Effects of dietary seaweed on sow and progeny performance and evaluation of an algae-clay complex-based feed additive and diet formulation on finishing pig growth performance

by

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B.S., Buenos Aires University, 2013

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Science and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2019

Approved by:

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Abstract

This thesis involved utilizing dietary feed additives in swine rations. Chapter 1 is a literature review of the need for added vitamins in growing and finishing rations for swine. Chapter 2 evaluated the effects of a dietary seaweed on 28 sows from day 30 of gestation until weaning and progeny from birth until market. Maternal Oceanfeed Swine supplementation did not improve ($P > 0.10$) sow or litter performance. No differences were observed in colostrum yield, or colostrum and milk composition between the two treatments. In the nursery period, there was no evidence ($P > 0.10$) for main effect or sow by nursery treatment interactions. There was no evidence for fecal score differences between treatments on d 0, 7 and 21 after weaning. On day 56 after weaning, there was an increased proportion of pigs with the families Peptostreptococcaceae and Veillonellaceae in those fed Oceanfeed Swine in the nursery and originating from Oceanfeed Swine-fed sows. Pigs from this treatment combination also had increased mean number of species detected within the families Ruminococcaceae and Lachnospiraceae and had lower mean number of species detected within the family Fusobacteriacea. In the finishing period, no evidence for main effects or interactions ($P > 0.10$) were observed on overall growth performance. Chapter 3 evaluated the effects of an algae-clay-complex-based (ACC) feed additive and diet formulation (High or Low diet energy and amino acids) regimen on growth performance and carcass characteristics of finishing pigs. Overall, ADG was greater *(*$P = 0.027$*)* for pigs fed added ACC diets compared with those fed diets without ACC. This was a result of late finishing (d 56 to 90) increases *(*$P < 0.019$) in ADG and G:F for ACC fed pigs compared with those fed no ACC. Overall, pigs fed High diets had improved growth performance and heavier live weighs than pigs fed Low diets. For carcass characteristics, pigs fed High diets tended to have greater *(*$P = 0.067$) loin depth and greater *(*$P <$

0.001*)* carcass weight than pigs fed Low diets. No evidence for differences was observed for carcass characteristics between the control and added ACC fed pigs.

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Acknowledgements

I would like to express mt sincere gratitude to my advisors Dr Joel M. DeRouchey and Dr. Jason C. Woodworth. Thank you for your guidance throughout my master's program and for pushing me to improve in a personal and professional way. I'm very lucky to have had you as mentors.

To my committee members, Dr. S. Steve Dritz, Dr. Mike D. Tokach and Dr. Robert D. Goodband, thank you for your mentorship throughout my graduate studies. I greatly appreciated your participation in my projects and advice during my committee meetings.

To my fellow graduate students and colleagues, I'm very grateful for your support and friendship.

To my family, and friends, thanks for the incessant encouragement and for always supporting my life decisions.

Chapter 1 - **Vitamins for growing and -finishing pigs**

Introduction

Vitamins are organic micronutrients that are primarily required as coenzymes to maintain health and performance (NRC 2012). Vitamins are classified as either fat-or water soluble. Fat soluble vitamins include A, D, E and K, which are required for the synthesis and maintenance of body tissues. Because they are hydrophobic in nature, they can be stored in organs such as liver, kidneys, brain, and fat or muscle tissue. On the other hand, water soluble vitamins include B-vitamins and vitamin C and are involved in catalytic reactions where they act as co-enzymes for different metabolic functions. In general, water soluble vitamins are not stored in the body and small quantities are required daily.

Vitamin requirements for swine are express as total amount present in the diet and are considered as minimum requirements, with no margin of safety included (NRC, 1988). However, it is a common swine industry practice to use added vitamin concentrations to meet or exceed the minimum requirements and disregard vitamin contributions from diet ingredients. This can be explained because of the wide variety of feed ingredients used in diet formulation with a high variability in their concentration and availability of vitamins. Moreover, vitamin levels in ingredients and feed decrease with storage and the rate of this may be affected by humidity, light, environmental temperature, pH, pelleting, extruding, storage time and presence of inorganic minerals in the premix (Coehlo, 1991; Shurson et al., 2011). Also, vitamins have historically been relatively inexpensive which has allowed nutritionists to over-fortify without considerably affecting production costs. However, recently, due to changes in manufacturing and availability, some vitamin prices have dramatically increased requiring the need to re-evaluate these wide margins of safety typical used.

There is limited information regarding vitamin requirements for modern genetics and under high rates of production. Many of the NRC requirement estimates are based on studies conducted in the 1980s or earlier. These studies may not represent present needs of pigs, as in the period between 1990 and 2015, genetics, nutritional, and management improvements have led to an average increase of 26% in ADG, 22% in G:F, and a 2% reduction in feed intake in the finishing period in the US (1990 PigChamp Summary; 2010-2015 U.S. Pork Industry Productivity Summary). Therefore, the objective of this review is to describe the latest information on vitamins that are typically supplemented in finishing pig's diets.

Vitamin A

Vitamin A is commonly referred as retinol, which is its active form. It is required for normal vision, maintenance of epithelial cells, reproduction, and mucus secretions. Vitamin A has also been demonstrated to have a key role in gene transcription, embryonic development, bone metabolism, and the immune system (Combs, 1999).

Vitamin A requirements can be met by either vitamin A or vitamin A precursors, such as B-carotene pigments. In the swine industry, vitamin A is commonly added to diets as esters of acetate or palmitate. Its concentration is expressed in international units (IU), with one IU equivalent to 0.3 ug of all trans retinol or 0.344 of all-trans retinyl acetate. To increase the stability of vitamin A, retinoids are normally encapsulated in the form of beadlets for manufacturing vitamin premixes. The most common protein encapsulant used in the industry is gelatin, but also collagen and gliadin can be utilized. In the process, an emulsion containing the protein encapsulant, antioxidants, sugar and starch is sprayed and the beadlets are cross linked by thermal or chemical reaction. Despite the efforts to increase the stability of vitamin A in the manufacture process, moisture present in premixes and feedstuff may soften vitamin A beadlets

predisposing to oxidation with the consequent loss of activity. Moreover, the presence of inorganic trace minerals and pH below 5 exacerbate losses of vitamin A in premixes exposed to moisture (NRC 2012).

Vitamin A requirements for swine may depend on the response criteria evaluated. Growth responses are less sensitive than liver storage, plasma concentrations, or cerebrospinal fluid pressure (NRC, 2012). Pigs can use liver reserves when dietary vitamin A is low and deficiency symptoms, such as reduced weight gain, incoordination, blindness, and posterior paralysis, are only observed after long periods of deprivation (Braude et al., 1941). Toxicity signs, such as sudden lameness, and periodic tremors, can be observed three to four days after administration of 195,000 IU/kg (Reiner et al., 2004). The NRC (2012) total vitamin A requirement estimates is 1,300 IU/kg of diet for pigs from 25 to 135 kg when daily gain is used as the criterion. This total requirement estimate is based on studies from more than 50 years ago. In a recent industry survey, the average added vitamin A concentration used in the growing (55 to 100 kg) and finishing (100 kg to market) period is approximately 3.7 and 3.2 times the recommended by the NRC, respectively (Flohr et al., 2016).

The lack of updated information can be explained because dietary requirements for vitamin A are hard to assess. High levels of added vitamin A (10,000. 20,000, or 40,000 IU vitamin A/kg feed) were evaluated in growing pigs with no significant differences in growth performance (Hoppe et al., 1992). Recently, low, medium, and high additions of a vitamin premix were evaluated in finishing pigs from 50 to 107 kg (Cho et al., 2017). The vitamin A concentrations were 2.5, 5, and 7.6 times the NRC (2012) recommendations in the low, medium, and high vitamin premix, respectively. No evidence for differences in growth or carcass characteristics were observed. Moreover, Del Tuffo (2018) evaluated a high and a low vitamin

concentration fed to pigs from 16 to 130 kg under commercial conditions. In the high vitamins concentration diet added vitamin A ranged from approximately 9,000 to 2,650 IU/kg of final feed. The low vitamins concentration diet had approximately half the added vitamin A of the high vitamin premix diet. No evidence for differences were observed in growth performance and carcass characteristics among dietary treatments.

Vitamin D

Vitamin D compounds are essential for biological functions that include synthesis of calcium binding proteins, absorption of calcium, and phosphorus in the small intestine and mobilization of calcium from bones. Moreover, vitamin D is involved in many biological functions related with normal growth of soft tissue, reproduction, and regulation of the immune system. The two main sources of vitamin D are ergocalciferol (Vitamin D_2) and cholecalciferol (Vitamin D_3). Ergocalciferol is synthesized in plant's tissue from ergosterol after exposure to ultraviolet light. Vitamin D_3, 7-dehydrocholesterol, can be synthesized in the skin of the pigs from cholecalciferol after exposure to ultraviolet light. However, in intensive production systems, pigs are commonly fed corn-soybean meal diets with insignificant concentrations of ergocalciferol and are rarely exposed to sunlight. Therefore, vitamin D is fortified in swine diets mainly as vitamin D_3 and 25-OHD_3 in the form of crystals or resins. To increase stability, vitamin D can be also incorporated to gelatin beadlets in combination with vitamin A and antioxidants. Toxicity signs, such as reduced feed intake, reduced growth rate, and calcification of aorta, kidneys, heart and lungs, may be observed depending on the time of exposure and the source supplemented with vitamin D_3 more toxic than vitamin D_2. Toxicity sings were observed when vitamin D_3 was supplemented at 33,000 IU/kg and fed for less than 60 days or 2,200 IU/kg for periods longer than 60 days (McDowell, 2000).

Vitamin D deficiency in growing pigs results in reduced retention of calcium, phosphorus, and magnesium (Miller et al., 1965) and, consequently, lameness, bone fractures and tetany signs can be observed. The NRC (2012) vitamin D requirement estimates for grow-finish pigs are based on a study done in the mid 1940's (Bethke et al., 1946) and vary from 200 IU/kg for pigs between 10 and 25 kg and 150 IU/kg for pigs between 25 and 135 kg. While early work reported that it takes 4 to 6 months for pigs fed vitamin D free diets to present signs of deficiency when housed in the absence of sunlight (Quarterman et al., 1964), a recent study has shown that deficiency signs can be observed when vitamin D-free diets are fed over a 5-week period (Amundson et al., 2016). Although growth rate has significantly increased since the 1960s, it is unknown if this is the reason for the change in rate of depletion of vitamin D reserves.

The average vitamin D supplementation rate used in the industry is approximately 860 and 745 IU/kg for pigs from 50 to 100 kg and 100 kg to market, respectively (Flohr et al 2016). These added levels represent approximately 5 times the NRC (2012) requirement estimates. In addition, BSAS (2003) recommends 800 IU/kg for pigs until 60 kg live weight and 600 IU/kg thereafter. In research evaluating vitamin D addition to finishing pig diets from 50 to 107 kg at 2.9, 5.9, and 8.8 times the NRC (2012) requirement, no differences in growth or carcass characteristics were observed (Cho et al., 2017).

Cholecalciferol is moderately resistant to oxidation and its average monthly losses when stored in a vitamin premix, a vitamin-inorganic trace mineral premix, and a vitamin-complex trace mineral premix are 3.0, 4.5, and 2.7%, respectively (Shurson et al., 2011).

Several studies have evaluated the effects of feeding high levels of vitamin D to finishing pigs to improve meat tenderness. It is hypothesized that feeding high concentrations of vitamin D

before slaughter may increase meat calcium levels and, thus, the activity of calpains, intracellular proteases involved in improving meat tenderness. Some studies have shown that high levels of Vitamin D improve the color of meat (darker) with no significant differences on tenderness (Enright et al., 1998; Wiegand et al., 2002; Wilborn et al., 2004). Moreover, several studies evaluating high vitamin D concentrations fed to pigs reported a significant reduction in growth and feed intake (Enright et al., 1998, Sparks et al 1999; Wiegand et al., 2002) and it's been hypothesized that the color improvement could be a result of a severe reduction in feed intake for several days before slaughter and not a result of vitamin D supplementation directly.

Vitamin E

Vitamin E is the term to identify compounds based on tocopherol or tocotrienol with antioxidant activity. They play a key role in tissues as an antioxidant that prevents oxidative damage from the self-perpetuating production of lipid peroxides (NRC, 2012). They are also needed for the development of reproductive organs, regulation of gene expression, and stimulation of the immune system (NRC 2012). From the eight compounds (α-, β-, γ-, and σ-tocopherols and α-, β-, γ-, and σ-totrienols) with vitamin E activity, alpha tocopherol is the most commonly supplemented to feed and the most active. Alpha tocopherol has 8 stereoisomers with different biological activity that range from 21 to 100% (Weiser et al., 1996). Natural sources of alpha tocopherol comprise only RRR stereoisomers and it is known as D-α-tocopherol. Synthetic alpha tocopherol is a combination of all 8 stereoisomers and is known as DL-α-tocopherol. Based on rat studies it has been estimated that the relative biopotency of D-α- tocopherol to DL-α- tocopherol is 1.36 and then extrapolated to other species including swine (Harris and Ludwig, 1948). However, true potency of different sources of vitamin E are hard to assess because they can only be quantified under a deficiency state and bioavailability varies with different dosages

and durations (Hoppe and Krennrich, 2000; Hoppe, 2010). In addition, metabolism and transport of α-tocopherol stereoisomers varies among species and studies performed in pigs suggest that the relative value is higher than 1.36 (Mahan et al., 2000; Lauridsen et al., 2002; Yang et al., 2009). Recently, the relative biopotency of D-α- tocopherol to DL-α- tocopherol has been evaluated on sows and litters and results indicate that tissues have different affinities for α-tocopherol stereoisomers and, therefore, bioavailability depends on the response criteria. (Shelton et al., 2014). The bioavailability coefficients estimated ranged from 1.9 to 4.2 for sows and pig plasma, 2.9 to 3.6 for colostrum α-tocopherol, 1.6 for milk α-tocopherol, 1.8 for heart α-tocopherol, and 2.0 for liver α-tocopherol and confirm that in pigs, independently of the response criteria, the relative biopotency of D-α- tocopherol to DL-α- tocopherol is higher than 1.36. Natural vitamin E is rapidly destroyed when grains are artificially dried. Moreover, the presence of moisture, trace minerals, and rancid fat accelerates the oxidation of natural vitamin E (NRC, 2012). Therefore, natural alpha tocopherol present in feedstuff is insufficient and synthetic vitamin E is supplemented in swine diets to prevent deficiency signs.

Vitamin E is considered the least toxic vitamins and no toxicity signs were observed when levels as high as 550 mg/kg were fed to growing pigs (Bonnette et al., 1990).

Vitamin E requirements are affected by many dietary factors such as amounts of selenium, vitamin A, unsaturated fatty acids, sulfur amino acids, copper, iron and synthetic antioxidants. From these factors, selenium has a close relationship with vitamin E in which they share the same antioxidant effect and deficiency signs. Deficiency signs include sudden death, mulberry heart, mastitis, edema, white muscles, and liver necrosis. The NRC (2012) total vitamin E requirement estimate for grow-finish pigs is 11 IU/kg. To our knowledge, there is no evidence in the literature to use different vitamin E levels than recommended by NRC (2012). However,

as stated before, the composition and quality of the diet has an influence on vitamin E requirements, and high inclusion of unsaturated fatty acids or low concentrations of selenium may increase vitamin E requirements.

Of all fat-soluble vitamins, vitamin E typically has the lowest margin of safety used in the industry, with an average ratio of 2.1 times the NRC (2012) total requirement estimates (Flohr et al 2016). This can be explained by the relatively high cost of vitamin E compared with other vitamins and its higher stability during the storage period (Shurson et al., 2011).

Several studies have evaluated the effect of supra-nutritional vitamin E supplementation on pork quality. Shortly after slaughter, oxymyoglobin is converted into metmyoglobin with the consequence change of meat color from red to brown. Cell membrane phospholipids oxidize and lose integrity and there is an increase in drip loss of muscle fibers. When vitamin E is added at supra-nutritional levels, alpha-tocopherol is accumulated in the muscle tissue and its antioxidant properties play a significant role preventing lipid oxidation and improving shelf life of pork. The positive effect of vitamin E supplementation on meat quality varies with the response criteria evaluated being a reduction in fat oxidation more consistent among studies than redness and drip losses. Vitamin E supplementation level and duration of supplementation are important variables that determine the magnitude of the response on lipid oxidation prevention. Supplementation levels of 200 mg/kg of the diet showed significant differences in oxidation level, measured as thiobarbituric acid reactive substances (TBARS) when vitamin E was supplemented for a minimum of 72 days (Cannon et al., 1996; Houben et al., 1998; Guo et al., 2006). However, improvements in fat quality were also observed when vitamin E levels between 200 and 210 IU/kg were supplemented to finishing pigs for a period of 42 days (Isabel et al., 1999; Wang et

al., 2012). When the basal oxidation level is above 0.15 TBARS, a significant response is more likely to be observed (Pettigrew and Esnaola, 2001).

As stated before, vitamin E supplementation has no consistent effect on meat color characteristics with some studies showing an improvement on meat redness when vitamin E was added at 200 mg/kg of diet (Asghar et al., 1991; Monahan et al., 1994; Lanari et al; 1995) and other studies showing no differences (Isabel et al., 1999; Wang et al., 2012; Huang et al., 2019). Moreover, vitamin E has also shown inconsistent responses on drip loss and the positive effects observed in some studies (Wang et al., 2012; Cheah et al; 1995) were not observed in others (Guo et al., 2006; Kim et al., 2015; Huang et al., 2019). The variable response of vitamin E supplementation on drip loss and meat redness is not well understood. It doesn't seem to be related with the supplementation level nor the duration of supplementation (Pettigrew and Esnaola, 2001). However, it's been hypothesized that muscle fiber characteristics (white vs red), membrane phospholipid composition, and phospholipase A_2 concentration in tissues may be important factors to consider (Cheah et al., 1995). It's important to highlight that the vitamin E levels needed to improve meat quality and shelf life are economically unjustified with the current pork and vitamin E market price.

Vitamin K

Vitamin K is required for the activation of clotting factors II, VII, IX, and X after synthesis in the liver. After activation, these clotting factors become strong chelators of calcium ions with an essential role in blood coagulation. Vitamin K can be provided from vegetable sources (phylloquinones), from microbial fermentation (menaquinones), and from synthetic synthesis (menadiones). Phylloquinones present in soybeans and corn are at very low concentrations and not considered in diet formulation. Menaquinones production by the bacterial flora occurs in the

lower gut and the availability is very limited unless the animal practices coprophagy. Consequently, vitamin K is commonly supplemented to swine diets as soluble forms of menadiones. The main soluble sources of vitamin K used in the industry are menadione sodium bisulfite (MSB), menadione dimethylpyrimidinol bisulfite (MPB), and menadione sodium bisulfite complex (MSBC; NRC, 2012). The relative vitamin K activity of these molecules depends on the menadione content in the molecules which is 50% in MSB, 33% in MSBC and 45% in MPB ((NRC, 2012)). Menadione sodium bisulfite is highly unstable under storage conditions and when it is exposed to manufacturing processes such as pelleting and extrusion (Laffi et al., 1989; Marchetti et al., 1999). To increase the stability of menadione, menadione nicotinamide bisulphite (MPB)is manufactured by substituting the sodium moiety with an organic base of nicotinamide and provides a more stable source of vitamin K with 45.7% relative vitamin K activity (Hughebaert, 1991; Marchetti et al., 1993). No toxicity signs were described when pigs where supplemented with vitamin K levels up to 1000 times the requirements.

Under vitamin K deficiency, liver stores are rapidly depleted and deficiency signs, such as internal hemorrhages and death, can be observed. According to the NRC (2012), total vitamin K requirements estimates in the wean-to-finish period is 0.5 mg/kg diet. The presence of mycotoxins or excess calcium in the diet may increase the requirements through the destruction of vitamin K, reduction of microbial vitamin K synthesis or reduction in the absorption of vitamin K. Moreover, the use of antibiotics or an imbalance in fat-soluble vitamins may have a negative effect in vitamin K activity increasing the pigs' requirements. The average vitamin K inclusion in the swine industry is 4.0 and 3.6 times the NRC (2012) requirement estimates for pigs between 55 and 100 kg and from 100 kg to market respectively (Flohr et al., 2016). These high safety margins can be explained because vitamin K is the most sensitive vitamin to

environmental conditions and significant losses can be expected during the storage period. The average monthly loss when stored in a vitamin premix, a vitamin-inorganic trace mineral premix, or vitamin-complex trace mineral premix was stated to be approximately 6, 10 and 2% respectively (Shurson et al., 2011). Moreover, the presence of choline chloride significantly increases vitamin K monthly activity losses (Coelho et al., 1991).

Vitamin B_{12}

Vitamin B_{12} is required as a coenzyme in reactions that involve transfer or synthesis of one carbon unit, such as methyl groups, and plays a key role in the metabolism of nucleic acids, proteins, lipids, and carbohydrates. Plant based ingredients are devoid of vitamin B_{12}, but microorganisms in the environment and within the gastrointestinal tract of the pig may provide enough vitamin B_{12} to meet requirements. Vitamin B_{12} can be stored in the body, primarily in liver tissue, and deficiency signs may take several months to be observed (Combs, 1999). However, because we cannot rely in coprophagy, cyanocobalamin, a microbial fermented vitamin B_{12} is normally added to swine diets. No toxic effects have been reported when high levels of vitamin B_{12} were fed to swine (NRC, 1988). The NRC (2012) recommendations for growing (25 to 50 kg) and finishing pigs (50 to 135 kg) is 10 and 5 ug/kg of feed, respectively. Deficiency signs include loss of appetite, impaired growth, incoordination, anemia, reproductive failure, rough skin, hair coat, vomiting, and diarrhea. For many years, cyanocobalamin was considered to be highly resistant to light, moisture, heat, and oxygen under storage conditions (Verbeeck, 1975; Gadient, 1986; Coehlo, 1991). However, Shurson (2011) reported that vitamin B_{12} monthly losses are approximately 2 and 5.4% when stored in a vitamin premix containing choline chloride and inorganic trace minerals, respectively. These monthly losses are more than 4 and 10 times higher than those reported by Coehlo (1991) in the vitamin premixes and the

vitamin-inorganic trace mineral premix, respectively. The average vitamin B_{12} inclusion in the swine industry for pigs between 55 and 100 kg and from 100 kg to market is 3.8 and 3.4 times the NRC (2012) recommendations (Flohr et al., 2016).

Niacin

Niacin plays a key role in the metabolism of carbohydrates, lipids, and proteins as a component of the two coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). These coenzymes are especially important in metabolic reactions that provide energy to the animal and maintained normal integrity of epithelial and nervous system tissue. Niacin is widely distributed in feedstuffs. However, much of the niacin present in vegetable sources is in bound form which is unavailable for the pigs. The niacin present in soybean meal is 100% available and availability in corn varies from 0 to 30%. Niacin can be synthesized from tryptophan when fed in excess. However, pigs are not very efficient at synthesizing niacin from tryptophan, and it's been stated that 50 mg of tryptophan are needed to synthesize 1 mg of niacin (Firth and Johnson, 1956).

Because in the US, diets are mostly corn-soybean meal based, which are low in niacin and tryptophan, niacin is commonly added as niacinamide and nicotinic acid. Niacinamide and nicotinic acid are chemical synthesized and commercially available in crystalline forms. Niacin is highly resistant to heat, humidity, oxygen, and light under storage conditions (Shurson et al., 2011). Niacin requirements depend on multiple factors such as bioavailability of niacin in feed, health status of the herd, tryptophan level in the diet, growth rate, and opportunity for coprophagy. In the 2012 edition, NRC increased the niacin requirements from 10 to 30 mg/kg in growing pigs (25 to 50 kg) and from 7 to 30 mg/kg in finishing pigs (50 to 135 kg). This increment was based on a study performed by Real et al (2002) were niacin levels up to 55

md/kg in corn-soybean meal-based diets improved growth performance of finishing pigs raised under commercial conditions. Moreover, the author stated that niacin levels up to 110 and 550 mg/kg continue to show improvements in pork quality traits such as 24-h pH and meat color. However, in a recent study, no consistent differences in growth performance, carcass characteristics and meat quality parameters were observed when niacin was supplemented at 30, 380, 730 and 1080 mg/kg of complete feed (Flohr et al., 2014). Because in the last NRC edition, niacin requirements were only based in one study (Real et al., 2002) and other studies available contradict that improvement in growth rate (Copelin et al., 1980; Ivers et al., 1993; Flohr et al., 2014), niacin is the only vitamin added in the US industry at lower rates than the recommended in the last edition of the NRC (Flohr et al., 2016). The average niacin added as a ratio of the NRC (2012) recommendations is 0.8 for pigs between 55 and 100 kg and 0.7 for pigs from 100 kg to market.

Riboflavin

Riboflavin can be found in nature as free dinucleotide riboflavin or as a component of many enzymes under the form of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Riboflavin in FMN and FAD form are called flavoproteins and play an important role in metabolism of carbohydrates, lipids, and proteins as intermediates in the energy transactions of electrons in oxidation-reduction reactions. Riboflavin is widely distributed in plants. However, corn-soybean diets are deficient in riboflavin and, therefore, riboflavin is commonly added to swine diets as crystalline riboflavin. Crystalline riboflavin is produced by chemical synthesis or fermentation and it can be found in powder form, spray dry powder and dry dilutions. No reports of riboflavin toxicity have been reported in swine.

The NRC (2012) total riboflavin requirement estimates are 2.5 mg/kg for pigs between 25 and 50 kg and 2.0 mg/kg for pigs between 50 and 135 kg. These estimates are based on studies performed in the 1950's. It's been hypothesized that under modern conditions, higher inclusions of B-group vitamins may be needed to support the higher growth rate (Weib and Quanz, 2002). However, riboflavin supplementation levels of 9.8 and 18.4 mg/kg of diet did not influence growth performance of grow-finish pigs from approximately 33 to 107 kg (Bohmer and Roth-Maier, 2007). Moreover, no differences were observed when low, medium, and high inclusions of a vitamin premix were evaluated in grow-finish pigs with added riboflavin of 4.4, 8.8, and 13.2 mg/kg. To our knowledge no data has been published suggesting that the requirements estimates should be different than recommended by NRC (2012). The average riboflavin inclusion in swine diets in the US is 4.2 and 3.7 mg/kg for pigs between 50 and 100 kg and 100 kg to market, respectively (Flohr et al., 2016), which represents approximately 1.9 times the NRC (2012) requirement estimates for final feed. The relatively low safety of margin used can be explained by the high stability of riboflavin under storage conditions (Shurson et al., 2011).

Pantothenic acid

Pantothenic acid acts as a component of two enzymes, coenzyme A (CoA) and acyl carrier protein (ACP), which play an important role in the metabolism of carbohydrates, proteins, and fat. Pantothenic acid is widely distributed in foods of plants and animal origin. Its biological activity is high in corn and soybean meal (Southern and Baker, 1981). Pantothenic acid is commercially available and commonly supplemented to swine diets as d- or dl- calcium pantothenate. The d-form has 92% of activity and the dl-form has 46% of activity. The NRC (2012) total pantothenic acid requirements estimates for pigs from 25 to 50 kg and 50 to 135 kg are 8 and 7 mg/kg, respectively. It's been hypothesized that pantothenic acid supplementation

above the requirements would modify body tissue composition from a partition of energy from fat synthesis to protein deposition (Stahly and Lutz, 2001; Santoro et al., 2006). However, the effects of pantothenic acid in body composition is controversial and other studies showed no differences when supplementation levels between 30 and 90 mg/kg were added to grow-finish pigs diets (Radcliffe et al., 2003; Yang et al., 2004; Saddoris et al., 2005). Moreover, Groesbeck (2007) evaluated the effect of pantothenic acid added at 0, 22.5, and 44 ppm on grow-finish pig performance and carcass characteristics and found no differences among the treatments. The pantothenic acid present in the corn-soybean meal diets was very close or above NRC (2012) requirements estimates and sufficient to maximize growth. The average pantothenic supplementation to swine diets is 14.5 mg/kg in pigs between 50 and 100 kg and 12.5 mg/kg in pigs from 100 kg to market which represents approximately 2 times NRC requirements estimates for final feed (Flohr et al., 2016).

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Chapter 2 - **Effects of a dietary blended seaweed product on sow and progeny performance, fecal consistency, and fecal microbiota during gestation, lactation, nursery and grow-finish periods**

Abstract

This study evaluated the effects of providing a selected mix of brown, red and green seaweeds (Oceanfeed Swine; Ocean Harvest Technology, Galway, Ireland) to sows during gestation and lactation and to progeny during nursery and grow-finish periods on growth performance, fecal consistency, and microbiota composition. For the sow portion, 28 sows and litters were used from d 30 of gestation until weaning. Sow treatments consisted of control diet or a diet supplemented with Oceanfeed Swine at 0.50% in gestation and 0.66% in lactation. At weaning, 360 pigs from the same sows were used from d 0 to 56 and 57 to 156 in the nursery and grow-finish periods, respectively. Treatments consisted of control diet or a diet supplemented with Oceanfeed Swine at 0.75% in the nursery and grower phase (5.5 to 34 kg and 34 to 59 kg respectively) and 0.5% in the finisher phase (59 to 127 kg). Treatments were arranged in a split-plot design with sow treatment (control or Oceanfeed Swine diet) as the main plot and nursery-grow-finish treatment (control or Oceanfeed Swine diet) as the subplot. Maternal Oceanfeed Swine supplementation did not improve ($P > 0.10$) sow or litter performance. Nor were there differences in colostrum yield, or colostrum and milk composition between the two treatments. In the nursery period (5.5 to 34 kg), there was no evidence ($P > 0.10$) for main effect or sow by nursery treatment interactions. There was no evidence for fecal score differences between treatments on d 0, 7 and 21 after weaning. On day 56 after weaning, there was an increased proportion of pigs exhibiting the families Peptostreptococcaceae and Veillonellaceae when fed

Oceanfeed Swine in the nursery and originating from Oceanfeed Swine-fed sows. Pigs from this treatment combination also had increased mean number of species detected within the families Ruminococcaceae and Lachnospiraceae and had lower mean number of species detected within the family Fusobacteriacea. In the finishing period, no evidence for main effects or interactions ($P > 0.10$) were observed on overall growth performance. In summary, addition of Oceanfeed Swine in gestation, lactation, and nursery-finishing phases had no consistent effect on sow or litter performance. However, differences were observed in the microbiota composition, with a relative increase of bacterias considered beneficial including Ruminococacea and Lachnospiraceae and a decrease of Fusobacteriaceae, which is generally considered pathogenic; which warrants further investigation.

Key words: Seaweeds, feed additive, microbiota, swine, growth performance

Introduction

There is an increased concern associated with the use of antibiotics in animal production (Williams et al., 2001). For that reason, different alternatives to replace antibiotics for growth promotion are being explored in swine production. One challenge to replace antibiotic use in feed is at weaning where the pig's microbiota is unstable and highly susceptible to external factors, such as diet composition and bacteria load in the environment. Moreover, weaning represents one of the most stressful periods in a pig's life and is associated with changes in their gut barrier integrity and immune system (Kyriakis et al., 1999). This can be observed by the increased occurrence of diarrhea and poor growth performance (Bouwhuis et al., 2016).

Seaweeds have gained great interest in recent years due to their bioactive compound content and thus potential to help overcome the challenge that weaning represents (Bouwhuis et al., 2016). Seaweeds are divided into three categories: red (Rhodophyta), brown (Phaeophyta)

and green (Chlorophyta). They are rich in many biologically active compounds such as laminarian and fucoidan polysaccharides. These compounds have been shown to improve pig growth performance by their antimicrobial, prebiotic and immunomodulatory properties (Leonard et al., 2011; Ruiz et al., 2018; Heim et al., 2014a).

Oceanfeed Swine (Ocean Harvest Technology, Galway, Ireland) is a newly introduced product that is created by drying and blending a selected mix of brown, red and green seaweeds harvested from the cold waters of Europe and warm waters in Southeast Asia. It is hypothesized that bioactive molecules present in these seaweeds have positive effects on colostrum immunoglobulins output, the gut environment, and growth performance of pigs (Leonard et al., 2010; Heim et al., 2015). Therefore, the objective of this study was to investigate the effect of dietary addition of Oceanfeed Swine on sow performance, growth performance of their offspring during nursery and grow finish, and fecal microbiome.

Materials and Methods

The Kansas State University Institutional Care and Use Committee approved the protocol used in this experiment. The experiment was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Sows and progeny used in this study were divided into a sow portion, from d 30 of gestation to weaning, a nursery portion, from weaning to d 56, and a grow-finish portion, from day 56 until market.

Sow portion

A total of 28 sows (DNA 241, DNA Genetics, Columbus, NE) and litters (360 piglets, DNA 241× 600) were used for the sow portion of the study. On day 30 of gestation, sows with confirmed pregnancy were assigned to one of two dietary treatments in a randomized complete block design based on sow parity and initial body weight (BW). Sows were individually housed

in stalls (0.6 × 2.1 m) and had ad libitum access to water. Sow dietary treatments consisted of providing a control diet or a diet containing 0.50 and 0.66% Oceanfeed Swine (Ocean Harvest Technology, Galway, Ireland) during gestation and lactation, respectively.

Gestation diets were fed from d 30 to 112 of gestation. On a daily basis, treatments were top dressed in a common gestation diet according to daily feed allowance. Sows were fed 2, 2.5, or 3 kg/day of gestation diet according to body condition and BW. For the control diet, the top dress contained only ground corn. In the Oceanfeed Swine diet, the top dress contained a mixture of 80% ground corn and 20% Oceanfeed Swine. To achieve an equivalent of 0.50% of the sow's daily feed allowance, sows with a daily feed allowance of 2 kg were provided with 50 g of the Oceanfeed Swine top dress. Sows with a daily feed allowance of 2.5 kg were top-dressed with a 62 g and sows with a daily feed allowance of 3 kg were provided with 75 g of top dress.

During lactation, sows were individually housed in stalls (0.6 × 2.10 m) for the sow with area (0.46 × 2.10 m) for piglets on both sides of the stall in an environmentally-controlled, mechanically-ventilated barn. Farrowing stalls were equipped with an individual nipple waterer and an electronic feeding system (Gestal Solo Feeders, Jyga Technologies, St-Lambert-de-Lauzon, Quebec, Canada) to provide ad libitum access to feed during lactation. Farrowing stalls were also equipped with a rubber mat and heat lamp for piglet comfort. All farrowings were supervised and immediately after birth, piglets were dried, identified with an ear tag, and weighed with a digital scale. Between 24 and 48 h after birth, piglets were administered 200 mg of iron injection (Gleptoforte, Ceva Animal Health, LLC., Lenexa, KS), tails were docked, males were castrated, and cross-fostering was performed within sow treatment group to equalize litter size within 24 hours of birth. Piglets had free access to water and no creep feeding was provided during lactation.

Lactation were fed from day 112 of gestation to weaning at approximately day 20 of lactation (Table 1). Sows were fed 2.7 kg/d from day 112 until farrowing and had ad libitum access to feed from farrowing to weaning. Sow performance was determined by recording feed intake on a daily basis and BW at day 30 and 112 of gestation and on day 1 and 20 after farrowing. Farrowing and litter performance were assessed by recording number of piglets total born, born alive, and stillborn, and individual piglet BW at birth. Litter size and pig weights were recorded on day 2 and 19. Pre-weaning survival was measured as the difference in pigs equalized on day 2 and those weaned on day 19 divided by the number of pigs on day 2.

In order to estimate colostrum yield, piglets were weighed at birth and 24 h later, according to the method described by Theil (2017). Additionally, colostrum and milk samples were collected during parturition and on day 10 of lactation. To facilitate milk collection on day 10, piglets were removed from the sow and milk ejection was induced by perivulvar administration of 2 ml oxytocin that provided 40 USP units (Bimeda-MTC Animal Health Inc, Cambridge, Ontario, Canada). Milk and colostrum samples were analyzed for fat and total solids content using the CEM SMART Tract II^{TM} Rapid Fat and Mositure/Solids Analyzer. Protein content was determined by combustion using a Leco TruMac N with TruMac operating software. Also, colostrum IgG concentration was determined by using a specific pig-Elisa kit (Bethyl Laboratories Inc. Montgomery, TX).

Nursery portion

A total of 360 weaned pigs (DNA 241 × 600), progeny of the sows in the study, were used in a 56-d study starting at weaning. Only twelve weaned pigs (eight from the control litters and four from the Oceanfeed Swine litters) were not included in the nursery portion of the study due to health or unthrifty issues. Weaned pigs were approximately 20 d of age and initially 5.44

kg. Weaned pigs were housed in an environmentally-controlled and mechanically-ventilated nursery barn with 1.5 × 1.5 m pens equipped with a four-hole, dry, self-feeder and one cup waterer. Pigs were placed in mixed-gender pens with 5 pigs per pen with numbers balanced for gender within block and 18 replications per treatment. At weaning, pigs were weighed and assigned to nursery pens in a split-plot design with lactation treatment as the whole-plot and nursery treatment as the sub-plot. The 4 treatments in the nursery phase consisted of: pigs from sows fed control diet in gestation and lactation, then fed either a control diet or a diet containing 0.75% Oceanfeed Swine. The remaining 2 treatments were pigs weaned from sows fed Oceanfeed Swine and fed a control diet or a diet containing Oceanfeed Swine diet.

Diets were based on corn and soybean meal and were fed in four dietary phases: Phase 1, fed from d 0 to 7 in pellet form; Phase 2, fed from d 7 to 21 in meal form; Phase 3, fed from d 21 to 42 in meal form and Phase 4, fed form d 42 to 56 in meal form (Table 2). Phase 1 diets were pelleted under the following parameters: 50.5° C average conditioning temperature, 71.7 °C average hot pellet temperature, 4.76 mm × 31.75 mm die size (L/D = 6.0), 708 kg/h production rate, 22 °C ambient temperature. Diets were formulated to meet or exceed NRC (2012) nutrient requirement estimates.

Nursery performance was assessed by recording BW and feed disappearance on d 7, 14, 21, and 56 to determine average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency (G:F). Also, fecal scores were determined on d 7, 14, and 21. Fecal scoring was assigned to pens and categorized as a numerical scale from 1 to 5: 1, hard pellet-like feces; 2, firm formed stool; 3, soft moist stool that retains shape; 4, soft unformed stool; and 5, watery liquid stool. Fecal scoring was performed by 3 trained individuals and the combined score was considered as the pen score.

For microbiota determination, fecal samples were collected on d 56 directly from the rectum using sterile mini cotton tip swabs from 1 pig per pen and 12 pens per treatment for microbial analysis. Fecal samples were kept at -80 °C until analysis. Fecal samples were analyzed in pigs from sows fed control diet in gestation and lactation, then fed control diet in the nursery, and from pigs from sows fed Oceanfeed Swine diets, then fed Oceanfeed Swine diet in the nursery using the Lawrence Livermore Microbial Detection Array (LLMDA) as previously described (Niederwerder et al., 2016; Ober et al., 2017).

Finishing portion

At the end of the nursery period, pigs from two nursery pens within weight block and treatment were combined and placed in a single grow-finish pen with approximately 10 pigs per pen. There were 9 replications per treatment. The facility was totally enclosed and environmentally regulated, containing 36 pens. Each pen (3.00 × 2.44 m) was equipped with a dry, single-sided feeder (Farmweld, Teutopolis, IL) with two feeder spaces and a 1-cup waterer. Pens were located over a completely slatted concrete floor with a 1.22-m deep pit underneath for manure storage. A robotic feeding system (FeedPro; Feedlogic Corp., Wilmar, MN) was used to deliver and record daily feed additions to each individual pen. Pens were equipped with adjustable gates to allow space allowances per pig to be maintained if a pig died or was removed during the experiment.

Growth performance was assessed by recording BW and feed disappearance every 2 weeks and at the conclusion of the study (day 156). Then, pigs were individually tattooed with a unique ID number, and an RFID transponder was inserted into the left ear to allow carcass measurements to be recorded on a pig basis. On d 156, final pen weights and individual pig weights were taken, and pigs were transported approximately 2.5 h to a commercial packing

plant (Triumph Foods, St. Joseph, MO) and held in lairage for approximately 7 h before slaughter. At the plant, hot carcass weight (HCW) was determined immediately after evisceration. Backfat and loin depth were measured with an optical probe (Fat-O-Meter, SFK, Herlev, Denmark) inserted between the third and fourth rib (counting from the ham end of the carcass) at a distance approximately 7 cm from the dorsal midline. Percentage lean was calculated using proprietary equations from the packing plant. Carcass yield was calculated by dividing individual HCW obtained at the packing plant by the individual final live weight obtained at the farm.

Diets were based on corn and soybean meal and were fed in meal form in three dietary phases: Phase 5, fed from d 56 to 82 after weaning; Phase 6, fed from d 83 to 111, and Phase 7, fed from d 115 to 156 (Table 3). Diets were formulated to meet or exceed the NRC (2012) requirement estimates, and the Oceanfeed Swine was included at 0.75% of the diet in Phase 5 and 0.50% in phases 6 and 7. All experimental diets for the sow, nursery and finishing portions were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS.

Statistical Analysis

Statistical models were fit using the GLIMMIX procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC). For the sow portion of the study, data were analyzed using a linear mixed model. Treatment was included as fixed effect and block as random effect. Sow or litter were the experimental units. Response variables were fit assuming a normal distribution except for piglets born alive, stillborn, and mummified that were analyzed assuming a binomial distribution as a proportion of piglets total born. Also, pre-weaning mortality was analyzed assuming a binomial distribution as a proportion of number of dead piglets from birth to weaning

in relation to the number of piglets born alive. Moreover, fecal score was analyzed assuming a multinomial distribution and considering the frequency distribution of experimental units within each fecal score category. For normally-distributed response variables, the residual assumptions were met by evaluating studentized residuals.

In the nursery and finishing portion of the study, data were analyzed using a linear mixed model. Treatment was included as fixed effect and pen as the experimental unit. Preplanned contrast statements were built to evaluate the main effects and interactions of sow treatment by nursery/finishing treatment. Hot carcass weight was used as a covariate for analyses of backfat, loin depth, and lean percentage. Results were considered significant at $P \leq 0.05$ and marginally significant at $P > 0.05$ and $P \leq 0.10$.

For the microbiota analysis, diversity was calculated as the mean number of families and species detected in each group. The number of families and species in each sample were fit in a generalized linear model using a binomial distribution. The glmfunction from the lme4package in R (R Core Team, 2015) was used for the analysis. The proportion of individual families present within each treatment were fit in a generalized linear mixed model using a binomial distribution. The glmerfunction from the lme4package in R (R Core Team, 2015) was used in order to account for the nature of the binary response variable and the randomized complete block design structure. The mean number of species within family were recorded as counts and a generalized linear model following a poisson distribution was used. Since the blocking factor was not being estimated, the block term was dropped from the model. The glmfunction from the lme4package in R (R Core Team, 2015) was used for the analysis.

Results

Sow Portion

There was no evidence for difference ($P > 0.10$) on sow parity and BW on d 30 of gestation between dietary treatments (Table 4) validating the randomization process. No evidence for differences ($P > 0.10$) were observed on sow BW at the end of gestation or at weaning. In gestation and lactation, ADFI was similar ($P > 0.10$) among dietary treatments. There was no evidence for differences ($P > 0.10$) in the number of piglets total born, born alive, stillborn, or piglet birth weight between sows fed control or Oceanfeed Swine diets. Growth performance and pre-weaning mortality of the litters were not influenced ($P > 0.10$) by treatments in the lactation period. Colostrum yield was not influence by dietary treatment. Total solids, fat content, protein concentration, and IgG did not differ between treatments (Table 5).

Nursery Portion

There was no evidence for interaction ($P > 0.10$) between sow and nursery treatments for ADG, ADFI, or G:F of nursery pigs (Table 6). Therefore, the main effects of sow dietary treatment and nursery dietary treatment were reported (Table 7).

Pigs weaned from the sows fed the control diet were heavier ($P = 0.001$) at the beginning of the nursery period compared to pigs weaned from sows fed the Oceanfeed Swine diet. This significant difference is the consequence of allotting pigs in small, medium and heavy weight blocks that reduced the weight variability within blocks, resulting in a small difference of 200 g BW between treatment groups. In phase 1, from day 0 to 7 of the nursery phase, there was a marginally significant ($P = 0.060$) increase in ADG of pigs weaned from sows fed the control diet compared with those weaned from sows fed the Oceanfeed Swine diet. In phase 2, from day 7 to 21 of nursery, pigs fed the Oceanfeed Swine diet had poorer ($P = 0.006$) G:F driven by a

marginally significant ($P = 0.055$) increase in ADFI. In phases 3 and 4 (d 21 to 42 and 42 to 56 respectively), there was no evidence ($P > 0.10$) for effect of sow or nursery dietary treatments on growth performance. Overall (d 0 to 56 post-weaning), there was no evidence ($P > 0.10$) for effect of sow or nursery treatment on pig growth performance.

Fecal scores of nursery pigs are presented as the frequency distribution of pens within each fecal score category. There was a marginal significant ($P = 0.062$) sow × nursery treatment interaction for pig fecal scores. Pigs weaned from sows fed the control diet that were fed the Oceanfeed Swine diet or pigs weaned from sows fed Oceanfeed Swine then fed the control diet in the nursery had increased frequency distribution of unformed softer feces compared to pigs weaned from sows fed the control diet that were fed control diet in the nursery or pigs weaned from sows fed Oceanfeed Swine and remained on Oceanfeed Swine in the nursery (Figure 1). There was also a sow treatment × day interaction ($P < 0.007$) observed with pigs weaned from control sows initially (day 7) having firmer feces than those weaned from sows fed the Oceanfeed Swine. However, by day 21, there appeared to be no differences in fecal consistency among pigs weaned from either sow treatment group (Figure 2).

Microbiota diversity and composition was only analyzed in pigs from sows fed control diet, then fed control in nursery and pigs from sows fed Oceanfeed Swine diet, then fed Oceanfeed Swine in the nursery. Microbiome diversity of the fecal samples was measure by calculating the number of families and species detected in each sample. A wide number of families and species were detected in both groups (Figure 3). No significant differences were observed in family and species diversity between the two groups ($P > 0.05$). The mean number of families detected in the pigs from sows fed control diets during gestation and lactation, then fed control was 35.3 ± 1.72 while the pigs from sows fed Oceanfeed Swine diets, then fed

Oceanfeed Swine had 36.8 ± 1.75. The mean number of species detected in the pigs from sows fed control, then fed control was 61.4 ± 2.26 while the pigs from sows fed Oceanfeed Swine, then fed Oceanfeed Swine had 65.4 ± 2.33.

Microbiome composition was also analyzed using the LLMDA method by the proportion of samples with each family detected (Figure 3A) and the mean number of species within that family (Figure 3B). At the family level, a trend with higher proportion of pigs with Peptostreptococcaceae was detected in the pigs from sows fed Oceanfeed Swine diet, then fed Oceanfeed Swine compared with the control group (41 and 8% respectively; $P = 0.085$). A similar trend was observed for the Veillonellaceae family, detected at an increased prevalence rate in the pigs from sows fed Oceanfeed Swine diet, then fed Oceanfeed Swine compared with the control group (92 and 58% respectively; $P = 0.085$).

At a specie level, a greater mean number of species within the family Ruminococcacea was detected in the pigs from sows fed Oceanfeed Swine diet, then fed Oceanfeed Swine compared with the control (1.42 and 0.58 respectively; $P = 0.0482$). Additionally, a trend for a greater number of species within the family Lachnospiracea was detected in the pigs fed Oceanfeed Swine diet, then fed Oceanfeed Swine compared with the control (4 and 2.7 respectively; $P = 0.076$). Moreover, a trend for lower mean number of species within the family Fusobacteriacea was detected in pigs from sows fed Oceanfeed Swine diets, then fed Oceanfeed compared with the control (8 and 56% respectively; $P = 0.089$).

Finishing Portion

In the finishing period, a sow × nursery/finishing treatment interaction ($P = 0.037$) was observed for G:F from d 56 to 111 (Table 8). Pigs weaned from sows fed control diets and fed Oceanfeed Swine diets in the nursery/finishing period had improved G:F compared with pigs

weaned from sows fed control diets and fed control diets in the nursery/finishing period. Also, pigs weaned from sows fed Oceanfeed Swine diets and fed control diets in the nursery/finishing period had improved G:F compared with pigs weaned from sows fed Oceanfeed Swine diets and fed Oceanfeed Swine diets in the nursery/finishing period. No evidence for any interactive or main effect differences ($P > 0.10$) were observed for overall finishing pig growth performance (Tables 8, 9). A sow × nursery/finishing treatment interaction was observed for backfat depth and percentage lean. Pigs weaned from sows fed control diets and fed control diets in the nursery/finishing period had greater backfat depth ($P < 0.044$) and decreased ($P < 0.065$) percentage lean than pigs on other treatment combinations. No evidence for differences ($P > 0.10$) between treatments or interactions were observed for hot carcass weight or carcass yield. However, pigs weaned from sows fed the Oceanfeed Swine diet had greater ($P = 0.088$) loin depth than pigs weaned from sows fed control diet (Table 9).

Discussion

At birth, a piglet's gastrointestinal tract (GIT) is considered sterile (Katouli et al., 1995), and colonization from environmental microorganisms is essential to support growth and development of the immune system (Mukherjee and Hooper 2015). The close contact of newborn pigs with the sow is a fundamental factor for early microbial colonization of the gastrointestinal tract (Everaert et al., 2017) and determines long term characteristics in pigs described as microbial imprinting (Thompson et al., 2008; Mach et al., 2015). Dietary strategies that modulate the bacterial population of sows could increase the prevalence of beneficial microbes and consequently provide health benefits to sows and their progeny (Baker et al., 2013). Seaweed extracts have been suggested as a promising additive to modulate the intestinal microbiota of nursing piglets through the supplementation of the sow diet (Leonard et al., 2012).

Seaweed extracts are rich in bioactive polysaccharides, such as laminarin and fucoidan, that are resistant to endogenous mammalian enzymes and therefore, valuable for bacterial fermentation in the hindgut (Hoebler et al., 2000; Deville et al., 2004). Moreover, maternal supplementation with seaweed extracts have shown to have a positive immunomodulatory effect in colostrum immunoglobulin concentration and piglet immune function (Leonard et al., 2010, 2012). In our study, it was hypothesized that supplementation with Oceanfeed Swine would improve sow performance, colostrum and milk quality, resulting in and improved microbiota composition of their offspring, lower fecal score and improved growth performance from birth to market.

Lactation period

Leonard et al. (2010; 2011; 2012) reported that supplementation of a seaweed extract to sows during late gestation had no beneficial effects on neonatal piglet growth. Leonard et al (2012) hypothesized that an earlier start to implement treatment diets may be necessary to promote neonatal growth. However, in the present study, sows were supplemented from d 30 of gestation and no differences were observed during the lactation period. Our results indicate that maternal supplementation with Oceanfeed Swine had no effect on milk or colostrum composition or colostrum yield. In contrast with the current study, greater colostrum IgG concentration and higher percentage crude protein in milk were reported when sows were supplemented with seaweed extract from d 109 of gestation (Leonard et al., 2010; 2012). The reason why the present study failed to find a difference in milk and colostrum composition is hard to assess, but it might be due to different concentrations of the laminaria and fucoidan contained in the commercial product that was utilized in this study.

Nursery period

Literature is inconsistent on nursery performance when seaweed extracts are supplemented to the diet. Turner et al. (2002) found a positive linear effect of seaweed extract inclusion on ADFI and a negative linear effect on G:F ratio in nursery pigs challenged with Salmonella typhimurium. However, other studies observed an improvement in G:F ratio when seaweed extracts were supplemented to nursery diets (Gahan et al., 2009; Heim et al., 2014b; McDonnell et al., 2010; Ruiz et al., 2018). Some authors were only able to find a beneficial effect of seaweed supplementation in nursery diets under challenge conditions such as low lactose levels (Gahan et al., 2009) and health challenges (Turner et al., 2002, Allen et al., 2001 and Heim et al., 2014a). Others found a positive effect when laminarian was added to diets but found no differences when a combination of laminarian and fucoidan was added to the diet (McDonnell et al., 2010). In the present study, a blend of brown, red, and green seaweeds was supplemented to the diets and the concentration of laminaria, fucoidan were not determined. Moreover, it has been described that components which have been shown to reduce growth performance in pigs, such as alginates and phenolic compounds, may be present in seaweed (Gardiner et al., 2008). Unfortunately, in the present study the concentration of antinutritional factors was not analyzed so no conclusion can be made regarding to these components. Furthermore, Heim et al. (2014a; 2015) observed no improvement in growth rate of piglets whose dams were supplemented with seaweed extracts containing laminaria, fucoidan or a combination of both.

Fecal score

The sow × nursery treatment interaction on fecal scores was unexpected, it could be possibly explained by the adaptation to the addition or removal of Oceanfeed Swine from sow to

nursery diets. Differences in the microbiota during the nursery period may be driving marginally significant differences in the fecal score between treatments. The sow treatment × day interaction observed along the nursery period was driven by a higher, but variable proportion of pigs weaned from control sows having firmer feces on d 7. However, over the nursery period, this interactive difference became less variable between treatments in the overall period. The lack of main effect differences between dietary treatments may be a consequence of the high health status observed under these experimental conditions. In contrast, O'Shea et al (2016) observed an improved diarrhea score in nursery pigs supplemented with fucoidan and laminarian under dextran sodium sulfate challenge.

Microbiota analysis

At the end of the nursery period (d 56), fecal microbiota was analyzed and compared between pigs weaned from sows fed control, then fed control and pigs weaned from sows fed diets with Oceanfeed Swine, then fed Oceanfeed Swine-containing diets. An increased microbial diversity is associated with a more stable balance between microbiota and host and has showed several benefits for the host such as an increased capacity to metabolize complex carbohydrates and improve digestive capacity (Backhed et al., 2005; Sonnenburg and Backhed, 2016). However, beneficial effects in nursery pigs transplanted with fecal microbiota under challenged conditions have been observed with no increment in microbiota diversity (Niederwerder, 2018). In the present study, the Lawrence Livermore Microbial Detection Array failed to detect differences in diversity of families and species between treatments. However, several differences at a family and specie level were detected. Pigs from sows fed Oceanfeed Swine, then fed Oceanfeed Swine increased the relative abundance of Peptostreptococcaceae and Veillonellaceae. Unfortunately, there is a lack of research exploring the effects of these

microorganisms on the vertebrate gut microbiome. Moreover, an increase in the mean number of species within Ruminococcacea and Lachnospiraceae families was detected in pigs weaned from Oceandfeed Swine sows, then fed Oceanfeed Swine. Bacteria in the Ruminococacea family are commonly present the GIT of vertebrate animals and help the host to break down complex carbohydrates (Liu et al., 2008). The members of the Lachnospiraceae family are anaerobic, fermentative bacteria, with the ability to hydrolyze complex carbohydrates, such as xylanase, α- and β-glucosidase and α- and β-galactosidase (Stackebrandt, 2014). Both, Ruminococcacea and Lachnospiraceae families have been associated with fatness traits in pigs (He et al., 2016). Moreover, a positive association between Ruminococcaceae species and growth after co-infection was observed (Ober et al., 2017). In addition, the lower mean number of species within the family Fusobacteriaceae detected in pigs from sows fed Oceanfeed Swine diets, then fed Oceanfeed Swine could be considered an improvement in gut health because the family Fusobacteriaceae has been associated with a wide spectrum of diseases in human and animals (Rosenberg et al., 2014). It has also been observed that the relative abundance of species from the Fusobacteriacea family significantly increase in piglets with diarrhea (Yang et al., 2017). Although the microbiota differences didn't reflect and improvement in growth performance, the differences observed in microbiota composition are considered an improvement in gut health that could drive to an improvement in growth performance under more health challenging conditions.

Finishing period

The literature is not consistent regarding to the effects of seaweed supplementation in finishing pig performance. Positive effects on final body weight and feed efficiency have been reported when sows or their offspring are supplemented with seaweed (Heim et al., 2015; Ruiz et al., 2018 respectively). However, others reported a reduction in ADG in pigs supplemented with

seaweed extract (Gardiner et al., 2008). We observed no effects of sow treatment, grow-finishing treatment, or their interaction on overall growth performance.

For carcass characteristics, an unexpected greater backfat depth and decreased percentage lean were observed in pigs weaned from sows fed control diets and then fed control in the nursery/finishing period. This result contradicts Gardiner et al. (2008) data who found no differences in these parameters when feeding different ratios of *Ascophyllum nodosum* to grow-finish pigs. Moreover, a tendency for greater loin depth was observed in pigs weaned from sows fed Oceanfeed Swine. To our knowledge there is no other study that evaluated the effects of sow supplementation with seaweed on carcass characteristics of the progeny.

In conclusion, the study does not provide evidence that supplementation of sows and their litters with Oceanfeed Swine has a significant benefit related with growth performance or carcass characteristics. However, interesting differences were observed in the microbiota composition, with a relative increase of bacterias within families considered beneficial including Ruminococacea and Lachnospiraceae and a decrease of Fusobacteriaceae, which is generally considered pathogenic.

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Table 2-1. Composition of gestation and lactation diets (as-fed basis)[1]

Item	Gestation[2]	Lactation[3]
Ingredient, %		
Corn	80.40	63.37
Soybean meal, 47% crude protein	15.61	29.99
Choice white grease	---	2.50
Calcium carbonate	1.15	1.05
Monocalcium phosphate, 21.5% P	1.40	1.30
Sodium chloride	0.50	0.50
L-Lysine HCl	---	0.20
DL-Methionine	---	0.05
L-Threonine	0.03	0.10
L-Valine	---	0.03
Vitamin premix[4]	0.25	0.25
Trace mineral premix[5]	0.15	0.15
Sow add pack[6]	0.50	0.50
Phytase[7]	0.02	0.02
Oceanfeed Swine[8]	---	+/-
Total	100.0	100.0
Calculated analysis		
Standardized ileal digestible (SID) amino acids, %		
Lysine	0.56	1.08
Isoleucine:lysine	86	67
Leucine: lysine	209	139
Methionine:lysine	38	30
Methionine and cysteine:lysine	76	56
Threonine:lysine	79	67
Tryptophan:lysine	24	20
Valine:lysine	99	76
Total lysine, %	0.66	1.20
ME, kcal/kg	3,245	3,360
NE, kcal/kg	2,476	2,511
SID lysine:NE, g/Mcal	2.26	4.24
Crude protein, %	14.1	19.8
Calcium, %	0.91	0.90
STTD P, %	0.47	0.49

[1]Gestation diets were fed from d 30 to d 112 of gestation and lactation diets were fed from day 112 of gestation until weaning. Diets were fed in meal form.

[2]Treatments were top dressed in a common gestation diet. In the control diet, the top dress contained ground corn. In the treatment diet, the top dress contained ground corn and Oceanfeed Swine was added to equal a 0.50% of the total diet.

[3]In lactation, Oceanfeed Swine was added at 0.66% of the diet.

[4]Provided per kg of premix: 4,409,200 IU vitamin A; 551,150 IU vitamin D; 22,455 IU vitamin E; 1,764 mg vitamin K; 15 mg vitamin B_{12}; 19,841 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin; 88 mg biotin; 661 mg folic acid; 1,984 mg pyridoxine; 220,460 mg choline; 19,841 mg carnitine.

[5]Provided per kg of premix: 73 g Zn from Zn sulfate; 73 g Fe from iron sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.2 g I from calcium iodate; 0.2 g Se from sodium selenite; 0.08 g chromium picolinate.

[6]Provided per kg of premix: 220,450 mg choline, 88 mg biotin, 660 mg folic acid, and 1,984 mg pyridoxine

[7]HiPhos 2700 (DSM Nutritional Products, Inc., Parsippany, NJ), providing 405 FTU/kg and an estimated release of 0.10% available P.

[8]Oceanfeed Swine is produced by drying and blending a selected mix of brown, red, and green seaweeds.

ME = metabolizable energy.

NE = net energy.

STTD = standardized total tract digestible phosphorus.

Table 2-2. Composition of nursery diets (as-fed basis)[1]

Item	Phase 1	Phase 2	Phase 3	Phase 4
Ingredient, %				
Corn	47.08	56.97	63.21	67.48
Soybean meal, 47% crude protein	18.83	29.05	32.94	29.19
Whey powder	20.00	10.00	- - -	- - -
Enzymatically treated soybean meal[2]	5.00	- - -	- - -	- - -
Fish meal	4.50	- - -	- - -	- - -
Choice white grease	1.50	- - -	- - -	- - -
Calcium carbonate	0.55	0.90	1.00	0.90
Monocalcium phosphate, 21.5% P	0.40	1.00	1.00	0.90
Sodium chloride	0.30	0.55	0.60	0.50
L-Lysine-HCl	0.40	0.50	0.40	0.33
DL-Methionine	0.19	0.20	0.15	0.10
L-Threonine	0.16	0.19	0.15	0.11
L-Tryptophan	0.04	0.05	0.03	0.02
L-Valine	0.10	0.13	0.05	0.00
Vitamin premix[3]	0.25	0.25	0.25	0.25
Trace mineral premix[4]	0.15	0.15	0.15	0.15
Choline chloride 60%	0.04	- - -	- - -	- - -
Phytase[5]	0.08	0.08	0.08	0.08
Zinc oxide	0.39	- - -	- - -	- - -
Vitamin E, 20,000 IU	0.05	- - -	- - -	- - -
Oceanfeed Swine[6]	+/-	+/-	+/-	+/-
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Standardized ileal digestible (SID) amino acids, %				
Lysine	1.40	1.35	1.30	1.15
Isoleucine:lysine	58	55	59	62
Leucine:lysine	112	112	121	130
Methionine:lysine	36	35	34	32
Methionine and cystine:lysine	56	56	56	56

Threonine:lysine	62	62	62	62
Tryptophan:lysine	19.2	19.7	19.5	19.4
Valine:lysine	69	68	68	68
Histidine:lysine	34	35	39	41
Total lysine, %	1.54	1.48	1.45	1.29
ME, kcal/kg	3,401	3,283	3,267	3,278
NE, kcal/kg	2,551	2,436	2,403	2,432
SID lysine:NE, g/Mcal	5.49	5.54	5.41	4.73
Crude protein, %	21.5	20.6	21.6	20.0
Calcium, %	0.75	0.76	0.75	0.68
STTD P, %	0.56	0.53	0.48	0.45

[1]Nursery diets were fed in four dietary phases: Phase 1, from d 0 to 7 in pellet form; Phase 2, from d 7 to 21 in meal form; Phase 3, from d 21 to 42 in meal form; and Phase 4 from d 42 to 56 in meal form.

[2]HP 300, Hamlet Protein, Inc., Findlay, OH.

[3]Provided per kg of premix: 4,409,200 IU vitamin A; 551,150 IU vitamin D; 17,637 IU vitamin E; 1,764 mg vitamin K; 15 mg vitamin B_{12}; 19,841 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin.

[4]Provided per kg of premix: 73 g Zn from Zn sulfate; 73 g Fe from iron sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.2 g I from calcium iodate; 0.2 g Se from sodium selenite.

[5]Ronozyme® HiPhos (DSM Nutritional Products, Inc., Parsippany, NJ) provided 405 FTU/kg of feed and an estimated release of 0.14% available P

[6]Oceanfeed Swine is produced by drying and blending a selected mix of brown, red, and green seaweeds.

ME = metabolizable energy.

NE = net energy.

STTD = standardized total tract digestible phosphorus.

+/- Oceanfeed Swine was added 0.75% of the diet at the expense of corn.

Table 2-3. Composition of grow-finish diets (as-fed basis)[1]

Item	Phase 5	Phase 6	Phase 7
Ingredients, %			
Corn	74.24	80.55	84.63
Soybean meal, 47% crude protein	23.02	16.89	13.15
Calcium carbonate	0.90	0.90	0.85
Monocalcium phosphate, 21.5% P	0.50	0.40	0.30
Sodium chloride	0.50	0.50	0.50
L-Lysine-HCL	0.33	0.33	0.25
DL-Methionine	0.08	0.05	0.00
L-Threonine	0.10	0.10	0.09
L-Tryptophan	0.02	0.02	0.02
Trace mineral premix[2]	0.15	0.13	0.10
Vitamin premix[3]	0.15	0.13	0.10
Phytase[4]	0.02	0.02	0.02
Oceanfeed Swine[5]	+/-	+/-	+/-
Total	100	100	100
Calculated analysis			
Standardized ileal digestible (SID) amino acids, %			
Lysine, %	1.00	0.85	0.70
Isoleucine:lysine	61	60	64

Methionine:lysine	33	32	29
Methionine & cystine:lysine	58	58	59
Threonine:lysine	62	63	68
Tryptophan:lysine	18.9	18.8	19.2
Valine:lysine	68	68	74
Total lysine, %	1.12	0.96	0.80
ME, kcal/kg	3,302	3,313	3,322
NE, kcal/kg	2,482	2,522	2,548
SID Lysine:NE, g/Mcal	4.03	3.37	2.75
Crude protein, %	17.5	15.1	13.6
Calcium, %	0.58	0.53	0.48
STTD P, %	0.32	0.29	0.26

[1]Diets were fed ad libitum in meal form from 35.8 to 138.8 kg with phase 5 fed from d 56 to 82 after weaning, phase 6 fed from 83 to 111, and Phase 7 fed from d 115 to 156.

[2]Provided per kg of premix: 73 g Zn from Zn sulfate; 73 g Fe from iron sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.2 g I from calcium iodate; 0.2 g Se from sodium selenite.

[3]Provided per kg of premix: 4,409,200 IU vitamin A; 551,150 IU vitamin D; 17,637 IU vitamin E; 1,764 mg vitamin K; 15 mg vitamin B_{12}; 19,841 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin.

[4] Ronozyme Hiphos (GT) 2700 (DSM Nutritional Products, Inc, Parsippany, NJ) provided 405 phytase units (FYT) per kg of diet, with an assumed release of 0.10% available P.

[5]Oceanfeed Swine is produced by drying and blending a selected mix of brown, red, and green seaweeds.

ME = metabolizable energy.

NE = net energy.

STTD = standardized total tract digestible phosphorus.

+/- Oceanfeed Swine was added at the expense of corn at 0.75% of the diet in phase 5 and 0.50% in phase 6 and 7.

Table 2-4. Effect of added Oceanfeed Swine in gestation and lactation diets on sow and piglet performance until weaning[1]

Item	Control	Oceanfeed Swine[2]	SEM	Probability, *P* =
No. of observations, n	14	14	---	---
Parity	1.2	1.3	0.99	0.999
Gestation length, d	116.1	115.9	0.238	0.530
Lactation length, d	19.8	20.0	0.289	0.364
Sow weights, kg				
d 30 of gestation	206.2	206.3	7.208	0.975
Farrowing	237.5	236.4	8.716	0.839
Weaning	228.4	225.3	8.578	0.434
Change, farrow to wean	-8.9	-11.2	2.288	0.460
Sow ADFI, kg				
Gestation	2.56	2.49	0.105	0.185

Lactation	5.54	5.40	0.200	0.541
Number of pigs				
Total born, n	17.9	18.0	0.984	0.900
Stillborn, n	1.5	1.5	0.388	0.594
Mummies, n	0.7	0.6	0.315	0.874
Born alive, n	15.7	15.9	0.782	0.890
Day 2 litter size[3]	15.4	15.4	0.467	0.912
Day 19 litter size	13.4	13.2	0.354	0.778
Preweaning mortality[4]*, %	15.3	15.6	0.008	0.959
Average pig birth weight,	1.30	1.34	0.099	0.561
Day 2 pig weight, kg	1.39	1.43	0.049	0.579
Day 19 pig weight, kg	5.48	5.40	0.182	0.750
Day 2 litter weight, kg	21.5	22.2	1.109	0.666
Day 19 litter weight, kg	72.6	71.2	2.214	0.608
Colostrum yield, g[5]	6,335	6,588	331.5	0.527

[1]A total of 28 sows (DNA 241, DNA Genetics, Columbus, NE) and litters were used. Dietary treatments were fed to sows from d 30 of gestation until weaning on d 20 of lactation.

[2]The Oceanfeed Swine was top dressed to gestation diets to achieve the equivalent of 0.5% of the diet. In lactation, Oceanfeed Swine was added at 0.66% of the diet.

[3]Cross-fostering was performed within treatments on day 2 to equalize litter size.

[4]Percent pre-wean mortality = mortality day 2 to weaning ÷ number on day 2.

[5]Colostrum yield was estimated by using the equation described by Theil (2017).

* Variables analyzed using a binomial distribution.

Table 2-5. Effect of added Oceanfeed Swine in gestation and lactation diets on milk and colostrum profile[1]

	Control	Oceanfeed Swine[2]	SEM	P-value
Colostrum[3]				
Total solids %	27.27	26.00	0.855	0.308
Fat %	6.13	5.41	0.420	0.235
Protein %	15.42	15.67	0.708	0.804
IgG mg/ml	5.46	5.12	0.143	0.114
Milk[4]				
Total solids %	19.40	19.79	0.405	0.464
Fat %	8.66	8.83	0.373	0.727

Protein %	5.00	4.88	0.129	0.501

[1]A total of 28 sows (DNA 241, DNA Genetics, Columbus, NE) were used in the 100-d sow portion of the trial.

[2]In gestation, sows were top dressed with either ground corn or a combination of ground corn and Oceanfeed Swine to achieve the equivalent 0.5% of the diet. In lactation, Oceanfeed Swine was added at 0.66% of the diet.

[3]Aproximately 30 ml of colostrum was collected during parturition and stored at -20 C until analysis.

[4]Milk ejection was induced by a perivulvar administration of oxytocin on d 10.

Table 2-6. Interactive effects of Oceanfeed Swine in nursery pig dietary treatment on growth performance of nursery pigs[1]

Sow treatment[2]	Control		Oceanfeed Swine			Probability, *P* =		
Nursery treatment[3]	Control	Oceanfeed Swine	Control	Oceanfeed Swine	SEM	Sow × nursery treatment	Sow treatment	Nursery treatment
Weight, kg								
d 0	5.6	5.6	5.5	5.5	2.001	0.497	<0.001	0.717
d 56	35.9	36.2	36.0	36.2	2.384	0.906	0.911	0.505
d 0 to 7								
ADG, g	119	111	95	104	11.83	0.286	0.060	0.949
ADFI, g	152	135	133	138	14.67	0.153	0.316	0.446
G:F, g/kg	769	821	697	767	39.79	0.812	0.115	0.129
d 7 to 21								
ADG, g	334	330	324	327	41.54	0.678	0.431	0.923
ADFI, g	459	486	453	476	57.62	0.854	0.552	0.055
G:F, g/kg	740	683	715	683	15.48	0.421	0.424	0.006
d 21 to 42								
ADG, g	612	617	628	624	34.53	0.657	0.276	0.971
ADFI, g	945	942	956	961	70.30	0.809	0.363	0.956
G:F, g/kg	652	656	659	651	14.74	0.365	0.906	0.785
d 42 to 56								
ADG, g	842	860	851	866	25.55	0.885	0.603	0.229
ADFI, g	1557	1565	1531	1586	75.59	0.343	0.918	0.219
G:F, g/kg	544	552	558	547	13.22	0.171	0.558	0.863
d 0 to 56								
ADG, g	538	542	538	539	31.87	0.815	0.797	0.707
ADFI, g	876	882	865	881	63.79	0.706	0.681	0.418
G:F, g/kg	618	617	623	612	10.19	0.325	0.962	0.211

[1]A total of 360 pigs (DNA 241 × 600, Columbus, NE) with initial BW of 5.5 kg were used in a 56-d trial with 5 pigs per pen and 18 replicates per treatment. Pigs were weaned at approximately 20 d of age and divided into light, medium and heavy weight groups within sow treatment. Within each weight group pigs were allocated to either control or Oceanfeed Swine dietary treatment in a split plot design with sow treatment (control or Oceanfeed Swine) as the whole-plot and nursery pig treatment (control or Oceanfeed Swine) as the sub-plot.

[2]Sow treatment consisted of providing a control diet or a diet with Oceanfeed Swine to achieve the equivalent of 0.5% in gestation (d 30 to farrowing) and 0.66% in lactation (farrowing to weaning).

[3]Nursery treatment consisted of providing a control diet or a diet with Oceanfeed Swine at 0.75%.

ADG = average daily gain. ADFI = average daily feed intake. G:F gain to feed ratio.

Table 2-7. Main effects of sow and nursery diets supplemented with Oceanfeed Swine on nursery growth performance[1]

	Sow treatment[2]				Nursery treatment[3]			
Item	Control	Oceanfeed Swine	SEM	*P*-Value	Control	Oceanfeed Swine	SEM	*P*-Value
Weight, kg								
d 0	5.6	5.5	0.788	<0.001	5.5	5.5	0.788	0.717
d 56	36.0	36.1	2.367	0.911	35.9	36.2	2.367	0.505
d 0 to 7								
ADG, g	115	100	10.42	0.060	107	108	10.42	0.949
ADFI, g	144	136	13.63	0.316	143	137	13.63	0.446
G:F, g/kg	796	732	28.14	0.115	733	794	28.14	0.129
d 7 to 21								
ADG, g	333	326	41.08	0.431	330	329	41.08	0.923
ADFI, g	472	465	56.91	0.552	456	481	56.91	0.055
G:F, g/kg	711	699	10.95	0.424	727	683	10.95	0.006
d 21 to 42								
ADG, g	614	626	33.69	0.276	620	620	33.69	0.971
ADFI, g	943	959	69.30	0.363	950	951	69.30	0.956
G:F, g/kg	654	655	14.02	0.906	655	654	14.02	0.785
d 42 to 56								
ADG, g	852	859	23.70	0.603	847	863	23.70	0.229
ADFI, g	1561	1559	73.49	0.918	1544	1575	73.49	0.219
G:F, g/kg	548	552	12.22	0.558	551	549	12.22	0.863
d 0 to 56								
ADG, g	540	538	31.42	0.797	538	541	31.42	0.707
ADFI, g	879	873	63.05	0.681	870	881	63.05	0.418
G:F, g/kg	617	617	9.60	0.962	620	614	9.60	0.211

[1]A total of 360 pigs (DNA 241 × 600, Columbus, NE) with initial BW of 5.5 kg were used in a 56-d trial with 5 pigs per pen and 18 replicates per treatment. Pigs were weaned at approximately 20 d of age and divided into light, medium and heavy weight groups within sow treatment. Within each weight group pigs were allocated to either control or Oceanfeed Swine dietary treatment in a split plot design with sow treatment (control or Oceanfeed Swine) as the whole-plot and nursery pig treatment (control or Oceanfeed Swine) as the sub-plot.

[2]Sow treatment consisted of providing a control diet or a diet with Oceanfeed Swine to achieve the equivalent of 0.5% in gestation (d 30 to farrowing) and 0.66% in lactation (farrowing to weaning).

[3]Nursery treatment consisted of providing a control diet or a diet with Oceanfeed Swine at 0.75%.

ADG = average daily gain. ADFI = average daily feed intake. G:F ratio.

Table 2-8. Interactive effects of Oceanfeed Swine on growth performance of grow-finish pigs[1]

Sow treatment[2]	Control	Oceanfeed Swine	Probability, *P* =

Grow-finish treatment[3]	Control	OceanfeedSwine	Control	Oceanfeed Swine	SEM	Sow treatment × nursery treatment	Sow treatment	Finishing treatment
Weight, kg								
d 56	35.8	36.2	35.9	36.2	1.244	0.965	0.979	0.810
d 111	93.4	94.5	94.2	92.9	1.632	0.465	0.801	0.934
d 156	138.8	139.8	139.7	138.6	1.661	0.535	0.916	0.975
d 56 to 111								
ADG, kg	1.05	1.06	1.06	1.03	0.001	0.118	0.425	0.530
ADFI, kg	2.59	2.58	2.53	2.52	0.046	0.973	0.209	0.847
G:F, g/kg	405	411	418	408	6.00	0.037	0.195	0.572
d 111 to 156								
ADG, kg	1.01	1.01	1.00	1.02	0.017	0.721	0.972	0.747
ADFI, kg	3.35	3.29	3.29	3.33	0.047	0.267	0.817	0.831
G:F, g/kg	301	307	305	305	4.71	0.582	0.785	0.535
d 56 to 156								
ADG, kg	1.03	1.04	1.03	1.02	0.008	0.314	0.525	0.806
ADFI, kg	2.93	2.90	2.87	2.88	0.039	0.506	0.310	0.823
G:F, g/kg	352	358	360	355	4.60	0.142	0.459	0.914
Carcass data								
HCW, kg	105.1	106.4	106.6	105.6	1.935	0.424	0.784	0.885
Carcass yield, %	75.71	76.06	76.24	76.10	0.207	0.247	0.193	0.613
Backfat depth, mm	18.0	16.83	16.68	17.05	0.363	0.044	0.148	0.286
Loin depth, mm	63.7	64.6	65.6	65.0	0.610	0.243	0.088	0.766
Lean, %	53.1	53.6	53.8	53.6	0.190	0.065	0.103	0.363

[1]A total of 347 pigs (DNA 241 × 600, Columbus, NE) with initial BW of 36.0 kg were used in a 100-d grow-finish trial. At the end of the nursery period, pigs from two nursery pens within weight block and treatment were combined and placed in each grow-finish pen with approximately 10 pigs per pen and 9 replicates per treatment.

[2]Sow treatment consisted of providing a control diet or Oceanfeed Swine to achieve the equivalent of 0.5% in gestation (d 30 to farrowing) and 0.66% in lactation (farrowing to weaning).

[3]Grow-finish treatment consisted of providing a control diet or a diet supplemented with Oceanfeed Swine at 0.75% and 0.50% for grower (34 to 59 kg) and finisher (59 to 127 kg) periods, respectively.

ADG = average daily gain. ADFI = average daily feed intake. G:F ratio.

Table 2-9. Main effects of Oceanfeed Swine on growth performance of grow-finish pigs[1]

	Sow treatment[2]				Nursery treatment[3]			
Item	Control	Oceanfeed Swine	SEM	*P*-Value	Control	Oceanfeed Swine	SEM	*P*-Value
Weight, kg								
d 56	36.0	36.1	2.374	0.965	35.9	36.2	2.374	0.810
d 111	94.0	93.6	1.154	0.465	93.8	93.7	1.154	0.934
d 156	139.1	139.3	1.174	0.535	139.2	139.2	1.174	0.975
d 56 to 111								
ADG, kg	1.05	10.4	0.009	0.425	1.05	1.04	0.009	0.530
ADFI, kg	2.59	2.53	0.032	0.209	2.56	2.55	0.032	0.847
G:F, g/kg	408	413	5.41	0.195	412	410	5.41	0.572
d 111 to 156								
ADG, kg	1.01	1.01	0.019	0.972	1.01	1.01	0.019	0.747
ADFI, kg	3.31	3.32	0.033	0.817	3.31	3.32	0.033	0.831
G:F, g/kg	304	305	3.33	0.785	303	306	3.33	0.535
d 56 to 156								
ADG, kg	1.03	1.03	0.006	0.525	1.03	1.03	0.006	0.806
ADFI, kg	2.92	2.88	0.027	0.310	2.90	2.89	0.027	0.823
G:F, g/kg	355	358	3.69	0.459	356	369	3.69	0.914
Carcass data								
HCW, kg	105.7	106.1	0.980	0.784	105.8	106.0	0.980	0.885
Carcass yield, %	75.9	76.2	0.188	0.193	76.0	76.1	0.188	0.613
Backfat depth, mm	17.42	16.87	0.329	0.148	17.34	16.94	0.329	0.286
Loin depth, mm	64.18	65.24	0.554	0.088	64.62	64.80	0.554	0.766
Lean, %	53.4	53.7	0.171	0.099	53.4	53.6	0.171	0.372

[1]A total of 347 pigs (DNA 241 × 600, Columbus, NE) with initial BW of 36.0 kg were used in a 100-d grow-finish trial. At the end of the nursery period, pigs from two nursery pens within weight block and treatment were combined and placed in each grow-finish pen with approximately 10 pigs per pen and 9 replicates per treatment.

[2]Sow treatment consisted of providing a control diet or Oceanfeed Swine to achieve the equivalent of 0.5% in gestation (d 30 to farrowing) and 0.66% in lactation (farrowing to weaning).

[3]Grow-finish treatment consisted of providing a control diet or a diet supplemented with Oceanfeed Swine at 0.75% and 0.50% for grower (34 to 59 kg) and finisher (59 to 127 kg) periods, respectively.

ADG = average daily gain. ADFI = average daily feed intake. G:F gain to feed ratio.

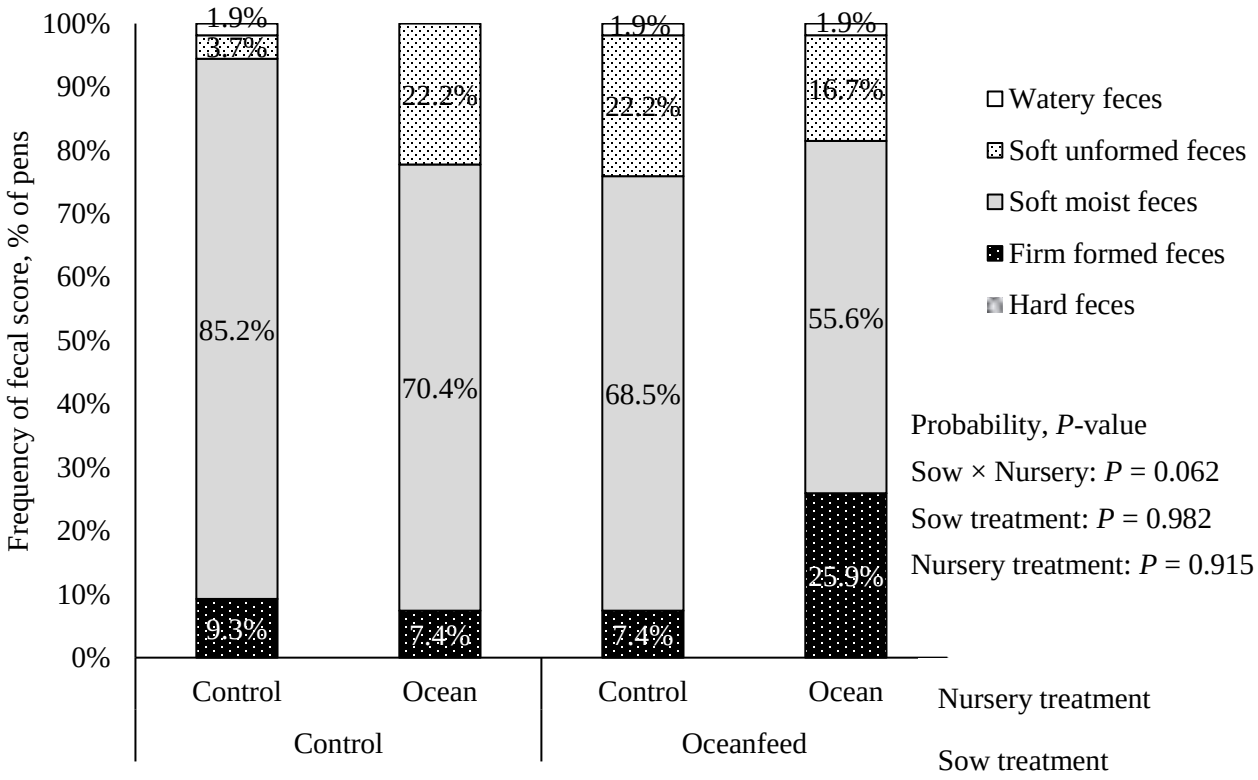

Figure 1. Overall frequency distribution of fecal score as a percentage of pens in each category. Fecal scores were conducted on d 7, 14, and 21 of the nursery period and the average consistency of feces was determined per pen (n = 18 per treatment). Fecal scoring was categorized as a numerical scale from 1 to 5: 1, hard feces like pellet; 2, firm formed stool; 3, soft moist stool that retains shape; 4, soft unformed stool; and 5, watery liquid stool.

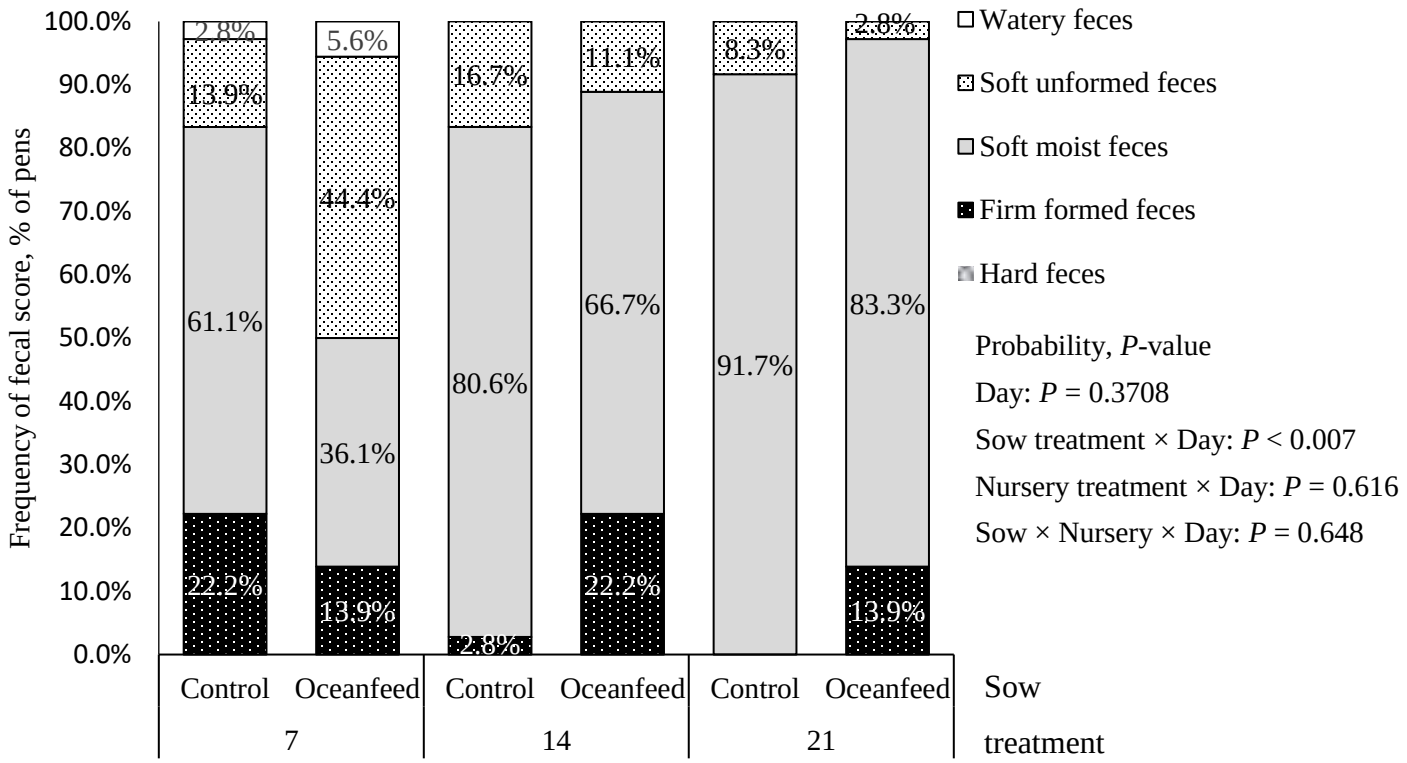

Figure 2. Frequency distribution of fecal score in nursery pigs by sow treatment by day. Fecal scores were conducted on d 7, 14 and 21 to determine the consistency of nursery pigs feces per pen (n = 36 per sow treatment). Fecal scoring was categorized as a numerical scale from 1 to 5: 1, hard feces like pellet; 2, firm formed stool; 3, soft moist stool that retains shape; 4, soft unformed stool; and 5, watery liquid stool.

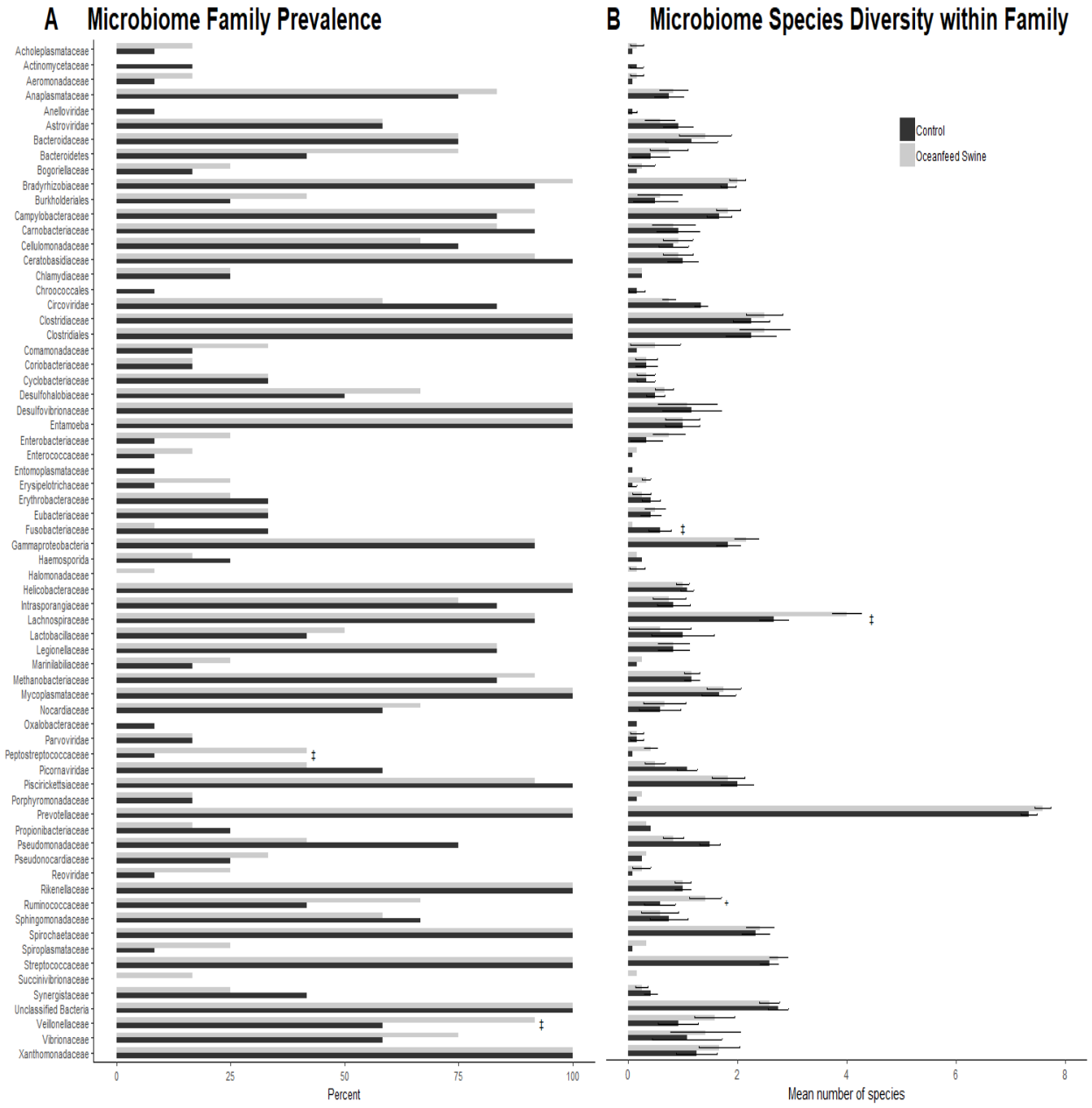

Figure 3**.** Fecal microbiome composition detected by the Lawrence Livermore Microbial Detection Array (LLMDA) from feces collected on day 56 in nursery pigs from sows fed control diets, then fed control and pigs from sows fed Oceanfeed Swine diets, then fed Oceanfeed Swine. (A) Microbiome family composition as a percentage of control pigs (n = 12) and Oceanfeed Swine pigs (n = 12) with that family detected. (B) Mean number of species detected ± 1 standard deviation in each group. + Indicates $P < 010$. ‡ Indicates $P < 0.05$.

Chapter 3 - **Effects of an algae-clay complex-based feed additive and diet formulation regimen on finishing pig growth performance and carcass characteristics**

Abstract

This study evaluated the effects of an algae-clay-complex-based feed additive (ACC, Olmix Group, Brehan, France) and diet formulation regimen on growth performance and carcass characteristics of finishing pigs. A total of 1,188 pigs (PIC 337 × 1050; initially 50.6 kg) were used in a 90-d study. There were 27 pigs per pen and 11 replications per treatment. Dietary treatments were arranged in a 2 × 2 factorial with main effects of added ACC (none or 0.10% until 100 kg body weight and 0.05% thereafter) and dietary formulation regimen (High vs. Low). High diets were formulated to maximize growth performance with added fat and no dried distillers grains with solubles (DDGS). Low diets were formulated to contain approximately 150 kcal per kg less net energy, 30% DDGS, no added fat, and were formulated 0.10% below the standardized ileal digestible lysine requirement based on the SID Lys:NE ratio as estimated in the High diets. Overall, there were no ACC × formulation interactions *(P* > 0.220) for growth or carcass characteristics. However, ADG was greater *(P* = 0.027*)* for pigs fed added ACC diets compared with those fed diets without ACC. This was a result of late finishing (d 56 to 90) increases *(P* < 0.019) in ADG and G:F for pigs fed diets with ACC compared with those fed no ACC. Also, pigs fed High diets had improved growth performance and final body weight than pigs fed Low diets. For carcass characteristics, pigs fed High diets tended to have greater *(P* = 0.067) loin depth and had greater *(P* < 0.001*)* carcass weight than pigs fed Low diets. No evidence for differences was observed for carcass characteristics between the control and added

ACC fed pigs. For economic analysis, pigs fed High diets had greater feed cost and feed cost per kg gain, but also had greater revenue with no differences on income over feed cost (IOFC) compared with pigs fed Low diets. The addition of ACC increased ($P > 0.001$) feed cost per pig but no evidence for differences *(*$P > 0.05$*)* were observed for feed cost per kg of gain, revenue, and IOFC compared with pigs not fed ACC. In conclusion, the addition of ACC resulted in an improvement in growth performance, but did not affect carcass characteristics or IOFC. High diets improved growth performance and feed cost with similar IOFC compared with pigs fed low-energy, low lysine diets.

Key words: Algae-clay, carcass, feed additive, finishing pigs, growth performance

Introduction

Inert clays have gained considerable attention as possible feed additives for use in swine diets to increase nutrient digestibility (Turner et al., 2001; Vondruzkova et al., 2010). Several studies have shown a significant improvement in growth performance in pigs fed diets supplemented with clay-derived compounds (Prvulovic et al., 2007; Yu et al., 2008; Li and Kim, 2013). As a mechanism to explain the positive effect, it has been proposed that clays reduced the transit time of nutrients along the gastro intestinal tract (GIT) and allows more time for digestion (Papaioannou et al., 2004; Vondruskoba et al., 2010; Trckova et al., 2009). It is also speculated that because pancreatic enzymes bind to the surface of clay molecules, they form complexes that are more stable under a wide range of pH in the GIT, which also might improve energy and protein digestibility (Cabezas et al., 1991). Finally, it has been reported that adding clays to diets can improve intestinal mucosa characteristics (Xia et al., 2004, 2005). However, literature has shown variability in the effects of clay products depending on the mineral type and pig weight.

Seaweeds are valuable source of bioactive compounds, such as the sulfated polysaccharides laminarin and fucoidan, which have shown to have antimicrobial, prebiotic and immunomodulatory properties in pigs (Leonard et al., 2011; Ruiz et al., 2018; Heim et al., 2014). In this study, we evaluated an algae-clay complex-based feed additive (ACC, Olmix Group, Brehan, France). This algae-clay-based complex is made from Ulva sp and Solieria chordalis macroalgae and montmorillonite clay that has elicited an increased ileal digestibility of energy and essential amino acids when added to diets of growing pigs (Suarez and Gallisot, 2016). Therefore, the objective of this study was to determine the effects of adding ACC in diets formulated in two different regimes based on energy and amino acid concentrations (High and Low) on growth performance and carcass characteristics of grow-finish pigs housed in a commercial research facility.

Materials and Methods

General

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment (IACUC protocol number 4033). The experiment was conducted in a commercial research facility in southwestern Minnesota. The barn was double-curtain-sided with completely slatted concrete flooring and deep pits for manure storage. Each pen was equipped with a 5-hole conventional dry self-feeder (Thorp Equipment, Thorp, WI) and 1 cup-waterer, providing ad libitum access to feed and water. Pigs were placed in mixed sexed pens and stocked to allow 0.61m^2 per pig. Daily feed additions to each pen were accomplished through a robotic feeding system (FeedPro; Feedlogic Corp., Willmar, MN).

A total of 1,188 pigs (PIC 337 × 1050; initially 50.6 kg) were used in a 90-d study. On day 0 of the experiment, pens of pigs were weighed, blocked by weight and randomly assigned

to 1 of 4 dietary treatments with 11 pens per treatment and 27 pigs per pen. Dietary treatments were arranged in a 2 × 2 factorial with main effects of ACC addition (none or 0.10% until 100 kg of BW and 0.05% thereafter; MFeed+, Olmix Group, Brehan, France) and diet formulation regimen based on energy and amino acid concentrations (High vs. Low).

The diets were corn-soybean based and provided in three phases from d 0 to 28, 28 to 56, and 56 to 90 (Table 1). High diets were formulated to maximize growth performance of this specific genetic line (Goncalves et al., 2017). These diets contained 3% added fat with no dried distillers grains with solubles (DDGS). The Low treatment diets were formulated to contain approximately 150 kcal per kg less net energy, contained 30% DDGS, no added fat, and were formulated 0.10% below the standardized ileal digestible (SID) lysine requirement based on the SID Lys:NE ratio as estimated in the High diets. Our hypothesis was that the improved nutrient digestibility suggested to be associated with ACC would be more likely to be observed in a lower energy and amino acid containing diet compared to the diet designed to maximize pig performance.

Pens of pigs were weighed, and feeder measurements were recorded on d 0, 13, 28, 42, 56, 75, and 90 to calculate ADG, ADFI, and G:F. On day 75, the 3 heaviest pigs in each pen were selected and marketed following routine farm marketing protocol. These pigs were included in the growth performance data, but not in carcass data collection. On d 90, final pen weights were recorded and pigs were tattooed with a pen identification number and transported 95 km to a USDA-inspected packing plant (JBS Swift and Co., Worthington MN) for processing and carcass data collection. Carcass measurements included: hot carcass weight (HCW), backfat depth, loin depth, and percentage lean. Backfat and loin depth were measured with an optical probe (Fat-O-Meter, SFK, Herlev, Denmark) inserted between the third and fourth last rib

(counting from the ham end of the carcass) at a distance approximately 7 cm from the dorsal midline. Percentage lean was calculated from a plant proprietary equation based on weight, backfat and loin depth. Carcass yield was calculated by dividing average HCW by average final live weight obtained at the farm.

For the economic analysis, feed cost per pig, feed cost per kg of gain, revenue per pig, and income over feed cost (IOFC) were calculated on a pen basis. Corn was valued at $127/tonne, soybean meal at $319/tonne, DDGS at $143/tonne, L-lysine HCL at $1.52 kg, DL-methionine at $2.65/kg, L-threonine at $1.96/kg, L-tryptophan at $8.60/kg and ACC at $4.12/kg. Feed cost per pig was calculated by multiplying the feed cost per kg by ADFI and by the number of days in each phase, then adding the values of each phase. Feed cost per kg of gain was calculated by dividing the feed cost per pig by the overall weight gain. Revenue was obtained by multiplying carcass gain by an assumed value of $1.54 per kg. The IOFC was calculated by subtracting the feed cost per pig from revenue per pig.

Chemical analysis

Complete diet samples were collected from a minimum of 6 feeders and combined to make one composite sample per treatment within dietary phase. Each sample was then split, subsampled, ground, and analyzed (Ward Laboratories Inc., Kearney, NE) for dry matter (method 935.29, AOAC, 2000) Ca, and P concentrations using the method described by Kovar (2003). Moreover, subsamples were shipped to Missouri State University, Experiment Station Chemical Laboratories and analyzed for total lysine (Method 982.30;AOAC) and to North Dakota State University Veterinary Diagnostic Laboratory, Fargo, ND and analyzed for mycotoxin concentrations through extraction in acetonitrile and water followed by chromatography/mass spectrometry/mass spectrometry (LC/MS/MS) detection.

Statistical analysis

Data were analyzed using the GLIMMIX procedure of SAS version 9.4 (SAS Institute, Inc., Cary, NC) in a randomized complete block design with pen as the experimental unit. The treatments were analyzed as a 2 × 2 factorial with main effects of ACC addition (none or 0.1% until 100 kg and 0.05% thereafter), diet regimen (High or Low), and their interactions on growth performance and carcass characteristics. A linear mixed model was used with treatment as fixed effect and block as random effect. Hot carcass weight was used as a covariate for analyses of backfat depth, loin depth, and percentage lean. Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 < P < 0.10$.

Results

Chemical analysis

The chemical analysis of dry matter, total lysine, Ca, and P content of the experimental diets (Table 2) supported the calculated values based on diet formulation. Mycotoxins were under detectable values except for deoxynivalenol, which ranged from 416 to 747 ppb for all treatment diets. The U.S. Food and Drug Administration recommends a maximum of 1,000 ppb deoxynivalenol in complete feed (FDA, 2010), thus we do not feel this would have impacted our findings.

Growth Performance, Carcass Characteristics and Economics

There was a significant diet formulation × ACC treatment interaction *(P* = 0.014) for ADFI from d 0 to 28 (Table 3). Pigs fed High diets without ACC had greater ADFI than pigs fed High diets with ACC. However, pigs fed Low diets with ACC had greater ADFI than pigs fed Low diets without ACC. There was no evidence for interactions *(P* > 0.05*)* between ACC and

formulation regimen were observed for any other growth performance criteria, carcass data or economic analysis.

From d 0 to 28 and 28 to 56, there was no evidence *(P* > 0.05) for differences for an ACC effect on growth performance (Tables 3 and 4). However, from d 56 to 90, pigs fed the ACC diets had increased *(P* < 0.001*)* ADG and *(P* = 0.019) G:F compared with pigs fed diets without ACC. The increase in ADG from day 56 to 90 resulted in an increase in overall (d 0 to 90) ADG *(P* = 0.027*)* for pigs fed ACC diets compared with pigs fed diets without ACC There was a tendency (*P* = 0.063) for heavier pig weights on d 75 for pigs fed ACC diets compared to those fed diets without ACC. Also, a tendency (*P* = 0.070) for heavier final weight (d 90) was observed for pigs fed ACC diets.

During the entire study (d 0 to 90), pigs fed High diets had greater *(P* < 0.001) ADG and G:F than pigs fed Low diets (Table 3 and 4). From d 56 to 90 pigs fed High diets had decreased ADFI (*P* = 0.001) compared with those fed Low diets. Overall, pigs fed High diets had greater (*P* < 0.001) ADG and G:F and decreased (*P* < 0.047) ADFI than pigs fed Low diets. As a result of the increased ADG, pigs fed High diets were heavier (*P* < 0.001) than pigs fed Low diets on d 28, 56, 90, and overall.

No evidence for differences (*P* > 0.05*)* were observed for carcass weight, carcass yield, backfat depth, loin depth, or percentage lean in pigs fed ACC compared with those fed diets without ACC. Pigs fed High diets had greater *(P* < 0.001*)* hot carcass weight and marginally *(P* = 0.067*)* greater loin depth than pigs fed Low diets.

In the economic analysis, the addition of ACC to diets increased (*P* > 0.001) feed cost per pig but no evidence for differences *(P* > 0.05*)* was observed for feed cost per kg of gain, revenue, and IOFC between pigs fed diets with and without ACC. Pigs fed High diets had greater *(P* <

0.001*)* feed cost and feed cost per kg of gain, but also greater *(*$P < 0.001$*)* revenue per pig than pigs fed Low diets with no evidence for differences ($P > 0.05$) in IOFC.

Discussion

The algae-clay complex used in this study is based on Ulva sp and Solieria chordalis macroalgae and montmorillonite clay that has been shown to improve digestibility of energy and amino acids in swine and poultry (Suarez and Gallissot 2016). The exact mechanism by which ACC increases the utilization of nutrients when added to diets is not well defined. It's been reported that montmorillonite clay can absorb organic compounds on its surface or within its inter-laminar spaces inactivating toxic compounds such as lead and mycotoxins (Yu et al., 2008; Duan et al., 2013). However, in this study, improvement in ADG observed in late finishing cannot necessarily be attributed to the mycotoxins level in feed since all mycotoxins tested were in relatively low concentrations. It has been proposed that montmorillonite can increase growth hormone concentration (Yu et al., 2008), and improve intestinal health (Xia et al., 2005). Moreover, the Ulva sp and Solieria chordalis macroalgae present in the ACC belong to a group of seaweeds that have shown positive effects on final body weight and feed efficiency when supplemented to finishing pigs (Ruiz et al., 2018). It's been hypothesized that the greater ADG and feed efficiency may be associated with a positive effect on intestinal health and a better absorption of nutrients (Gardiner et al, 2008). However, dietary addition of seaweed feed additives has inconsistent results that may be associated with the different algae species utilized, stage of life of the pigs, and concentration of the bioactive compounds supplemented (Ruiz et al., 2018).

In the present study, the increased ADG of pigs fed the ACC diets seems to be driven by the improvement in G:F from day 56 to 90. Other studies have shown a similar response when

pigs were fed diets supplemented with montmorillonite clay (Yu et al., 2008; Duan et al., 2013). A possible explanation of this improvement in G:F may be related to the greater stability of digestive enzymes that bind to clay minerals increasing their activity and nutrient digestibility (Yan et al., 2010; Li and Kim 2013; Chen et al., 2005). However, the lack of response from d 0 to 28 and 28 to 56 contradicts the literature because in diets supplemented with clay, a greater response is usually observed in younger pigs rather than older pigs (Papaioannou et al., 2004; Alexopoulos et al.,2007; Prvulovic et al., 2007). The greater overall ADG of pigs fed ACC led to a 1.4 kg difference in final weight that was not reflected in the carcass weight due to numerically decreased carcass yield of the ACC pigs compared with control fed pigs (72.5 vs 73.2%). To our knowledge, no other studies have observed differences in carcass yield when various clays were added to finishing pig diets (Thacker 2003; Han and Thacker 2006; Yan et al., 2010).

The objective of using High and Low diets based on energy and amino acid concentrations was to be able to detect an increase in growth due to increased nutrient digestibility caused by added ACC. High diets were formulated to meet the specific genetic line nutrient requirements to maximize growth performance (Goncalves et al., 2017). The reduction in ADG and G:F for pigs fed Low diets compared with those fed High diets can be explained by the lower NE content and amino acid concentration of Low diets. So, a difference in growth performance was expected between pigs fed High and Low diets as well as anticipated interactions between ACC addition and dietary regimen. However, there were no overall interactions observed indicating the ACC affected both diet regimens equally.

Surprisingly, no difference was observed in percentage carcass yield between pigs fed High and Low diets. This is in contrast with other trials were pigs fed high fiber diets (30%

DDGS) had reduced yield due to an increase in the gastrointestinal tract weight compared with pigs fed a corn-soybean meal-based diet (Asmus et al., 2014; Coble et al., 2018).

As expected, High diets had greater feed cost and feed cost per kg gain. However, because they grew faster, the revenue was also higher and no differences in IOFC were observed indicating that different nutritional approaches can be equally profitable.

In conclusion, the addition of ACC resulted in an improvement in growth performance, especially in the later stages of the finishing period. However, because of numerical reductions in carcass yield, this difference was not reflected in hot carcass weight. The economic analysis showed a higher feed cost of the ACC diets with no differences in IOFC compared with pigs not fed ACC. Feeding High diets improved growth performance and increased feed cost with similar IOFC compared with pigs fed low-energy-low lysine diets.

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Table 3-1. Diet composition (as fed basis)[1]

	Dietary phase					
	1		2		3	
Treatment	High[2]	Low[3]	High	Low	High	Low
Ingredient, %						
Corn	68.13	59.52	74.91	65.38	77.79	67.55
Soybean meal	26.47	8.01	19.8	2.27	16.99	0.22
DDGS, 8% oil[4]	---	30.0	---	30.0	---	30.0
Beef tallow	3.00	---	3.00	---	3.00	---
Calcium phosphate	0.50	0.09	0.40	---	040	---
Limestone	0.97	1.23	0.98	1.23	0.88	1.10
Sodium chloride	0.35	0.35	0.35	0.35	0.35	0.35
L-Lysine-HCl	0.28	0.53	0.28	0.50	0.29	0.50
DL-Methionine	0.06	---	0.03	---	0.03	---
L-Threonine	0.09	0.09	0.09	0.07	0.11	0.08
L-Tryptophan	0.001	0.04	0.01	0.04	0.01	0.04
Phytase[5]	0.01	0.01	0.01	0.01	0.01	0.01
Vitamin and mineral	0.15	0.15	0.15	0.15	0.15	0.15
ACC[7]	+/-	+/-	+/-	+/-	+/-	+/-
Total	100	100	100	100	100	100
Calculated analysis						
Standard ileal digestible (SID) amino acids %						
Lysine, %	1.04	0.88	0.88	0.72	0.82	0.67
Isoleucine:lysine	64	60	62	60	61	60
Methionine:lysine	31	31	29	35	29	36
Methionine & cystine:lysine	56	60	56	66	56	69
Threonine:lysine	63	63	64	64	66	66
Tryptophan:lysine	18.5	18.5	18.5	18.5	18.5	18.5
Valine:lysine	70	74	70	78	70	79
Total lysine, %	1.17	1.04	0.99	0.86	0.93	0.81
Net energy, kcal/kg	2,665	2,511	2,687	2,530	2,700	2,539
SID Lysine:NE, g/Mcal	3.90	3.51	3.27	2.85	3.04	2.64
Crude protein, %	17.7	17.0	15.0	14.6	13.9	13.8
Calcium, %	0.53	0.53	0.50	0.50	0.45	0.45
STTD Phosphorus, %	0.33	0.33	0.30	0.30	0.29	0.29

[1] Phase 1, 2 and 3 were fed in meal form from d 0 to 28, 28 to 56 and 56 to 90 respectively.

[2] Diets formulated to maximize growth performance with 3% added fat and no DDGS.

[3]Diets were formulated to contain approximately 150 kcal per kg less net energy, 30% DDGS, no added fat, and were formulated 0.10% below the standardized ileal digestible lysine requirement based on the SID Lys:NE ratio as estimated in the High diets.

[4]DDGS = distillers dried grains with solubles.

[5]Optiphos 2000 (Huvepharma, Sofia, Bulgaria) provided an estimated release of 0.10% digestible P for phase 1, 2 and 3.
[6]Vitamin and trace mineral premix provided per kg of diet: 111 mg Zn, 111 mg Fe, 33 mg Mn, 17 mg Cu, 0.33 mg I, 0.30 mg Se, 2,400 IU vitamin A, 600 IU vitamin D, 12 IU vitamin E, 1.2 mg vitamin K, 22.5 mg niacin, 7.5 mg pantothenic acid, 2.25 mg riboflavin, and 10.5 μg vitamin B12.
[7]ACC (Olmix Group, Brehan, France) was added at 0.1% in phase 1 and 2 and 0.05% in phase 3.

3. Chemical analysis of experimental diets (as-fed basis), %[1]

	Phase 1				Phase 2				Phase 3			
	High[2]		Low[3]		High		Low		High		Low	
Item	No[4]	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
Dry matter	88.	88.	89.	89.	88.	87.	89.	89.	88.	88.	88.	88.
Total lysine	1.0	1.0	1.0	1.0	1.0	0.9	0.8	0.8	0.9	0.8	0.8	0.8
Calcium	0.8	0.7	0.5	0.7	0.5	0.6	0.5	0.4	0.5	0.6	0.4	0.5
Phosphorus	0.4	0.4	0.4	0.4	0.3	0.3	0.4	0.4	0.3	0.3	0.3	0.4

[1]Diet samples were collected at manufacturing and composite samples were submitted for proximate analysis to Ward Laboratories (Kearney, NE) for dry matter, calcium and phosphorus and to University of Missouri, Experimental Station for total lysine.

[2] Diets formulated to maximize growth performance with 3% added fat and no DDGS.

[3]Diets were formulated to contain approximately 150 kcal per kg less net energy, 30% DDGS, no added fat, and were formulated 0.10% below the standardized ileal digestible lysine requirement based on the SID Lys:NE ratio as estimated in the High diets

[4]Diets with no addition of ACC.

[5]ACC (Olmix Group, Brehan, France) was added to the ACC diets at 0.10% until 100 kg and 0.05% thereafter.

Table 3-2. Effects of diet formulation and ACC on growth and carcass characteristics of finishing pigs[1]

Item	High[2]		Low[3]		SEM	Probability, $P <$		
	No[4]	Yes[5]	No	Yes		ACC × Diet type	Diet type	ACC
Weight, kg								
d 0	49.3	49.3	49.6	49.6	0.88	0.815	0.093	0.938
d 28	76.5	76.3	74.2	74.2	1.03	0.234	<0.001	0.548
d 56	102.9	103.4	98.0	98.4	1.12	0.924	<0.001	0.481
d 90	134.5	135.9	126.8	128.1	1.19	0.907	<0.001	0.070
D 0 to 28								
ADG, kg	0.97	0.96	0.88	0.90	0.011	0.167	<0.001	0.565
ADFI, kg	2.11	2.08	2.06	2.12	0.027	0.014	0.848	0.486
G:F g/kg	459	462	427	424	4.152	0.241	<0.001	0.958
D 28 to 56								
ADG, kg	0.94	0.96	0.84	0.84	0.016	0.456	<0.001	0.596
ADFI, kg	2.48	2.50	2.50	2.54	0.029	0.923	0.317	0.303
G:F g/kg	379	384	336	330	4.980	0.320	<0.001	0.900
D 56 to 90								
ADG, kg	0.95	0.99	0.89	0.92	0.012	0.895	<0.001	0.001
ADFI, kg	2.99	3.04	3.10	3.12	0.029	0.555	0.001	0.246
G:F g/kg	319	325	286	296	4.080	0.520	<0.001	0.019
D 0 to 90								
ADG, kg	0.95	0.97	0.87	0.89	0.007	0.911	<0.001	0.027
ADFI, kg	2.55	2.56	2.57	2.61	0.021	0.559	0.047	0.228
G:F g/kg	375	379	338	340	3.123	0.643	<0.001	0.180
Carcass data								
HCW, kg	98.3	98.6	92.9	92.8	0.922	0.755	<0.001	0.838
Yield, %	73.1	72.6	73.3	72.5	0.41	0.715	0.908	0.146
Backfat, mm[6]	17.2	16.7	16.9	17.2	0.319	0.220	0.743	0.896
Loin depth, mm[6]	72.2	72.0	71.1	71.0	0.536	0.886	0.067	0.698

Lean, %[6]	57.1	57.3	57.1	56.9	0.23	0.319	0.424	0.990
Economics, $ per pig[7]								
Feed cost	45.10	46.08	37.31	38.57	0.647	0.668	<0.001	0.001
Feed cost per kg gain[8]	0.53	0.53	0.48	0.48	0.004	0.625	<0.001	0.261
Revenue[9]	94.5	95.1	86.0	85.9	1.04	0.713	<0.001	0.841
IOFC[10]	49.4	49.0	48.6	47.2	0.95	0.570	0.177	0.321

[1]A total of 1,188 pigs (initial weight = 50.6 kg) were used in a 90-d study with 27 pigs per pen and 11 replicates per treatment.
[2]Diets formulated to maximize growth performance with 3% added fat and no DDGS.
[3] Diets were formulated to contain approximately 150 kcal per kg less net energy, 30% DDGS, no added fat, and were formulated 0.10% below the standardized ileal digestible lysine requirement based on the SID Lys:NE ratio as estimated in the High diets.
[4]Diets with no addition of ACC.
[5]ACC (Olmix Group, Brehan, France) was added at 0.10% until 100 kg and 0.05% thereafter.
[6]Adjusted for hot carcass weight (HCW).
[7]Corn was valued at ($127/tonne), soybean meal at $319/tonne, DDGS at $143/tonne, and L-lysine at $1.52/kg, DL-methionine at $2.65/kg, L-threonine at $0.1.96/kg, L-tryptophan at $8.60/kg and ACC at $4.12/kg.
[8]Feed cost per kg gain = feed cost per pig ÷ overall gain per pig.
[9]Revenue = (HCW × $1.54) – (d 0 BW × 0.75 × $1.54).
[10]Income over feed cost = revenue – feed cost.

Table 3-3. Main effects of diet formulation and ACC on growth and carcass characteristics of finishing pigs[1]

Item[2]	High[3]	Low[4]	SEM	*P*-Value	No[5]	Yes[6]	SEM	*P*-Value
Weight, kg								
d0	49.3	49.6	0.875	0.093	49.5	49.5	0.874	0.938
d13	62.0	60.7	0.956	<0.001	61.5	61.3	0.956	0.442
d28	76.4	74.5	1.000	<0.001	75.4	75.6	1.000	0.548
d42	89.0	85.0	0.999	<0.001	86.7	87.4	0.999	0.202
d56	103.1	98.2	1.033	<0.001	100.5	100.9	1.033	0.481
d75	122.2	115.8	1.020	<0.001	118.4	119.6	1.020	0.063
d90	135.2	127.5	1.077	<0.001	130.6	132.0	1.077	0.070
D 0 to 28								
ADG, kg	0.96	0.89	0.009	<0.001	0.93	0.93	0.009	0.565
ADFI, kg	2.09	2.09	0.024	0.848	2.08	2.10	0.024	0.486
G:F g/kg	461	425	3.634	<0.001	443	443	3.634	0.958
D 28 to 56								
ADG, kg	0.95	0.84	0.011	<0.001	0.89	0.90	0.012	0.598
ADFI, kg	2.49	2.52	0.020	0.317	2.49	2.52	0.021	0.303
G:F g/kg	382	333	3.678	<0.001	358	357	3.678	0.900
D 56 to 90								
ADG, kg	0.96	0.91	0.009	<0.001	0.92	0.96	0.008	0.001
ADFI, kg	3.01	3.12	0.022	0.001	3.04	3.08	0.02	0.246
G:F g/kg	322	291	3.370	<0.001	303	311	3.370	0.019
D 0 to 90								
ADG, kg	0.96	0.88	0.005	<0.001	0.91	0.93	0.006	0.027
ADFI, kg	2.55	2.59	0.016	0.047	2.56	2.59	0.016	0.228
G:F g/kg	377	339	2.612	<0.001	3.57	360	2.612	0.180
Carcass data								
Backfat, mm	16.9	17.1	0.225	0.743	17.1	16.9	0.227	0.896
Loin depth, mm	72.1	71.1	0.377	0.067	71.7	71.5	0.383	0.698
Lean, %	57.2	56.8	0.177	0.116	57.1	57.0	0.173	0.908
HCW, kg	98.4	92.9	0.781	<0.001	95.6	95.7	0.781	0.838
Yield, %	72.8	72.9	0.292	0.908	73.2	72.5	0.292	0.143
Economics, $ per pig[7]								
Feed cost	45.6	38.0	0.262	<0.001	41.2	42.4	0.262	0.002
Feed cost per kg gain[8]	0.53	0.48	0.004	<0.001	0.50	0.51	0.003	0.261
Revenue[9]	94.8	85.9	0.762	<0.001	90.3	90.5	0.762	0.841
IOFC[10]	49.2	48.0	0.705	0.174	49.0	48.1	0.705	0.321

[1]A total of 1,188 pigs (initial weight = 50.6 kg) were used in a 90-d study with 27 pigs per pen and 11 replicates per treatment.
[2]ADG = average daily gain. ADFI = average daily feed intake. G:F = gain-to-feed ratio. HCW = hot carcass weight.
[3] Diets formulated to maximize growth performance with 3% added fat and no DDGS.
[4]Diets were formulated to contain approximately 150 kcal per kg less net energy, 30% DDGS, no added fat, and were formulated 0.10% below the standardized ileal digestible lysine requirement based on the SID Lys:NE ratio as estimated in the High diets.
[5]Diets with no addition of ACC.
[6]ACC (Olmix Group, Brehan, France) was added at 0.10% of inclusion until 110 kg and 0.05% thereafter.
[7]Corn was valued at $127/tonne, soybean meal at $319/tonne, DDGS at $143/tonne, and L-lysine HCL at $1.52/kg, DL-methionine at $2.65/kg, L-threonine at $0.1.96/kg, L-tryptophan at $8.60/kg and ACC at $4.12/kg.
[8]Feed cost per kg gain = feed cost per pig ÷ overall gain per pig.
[9]Revenue = (HCW × $1.54) – (d 0 BW × 0.75 × $1.54).
[10]Income over feed cost = revenue – feed cost.