary Feed Directive that restricts the use of antibiotics in feed (AVMA, 2017). Beta-adrenergic agonists pose a more complicated risk. Consumers and industries have

become increasingly concerned about the health risks associated with the use of β-AA such as RAC. Controversy exists relating to the acceptable daily intake and maximum residue limit for RAC in both cattle and pigs. While the Codex Alimentarius Commission and the Joint Expert Committee for Food Additives did establish an acceptable daily intake and maximum residue limit, the European Food Safety Authority was unable to establish any maximum residue limit or acceptable daily intake because they felt the data was insufficient to draw any conclusions (Bories et al., 2009). Countries such as Russia and China remain apprehensive and refuse to accept any traces of RAC in certain organs due to these organs being important to Russian and Chinese diets (Centner et al., 2014). As a result, some export markets are closed or extremely restrictive to U.S. product.

As US consumers become increasingly involved in dictating agricultural policy, an alternative to using antibiotics and β-AA for improving feed efficiency is important. One alternative that is currently being investigated is the use of seaweed and seaweed extracts (**SWE**) because they have been shown to have similar effects on growth performance and feed efficiency as RAC as well as improving immune response to infection positive effect on the immune system (Mukhopadhya et al., 2012). Consequently, research related to the impacts of seaweed and SWE on immunity, performance, and feed efficiency could unveil novel techniques to maximize profitability and minimize financial losses. These compelling characteristics of seaweed have led to this study. Hence, the objectives of this thesis project were to compare feed efficiency, growth performance of pigs fed SWE, RAC, and control diets, and to determine if feeding SWE results in a lower incidence of disease, which would indicate a stronger immune system because all pigs were exposed to the same environment. There are many factors affecting performance, meat quality traits as well as the overall health status of swine.

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The objective of the below literature review is to examine the impacts of feed additives such as ractopamine and antibiotics on various aspects of pork production. It also gives a brief overview of seaweed and seaweed extracts as alternatives to these feed additives as well as some of the economic and political climate that currently exist due to controversy over the use of these additives in the diets of pigs.

Traditional Feed Additives

Swine producers have been using feed additives as a means of improving performance traits for decades. Traditional feed additives that have been used are β-AA and antibiotics. These additives are used as a method of improving feed efficiency, growth, carcass characteristics, and prevention of disease. They can be very beneficial; however, controversy is arising regarding their use.

Beta-adrenergic agonists

Beta-adrenergic agonists are repartitioning agents that divert nutrients from adipose to muscle tissue accretion, resulting in them acting as growth promoters that stimulate skeletal muscle development (Mersmann, 1998). Wray-Cahen (2001) reported that epinephrine and norepinephrine secreted by chromaffin cells of the adrenal medulla are naturally occurring β-AA or catecholamines. While there are naturally occurring β-AA, the use of β-AA in agriculture were not given much thought until the development of synthetic β-AA in the 1980s (Wray-Cahen, 2001). These synthetic β-AA have similar pharmaceutical properties and structures as naturally occurring adrenergic agonists. Although synthetic β-AA were reported to increase protein synthesis and limit protein degradation in meat animals, the effects are variable within and among species (Mersmann, 1998).

Currently, the most widely used β-AA in the swine industry is RAC (Paylean, Elanco Animal Health, Greenfield, IN) and the most widely used in the cattle industry are zilpaterol hydrochloride (Zilmax, Merck Animal Health, Summit, NJ) and RAC (Optaflexx, Elanco Animal Health, Greenfield). Ractopamine hydrochloride has been approved by the FDA for use in both swine and cattle while zilpaterol hydrochloride has been approved for use in cattle only (FDA, 2000, 2003, 2006). According to the European Food Safety Authority, a warning statement that RAC may result in an "increased risk for exhibiting the downer pig syndrome, synonymous to fatigued pig syndrome" must be written on the label if the product is intended to be used for pigs (Bories et al., 2009).

Mechanisms of action

Beta-adrenergic receptors (**β-AR**) are present on the surface of almost all mammalian cells. When a β-AA binds to a β-AR, a series of biochemical reactions occurs, starting with the activation of the G_S protein, which in turn activates adenylyl cyclase to produce cyclic adenosine monophosphate (Mersmann, 1998). The distribution of the three sub-types of the β-AR on the surface of the cell contributes to the physiological function regulated by β-AR, with the range of physiological functions being so broad that the mechanism behind any single effect may be extremely complex (Mersmann, 1998).

Gastrointestinal tract health

Several research studies have shown that the microbial community within the gastrointestinal (**GI**) tract play a key role in maintaining health (van der Waaij and Nord, 2000; Cromwell, 2002; Gaskins et al., 2002). Montagne and others (2003) defined GI health as a state of equilibrium in which the commensal flora, the mucosal layer, and the diet work together to protect the animal against pathogen invasion and maintain the functionality of the digestive

system. In pigs, the GI tract of neonates is generally sterile and the sow's milk contains many important nutrients that are needed for cell growth, differentiation, function, and development of the small intestine (Pluske et al., 1997). Bacteria from the environment and the dam will colonize the GI tract and result in histological and biological changes in the GI tract (Pluske et al., 1997). Weaning has been shown to affect GI health and, subsequently, the piglet's capability to mitigate the effects of enteric infections caused by invading pathogens (Pluske et al., 1997; Montagne et al., 2003; Reilly et al., 2008; Lynch et al., 2010; O'Doherty et al., 2010; Smith et al., 2011). Additionally, microorganisms present in the GI tract come in direct contact with a wide range of toxins, hormones, antibiotics, and other substances to which the host animal is exposed (Collington et al., 1990; Guarner and Malagelada, 2003; Sekirov et al., 2008; Antonopoulos et al., 2009). These substances have the capacity to interfere with the natural populations of GI tract flora, causing an imbalance within the microbial community (Collington et al., 1990; Wray-Cahen, 2001; Sekirov et al., 2008). These substances could also cause disruptions to the ability of the mucosal layer to interact with intestinal epithelial cells, commensal microbiota, and the host's innate immune system, resulting in a defective mucosal layer, which would allow microbes to permeate the barrier and cause inflammation (Kim and Ho, 2010). The composition of the diet, as well as the feeding strategy, play a major role in microbial diversity and establishment within the GI tract (Rist et al., 2012).

Because of the presence of β-AR on the surface of almost all mammalian cells, β-AA can have a direct physiological impact on the GI tract (Walker and Drouillard, 2012). Previous research has shown that various β-AA increase the growth of gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* (Walker and Drouillard, 2012). This is important

because *Escherichia coli* is the major cause of diarrhea and economic loss worldwide in the swine industry (Pluske et al., 1997).

Stress and immune regulation

Pigs are confronted with many stressors, including noise, poor handling, transport, temperature, feed, and confinement that may cause a change in their behavior, physiological, and biological functions as they try to cope and adapt to the changes within their environment (Barnett and Hemsworth, 1990). Stressors typically activate the endocrine system to release natural β-AA (epinephrine and norepinephrine) that are associated with the fight-or-flight response (Cannon, 1929). Ractopamine hydrochloride has been shown to increase heart rate and to cause vasodilation of blood vessels (Bottemiller, 2012), which would be part of the fight-or-flight response. Furthermore, when the animal is in a stressful situation, like fear or excitement during transport or handling, RAC can increase glycogenolysis and creatine phosphate in the muscle, resulting in cramps and injuries due to muscle fatigue (Ferreira et al., 2013). This is evident in the increased number of downer and dead-on-arrival pigs that have been reported by United States Department of Agriculture (**USDA**) meat inspectors since the approval of RAC (Bottemiller, 2012).

The physiological β-AA are norepinephrine and epinephrine. Ractopamine has similar pharmaceutical function and structures to these natural β-AA, which means RAC can affect the immune function in a similar way. Because RAC can induce stress, it has the potential to regulate immune cells, such as natural killer cells that have β-AR, and, consequently, lead to suppression of host resistance to diseases (Lotzová, 1991; Irwin, 1993). Previous research by Shakhar and Ben-Eliyahu (1998) demonstrated that in vivo administration of a β-AA, metaproterenol, in rats suppressed of natural killer cell activities, thereby increasing

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susceptibility of tumor development due to reduced cellular resistance. Natural killer cells plays a key role in preventing viral diseases, and human studies have found a strong correlation with the levels of natural killer cell and development of tumor (Lotzová, 1991). The effects of β-AA may be directed through the stress response as Benschop and others (1996) found that various stress hormones affect the number of natural killer cells per milliliter of blood. Additionally, Ben-Eliyahu and others (1999) found that rats subjected to various stressors developed tumors and had higher mortality rates due to suppression of immune cells needed to develop resistance.

Performance and carcass characteristics

Synthetic analogs of β-AA such as RAC have been widely studied for their ability to stimulate muscle accretion and reduce fat deposition. Numerous studies have shown that feeding RAC results in increased ADG and G:F in finishing swine (Dunshea et al,. 1993; Barker et al., 2002; Stoller et al., 2003; Armstrong et al., 2004, 2005; Carr et al., 2005; Potter et al., 2009; Scramlin et al., 2010; Garbossa et al., 2013; Hinson et al., 2014). Dietary inclusion studies using both RAC and zilpaterol hydrochloride in beef steers on performance, carcass traits, and cutability have also shown improvements (Arp et al., 2014).

Dunshea and others (1993) reported a significant correlation between RAC and ADG; however, this was predominantly evident among pigs supplemented with higher protein levels. Similarly, Ferreira and others (2013) also saw a linear relationship between RAC and dietary protein and contended that pigs fed a RAC diet had increased dietary requirements for lysine. Additionally, pigs fed RAC diets tended to be deficient in calcium and phosphorous and display poor skeletal development (Ferreira et al., 2013).

Because of improved muscle deposition, pigs fed diets containing RAC had increased hot carcass weight, dressing percent, and loin muscle area (Warriss et al., 1990; Armstrong et al.,

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2004; Carr et al., 2005; Potter et al., 2009). Potter and others (2009) reported that RAC reduced back fat, increased loin depth, and improved carcass lean when compared to controls. Previous research indicates that high levels of epinephrine in animals exposed to stress prior to slaughter may affect postmortem metabolism (Lawrie, 1966). For example, pale, soft, and exudative (**PSE**) meat in pork and dark, firm, and dry (**DFD**) meat in beef are commonly associated with animals being stressed right before slaughter (Kannan et al., 1997). While Garbossa and others (2013) found no treatment differences in both initial and final pH, Warris and others (1990) reported higher ultimate pH values in the semimembranosus, adductor, and supraspinatus muscles of pigs treated with albuterol, an inhaled β-AA. However, albuterol showed no evidence of increased propensity of PSE meat, as there were no observed differences in the pH of longissimus and semimembranosus muscles at 45 min postmortem (Warris et al., 1990).

The longissimus dorsi of pigs fed RAC were lighter (greater L* and lower subjective color score) and less red (lower a*) than those of pigs fed a control diet (Armstrong et al., 2004). Evaluating the supplementation of RAC on steers, Bohrer and others (2016) found no treatment effect on tenderness and cook loss for animals receiving the additive. Similarly, Bridi and others (2006) and Agostini others (2011) found no effect of RAC on tenderness in pigs fed a RAC diet. However, Warris and others (1990) and Wood and others (1994) reported differences in tenderness in pigs supplemented with RAC. Higher shear force values in RAC supplemented pigs was attributed to increased muscle fiber type and reduction in calpain activities (Warris et al., 1990; Wood et al., 1994). Varying results were reported regarding drip loss. Garbossa and others (2013) reported a linear decrease in drip loss with increasing doses of RAC; however, Agostini and others (2011) found no effect of RAC dose on drip loss.

Ractopamine hydrochloride has sparked a debate among consumers in key international markets that has led to a ban in 160 countries, including Taiwan, China, Russia, and the European Union (Bories et al., 2009; Garina, 2012; Franzetta and Backus, 2012). While the US, Canada, Brazil, and Australia continue to use RAC in cattle and swine production, consumers in these countries are progressively becoming intolerant due to concerns with possible associated health risks, particularly for people who suffer from asthma or cardiovascular diseases (CFS, 2013). According to a recent report, exposure to RAC may result in toxicity, behavioral changes, cardiovascular, musculoskeletal, reproductive and endocrine problems (CFS, 2013).

Based on the health concerns mentioned above, consumers are concerned with the ways in which members of Codex Alimentarius Commission and the Joint Expert Committee for Food Additives made their decisions in relation to establishing an acceptable daily intake and a maximum residue limit for RAC in both cattle and pigs (Bories et al., 2009). The European Food Safety Authority conducted a human study and, while the results obtained from these studies were satisfactory for the Joint Expert Committee for Food Additives to conclude that the use RAC is safe for human consumption, the European Food Safety Authority was not able to establish any maximum residue limit or acceptable daily intake. This is because they felt that these results were insufficient to conclude that RAC was safe for human consumption (Bories et al., 2009). For these reasons, countries such as Russia and China remain apprehensive and refuse to accept any traces of RAC in organs of the heart, kidney, lungs, and liver, organs that are of vital importance to their diets (Centner et al., 2014). Additionally, a consumer report revealed that 20 % of pork products tested for RAC were positive. This same report suggested that RAC decreased the quality of meat because of its negative impact on taste and tenderness (CFS, 2013).

The Center for Food Safety reported that the US has a bad history for testing for RAC in pork, cattle, and turkey production. This was evident in 2010, which saw 22 billion pounds of pork go untested, and only 712 samples for the 26 billion pounds of beef produced were tested for RAC residue (CFS, 2013). In 2012, public interest groups challenged the FDA to provide information and to reduce maximum intake levels to match the standards of the Codex or lower in meat products in the US regarding the safety of RAC in human consumption (CFS, 2013).

International trade impact

Although the FDA approved the use of β-AA, which assures continuous production of pork to meet the current growing demands, some of these β-AA are not approved in major US exports markets, including China, European Union, and Russia. Therefore, this could have some negative effects on the economic viability of the US pork export market (Seng, 2015). For example, China and Hong Kong imported approximately 17 % of the pork the US exported which represents approximately $775 million of the US pork export market (USMEF, 2015a). In terms of value, this was the fourth largest market for US pork including variety meat in 2014.

Exports from the US to Russia accounted for 100,000 metric tons of pork, valued at more than $280 million in 2012 prior to a ban related to β-AA, making Russia the sixth largest pork export market for the US in both volume and value (USMEF Staff, 2017). Although US pork is currently banned from Russia for political reasons (2014 economic sanctions due to the conflict in Ukraine), they have the potential to be an important market for pork from pigs never fed β-AA, with two US plants having been approved for export to Russia in 2013 (USMEF Staff, 2017). As of 2016, Brazilian pork accounts for 95 % of pork imports to Russia (Vorotnikov, 2017). If political relations are restored with Russia, the US is in a prime position to regain some of the Russian market from Brazil because of the US exporting pork at $0.40/lb lower than

Brazil (Vorotnikov, 2017). The US also stands to lose strong economic trade ties with Taiwan, the 12th largest trading partner in 2012 and 13th largest pork export market for the US (Kan and Marrison, 2014).

Antibiotics

Antibiotics have been widely used in livestock industries to prevent and treat diseases, as well as a growth promotant. As animal agriculture transitioned from small farms to large commercial operations, the use of antibiotics has increased (Smith and Crabtree, 2005). Antibiotics as a feed additive have been widely used to prevent disease and as a growth promotant. With the increase in cases of infections by antibiotic resistant bacteria, consumers are becoming increasingly concerned about the use of antibiotics in livestock industries (O'Neil, 2014).

<u>*Mechanism of action*</u>

The exact mechanism by which antibiotics work is not fully understood; however, three modes of actions have been proposed (Cromwell, 1991). The first is the nutrient effect as a growth promotant, antibiotics may enhance nutrient uptake by shifting the population of bacteria from those that compete with the host for amino acids and vitamins to those that can synthesize amino acid and vitamins (Cromwell, 1991). The second is the metabolic effect .Though different antibiotics have different mode of actions, they are able to target specific sites in the bacteria and suppress metabolic activities critical for survival, i.e. inhibition of cell wall and cell membrane synthesis (Cromwell, 1991). The third is a diseases control effect. Pigs are exposed to pathogens in the environment that may cause sub-clinical disease. By administering antibiotics, the pathogen load is reduced and the overall health of the animal increased so that they can perform as close as possible to their genetic potential (Cromwell, 1991).

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The use of antibiotics as a feed additive can have detrimental effects on the normal GI flora and subsequently reduce protection to the host animal (Fuller, 1989; Waaij et al., 2000; Sørum and Sunde, 2001; Gaskins, 2002). While antibiotics are very effective in treating disease, antibiotics can have negative impacts on indigenous microflora (Fuller, 1989). Antibiotics can disturb the normal colonization pattern of microflora in the GI tract and lead to the spread of bacteria that may become resistant to antibiotics (van der Waaij and Nord, 2000). Additionally, disturbance of the indigenous microflora can lead to secondary infections, which would require more antibiotic usage (Sørum and Sunde, 2001).

Previous research has shown antibiotics have the potential to alter the intestinal microbial community, particularly with prolonged use, and could negatively impact immune function (Gaskins et al., 2002; Croswell et al., 2009; Russell et al., 2012). According to Russell and others (2012), the colonization of microbes in the gut and the role they play in the functionality of the immune system has been evaluated in many human and mouse studies over the years. Sørum and Sunde (2001) suggested that normal microflora are able to activate the development of lymphatic tissues in the GI tract, and these tissues produce immunologic agents to protect the host animal from infection. For example, Russell and others (2012) demonstrated that vancomycin significantly reduce immunologic T-cells in the colon of mice and resulted in an increased predisposition to allergy-induced asthma. Antibiotics administered to rainbow trout negatively impacted mitogenic and allogeneic responses of leuckocytes (Grondel et al., 1985). Some intestinal bacteria stimulate the secretion of immunoglobulins such as immunoglobulin A, which helps to limit the entry of pathogens into the epithelia (Che et al., 2014). Antibiotics reduced the activities of various enzymes such as sucrase and lactase in the intestines of pigs before weaning

(Collington et al., 1990). Some antibiotics may up-regulate pro-inflammatory cytokines that are associated with inflammatory diarrhea (Lee et al., 2016).

Growth and feed efficiency

Pork producers have commonly used antibiotics to prevent infection and to improve growth performance and feed efficiency (Cromwell, 2002; Allen et al., 2013). Between 1950 and 1985, extensive research was performed documenting the effectiveness of antibiotics on efficiency and growth in pigs was done (Stahly et al., 1980; Edmonds et al., 1985; Cromwell, 2002). Later, other researchers demonstrated the efficacy of antibiotic inclusion in pig feed and found live weight gain improved by an estimated 3.3 % to 8.8 % and feed efficiency by 7% (Van Lunen, 2003). However, more recent research distinguished the effects on pigs based on their age and found the improvement in growth and feed efficiency was greater significant in younger pigs treated with antibiotics when compared to older pigs (Gaskins et al., 2002). These results were in agreement with those of Cromwell (2002), which demonstrated that young pigs fed antibiotics had an estimated 16.4 % increase in growth rate and 9.6 % increase in feed efficiency while older and slightly heavier pigs had an estimated 10.6 % increase in growth rate and 4.5 % increase in feed efficiency. Although antibiotics are more effective in in younger pigs, they have an overall positive benefit in terms of maximizing production efficiency (Cromwell, 2002). When feed intake was held constant, administration of antibiotics still had a favorable effect on feed efficiency and body weight gain, which was attributed to improved nitrogen metabolism, apparent nitrogen digestibility, and nitrogen retention of pigs supplemented with antibiotics (Gaskins et al., 2002).

Furthermore, microbial fermentation in the large intestine provides an estimated 16 % of the total energy supply for pigs, but certain strains of gram-positive bacteria use as much as 6 %

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of the net energy in the pig's diet during microbial fermentation in the stomach and small intestine (Jensen, 1998). Additionally, bacteria use glucose to produce lactic acid, which not only increases the velocity of nutrient along the intestine, but also reduces the available energy to the host epithelial cells (Gaskins et al., 2002). Moreover, bacteria degrade amino acids and converts them into toxic metabolites such as amines, ammonia, phenols, and indoles; therefore, antibiotics are used to target these bacteria and increase the availability of amino acids (Macfarlane and Macfarlane, 1995).

Consumer opinion

The benefits derived from antibiotics have not been without public health concerns due to the associated risk of antibiotic resistant bacteria (Cromwell et al., 2002). The development of antibiotic resistant bacteria has led to human health concerns. Antibiotic resistant bacteria, or "superbugs," are on the increase (Gillies, 2016), which has resulted in an increased focus on alternatives to antibiotics in order to protect the health of the public as well as the health of livestock. Approximately 2 million people are infected annually with antibiotic resistant bacteria in the US with over 23,000 deaths attributed to these infections (CDC, 2017).

Many countries have seen a rapid increase in organic animal production due to the increase demand from consumers for meat they consider wholesome, fresh, free of hormones, antibiotics, and other toxic chemicals that may also negatively impact the environment (Smith and Crabtree, 2005; Blair, 2007). Multiple studies found consumers are willing to pay a premium for meat marketed with some form of safeguards relating to additives used (Smith and Crabtree, 2005; Blair, 2007; Consumer Reports, 2012). Within this context, antibiotic free labeling on food derived from animals is becoming increasingly popular, and this has prompted producers to look

for natural alternatives to antibiotics to maintain animal health, growth performance, and feed efficiency (Consumer Reports, 2012).

Veterinarian Feed Directive

The increasing incidence of antibiotic resistant bacteria is the driving force behind the Food and Drug Administration's decision to implement the Veterinary Feed Directive (**VFD)** rule, which became effective on January 1, 2017. The VFD rule creates stricter guidelines for the use of antibiotics the Food and Drug Administration deems "medically important" (AVMA, 2017). The initiative by the FDA to transition over the counter antibiotics to VFD was a 3 year process because animal health companies were given time to voluntarily remove medically important antibiotics from their labels (NPB, 2016) . Farmers were also given the time to develop a veterinary-client-patient relationship. Previously, only three products labelled for use in swine required approval by a veterinarian, but under the new VFD provisions, most antibiotics labelled for use in swine are affected (NPB, 2016). Swine producers are now required to obtain a VFD before administering any antibiotics that falls under the new VFD and the issuing veterinarian, distributing feed mill, as well as producers must keep a copy of the VFD for a period of two years (NPB, 2016). Furthermore, each VFD has an expiration date, which means that farmers cannot administer feed to animals that has been expired. Instead, they must obtain a new VFD irrespective of the amount of feed remaining in feeder bins (NPB, 2016).

Alternative Forms of Feed Additives

Seaweed and seaweed extracts

Seaweed is not just the wrap on sushi. Seaweeds are dynamic forms of algae that possess many biologically active compounds, which are currently being exploited for their applications in medicine, pharmaceutical industries, livestock feed additives, and a source of food, especially

in Asian countries (Wong and Cheung, 2000; Dharmanada, 2002; McDonnell et al., 2010; Holdt and Kraan, 2011; Li et al., 2011; Fleurence et al., 2012; Jiao et al., 2012; Liu et al., 2012; Rohani-Ghadikolaei et al., 2012; Yu et al., 2014). By definition, seaweeds are photosynthetic, multicellular marine macro algae that grow in abundance in rivers, lakes, and oceans of the world (Yu et al., 2014). Typically, seaweeds only occupy littoral or intertidal zones, seldom inundated with people or floodwaters (Gupta and Abu-Ghannam, 2011a). Littoral zones are close to the shoreline where sunlight can easily penetrate and are most suitable for the growth and development of seaweed because of the specific growing conditions required (Beames, 1990). Although seaweeds resemble a plant, they do not have some basic structures associates with terrestrial plants. For example, they lack vascular tissues such as roots, stems, and leaves (Yu et al., 2014). Instead, some species of seaweeds have a holdfast, stipe, and blade (Fig. 1.1). The holdfast is a root root-like structure that anchors the plant to surfaces such as rocks or the ocean floor (Duddington, 1971; Beames, 1990). Unlike terrestrial plants that have a canopy, the section above the holdfast is called a frond (Beames, 1990). The frond consists of the blades, stipe, and gas bladders. The blades, or lamina, are the life-like structures that are similar to the leaves of a plant. The stipe, which resembles the stem of terrestrial plants and varies in length (Duddington, 1971), connects the upper section of the plant to the holdfast (Beames, 1990). There are also gas bladders called pneumatocytes incorporated within the frond to help the plant float in water and to access light for photosynthesis (Beames, 1990).
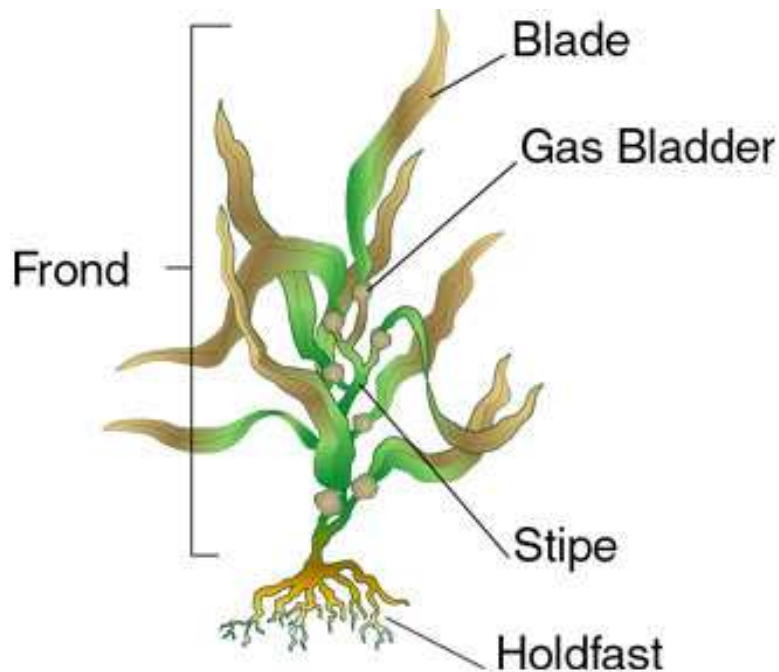
Figure 1.1. Structure of seaweed. (Gooding, 2011)

Seaweeds are classified by their biochemical constituents and pigmentation into three

major taxonomical classes: brown seaweed *(Phaeophyceae)*, green seaweed (*Chlorophyceae*),

and red seaweed (*Rhodophyceae*) (Gupta and Abu-Ghannam, 2011a). Red seaweeds contain

phycoerythrin and phycocyanin, which mask the reflection of other pigments and reflect a red

light, resulting in the seaweed appearing to be red (Gupta and Abu-Ghannam, 2011a). The

pigments that mask other pigments are fucoxanthin and xanthophyll in brown seaweeds and are

chlorophyll a and b, beta-carotene, and varying xanthophylls in green seaweeds (Gupta and Abu-

Ghannam, 2011a). There are many biologically active compounds that can be extracted from

seaweeds and include polysaccharides, phenolics, phlorotannins, proteins, peptides, amino acids,

terpenes, and lipids (Yu et al., 2014). However, the nutritional value of seaweed, which is

estimated from the chemical composition, may vary over the growing season as the chemical

composition can be influenced by factors such as time of harvest, sex, geographic area, season,

year, water temperature, species, family, and depth of immersion (Jenson, 1993; Murakami et al., 2011; Fleurence et al., 2012; Rohani-Ghadikolaei et al., 2012). Murakami and others (2011) observed seasonal and maturity variations in lipid, protein, and dietary fiber content of a brown seaweed species, *Sargassum horneri*. The authors concluded that early spring seemed to be the best time of year to harvest seaweed, as the seaweed would be fully mature and consist of the optimum lipid, protein, and dietary fiber content (Murakami et al., 2011).

Chemical composition of seaweed

Biochemical composition of seaweed is well studied. Of the three taxonomical classes, brown seaweeds, such as *Laminaria digitata*, *Ascophylum nodosum*, *Fucus vesiculoss*, and *Himanthalia elonggata*, seem to be the most studied (Fleurence, 1999). Brown seaweeds contain polysaccharides such as alginate, fucoidan, and laminarin (Chojnacka et al., 2012, Schiener et al., 2015). These polysaccharides have widespread applications including use as a strong antimicrobial (Nagayama et al., 2002), an antitumor (Soeda et al., 1994), an antioxidant (Ye et al., 2005), and an antiviral (Ponce et al., 2003). Agar, xylan, carrageenan, water-soluble sulfated galactan, floridean starch, and porphyrin are some of the common polysaccharides found in red seaweeds. Green seaweeds contain polysaccharides such as sulfuric acids, sulphated galactan, and xylan. The biochemical composition of seaweed is primarily influenced by factors such as species, sex, maturity, geographical location, and environmental conditions (Fleurence et al., 2012). Seaweeds that are found growing under adverse marine conditions will produce different bioactive compounds from those grown either on land or in tidal or subtidal zones where environmental conditions are not as severe (Yu et al., 2014).

The composition of laminarin varies seasonally, but laminarin forms about 10 to 32 % of the dry matter weight of seaweed (Holdt and Kraan, 2011). Fucoidan constitutes about 10 to 20

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% of dry matter of brown seaweed (Holdt and Kraan, 2011). The content of phenols in seaweeds ranges from 1 to 4 % and is lower in green and red seaweeds as compared to brown seaweeds (Holdt and Kraan, 2011).

Protein content of some seaweeds range from 5 to 35 % of the dry weight, with high protein seaweeds having a similar protein content to high protein content crops such as soybean, which contains about 35 % protein on a dry-matter basis (Rajauria, 2015). The amino acid profile of seaweed varies depending on species. Glutamic and aspartic acids in brown seaweeds represent 22 to 44 % of the total amino acids content. In green seaweeds, these 2 amino acids represent 26 to 32 % of the total amino acid content, but are present in much lower concentrations in red seaweeds, ranging from 14 to 19 % of the total amino acid content (Fleurence, 1999). Additionally, seaweed contain polysaccharides and low levels of lipids. On a dry matter basis, seaweeds contain 1 to 5 % lipids and the total content of polysaccharides is about 76 % of the dry weight (Rajauria, 2015). Seaweed mineral content is much greater than terrestrial plants. Seaweeds contain a rich content of macro-minerals and trace elements such as calcium, sodium, and magnesium, which are very important in the diet of humans and animals. Some brown seaweeds contain upwards of 3.25 g calcium/kg dry matter (Fleurence et al., 2012).

Traditional use of seaweed

Countries in the Far East such as Japan and Vietnam have a long history of using seaweed as a source of food, pest control, fertilizers, and medicine (Mabeau and Fleurence, 1993; Dharmananda et al., 2002; Hong et al., 2007; Yang et al., 2010; Craigie, 2011; Murakami et al., 2011; Wang et al., 2016). Seaweed seems to be a relatively low cost source of food, which makes it accessible to millions of people that use it to improve the quality of their lives. Wakame, or *Undaria pinnatifda*, is considered a delicacy by the Japanese and Koreans and,

despite the Japanese producing over 70,000 tons of raw Wakame, it was not enough to meet the high demand (Dharmanada, 2002). Kelp, or brown seaweed, is also commonly consumed in Asia because it is relatively inexpensive and has many nutritious benefits (Dharmanada, 2002). Porphyra, a type of red seaweed, is the most commonly used seaweed in Japan and is the seaweed used for wrapping sushi (Dharmanada, 2002). An analysis of the use of seaweed as a source of food showed that 221 species were used globally between 1994 and 1995 (Zemke-White and Ohno, 1999). Europe is rapidly consuming more seaweed despite lacking a regulatory body to govern the use of seaweed as a food (Rupérez, 2002). France is reported as the only country in Europe to have specific regulations on how to use seaweed (Mabeau and Fleurence, 1993). Some classes of seaweed, specifically *Sargassum*, *Gracilaria*, *Kappaphycus*, and *Euchema*, are widely used and have great economic benefits in Vietnam (Hong et al., 2007). The **FDA** permits the use of seaweed as a condiment as long as the concentrations of arsenic, heavy metals, lead, and iodine do not exceed toxic levels (Mabeau and Fleurence, 1993). It appears that seaweed is gaining popularity among Western countries, which are now exploring the nutritional benefits of including seaweed in the diet.

The phytochemicals present in seaweed have strong biological activities that are of potential benefit to human, animal, and plant health. For decades, agricultural industries worldwide have utilized seaweed to improve microbial populations in the soil, stimulate root growth, inhibit growth and proliferation of soil pathogens, treat plant diseases, and improve crop yields (Craigie, 2011; Wang et al., 2016). Seaweeds, when used as a fertilizer or for pest control, have been shown to increase the yield of marigolds and cabbages (Aldworth and van Standen, 1987), soybeans (Rathore et al., 2009), tomatoes (Hernández-Herrera et al., 2014), and apples (Wang et al., 2016). Stirk and Van Staden (1997) indicated that seaweed has the potential to

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protect plants from several environmental stressors such as insects (aphids and spiders), fungus, and nematodes that could be detrimental to plant growth. Wang and others (2016) conjectured that the process by which seaweed acted as a fertilizer was by suppressing soil pathogens, resulting in a more favorable soil microbial population, and by increasing soil enzymatic activity, resulting in increased breakdown of organic matter and increased water holding capacity. The use of pesticides and nitrogen-based fertilizers has grown and exposure of humans to these agrichemicals can have acute and chronic health effects, including acute and chronic neurotoxicity, lung damage, chemical burns, infant methemoglobinemia, cancer, immunologic abnormalities, and adverse reproductive and developmental effects (Weisenburger, 1993). Wang and others (2016) showed that application of a seaweed fertilizer resulted in apple (*Malus hupehensis* Rehd.) seedlings having an increased height and dry weight compared to controls (no fertilizer applied) in fields susceptible to apple replant disease, which is caused by a variety of biological agents with *Pratylenchus penetrans* being the main contributor.

Consumers are concerned with what food they eat not only because of the nutrition content but because food has bioactive compounds that may restore damaged cells, cure and prevent diseases, and improve the quality of life (Holdt and Kraan, 2011; Wang et al., 2016). Food can serve as a social marker and can provide relief from anxiety, depression, loneliness, and boredom (Berry, 2010). Seaweed contains polysaccharides that have strong anticancer, antimicrobial, and antibacterial properties that may be able to promote cell function and protect the body from disease (Li et al., 2011; Liu et al., 2012; Atashrazm et al., 2015). The phlorotannin faction in seaweed has been reported to reduce cancer cell formation in the human body by targeting key apoptotic molecules (Atashrazm et al., 2015). Fucoidan, a natural sulphated polysaccharide localized in cell wall matrix of different species of brown seaweed, has shown

both *in vivo* and *in vitro* anticancer properties (Kalimuthu and Kim, 2015). Specifically, fucoidan inhibited mouse breast cancer growth *in vitro* and *in vivo* by down regulating the expression of vascular endothelial growth factor (Xue et al., 2012). Jiao and others (2012) found that polysaccharides extracted from seaweed have an anti-H1N1 property. Particularly, an extract from *Fucus vesiculosus* inhibits an influenza virus by 77.9 % (Jiao et al., 2012).

Seaweed as a livestock feed additive

The use of seaweed as a feed additive in animal diets is gaining worldwide acceptance, with Europe, Canada, and Latin American countries relying heavily on sustainable production (Chojnacka et al., 2012; Rebours et al., 2014; Rajauria, 2015). It has become apparent that dietary inclusion of one or more species of seaweed maximizes the nutritional value of the feed and may lower the requirements for food crops used in human diets (Rajauria, 2015). Some of the driving forces being the increased interests to use seaweed as a livestock additive, relates to the high protein and fiber content, and relatively well-balanced amino acid profile (Rajauria, 2015). More importantly, producers have specific interests in feed additives that are organically derived, economically viable and globally underutilized, which has put an increased focus on seaweed as feed additive (Chojnacka et al., 2012; Rajauria, 2015).

Ascophyllum nodosum is currently the most extensively exploited seaweed for its bioactive compounds, which are used as raw materials in seaweed meal for a wide range of domesticated farm animals (McHugh, 2003). In fish diet, seaweed meal as an additive improved performance through better feed efficiency and greater lipid metabolism (Vadher et al., 2016). It also improved diseases resistance and meat quality traits without negatively impacting performance and feed efficiency, and indicates potential for its use as feed additive (Vadher et al., 2016).

In sheep, seaweed meal increased wool production and regulated body weight better during winter feeding months (McHugh, 2003). Additionally, the use of seaweed meal in dairy cattle increased milk production by an estimated average of 6.8 %, resulting in an increase of income rates by 13 %. However, only minimal benefits were obtained in poultry diets (McHugh, 2003).

Gastrointestinal tract health

Improving and maintaining GI tract health is fundamental to feed efficiency as well as the overall health of pigs since the GI tract is home to a highly diverse and densely populated commensal microflora, which regulate growth and development (Rastall, 2004; Tlaskalová-Hogenovà et al., 2004; Reilly et al., 2008; Sekirov et al., 2008; Yap et al., 2008; Antonopolos et al., 2009; de Lange et al., 2010; McDonnell et al., 2010; O'Doherty et al., 2010; Jandhyala et al., 2015). Seaweed-derived soluble dietary fibers such as laminarin, fucoidan, and alginates have a positive impact on the gut microflora due their strong antimicrobial, antibacterial and antiviral properties that helps to maintain a healthy microbial environment (Brownlee et al., 2003; Thomsen et al., 2006; Lynch et al., 2010; McDonnell et al., 2010; O'Doherty et al., 2010; Smith et al., 2011).

In addition, dietary fibers such as laminarin interact directly with the mucosal layer to increase the production of mucin from goblet cells and to strengthen mucosal stability in order to maintain intestinal hemostasis (Montagne et al., 2003; Kim and Ho, 2010; Smith et al., 2011; Heim et al., 2014). Although the mechanisms by which laminarin stimulates the production of mucin is not yet known, it is hypothesized that laminarin either provides a source of substrate for intestinal microbes or stimulates mucosa cells, which corresponds with increase expression of mucin 1 and 4 in the ileum and colon (Smith et al., 2011). Furthermore, Smith and others (2011)

found laminarin affects the quality and quantity of mucin production in the jejunum, ileum, caecum, and colon of piglets.

Mucin is comprised of several defensive compounds, which protect against microbial attachment and invasion (Devillé et al., 2007; Linden et al., 2008; Heim et al., 2014). Therefore, *Ascophyllum nodosum*, which is a soluble fiber stimulating mucin production, was found to reduce coliform counts in the ileum and caecum of finishing pigs (Gardiner et al., 2008). Similarly, Reilly and others (2008) reported a reduction of bacteria considered bad in the intestines of piglets fed different varieties of seaweed extracts. Although seaweed extracts demonstrated positive effects on GI health, deliberate challenge studies are required in order to determine the efficacy of seaweeds on pathogen reduction (Gardiner et al., 2008). Furthermore, this would have some implications for feed efficiency, which has a strong correlation with pathogen load (Reilly et al., 2008; Jiao et al., 2012). It would also increase the understanding about how pathogens may result in more energy being directed toward disease prevention and not production.

Immune regulation

Extensive experiments were conducted to test immunostimulant properties of seaweed-based polysaccharides (laminarin and fucoidan) on the immune status of pigs (Turner et al., 2000; Reilly et al., 2008; McDonnell et al., 2010; O'Doherty et al., 2010; Smith et al., 2011; Leonard et al., 2012; Heim et al., 2014). Plant based polysaccharides exhibiting immunomodulatory properties were found to stimulate a strong immune response, modulating humoral and cellular immunity thus controlling control pathogenic infections (Brown and Gordon, 2003; Chen and Seviour, 2007; Volman et al., 2008; Soltanian et al., 2009). In weaner pigs, dietary laminarin stimulated the release of cytokines and chemokines, which intern

increased leukocytes counts to improve a strong immune response (Reilly et al., 2008). Piglets from sows supplemented with laminarin, produced more phagocytes that eliminated *Escherichia coli*, which typically increases susceptibility to infections (Leonard et al., 2010). Additionally, laminarin and fucoidan were found to inhibit the replication of viruses and bacteria due to their antiviral, antimicrobial, and immunomodulatory properties (O'Doherty et al., 2010). They elicit the expression of pro-inflammatory cytokines (TNF-α, TGF-α, IL-6, and IL-1α) and anti-inflammatory cytokines (IL-10) (Leonard et al., 2012; Heim et al., 2014), which are necessary to increase resistance to diseases and restore balance after immune activation in immune compromised pigs.

Laminarin is a beta glucan and, although the mechanisms by which it regulates immune system is not yet fully elucidated, there are some suggestions presented in the literature. For example, beta glucans bind to a number of receptors present on cell surfaces and activate a cascade of events that cause these cells to increase their abilities to phagocytize, eliminate, and digest bacteria that may negatively impact immune system (Brown and Gordon, 2003). More specifically, laminarin binds to complement receptor 3, lactosylceramide, dectin-1, and scavenger and non-toll-like receptors resulting in the recognition, cytotoxicity, and phagocytosis abilities of these cells to target invaders that may affect immune system (Brown and Gordon, 2003; Brown, 2006). The dectin-1 pathway seems to be activated by laminarin and this activation results in an increased production of interleukin-6 and -8 cytokine gene expression in pigs challenged *ex vivo* by bacterial lipopolysaccharide challenge (Smith et al., 2011). However, the spleen tyrosine kinase pathway is just as important because it is needed for collaborative signaling of dectin-1 and toll-like receptor 2 (Dennehy et al., 2008). Dectin-1 receptors use the

spleen tyrosine kinase pathway and intercellular adaptors to induce secretion of cytokines and chemokines necessary to fight pathogens (Dennehy et al., 2008).

Immunoglobulins also known as antibodies are specialized glycoprotein molecules produced by plasma cells. They are used by immune cells to recognize and destroy foreign invaders. Seaweed extracts enhanced secretion of serum immunoglobins G, A, and M concentrations in piglets during lactation from sows supplemented with the extract, which suggests enhanced humoral immunity that creates an environment for better growth and development due to greater protection against pathogens (Leonard et al., 2010)

Stress regulation

Literature regarding how seaweed regulates stress in animals and plants is very limited. However, studies using seaweed to mitigate stress are populous and have been performed in lambs (Saker et al., 2004; Archer et al., 2008; Abdoun et al., 2014), goats (Kannan et al., 2007a, b), rats (Spiers et al., 2004), beef steers (Spiers et al., 2004), poultry (Sheikh-Hamad et al., 1994), spinach (Fan et al., 2011), and wheat (Wang et al., 2003; Ibrahim et al., 2014). In lambs, seaweed supplementation alleviated heat stress and reduced basal plasma cortisol concentrations (Archer et al., 2008). Though the mechanism by which seaweed supplementation works is not fully understood, sodium chloride and potassium gluconate salt contents are speculated to be responsible for the effects found in this study (Archer et al., 2008). Contrary to these findings, Kannan and others (2007a) reported no effects on plasma cortisol concentration in goats.

Multiple students found heat and transport stress as major environmental factors impacting physiological changes such as, increased respirator rate, body temperature, and oxidative stress (Sheikh-Hamad et al., 1994; Spiers et al., 2004; Archer et al., 2008; Fan et al., 2011). In beef steers and lambs exposed to heat stress, supplementation of seaweed reduced

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respiratory rate significantly lower variation in body temperature during transport (Spiers et al., 2004; Abdoun et al., 2014). Because seaweed is able to improve health conditions and moderate body temperature under stressful conditions, heat stressed lambs would be better able to regulate stress (Abdoun et al., 2014).

Performance and carcass characteristics

The use of seaweed extracts to improve performance, feed efficiency, and carcass characteristics in pigs is well studied and variable results have been reported (Grinstead et al., 2000; Turner et al., 2002; Gardiner et al., 2008; Gahan et al., 2009; Leonard et al. 2010; Michiels et al., 2012). The magnitude of response associated with the use of seaweed on growth performance and carcass characteristics has a strong correlation with variety (red, brown, green or yellow), bioactive compounds, and the level or rate of inclusion in the diet (Galland-Irmouli et al., 1999; Norziah and Ching, 2000).

For example, inclusion of 300 ppm of laminarin significantly improved performance of weaned pigs, with laminarin supplemented pigs having a higher ADG and G:F than non-supplemented pigs (McDonnell et al., 2010). These results concur with those of O'Doherty and others (2010) and Dillon and others (2010), who supplemented pigs with 112 g laminarin and 89 g fucoidan per kilogram of feed. Growth (ADG) and G:F were improved in supplemented pigs compared to non-supplemented pigs (Dillon et al., 2010; O'Doherty et al., 2010). In all of these experiments, the authors speculated that laminarin was mainly responsible for the improved ADG and G:F due to its effects on nutrient digestibility, as well as reducing the population of *Escherichia coli* in the feces, which could cause infection and negatively impact growth (Dillion et al., 2010; McDonnell et al., 2010; O'Doherty et al., 2010).

In contrast, *Laminaria hyperborea* and *Laminaria digitata* seaweed extracts had no significant effects on performance (feed intake, ADG, and G:F) when compared to non-supplemented animals (Reilly et al., 2008). Other varieties, such as dried *Ascophyllum nodosum* gave variable results depending on the processing techniques used to remove certain compounds (phenols and alginates) found to inhibit growth (Turner et al., 2002; Gardiner et al., 2008; Gahan et al., 2009). Therefore, the use of *Ascophyllum nodosum* as a feed additive in a commercial setting would be limited if these compounds are not removed during the extraction process (Gahan et al., 2009). Furthermore, seaweed increased colostrum and serum immunoglobulin G concentrations in piglets from mothers fed the extract. This is important because the immunomodulating effects of immunoglobulins improved overall health resulting in increased utilization and absorption of nutrients to improved efficiency of production (Leonard et al., 2010). Moreover, seaweed extract significantly increase villous height and villous height to crypt depth ratio in the ileum and jejunum of pigs fed the extract (Leonard et al., 2010). An increase in villous height and crypt depth ratio is important because it could increase the potential for better utilization of feed, which would lead to improved production efficiency.

Previous research found seaweed contain bioactive compounds, which affect major carcass characteristics such as marbling, color, and hot carcass weight (Allen et al., 2001; Branden et al., 2007; Holdt and Kraan, 2011). For example, greater marbling scores and percentage of USDA choice carcasses were observed in cattle supplemented with *Ascophyllum nodosum* (Branden et al., 2007). These results concurred with those reported by Allen and others (2001), who found increased USDA quality grades and marbling scores in supplemented cattle. Researchers evaluated the effects of seaweed on loin muscle area, hot carcass weight, kidney-

pelvic-heart fat, and final yield in *Ascophyllum nodosum* supplemented steers and pigs and found no differences (Branden et al., 2007; Gardiner et al., 2008).

Furthermore, seaweed contains many bioactive compounds that act as flavor and moisture enhancers (Holdt and Kraan, 2011). For example, mannitol, a sugar alcohol similar to mannose, is found in many species of brown seaweed, especially in *Laminaria* and *Ecklonia* species, and is used in a wide variety of foods such as candies and chocolate to enhance flavor and taste (Holdt and Kraan, 2011). Because of its absorptive nature, mannitol increases moisture level, shelf life, and stability of food products. Increased juiciness and flavor is observed in seaweed fed pigs due to the presence of mannitol (Holdt and Kraan, 2011). Phycobiliproteins, particularly phycoerythrin, are natural compounds present in red seaweeds, which may influence meat color (Chronakis et al., 2000; Holdt and Kraan, 2011). For example, phycobiliproteins have diverse applications and are currently used as a natural colorant in the dairy and cosmetic industries (Sekar and Chandramohan, 2008).

Consumer opinion

Consumers are health conscious and are very particular about the food they eat (Smith and Crabtree, 2005). Consumers are also willing to pay more for food that is high in quality and have good appearance. Lately, consumers have been restrictive to chemical preservatives because research have shown that chemical preservatives such as benzoic acid, nitrates, butylated hydroxytoluene, butylated hydroxyanisole, and monosodium glutamate are associated with diseases that may have detrimental effects (Anand and Sati, 2013). Because of this, the need for natural preservatives containing organic compounds that are non- toxic in nature are highly demanded by consumers (Chojnacka et al., 2012).

Seaweed has been getting considerable attention and its popularity as a potential natural alternative to synthetic preservatives is increasing in the western world (Bouga and Combet, 2015). Particularly, there is a growing interest, in the food and pharmaceutical industries for the rich and diverse bioactive compounds found in seaweed and their potential applications to increase quality and safety (Gupta and Abu-Ghannam, 2011b; Holdt and Kraan, 2011). Seaweed is a rich source of natural antioxidant compounds such as fucoxanthin and astaxanthin, carotenoids, vitamins, phospholipids, terpenoids and peptides, which could reduce lipid oxidation in foods (Gupta and Abu-Ghannam, 2011b; Holdt and Kraan, 2011). Lipid oxidation is a major concern in the meat industry because it is the main reason for deterioration of meat in storage containing lipids (Castillo et al., 2013). Lipid oxidation occurs as early as 4 or 7 d of storage, resulting in reduced shelf life, rancidity and color changes, which are not appealing to consumers. Recently, concerns have arisen over the toxicity of synthetic antioxidant widely used in the meat industry (Castillo et al 2013), as such, consumers are demanding natural antioxidants that could potentially improve quality and safety (Gupta and Abu-Ghannam, 2011b).

Furthermore, consumers prefer organic compounds that may benefit their and seaweed has been linked to health benefits such as, reduced incidences of cancer, coronary heart diseases, human immunodeficiency virus, and herpes due to its antiviral properties and ability to enhance a strong immune function (Jiao et al., 2012; Brown et al., 2014; Bouga and Combet, 2015). Because of this, there is an increased consumption of seaweed, especially in Asian countries to improve health and wellbeing. Additionally, seaweed is now readily available to consumers in United Kingdom as a whole food and ingredient (Bouga and Combet, 2015). Moreover, careful selection processing and techniques mitigate concerns regarding toxicity due to high concentrations.

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CHAPTER 2: EFFECT OF FEEDING A SEAWEED EXTRACT TO COMMERCIAL PIGS[1]

Abstract

The purpose of this study was to investigate the effects of seaweed extract in the diet on performance, carcass characteristics, and pork quality of swine. Crossbred weaned pigs (n=120) were randomly allotted to 3 treatments (CON, SWE, and RAC). There were 8 pigs per pen and 5 pens per treatment. The CON group was fed a basal diet representative of a typical industry diet. The SWE group was fed the basal diet plus 1.5% seaweed extract during nursery and the first 37 days of grow/finish and fed the basal diet plus 0.5% seaweed extract thereafter. The RAC group was fed the basal diet until 28 days prior to market when they were fed ractopamine (Paylean, Elanco Animal Health, Greenfield, IN) at 7.4 mg/kg feed. No differences were found between treatments for growth, feed intake, or feed efficiency. Pigs on the RAC treatment had greater hot carcass weight and dressing percentage ($P < 0.05$). Chops from RAC pigs were lighter ($P = 0.05$), less red ($P < 0.05$), and tougher as measured by Warner-Bratzler shear force ($P = 0.08$). Therefore, no significant positive or negative effects of feeding seaweed extract to pigs on performance, carcass characteristics, or pork quality were found.

Key Words: Feed efficiency, Natural growth promotant, Ractopamine

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[1] The material in this chapter was co-authored by R. Somers, J. M. Young, J. Flaten, P. Borowicz, A. Ward, and D. J. Newman. R. M. Somers was responsible for feeding pigs and data collection, performing all laboratory experiments relating to project, and writing of this chapter. D. J. Newman designed the study, assisted with animal bleeding, data collection at slaughter, and carcass evaluation. J. M. Young assisted with all data collection, performed statistical analyses, and assisted with writing of this chapter. J. Flaten assisted with data collection at slaughter and histology and imaging analyses. P. Borowicz assisted with staining and imaging analysis. A. Ward assisted with data collection at slaughter and laboratory analysis of tissue samples.

Introduction

Ractopamine hydrochloride is a β-adrenergic agonist that has been widely used in the United States (US) swine industry to improve growth performance and feed efficiency. However, consumers have become increasingly concerned about the health risks associated with the use of β-agonists such as ractopamine. Countries such as China and Russia remain apprehensive and refuse to accept any traces of ractopamine in organs of the heart, kidney, lungs, and liver owing to the fact that these organs are of vital importance in their diets (Centner et al., 2014). As a result, some export markets are closed or extremely restrictive to product from the US. In 2014, China and Hong Kong imported approximately 17 % of pork exported from the US, which represents approximately $775 million of the US pork export market (USMEF, 2015). In terms of value, this was the fourth largest market for US pork including variety meat in 2014. Losing this market due to conflicts over the use of ractopamine could be detrimental to the US pork industry. Therefore, producers are investigating alternatives to ractopamine use in order to protect the health of the public and maintain US pork export markets.

Seaweed extracts are being investigated as a natural alternative because they contain bioactive compounds that are able to improve growth performance and carcass characteristics Allen et al., 2001; McDonnell et al., 2010). By decreasing the population of *Escherichia coli* in the intestines, feeding seaweed extracts results in increased nutrient digestibility and absorption Dillon et al., 2010). Because of this, seaweed extracts have the potential to serve as a replacement to ractopamine use in the US pork industry.

Therefore, the objective of this study was to investigate the effects of feeding seaweed extracts to pigs from weaning to market on performance, carcass characteristics, and pork quality.

Materials and Methods

All live animal procedures were approved by the North Dakota State University Institute of Animal Care and Use Committee (Protocol #A16033).

Table 2.1. Ingredient composition of the basal diet (as-fed basis).

Ingredients, % total	Phase[1] I	II	III	IV	V	VI
Corn, ground	28.80	52.42	70.48	76.66	81.33	84.57
Soy bean meal, 46%	16.20	23.90	26.40	20.70	16.50	13.54
Sun oil		1.50				
EnMax						
Nursery	55.00	20.00				
Sow		0.30	0.25			
G/F			0.19	0.38	0.38	0.38
Mono-cal, 21%		0.55	0.72	0.48	0.22	0.09
Limestone		0.67	1.06	0.95	0.89	0.86
Salt		0.40	0.45	0.40	0.40	0.40
L-lysine	e	0.15	0.26	0.25	0.20	0.11
L-threonine		0.04	0.08	0.07	0.01	
DL-methionine		0.07	0.11	0.06	0.02	
ReganoEX				0.05	0.05	0.05

[1]Phase I, II, III, IV, V, and VI diets were fed to pigs from 18 to 22 d of age, 23 to 38 d of age, 39 to 59 d of age, 60 to 97 d of age, 98 to 138 d of age, and 139 to 167 d of age, respectively.

Crossbred pigs (n = 120) were weaned and allotted to one of fifteen pens by body weight, equalizing sex across pens. Pens were randomly assigned to one of three treatments (**CON**, **SWE**, and **RAC**). Pigs on the CON treatment were fed a basal diet representative of a typical industry diet. Pigs on the SWE and RAC diets were fed the basal diet (Table 2.1) which is the

diet typically fed at the North Dakota State University Swine Barn and formulated to meet National Research Council requirements (NRC, 1998). The addition of supplements by treatment is shown in Table 2.2. Feed offered to each pen was weighed. Weigh backs were recorded as necessary and at the end of each phase.

Table 2.2. Diet assignments for treatments by diet phase (basal diet compositions for each phase were presented in Table 2.1).

	Nursery			Grow/Finish		
Treatment	Phase I	Phase II	Phase III	Phase IV	Phase V	Phase VI
Control	Basal	Basal	Basal	Basal	Basal	Basal
SWE	Basal + seaweed[1]	Basal + seaweed[1]	Basal + seaweed[1]	Basal + seaweed[1]	Basal + seaweed[2]	Basal + seaweed[2]
RAC	Basal	Basal	Basal	Basal	Basal	Basal + Paylean[3]

[1]Seaweed extract included in diet at 1.5 % on a weight basis.

[2]Seaweed extract included in diet at 0.5 % on a weight basis.

[3]Paylean (Elanco Animal Health, Greenfield, IN) was used as source of ractopamine hydrochloride and included at a rate of 7.4 mg/kg of total ration.

Pigs from each treatment group were weighed every two weeks from weaning to market to monitor average daily gain (**ADG**). When pigs reached an average of 127 kg live body weight, they were transported to a commercial slaughter facility for humane harvest. At the pork processing plant, hot carcass weight (**HCW**) was recorded and dressing percentage was calculated as percentage HCW of live body weight. Backfat depth was measured at the 1st and 10th ribs and at the last lumbar.

A 2.54-cm thick chop was removed at the 10th rib and transported to North Dakota State University for further analysis. Subjective color and marbling scores were assessed using the US

National Pork Board standard cards (NPB, 2011). Loin muscle area, chop fat depth (assessed ¾ around the chop from the center line), pH, and objective color (L*, a*, b* using a Minolta colorimeter) were assessed. An approximately 20-g sample was weighed, hung in a refrigerated room (4 °C) for 24 h, and reweighed. Drip loss was calculated as the percent loss of original weight. Samples were weighed, cooked to an internal temperature of 65 °C using clam-shell grills, and reweighed. Cook loss was calculated as the percent loss of original weight. Six 1.27-cm diameter cores were taken from each chop parallel to the muscle fibers and then sheared perpendicular to the muscle fibers using a Warner-Bratzler machine (G-R Electrical Manufacturing Co., Manhattan, KS). Maximum force for each core was recorded and analyzed as the average of the cores removed from each chop (**WBSF**).

All data was analyzed using the mixed procedure of SAS (SAS Institute, Inc., Cary, NC). The pen was the experimental unit for average daily feed intake (**ADFI**) and gain:feed ratio (**G:F**). The pig was the experimental unit for all other traits. Treatment was fit as a fixed effect for all models. Pen was fit as a random effect for ADFI and G:F. Dam and sire were fit as random effects for traits where the pig was the experimental unit.

Fresh tissue samples were collected from the mesentery side of the ileum, jejunum, and duodenum of pigs at slaughter. Dissected samples were immersion-fixed in a 10 % solution of neutral buffered formalin for < 24 h and transferred to a 70 % ethanol solution. After fixation, the samples were embedded in paraffin and 5 μm tissue sections were cut, mounted on glass slides to prepare for staining procedures. After deparaffinization and rehydration, slides were incubated in antigen retrieval buffer (10mM sodium citrate, 0.05 % Tween 20, pH 6) in a 2100 Retriever (Electron Microscopy Sciences), for 15 min at 121 °C. Tissues samples from all pigs were treated with a blocking buffer of 1 % BSA normal horse serum (Vector Laboratories, Burlingame, CA);

for 1 h at room temperature, and then incubated with a Ki-67 rabbit monoclonal antibody (Abcam). To label the primary antibody, a secondary CF 633 goat anti-rabbit antibody (Abcam) was incubated on tissue sections for 1 h at room temperature. The background nuclear staining was done with DAPI for 5 min at room temperature. Images (n = 3/tissue section) were taken with an LSM 700 observer Z1 microscope (Carl Zeiss AG; Oberkochen, Germany). Cellular proliferation was quantified using Image-Pro Premier software (Media-Cybernetics Inc.). For each image, the crypt regions of the jejunum. Ileum and duodenum were distinguished, and the total jejunal crypt cells versus proliferating cells within the same crypt was quantified.

Tissue samples (n= 3 tissue/section) were collected from the mesentery side of the ileum, jejunum, and duodenum of pigs at slaughter. All samples were stored in RNAlater (Ambion, Inc., Austin TX, USA) and transported to the NDSU laboratory where it was stored at a -20 °C until time for RNA extraction. Total RNA was extracted from 50 mg tissue samples (disrupted and homogenized) using an RNeasy Plus Mini Kit (Qiagen) according to the manufacturer's instruction. To avoid RNAse contamination, all equipment and surfaces were sprayed using RNase Away Decontamination Reagent (ThermoFisher Scientific). The extracted RNA was quantified using the Take3 module of synergy H1 Microplate Reader (BioTek, Winooski, VT). The mRNA was reverse transcribed to cDNA using QuantiTech Reverse Transcription Kit (Quiagen).

Real-time quantitative PCR assays were performed on cDNA samples in a 96-well optical plate on a 7500 Fast, ABI sequence detection System (Applied Biosystems, Foster City, CA, USA). All reactions were performed in triplicate. The primers used for real-time PCR were designed using NCBI Primer Blast. Primer sequence data are presented in Table 2.3.

Table 2.3. Primers for gene expression analysis of ileum, jejunum and duodenum.

Gene	Genbank ID	Forward/ Reverse	Amplcon Length (BP)
FABP2	NM_001031780.1	CGGAACTGAACTCACTGGGA/ CTGGACCATTTCATCCCCGA	124
PPIA	NM_214353	GCGTCTCCTTCGAGCTGTTT/ ACTTGCCACCAGTGCCATTA	174
B2M	NM_213978	TCAACCACTTTTCACACCGC/ GGTGGCGTGAGTAAACCTGA	130
ACTB	XM_003124280	AGATCAAGATCATCGCGCCT/ ATGCAACTAACAGTCCGCCT	170
HPRTI	NM_001032376.2	GGGAGGCCATCACATCGTAG/ CGCCCGTTGACTGGTCATTA	167
GAPDH	NM_001206359	CATTGCCCTCAACGACCACT/ ATGAGGTCCACCACCCTGTT	84

Amplification efficiency was determined by performing reactions over a five-point serial dilution. The reaction was carried out in a total volume of 20 μl containing a 10 μl (2X) iTaq Universal SYBR Green Master Mix (Bio-Rad), 1 μl each of 10 μM forward and reverse primers, and 1 μl of cDNA (1:100 dilution). The PCR program followed was 95 °C for 10 min followed by 40 cycles of 95 °C for 15 sec and 60 °C for 1 min. Following the completion of the reaction a disassociation (melt) curve was performed to monitor primer specificity.

Gene Norm function of qBase+ (Biogazelle) was used to calculate the M value of 5 potential reference genes (GAPDH, ACTB, B2M, PPIA, and HPRTI) to choose the one that was most stably expressed. PPIA had the lowed M value and, therefore, was selected as the reference

gene. Relative expression calculated using delta-delta Ct method (Livak and Schmittgen, 2001) and normalized to the same control animal in each tissue.

Results and Discussion

Feed was sent to South Dakota State University (Brookings, SD, USA) for analysis. Results are presented in Tables 2.4 and 2.5. Results were similar between treatment diets.

Simple statistics for performance traits are presented in Table 2.6. No treatment effects ($P > 0.15$) were observed for ADFI, G:F, or ADG during the nursery or grow/finish phases of production (Table 2.7). However, there were treatment × sex effects found for some ADG traits, particularly ADG measured in the nursery and Phases II to IV (Fig. 2.1-2.4). These results are comparable to those obtained by Reilly and others (2008) who supplemented pig diets with seaweed extracts and found no significant effects on performance traits when compared to the control. However, numerous researchers (Dierick et al., 2008; Gahan et al., 2009; Dillon et al., 2010; Leonard et al., 2010; O'Doherty et al., 2010; Michiels et al., 2012) have reported significant improvements in ADG and ADFI in nursery and grower-finish pigs supplemented with seaweed extracts in the diet. A lack of response in the current study can be explained by the health status of the pigs in this study, which was considered high health. According to Pierce and others (2005), minimal or no response is expected when healthy animals are fed additives such as seaweed that have prebiotic properties. Additionally, Gardiner and others (2008) reported linear decreases in ADG with increasing levels of SWE and attributed this response to compounds such phenols and alginates present in certain varieties of seaweeds known to inhibit growth. Unless processed to remove these compounds, the benefits of improving feed efficiency and other production traits will be limited (Gahan et al., 2009). It is possible that the SWE used in the current study contained compounds that may inhibit growth; however, the SWE used was not

Table 2.4. Feed analysis of diets for phases I, II, and III.

Traits	I	I+SWE	II	II+SWE	III	III+SWE
			Diet[1]			
Ash, %	7.00	7.76	5.82	7.62	4.60	5.67
Crude fat, %	4.74	4.91	4.43	4.75	2.43	2.28
Crude fiber, %	2.80	2.75	2.77	2.71	5.53	3.14
Nitrogen free extract, %	55.75	55.45	58.64	59.75	61.84	61.43
Crude protein, %	21.71	21.39	18.96	19.21	17.62	17.64
Total dry matter, %	92.00	92.33	90.62	91.33	89.24	90.14

[1]Phase I, II, and III diets were fed to pigs from 18 to 22 d of age, 23 to 38 d of age, and 39 to 59 d of age, respectively. SWE = seaweed extract.

Table 2.5. Feed analysis of diets IV, V, and VI.

Traits	IV	IV+SWE	V	V+SWE	VI	VI+SWE	IV+RAC
				Diet[1]			
Ash, %	3.97	4.49	3.61	3.67	3.35	3.53	3.27
Crude fat, %	2.67	2.68	2.73	2.55	2.74	2.34	3.06
Crude fiber, %	2.52	2.50	2.39	2.50	2.34	2.21	2.35
Nitrogen free extract, %	65.34	65.11	67.30	67.07	68.23	68.28	68.43
Crude protein, %	15.54	15.34	13.32	13.52	12.42	12.07	12.46
Total dry matter, %	90.21	90.11	89.37	89.31	89.07	89.92	89.56

[1]Phase IV, V, and VI diets were fed to pigs from 60 to 97 d of age, 98 to 138 d of age, and 139 to 167 d of age, respectively. SWE = seaweed extract; RAC = ractopamine hydrochloride.

Table 2.6. Simple statistics of performance traits.

Trait[1]	N	Mean	SD	Minimum	Maximum
ADFI, kg/d					
Nursery	15	0.73	0.05	0.64	0.80
Grow/Finish	15	2.23	0.14	2.04	2.44
Overall	15	1.79	0.10	1.64	1.93
I	15	0.12	0.02	0.09	0.17
II	15	0.48	0.04	0.43	0.55
III	15	1.07	0.08	0.92	1.19
IV	15	1.75	0.17	1.38	2.08
V	15	2.40	0.15	2.21	2.58
VI	15	2.63	0.16	2.35	2.90
Gain:feed, kg:kg					
Nursery	15	0.60	0.02	0.55	0.63
Grow/Finish	15	0.35	0.01	0.33	0.38
Overall	15	0.51	0.02	0.47	0.56
I	15	1.83	0.42	1.32	2.50
II	15	0.71	0.06	0.58	0.80
III	15	0.61	0.04	0.52	0.68
IV	15	0.48	0.03	0.44	0.55
V	15	0.38	0.01	0.36	0.40
VI	15	0.36	0.03	0.29	0.40
ADG, kg/d					
Nursery	116	0.43	0.06	0.32	0.59
Grow/Finish	111	0.88	0.18	0.0005	1.14
Overall	111	0.75	0.15	0.0002	0.96
I	120	0.21	0.03	0.14	0.28
II	120	0.34	0.07	0.13	0.50
III	118	0.65	0.07	0.48	0.81
IV	116	0.84	0.11	0.56	1.12
V	112	0.91	0.12	0.61	1.16
VI	109	0.93	0.24	-0.88	1.46

[1]ADFI = average daily feed intake; ADG = average daily gain; Phase I, II, III, IV, V, and VI = 18 to 22 d of age, 23 to 38 d of age, 39 to 59 d of age, 60 to 97 d of age, 98 to 138 d of age, and 139 to 167 d of age, respectively.

Table 2.7. Least square means of performance traits by treatment and by sex.[1]

	Treatment[2]					Sex[3]			
	CON	SWE	RAC	SEM	*P*-value	Barrow	Gilt	SEM	*P*-value
ADFI, kg/d									
Nursery	0.73	0.72		0.03	0.60				
Grow/Finish	2.21	2.21	2.25	0.07	0.88				
Overall	1.78	1.79	1.80	0.05	0.97				
I	0.12	0.12		0.01	0.93				
II	0.49	0.48		0.01	0.60				
III	1.08	1.05		0.04	0.54				
IV	1.77	1.72		0.08	0.59				
V	2.40	2.40		0.07	0.96				
VI	2.62	2.62	2.66	0.08	0.92				
G:F, kg gain:kg feed									
Nursery	0.59	0.60		0.01	0.71				
Grow/Finish	0.35	0.34	0.35	0.005	0.83				
Overall	0.50	0.50	0.51	0.01	0.61				
I	1.84	1.80		0.20	0.88				
II	0.71	0.71		0.03	0.90				
III	0.61	0.62		0.02	0.67				
IV	0.48	0.49		0.01	0.78				
V	0.38	0.38		0.004	0.17				
VI	0.35	0.35	0.36	0.01	0.90				
ADG, kg/d									
Nursery	0.43	0.43		0.01	0.69	0.43	0.43	0.01	0.78
Grow/Finish	0.87	0.87	0.89	0.03	0.77	0.89	0.86	0.03	0.39
Overall	0.74	0.74	0.76	0.03	0.86	0.76	0.74	0.02	0.46
I	0.22	0.21		0.005	0.38	0.21	0.21	0.005	0.77
II	0.34	0.34		0.02	0.99	0.34	0.33	0.02	0.42
III	0.65	0.65		0.01	0.79	0.66	0.64	0.01	0.12
IV	0.85	0.83		0.02	0.25	0.88[a]	0.80[b]	0.02	<0.0001
V	0.92	0.89		0.02	0.26	0.95[a]	0.85[b]	0.02	<0.0001
VI	0.90	0.92	0.94	0.05	0.69	0.90	0.94	0.04	0.39

[1]CON = control treatment; SWE = seaweed extract treatment; RAC = ractopamine hydrochloride treatment; SEM = standard error of the mean; ADFI = average daily feed intake; G:F = gain-to-feed ration; ADG = average daily gain. Phase I, II, III, IV, V, and VI = 18 to 22 d of age, 23 to 38 d of age, 39 to 59 d of age, 60 to 97 d of age, 98 to 138 d of age, and 139 to 167 d of age, respectively.
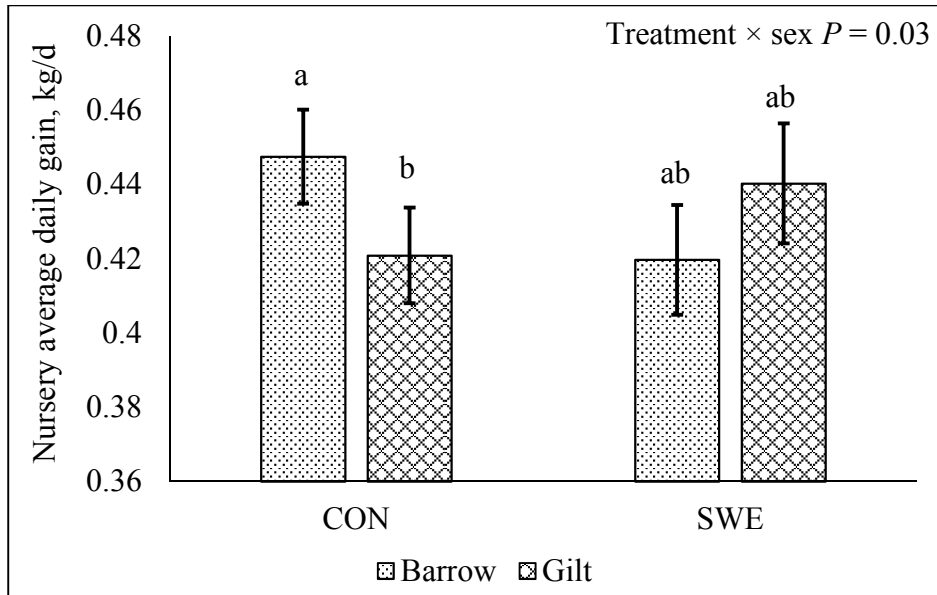
[2]Phases I-V are prior to feeding ractopamine hydrochloride so the RAC and CON treatment groups are the same.

[3]Feed intake was recorded on a pen basis and, since pens were mixed sex, ADFI and G:F cannot have a sex effect estimated.

Figure 2.1. Treatment (CON= control, SWE= seaweed extract supplement) × sex interaction for nursery average daily gain. Since the RAC (ractopamine hydrochloride supplement) and CON treatment pigs were on the same diet, RAC and CON were combined. Bars with different letters differ by $P < 0.05$.
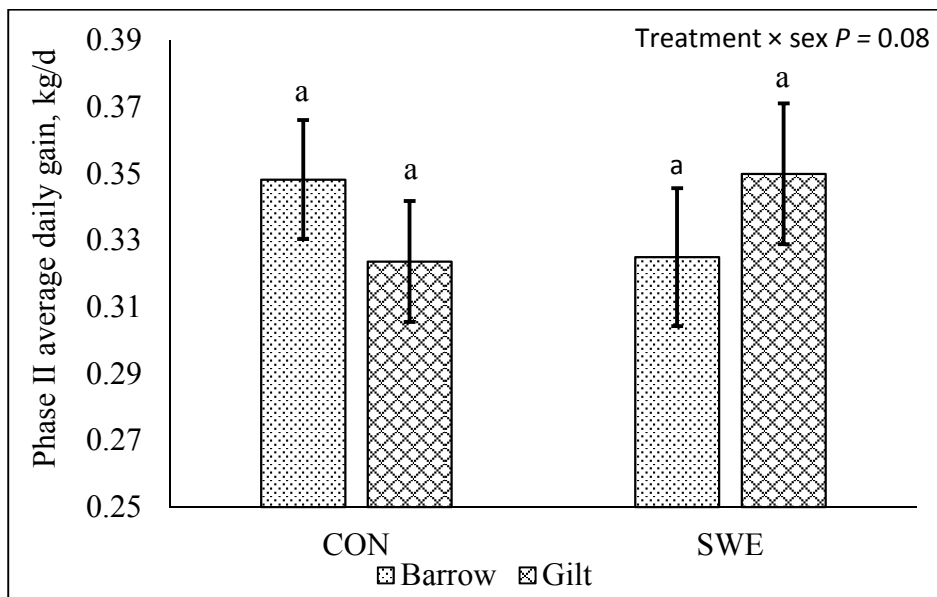
Figure 2.2. Treatment (CON= control, SWE= seaweed extract supplement) × sex interaction for Phase II (23 to 38 d of age) average daily gain. Since the RAC (ractopamine hydrochloride supplement) and CON treatment pigs were on the same diet, RAC and CON were combined. Bars with different letters differ by $P > 0.05$.
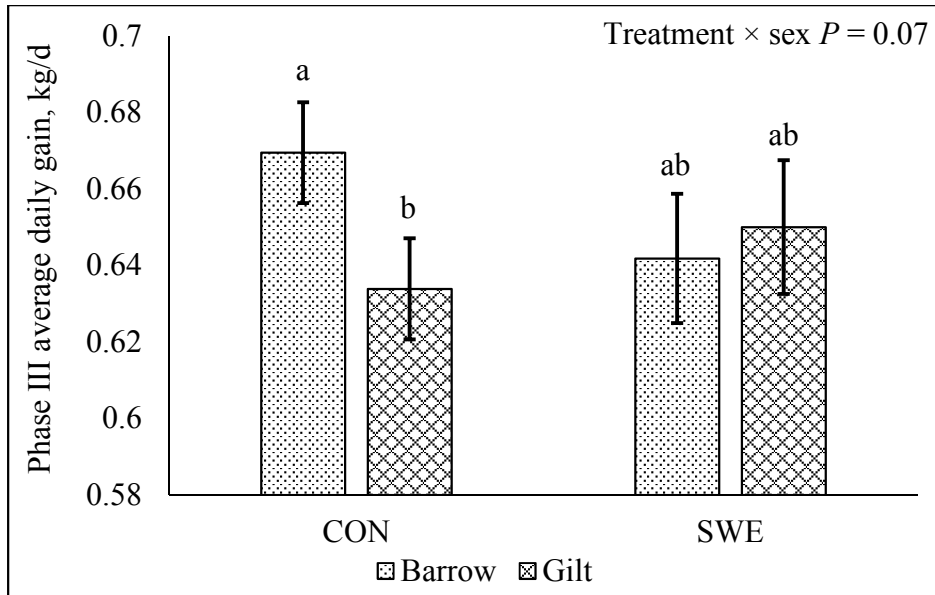
Figure 2.3. Treatment (CON= control, SWE= seaweed extract supplement) × sex interaction for Phase III (29 to 59 d of age) average daily gain. Since the RAC (ractopamine hydrochloride supplement) and CON treatment pigs were on the same diet, RAC and CON were combined. Bars with different letters differ by $P < 0.05$.
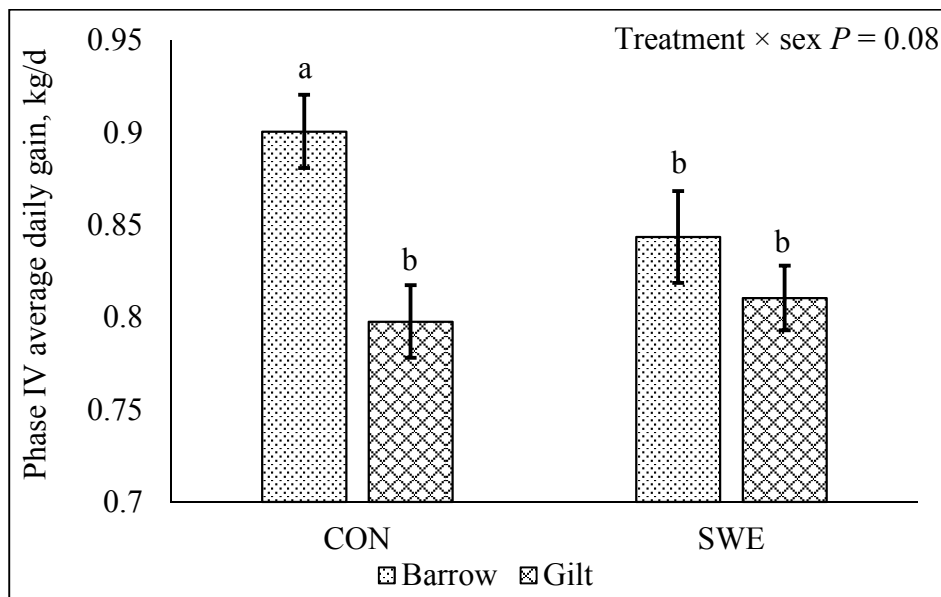
Figure 2.4. Treatment (CON= control, SWE= seaweed extract supplement) × sex interaction for Phase IV (60 to 97 d of age) average daily gain. Since the RAC (ractopamine hydrochloride supplement) and CON treatment pigs were on the same diet, RAC and CON were combined. Bars with different letters differ by $P < 0.05$.

analyzed for the presence of phenols and alginates. Time of harvest, geographic location, and maturity of seaweed have been shown to affect composition of bioactive compounds that play a key role in performance (Jenson, 1993; Murakami et al., 2011) and may also explain the observed results in the current study.

Simple statistics of carcass traits are reported in Table 2.8. Pigs fed RAC yielded carcasses with greater dressing percentages than CON or SWE pigs ($P < 0.05$), as a result of greater HCW ($P < 0.05$; Table 2.9). Improvement in dressing percentage and HCW is supported by previous studies (Dunshea et al,. 1993; Barker et al., 2002; Stoller et al., 2003; Armstrong et al., 2004, 2005; Carr et al., 2005; Potter et al., 2009; Scramlin et al., 2010; Garbossa et al., 2013; Hinson et al., 2014). Backfat depths, regardless of location of measurement, did not differ between treatments ($P > 0.18$). However, backfat depth at the 10th rib had a treatment × sex interaction with barrows having equal 10th rib backfat depths across treatments (Fig. 2.5). Gilts on the SWE treatment had the least amount of backfat at the 10th rib while gilts on the RAC treatment had the greatest amount of backfat at the 10th rib, with gilts on the CON treatment being intermediate to, but not different from, the other two treatments (Fig. 2.5). Previous research (Carr et al., 2005; Gardiner et al., 2008) showed no effects of RAC supplementation on first rib, last rib, or last lumbar backfat depths, which is consistent with the results in the present study. Pigs on the RAC treatment tended to have greater loin muscle area than CON ($P = 0.09$) and SWE ($P = 0.06$). While not significant in this study, these findings are consistent with the results of previous studies with pigs fed RAC (Crenshaw et al., 1987; Watkins et al., 1988; Armstrong et al., 2004; Carr et al., 2005), which reported greater loin muscle area with RAC supplementation.

**Table 2.8.** Simple statistics of carcass traits.

Trait	N	Mean	SD	Minimum	Maximum
Hot carcass weight, kg	97	93.67	9.35	75.28	117.01
Dressing percentage	97	76.63	2.24	64.16	85.08
Backfat depth, cm					
1st Rib	97	3.45	0.45	2.54	4.57
10th Rib	97	2.52	0.51	1.52	4.32
Last lumbar	97	1.80	0.51	0.76	2.79
Chop	97	1.62	0.50	0.76	3.30
Loin muscle area, cm^2	97	62.00	6.97	35.48	78.06

Table 2.9. Least square means of carcass traits by treatment and by sex.

	Treatment					Sex			
	CON	SWE	RAC	SEM	P-value	Barrow	Gilt	SEM	P-value
Hot carcass weight, kg	92.70^a	90.85^a	97.82^b	1.87	0.003	97.49^a	90.09^b	1.66	<0.0001
Dressing percentage, %	76.27^a	76.21^a	77.55^b	0.41	0.03	76.75	76.60	0.33	0.73
Backfat depth, cm									
1st Rib	3.46	3.42	3.50	0.08	0.78	3.47	3.44	0.07	0.75
10th Rib	2.51	2.41	2.59	0.11	0.36	2.65^a	2.35^b	0.10	0.005
Last lumbar	1.84	1.68	1.91	0.10	0.18	1.93^a	1.70^b	0.08	0.03
Chop	1.61	1.60	1.70	0.11	0.70	1.79^a	1.48^b	0.10	0.003
Loin muscle area, cm^2	61.06	60.77	64.15	1.38	0.12	61.48	62.51	1.15	0.47

[a,b] Values with different superscripts within a row and main effect differ by P < 0.05.
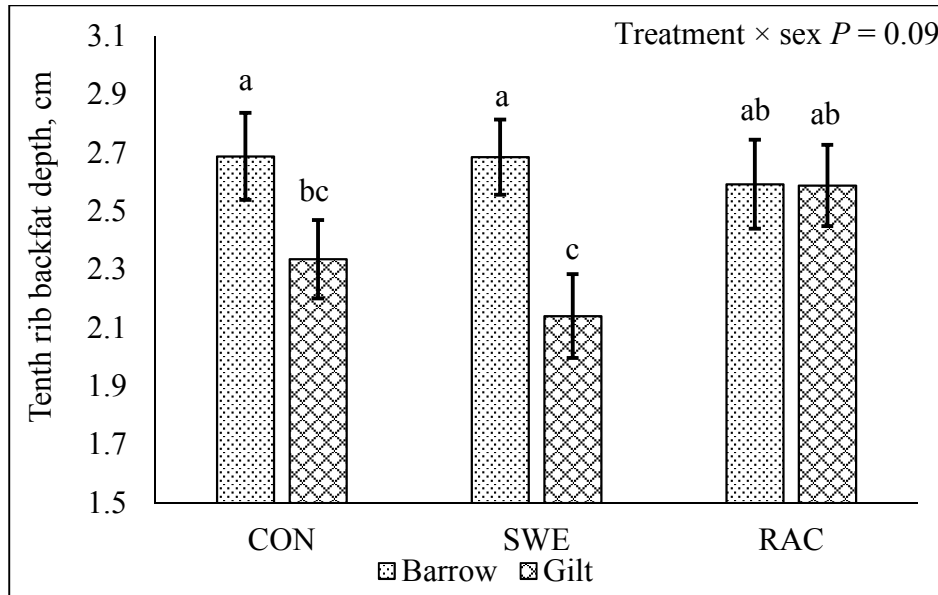
Figure 2.5. Treatment (CON= control, SWE= seaweed extract supplement, RAC = ractopamine hydrochloride supplement) × sex interaction for tenth rib backfat depth. Bars with different letters differ by $P < 0.05$.

Simple statistics for quality traits are presented in Table 2.10. No treatment effect was found for pH across treatment ($P = 0.58$; Table 2.11). These results are in agreement with those of (Warris et al., 1990; Moroney et al., 2012, 2013; Garbossa et al., 2013), who reported no effects of RAC and SWE on pH in freshly cut pork. These values are lower that those of Garbossa and others (2013), but are very close to what is considered optimal for fresh pork meat. Subjective color and marbling did not differ between treatments ($P > 0.19$; Table 2.11). However, chops from RAC pigs were lighter in color (higher L) and less red (lower a*) than those from CON ($P = 0.06$ and 0.003, respectively) or SWE pigs ($P = 0.02$ and 0.0004, respectively). Armstrong and others (2004) also reported lighter color (higher L* and less red (lower a*) in the longissimus dorsi from pigs fed a RAC supplement. Moroney and others (2012) observed no change in color scores in chops form seaweed supplemented pigs when compared to controls. In contrast to these results, previous research reported increased marbling in pork from

Table 2.10. Simple statistics of quality traits.

| Trait	N	Mean	SD	Minimum	Maximum
pH	97	5.48	0.17	5.11	6.43
Subjective color	97	3.01	0.34	2.00	4.00
Subjective marbling	97	2.33	0.73	1.00	5.00
Chop color					
L*	97	56.72	2.51	48.45	64.80
a*	97	18.12	1.14	14.86	20.78
b*	97	9.90	1.39	7.09	13.60
Drip loss, %	97	8.18	1.18	5.80	11.03
Cook loss, %	93	21.21	6.06	7.66	56.57
WBSF, N	96	25.39	5.85	15.56	40.01

Table 2.11. Least square means (S.E.) of quality traits by treatment and by sex.

	CON	SWE	RAC	SEM	P-value	Barrow	Gilt	SEM	P-value
pH	5.51	5.48	5.46	0.03	0.58	5.50	5.47	0.03	0.55
Subjective color	3.05	3.08	2.93	0.08	0.19	3.03	3.01	0.07	0.74
Subjective marbling	2.45	2.39	2.44	0.21	0.92	2.49	2.36	0.20	0.35
Japanese subjective color	3.20	3.18	3.05	0.11	0.60	3.04	3.25	0.09	0.096
L*	56.19^{a,b}	55.96^{b}	57.36^{a}	0.57	0.05	56.69	56.31	0.51	0.45
a*	18.30^{a}	18.43^{a}	17.50^{b}	0.30	0.0009	18.23	17.92	0.28	0.15
b*	9.58	9.69	9.57	0.36	0.92	9.79	9.44	0.34	0.19
Drip loss, %	8.02	8.45	7.92	0.21	0.14	8.02	8.24	0.17	0.36
Cook loss, %	20.03^{a}	23.09^{b}	20.04^{a,b}	1.24	0.07	20.36	21.74	1.07	0.29
WBSF, N	23.81^{a}	25.33^{a,b}	27.17^{b}	1.08	0.08	25.36	25.51	0.88	0.90

[a,b] Values with different superscripts within a row and main effect differ by P < 0.05.

pigs fed a seaweed diet (Allen et al., 2001; Branden et al., 2007). These findings are important because marbling and color significantly influence pork quality and consumer purchasing power.

Although marbling was unaffected in the current study, it is interesting to note inclusion of seaweed diet did not result in any detrimental effect on these variables. Although not significant, there was a numerical difference in color from seaweed chops compared to control. More research is needed to understand the effects of seaweed on color since there are indicators that seaweed may improve quality standards of pork. No main effect of treatment was observed for drip loss ($P = 0.14$). However, there was a treatment × sex interaction for drip loss (Fig. 2.6). Drip loss in gilts was not significant different across treatments. However, when looking at chops from barrows, drip loss was greatest in chops from SWE barrows compared to CON and RAC barrows ($P < 0.05$; Fig. 2.6). Cook loss followed the same pattern as drip loss in barrows and was greatest in chops from SWE pigs compared to chops from CON and RAC pigs ($P < 0.05$). Moroney and others (2013) found no differences in water holding capacity or cook loss in fresh
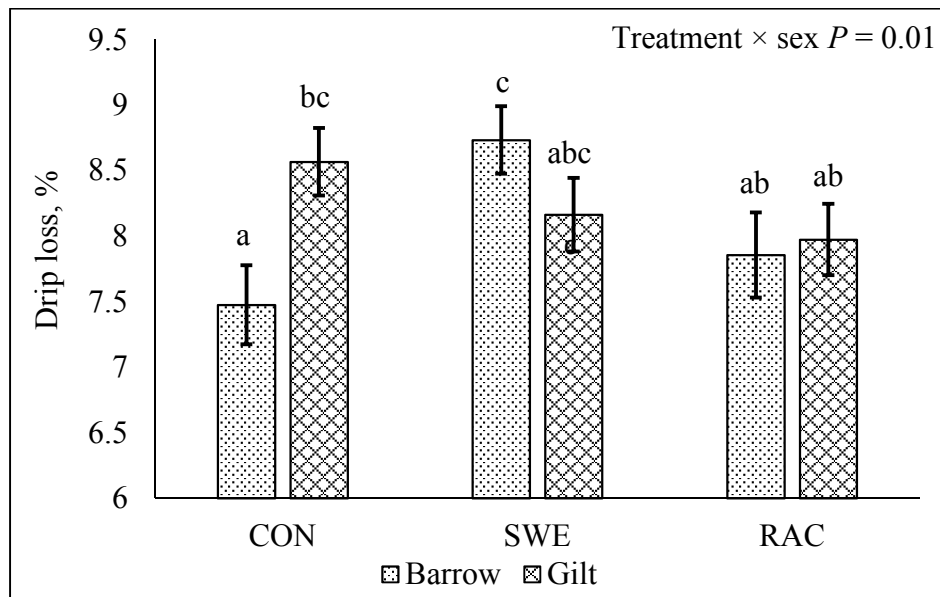
Figure 2.6. Treatment (CON= control, SWE= seaweed extract supplement, RAC = ractopamine hydrochloride supplement) × sex interaction for drip loss percentage. Bars with different letters differ by $P < 0.05$.

pork chop patties containing extracts form a brown seaweed species. Similarly, Choi and others (2012) found reduced-fat pork patties supplemented with *Laminaria japonica* and gave overall highest quality attributes in sensory evaluations. The effects of RAC on drip loss in the current study is consistent with those reported by Agostini and others (2011) who found no effect of RAC dose on drip loss. However, Garbossa and others (2013) found linear decrease in drip loss with increasing doses of RAC. These results indicates the effects of RAC on these variables might be dose dependent. Chops from RAC pigs had the highest WBSF values while chops from CON pigs had the lowest WBSF values. Chops from SWE pigs were intermediate to, but not different from, CON and RAC chops ($P > 0.05$). While some studies (Bridi et al., 2006; Agostini et al., 2011) found no difference of RAC on WBSF, indicating that tenderness was unaffected, other studies (Warris et al., 1990; Wood et al., 1994) showed higher shear force values for RAC treated pigs. This was attributed to hypertrophy in fiber types or a reduction in enzyme activities due to the effects of RAC.

Simple statistics for FABP2 and cell proliferation are reported in table 2.12. Least square means by treatment and by sex are presented in table 2.13. No significant ($P < 0.05$) differences between were observed for FABP2 and cell proliferation in the duodenum, ileum, or jejunum. However, numerically, SWE treatments had the lowest cell proliferation percentage when compared with other treatments. The lack of response to any changes in gut morphology including FABP2 and cell proliferation are often attributed to the health status of the animals. Reilly and others (2008) observed marginal effects on villous height:crypt depth in seaweed fed pigs and suggested good health could be the cause of no response. Although this study did not measure villous height:crypt depth ratio, the lack of response to seaweed supplementation for cell proliferation and FABP2 could be due to the high health status of the pigs in the study. Since

Table 2.12. Simple statistics for serum fatty acid binding protein 2 (FABP2) concentration, cell proliferation, and crypt depth.

	N	Mean	S.D.	Minimum	Maximum
FABP2, ng/mL					
Duodenum	18	0.09	0.13	0.00	0.56
Jejunum	18	0.90	0.68	0.09	2.85
Ileum	18	0.17	0.30	0.01	1.26
Cell proliferation, %					
Duodenum	28	3.07	2.57	0.00	9.25
Jejunum	26	4.60	5.75	0.01	23.96
Ileum	29	3.26	3.74	0.00	16.08
Crypt depth, μm					
Duodenum	30	337.54	67.32	204.52	473.38
Jejunum	30	300.30	63.11	185.08	467.19
Ileum	30	288.00	67.89	191.14	446.94

Table 2.13. Least square means (S.E.) of for serum fatty acid binding protein 2 (FABP2) concentration, cell proliferation, and crypt depth by treatment and by sex.

	CON	SWE	RAC	SEM	P-value	Barrow	Gilt	SEM	P-value
FABP2, expression fold change									
Duodenum	0.08	0.05	0.15	0.06	0.45	0.09	0.10	0.06	0.93
Jejunum	1.06	1.09	0.84	0.30	0.80	0.69	1.31	0.22	0.008
Ileum	0.36	0.17	0.07	0.13	0.29	0.09	0.30	0.11	0.096
Cell proliferation, %									
Duodenum	3.30	2.74	4.14	1.30	0.68	3.65	3.15	0.83	0.59
Jejunum	6.30	2.15	4.63	2.64	0.48	5.14	3.58	1.81	0.51
Ileum	4.73	2.37	3.94	1.83	0.49	3.31	4.06	1.12	0.58
Crypt depth, μm									
Duodenum	324.26	334.82	370.15	31.04	0.49	343.21	342.95	19.54	0.99
Jejunum	290.26	304.20	305.91	29.22	0.86	311.17	289.08	18.40	0.37
Ileum	320.64	280.96	251.91	30.77	0.21	288.84	280.17	19.25	0.73

FABP2 is an indicator of cell damage (Niewold et al., 2004), the results of this study indicate there was no difference in cell damage across treatments.

Mortality was low and equally spread across treatments. One SWE pig was killed by its pen mates, a CON pig was taken off-test due to severe tail-bite, a CON pig was taken off-test for failure to

thrive, a SWE pig was found dead after being caught wheezing, and a RAC pig, prior to RAC supplementation, was found dead and cause of death was determined as pneumonia based on a necropsy. Additionally, two pigs (a SWE and a RAC) were treated for wheezing. Because there was a low incidence of morbidity and mortality, no analyses were performed on the effect of SWE supplementation on overall health of the pigs.

Conclusions and Implications

In the current study, no differences were found for performance and carcass traits between seaweed-supplemented pigs and those pigs not fed a seaweed supplement. However, pork from pigs fed the seaweed extract had chops that were numerically darker in color and had greater cook loss and WBSF values than chops from control pigs. No differences were found for FABP2 gene expression, cell proliferation percentage, or crypt depth. Overall, no detrimental effects were found in this study when feeding seaweed extracts to pigs.

While no differences were found for performance traits for pigs fed seaweed and ractopamine supplements, there were differences in hot carcass weight and dressing percentage. Pigs fed ractopamine had greater hot carcass weights and dressing percentages than those fed seaweed. However, seaweed supplementation did not have the negative impact on pork quality that ractopamine supplementation had. Pork from ractopamine-supplemented pigs was paler, less red, and more tough than those from seaweed-supplemented pigs. Therefore, seaweed extracts would not be an alternative to ractopamine based on the results from this study. In this study, pigs were from a high health herd. Due to the low incidence of mortality (5 out of 120), there was not enough data to evaluate the ability of seaweed extracts to serve as an alternative to antibiotics.

The numerical differences observed in color and tenderness warrant further studies to elucidate the impacts of phytochemicals in seaweed on meat quality parameters. It would be interesting to delve into the specific components of seaweed and seaweed extracts to determine exactly which phytochemical elucidates the effect of seaweed on pork quality. Additionally, pigs in this study were from a high health herd. According to Pierce and others (2005), minimal or no response is expected when feeding seaweed to healthy animals. Therefore, it would be beneficial to conduct future research regarding seaweed as an alternative to antibiotics when pigs are diseased challenged. This could be done using three treatment groups: a control group, a seaweed-supplemented group, and an antibiotic-supplemented group. Furthermore, variable results have been reported on performance due to variations in dietary inclusion levels, bioactive compounds present, and variety of seaweed extract used in the diet. Therefore, studies that compare different levels of feeding seaweed extract or different seaweed extracts could be beneficial. Additionally, analyses of seaweed extracts fed could be conducted to determine the concentrations of bioactive compounds present in the extract

Acknowledgements

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