ISOQUINOLINE ALKALOIDS IN DIETS FOR YOUNG GROWING PIGS IMPROVE NUTRIENT DIGESTIBILITY AND GUT HEALTH

BY

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THESIS

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ABSTRACT

Three experiments were conducted to evaluate the effects of isoquinoline alkaloids (IQ) in corn-soybean meal (SBM) diets fed to young growing pigs on growth performance, gut health, energy and nutrient digestibility, and digestible energy (DE) and metabolizable energy (ME) concentrations in the diets. In the first experiment, 32 ileal cannulated barrows (12.19 ± 1.38 kg) were allotted to 4 diets with 8 replicate pigs per diet to determine effects of dietary inclusion of IQ on apparent ileal digestibility (AID) of amino acids (AA), crude protein (CP), starch, and acid hydrolyzed ether extract (AEE). The corn-soybean meal basal diet was supplemented with 0, 90, 180, or 360 mg IQ/kg complete feed. Diets were fed for 27 d and ileal digesta were collected on d 13 and 14 (period 1) and d 26 and 27 (period 2). Results indicated that dietary inclusion of IQ resulted in a quadratic increase ($P < 0.05$) in the AID of starch, Thr, Trp, Val, Pro, and Tyr in period 1. Additionally, the AID of starch was greater ($P < 0.05$) in period 1 than in period 2. The AID of CP, Arg, His, Ile, Leu, Met, Phe, Thr, Trp, Val, Pro, and Tyr in period 2 was greater ($P < 0.05$) than in period 1. There were no differences among treatments or periods observed for AID of AEE. The second experiment tested the hypothesis that including IQ in diets fed to young growing pigs increases the apparent total tract digestibility (ATTD) of gross energy (GE) and concentrations of DE and ME of the diets. Twenty-four gilts and 24 barrows (13.67 ± 1.35 kg) were allotted to 8 diets and 6 replicate pigs per diet. A basal diet consisting of corn and SBM and a second basal diet consisting of corn, SBM, and distiller's dried grains with solubles (DDGS) were prepared. Six additional diets were prepared by adding 0, 90, 180, or 360 mg/kg IQ to each of the 2 basal diets. Pigs were housed in individual metabolism crates for the 12 d experimental period, which included a 5 d adaptation period and a collection period from d 6 to 11. Dietary inclusion of IQ in corn-SBM and corn-SBM-DDGS diets had no effect on overall energy

digestibility. Addition of IQ to corn-SBM-DDGS diets quadratically increased ($P < 0.05$) average daily feed intake (ADFI), dry feces output, and fecal GE output of pigs with the greatest values observed in the 90 and 180 mg/kg diets. Additionally, IQ linearly decreased ($P < 0.05$) the ME of corn-SBM diets, with the highest ME value in the 90 mg/kg diet. The third experiment tested the hypothesis that dietary inclusion of IQ improves growth performance and gut health of weanling pigs. A total of 160 pigs (6.33 ± 0.61 kg) were allotted to 4 corn-SBM based diets with 4 pigs per pen and 10 replicate pens per treatment. A 3-phase feeding program was used with d 0 to 8 as phase 1, d 8 to 21 as phase 2, and d 21 to 34 as phase 3. Within each phase, the 4 diets were supplemented with 0, 90, 180, or 360 mg IQ/kg complete diet. There were no effects of IQ on overall growth performance of weanling pigs; however, ADFI quadratically ($P < 0.05$) decreased in phase 1 and linearly decreased ($P < 0.05$) in phase 2, G:F quadratically increased in phase 3 ($P < 0.05$), and ADG decreased (quadratic; $P < 0.05$) during phase 1. Plasma urea nitrogen tended to increase in phases 2 and 3 (linear; $P < 0.10$) if IQ was added to the diet and total plasma protein quadratically increased ($P < 0.05$) in phase 1. In the ileum, crypt depth and lamina propia thickness decreased (quadratic; $P < 0.05$) and there was a tendency for the villus height: crypt depth ratio to increase (linear; $P < 0.10$). Neutrophil infiltration tended to increase in the jejunum and decrease in the ileum (quadratic; $P < 0.10$) with the greatest response in the tissues of pigs fed the diet with 180 mg IQ/kg diet. Results indicate that dietary inclusion of IQ improves gastrointestinal health resulting in an increase in both AA absorption and apparent ileal digestibility of AA and starch, with the greatest response observed at inclusions between 90 and 180 mg IQ/kg complete diet.

Key words: apparent ileal digestibility, energy concentration, growth performance, gut health, isoquinoline alkaloids, pigs

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CHAPTER 1: INTRODUCTION

Post-weaning stress often results in poorer health and reduced growth of the young piglet (Pluske et al., 1997). As a result, antibiotic growth promoters have been provided at sub-therapeutic levels to minimize symptoms of post-weaning stress and maximize growth potential (van den Bogaard and Stobberingh, 1999). Restrictions in both the European Union and the United States have been introduced to limit the use of sub-therapeutic doses of antibiotics in livestock animals due to concerns of antibiotic resistance. Hence, natural alternatives for growth promotion are being investigated including organic acids, prebiotics, probiotics, and plant extracts.

Isoquinoline alkaloids (IQ) comprise a phytobiotic feed additive derived from *Macleaya cordata*, commonly referred to as the plume poppy. Specifically, these alkaloids include sanguinarine, chelerythrine, protopine, and allocryptopine; each with significant physiological effects including antimicrobial activity (Walker, 1990a; Kosina et al., 2010), immuno-modulation (Agarwal et al., 1991; Chaturvedi et al., 1997b; Soler et al., 2016), and improvement of intestinal barrier function (Robbins et al., 2013). These physiological effects reduce inflammation, improve intestinal health, and consequently improve growth performance of production animals (Kosina et al., 2010).

However, existing data regarding the influence of IQ on the digestibility of nutrients by young growing pigs as well as growth performance of weanling pigs is limited. Therefore, the objectives of this work were:

1. To determine the impact of IQ on the apparent ileal digestibility of crude protein, amino acids, starch, and acid hydrolyzed ether extract of corn-soybean meal diets fed to young growing pigs.

2. To determine the apparent total tract digestibility of energy and concentrations of digestible and metabolizable energy of diets consisting of corn and soybean meal or corn, soybean meal, and distiller's dried grains with solubles supplemented with IQ fed to young growing pigs.

3. To examine effects of IQ on intestinal health, blood characteristics, and growth performance of weanling pigs fed corn-soybean meal diets.

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CHAPTER 2: REVIEW OF THE LITERATURE - ALKALOIDS

INTRODUCTION

Antimicrobial growth promoters (AGP) are defined as antibiotics used as growth promoters in livestock feed to enhance feed efficiency and weight gain. The main mode of action of antibiotics used in this way is to regulate the intestinal microbiota to prevent pathogenic bacteria from colonizing. In turn, AGPs increase growth performance by providing an ideal intestinal microbiota to maximize nutrient absorption and are most effective in young animals at sub-therapeutic levels (van den Bogaard and Stobberingh, 1999). Weaning exposes young pigs to a variety of environmental and psychological stressors that result in changes to gut physiology and immunology that ultimately lead to diarrhea and other disorders (Pluske et al., 1997; Williams, 2003; Lalles et al., 2004). Reducing the effects of these changes is crucial to maximizing growth performance in weanling pigs.

Since January 1, 2006, there has been a ban on AGP in animal feed in the European Union. The United States Food and Drug Administration has moved all human medically important feed-grade antibiotics to the Veterinary Feed Directive drug process as of January 1, 2017. This means that the drugs on this list are critical for maintaining human health and will only be allowed in animal feed under the supervision of a licensed veterinarian (FDA, 2013). As a result, the demand for antibiotic alternatives has led to expanded research on possible substitutes in feed for production animals to increase growth performance and maintain herd health.

Commonly used alternatives to AGP include acidifiers, enzymes, essential oils, minerals, immune modulators, prebiotics, probiotics, and plant extracts (Thacker, 2013; Gleeson and Collins, 2015). Other, lesser researched, alternatives include competitive exclusion cultures,

bacteriophage therapy, and immunopotentiators such as egg yolk antibodies (Thacker, 2013). Changes in farming practices including enhanced biosecurity and disinfection, improved air quality, lower stocking rates, temperature regulation, and controlled production flow are also considered (Kil and Stein, 2010; Gleeson and Collins, 2015). Of these alternatives, the use of plant extracts, specifically alkaloids, to increase digestibility and promote gut health will be the focus of this review.

Secondary metabolites, active components of plant extracts, are compounds not directly involved with an organism's growth, development, or reproduction, and are often only produced by certain groups of plants (Briskin, 2000; Greathead, 2003). Metabolites may protect the plant against many environmental stressors including pathogens, herbivores, competition, dehydration, sun damage, and temperature changes (Kaufman, 1999; Briskin, 2000). It was hypothesized that plant extracts act much in the same way as AGP, limiting the growth of bacteria in the intestine and preventing pathogens from propagating (Baydar et al., 2004). In a study using Ross male broilers, both AGP and plant extracts increased total tract and ileal digestibility of nutrients (Hernandez et al., 2004). In recent studies, it was observed that plant extracts improved the immune response of weanling pigs, reduced the negative effects of porcine reproductive and respiratory syndrome virus, and limited the frequency of diarrhea in weanling pigs exposed to an *E. coli* challenge (Liu et al., 2013a; 2013b).

ALKALOIDS

Alkaloids are a group of naturally occurring chemicals containing basic N atoms present in plants, animals, bacteria and fungi. They occur in about 20% of all plant families and can be

found in all parts of the plant (Cushnie et al., 2014). Alkaloids include many drugs and poisons and have a variety of physiological functions including acting as an anticholinergic, analgesic, antiprotozoal, antihypertensive, stimulant, depressant, vasodilator, and antiarrhythmic (Babbar, 2015). There are 2 broad categories of alkaloids: heterocyclic alkaloids, which are mainly characterized by their ring structure, and non-heterocyclic alkaloids, which are characterized by their biochemical origin (Evans, 2009). However, there are many ways to classify alkaloids including functionally, taxonomically, biochemically, and chemically (Aniszewski, 2015). One of the most accepted ways is to organize alkaloids by their carbon skeleton, which results in the following groups: true alkaloids, protoalkaloids, polyamine alkaloids, peptide and cyclopeptide alkaloids, and pseudoalkaloids (Aniszewski, 2015).

True alkaloids, the largest group of alkaloids, are defined as alkaloids with N heterocycles derived from amino acids (**AA**; Hegnauer, 1988). These alkaloids can be classified by determining N supplying AA and can be classified in the following categories: phenylalanine-tyrosine family, tryptophan family, ornithine and arginine derived family, lysine family, anthranilic acid family, histidine family, nicotinic acid derived family, and mixed AA origin family (Hegnauer, 1988). Members of the true alkaloid class include pyrrolidines, tropanes, pyrrolizidines, piperidines, quinolizidines, indolizidines, pyridines, isoquinolines, oxazoles, isoxazoles, thiazoles, acridines, quinazolines, indoles, imidazoles, and purines (Aniszewski, 2015).

The phenylalanine-tyrosine family includes isoquinolines, oxazoles, and thiazoles. One proposed mechanism of action for the isoquinoline class, which contains alkaloids such as sanguinarine and chelerythrine, is that they disturb the Z-ring and inhibit cell division of bacteria.

These alkaloids bind to FtsZ, inhibit GTPase activity, inhibit Z-ring formation, and elongate the cell without damaging the DNA or membrane structure of the cell (Beuria et al., 2005). Isoquinoline alkaloids may also inhibit type I topoisomerases by inhibiting nucleic acid synthesis (Casu et al., 2011) or inhibition and relocation of cyclin D and type II topoisomerases, thereby disrupting DNA synthesis (Holy et al., 2006). The group of quaternary benzophenanthridine alkaloids is characterized by a full aromatic C ring. These alkaloids require a methylenedioxy functional group at C-2 and C-3 for activity (Parhi et al., 2012). The sanguinarine ring may be reduced or oxidized to form dihydrosanguinarine through sodium borohydride reduction or oxysanguinarine through potassium ferricyanide oxidation.

Oxazoles and thiazoles are 5 ring heterocycles formed from a 2-electron oxidation system which can contain cysteine, serine, and threonine (Roy et al., 1999). Oxazoles contain an N and O separated by one C on the azole ring. Many anti-tumor, antibacterial, and anti-malarial properties have been documented in this group of alkaloids. Thiazoles, found in bacitracin, and oxazoles can undergo redox reactions (Roy et al., 1999).

The tryptophan family of alkaloids consists of simple indoles, terpenoid indoles, pyrroloindoles, and ergots. Simple indoles are commonly found in many invertebrate marine species (Gul and Hamann, 2005). These indole derivatives have many physiological effects including antimicrobial, antiviral, antiparasitic, anti-inflammatory, and antiserotonin properties (Gul and Hamann, 2005). Ergot alkaloids are characterized as chemicals containing a tetracyclic ergolene or ergoline- ring (Berde and Sturmer, 1978), are derivatives of lysergic acid (van Dongen and de Groot, 1995) and are commonly known as the cause of ergotism. Ergot poisoning, caused by the *Claviceps purpurea* fungus that infects rye and other cereals, results in

symptoms including convulsion, seizures, diarrhea, mania or psychosis, headaches, nausea and vomiting, and gangrene as a result of vasoconstriction (van Dongen and de Groot, 1995). In recent history, ergot alkaloids have been used therapeutically to treat migraines and Parkinson's disease because of the vasoconstrictive properties of these compounds (Berde and Sturmer, 1978).

Pyrroloindole alkaloids are alkaloids in which the C-2 and C-3 of the indole ring are nucleophilic and contain reactive side chains. Physostigmine, one pyrroloindole alkaloid, acts as a reversible inhibitor of cholinesterase and is used as a parasympathomimetic drug to treat neurological disorders (Liu et al., 2013a). Simple β- carbolines are characterized as products of tryptophan, indole alkylamine, and aldehyde condensation and can be found in human plasma and urine, in many plants that are used as hallucinogens, and in tobacco smoke (Torreilles et al., 1985). Terpenoid indole alkaloids are derived from tryptophan and the terpene secloganin (O'Connor and Maresh, 2006). There are more than 3,000 terpenoid indole alkaloids (Geerlings et al., 2000). These terpenoid indoles are important due to their antitumor properties and are most commonly used in anticancer drugs (Suttipanta et al., 2011), but can also be used to improve cerebral circulation (Verpoorte et al., 1997) and as an antimalarial or anti-arrhythmic agent (O'Connor and Maresh, 2006). The different classes of terpenoid indoles include andajmalan, aspidosperma, bisindole, corynanthe, iboga, sarpagan, strychnos groups (O'Connor and Maresh, 2006). All indoles originate from strictosidine, and can be arranged by their central intermediates (O'Connor and Maresh, 2006). Sarpagan and ajmalan type alkaloids are likely a result of the cytochrome P450 enzyme interacting with deglycosylated strictosidine (O'Connor and Maresh, 2006). Corynanthe type indoles are a result of the reduction of an intermediate, cathenamine (O'Connor and Maresh, 2006). Aspidosperma, strychnos, and iboga alkaloids are thought to be

derivatives of the corynanthe alkaloids, whereas bisindole type alkaloids are formed from oxidized catharanthine, an iboga indole (O'Connor and Maresh, 2006).

The lysine family of alkaloids includes piperidines, quinolizidines, and indolizidines. The members of the lysine family are derived from either one or 2 mols of cadaverine (Hegnauer, 1988). Indolizidine alkaloids are found in fungi and plant sources and have a fused 5- and 6-membered carbon ring. Piperidine alkaloids are derived from lysine in combination with acetate/malonate, with cadaverine acting as an asymmetrical intermediate (Seigler, 1998). Quinolizidine is formed as a result of lysine decarboxylase action (Bunsupa et al., 2012).

Anthranilic acid is an intermediate in the biosynthesis of tryptophan and members of this family include quinazolines, quinolones, pyrroloquinazolines, furoquinolines and acridones. Most of the alkaloids derived from anthranilic acid are from Rustaceae, the citrus family (Seigler, 1998). Quinazolines and pyrroloquinazolines have been identified in several plants, animals, and microorganisms, and about 60 of these alkaloids have been isolated (Groger, 1980). Quinolones are defined as an addition of acetate or malonate to the carboxyl group of anthranilic acid followed by cyclization; whereas, prenylation of quinolones leads to furoquinoline and pyranoquinoline alkaloids (Seigler, 1998). Furoquinoline alkaloids are produced from first forming a quinoline ring system followed by introduction of side chains.

Imidazoles are derived directly from L-histidine and make up one of the smallest groups of alkaloids, and include histamine, pilocarpine, and pilosine. Histamine may be present in fungi, marine, and plant species and is formed by decarboxylation of L-histidine by histidine decarboxylase (Santos and Moreno, 2013). Histamines function to stimulate contraction of smooth muscles, increase the permeability of the vascular system, stimulate gastric acid

secretion, and may alter neurotransmission, and therefore, may be used for treatment of allergic diseases and peptic ulcers (Ohtsu and Watanabe, 2003).Other functions of histamine include roles in anaphylaxis, neutrophil recruitment, airway eosinophilia, bacterial peritonitis, and angiogenesis (Ohtsu and Watanabe, 2003). The majority of pilocarpus alkaloids, including pilocarpine and pilosine, have an imidazole and a γ-lactone ring (Santos and Moreno, 2013). Pilocarpine is mainly used as a parasympathomimetic treatment or ophthalamic-cholinergic drug for health conditions, and has been used in treating glaucoma, mouth ulcers, and xerostomia by stimulating the bladder, tear ducts, and sudoriferous and salivary glands (Santos and Moreno, 2013). Pilocarpine acts as a muscarinic-cholinergic agonist with slight β- adrenergic activity (Fox et al., 1991). Pilosine is an isomer of pilocarpine, with an α-hydroxylbenzyl group replacing the ethyl at C-2 in the lactone ring (Santos and Moreno, 2013).

Alkaloids of mixed AA origin include purines and isoxazoles. Examples of purines include caffeine and theobromine, whereas examples of isoxazoles include ibotenic acid and muscimol. Caffeine and theobromine are present in *Coffea arabica* leaves with higher concentration in the buds and young leaves compared with fully developed leaves (Zheng and Ashihara, 2004). Caffeine in coffee and tea is synthesized through a number of steps including 3 methylation steps catalyzed by S-adenosyl-L-methionine – dependent N-methyltranferases and a cleavage step catalyzed by 7-methylxanthosine nucleosidase (Ashihara and Crozier, 2001). Theobromine, commonly found in the cacao plant and tea leaves, is also an intermediate in the synthesis of caffeine (Ashihara and Crozier, 2001). Caffeine and theobromine primarily function as vasodilators, diuretics, and stimulants. One type of isoxazole, R-11, improves the immune response in immunocompromised mice by increasing the number of leukocytes and splenocytes in the blood stream, and increases the rate of production of interleukin-6 in blood stimulated with

lipopolysaccharide (LPS; Zimecki et al., 2012). Another isoxazole, RM-11, increases T cell concentration in the spleen of cyclophosphamide-treated mice while simultaneously decreasing the expression of B cells (Zimecki et al., 2008). Muscimol interacts through γ- aminobutyric acid (GABA) receptors, and is lethal to mice at certain doses. Ibotenic acid, also known as ibotenate, is a psychoactive drug or neurotoxin that is present in *Amanita muscaria*, is an analogue of glutamate, can act as a glutamate receptor agonist, and can create lesions in the brain (Newsome et al., 1985).

The nicotinic acid family includes pyridines. Examples of pyridines include nicotine, actinidine, evonine, and trigonelline. Trigonelline was first located in *Trigonella foenumgraecum*, but has since been discovered in many plant and animal species including various coffee species (Zheng and Ashihara, 2004). Trigonelline is synthesized in all parts of coffee seedlings (Zheng and Ashihara, 2004), and has been used as an antidiabetic drug by using the seed powder form in cooking (Khosla et al., 1995). Nicotine is produced in the roots of tobacco and transported to the leaves, where it is commonly used for production of various tobacco products. Nicotine is highly toxic to herbivores due to its interaction with acetylcholine receptors in the nervous system and has been used as an insecticide (Steppuhn et al., 2004). Evonine is a highly oxygenated sesquiterpene with acetic acid and evoninic acid playing a role in its esterification (Shizuri et al., 1973). Evonine alkaloids have been used as insecticides and cancer treatments (Duan et al., 2000).

Members of the ornithine and arginine derived family, pyrrolidines, pyrrolizidines, and tropanes, originate from AA in the L- configuration (Aniszewski, 2015). Pyrrolidine alkaloids are derived from ornithine or arginine with acetate/malonate included (Seigler, 1998). The R-

enantiomers of pyrrolidine alkaloids have a strong binding affinity to DNA and also are hepatoxic in rats and humans (O'Hagan, 2000). Many pyrrolidines act by inhibiting β-glucosidase, β-galactosidase, and β-mannosidase enzymes, which causes their toxic effect. Pyrrolidines are found in bluebells, tubers, himekouzo trees, and ant venom, all of which are toxic to those that consume them (O'Hagan, 2000). Tropane alkaloids have been extracted from roots, bark and leaves of medicinal plants. The tropane alkaloids are formed from a 5-membered ring with a three-carbon section derived from acetate or malonate (Seigler, 1998). One anatoxin, a bacterial toxin, found in blue-green alga has been observed to depolarize acetylcholine receptors and is known as 'very fast death factor' as it is extremely toxic (O'Hagan, 2000). Whereas some alkaloids have demonstrated beneficial antibacterial and antivirulent properties, many are also classified as toxic to mammals and some are considered carcinogenic (Stegelmeier et al., 1999). Pyrrolizidines exhibit toxic effects. Plants containing pyrrolizidines are present in open ranges and fields and in prepared feeds and grains, which are avoided by wild animals and livestock alike (Stegelmeier et al., 1999). Pyrrolizidine alkaloid plants may be consumed by humans through contaminated milk and animal products and are known to be hepatoxic, tumorigenic, and carcinogenic (Schoental, 1968). Some of the hepatoxic pyrolizidines are allylic esters of amino alcohols with branched chain acids, whereas the most toxic pyrrolizidines are cyclic diesters of retronecine containing adipic and glutaric acids (Schoental, 1968). One group of pyrrolizidines is the senecio group, which contains the chemical senecionine. The double bond in senecionine is crucial for its hepatoxic action as platyphylline has no effects on the liver and differs from senecionine only in the absence of the double bond (Schoental and Head, 1957).

MECHANISMS OF ACTION: ISOQUINOLINE ALKALOIDS

The main isoquinoline alkaloids (IQ) derived from the plant species *Macleaya cordata*, or plume poppy, include sanguinarine, chelerythrine, protopine, and allocryptopine in addition to small amounts of various other alkaloids (Kosina et al., 2010). These alkaloids have a variety of pharmacological activities including immunomodulatory and anti-inflammatory effects. When supplemented to diets fed to model animals, all animals maintained health with high levels of sanguinarine in almost all tissues in a study conducted in the Czech Republic (Kosina et al., 2003). Likewise, no significant changes in rat health except in rats fed diets containing 14,000 mg/kg of isoquinoline alkaloids where reduced glutathione levels and superoxide dismutase activity in the liver were observed (Zdarilova et al., 2008). Thus, it appears that unless extremely high inclusion rates are used, isoquinoline alkaloids are safe to include in diets for animals.

An immunomodulator is a chemical agent that modifies the immune response or the functioning of the immune system. This can be done by either influencing antibody formation or inhibiting white blood cell activity. Sanguinarine may inhibit nuclear factor kappa-light-chain-enhancer of activated B cell (NF-kB) activation (Chaturvedi et al., 1997). NF-kB is a cytoplasmic nuclear transcription factor and regulates the expression of cytokines and their receptors, histocompatibility complex genes, cell adhesion proteins, and genes involved in inflammation (Chaturvedi et al., 1997). This inhibition of NF-kB leads to a lower inflammatory response. Results of an in vitro study focusing on *Macleaya cordata* extract effects on adherent enterotoxigenic *E. coli* and non-adherent *E. coli* demonstrated that incubation with the *Macleaya cordata* extract and acetylsalicylic acid significantly reduced the inflammatory response, specifically reducing IL-1β and TNF-α (Soler et al., 2016). *Macleaya cordata* extract also quadratically increased the expression of IL-1β and decreased the iNOS gene expression

(Khadem et al., 2014). Heme oxygenase-1, a cytoprotective enzyme, was significantly increased in response to sanguinarine in human hepatocytes (Vrba et al., 2012). NADPH oxidase, a membrane-bound enzyme used in neutrophils to absorb microrganisms, was inhibited by direct sanguinarine activity, which prevents the configuration of the NADPH oxidase protein complex (Vrba et al., 2004). Sanguinarine and chelerythrine may also irreversibly inhibit amino acid decarboxylase (Drsata et al., 1996) and induce cell apoptosis (Malikova et al., 2006).

Chelerythrine has inhibitory effects on protein kinase C. It mainly acts as a competitive inhibitor to the phosphate receptor and a non-competitive inhibitor of ATP (Herbert et al., 1990). Protein kinase C is a signaling protein that is responsible for tight junction regulation in the intestine and that plays a role in the Toll-like receptor 2 pathway, which functions to identify microbes and modulate immune responses (Cario et al., 2004; Farhadi et al., 2006).

Protopine and allocryptopine reduce inflammation and blood clotting (Teng et al., 1991), and contributes to the role of *Macleaya cordata* as an antimicrobial agent (Kosina et al., 2010). Protopine also inhibits K(ATP) channels (Jiang et al., 2004), whereas allocryptopine reduces K current in rabbit myocardium (Fu et al., 2016). Combined, these four IQ have the potential to improve the health of the gastrointestinal system and increase growth when supplemented to diets for livestock.

GASTROINTESTINAL HEALTH

Weaning is associated with inflammation in the small intestine and results in a decrease in nutrient absorption and digestive capacity (Pluske et al., 1997; Pickler et al., 2013). Whereas the villi function to absorb nutrients, the crypt is the location of cell differentiation and secretory

function (Kiela and Ghishan, 2016). The villus height to crypt depth ratio is a representation of the balance of cell production in the intestine (Pluske et al., 1997). During the weaning period, villi are blunted and crypts are elongated, which is the cause of reduced nutrient absorption (Pluske et al., 1997). Consequently, dietary strategies during the nursery phase primarily focus on mitigating the symptoms of post weaning diarrhea and promoting gastrointestinal health.

Supplementing diets for broilers with 20 mg/kg IQ resulted in a decrease in villus height in the duodenum and in the mass of the small intestine (Jankowski et al., 2009). Cobb broiler chickens challenged with *Salmonella enteritidis* fed a control diet supplemented with 100 g/1,000 L sanguinarine in distilled water had reduced villi height in the duodenum, jejunum, ileum, and cecum and greater crypt depth in the jejunum and ileum compared with broilers fed only the control diet (Pickler et al., 2013). Ileal samples from pigs fed 1.5 g/kg IQ supplemented diets had a greater transepithelial resistance, indicating better health of the mucosal barrier compared with ileal tissue of pigs fed the control diet (Robbins et al., 2013). In contrast, no differences were observed in villus height or crypt depth in broilers fed 50 mg/kg IQ from d 1 to 21 and 25 mg/kg IQ from d 22 to 42 compared with broilers fed the control diet (Vieira et al., 2008b). Similarly, inclusion of 60 and 120 mg/kg IQ in nursery and grower diets fed to pigs did not result in differences in villus height, crypt depth, or villus height: crypt depth ratio (Goodarzi Boroojeni et al., 2018).

DIGESTIBILITY

Digestibility is defined as the percentage of a nutrient that is absorbed in the gastrointestinal tract of an animal, and therefore, indicates the availability of a nutrient for the animal to use for growth and maintenance. Total tract digestibility experiments are conducted by

analyzing nutrient concentration in the diet and in fecal output, whereas ileal or duodenal digestibility experiments involve collecting samples from the ileum or duodenum and subtracting the output from the dietary intake (Adeola, 2001; Stein, 2017). Nutrient digestibility can be expressed as apparent, standardized, or true digestibility depending on how endogenous losses are accounted for (Stein et al., 2007). Nutrient digestibility studies focusing on IQ in livestock diets are sparse and primarily focus on total tract digestibility or ileal digestibility of nutrients.

In an experiment examining effects of IQ supplementation to high-energy diets fed to steers, it was observed that the digestible energy (DE) of the diets tended to increase as dietary IQ increased (Aguilar-Hernandez et al., 2016). Likewise, supplementing diets fed to finishing ewes with 0.50 g/d IQ increased the dietary net energy (Estrada-Angulo et al., 2016). However, at this point, there is no information about effects of IQ on DE or net energy in diets fed to pigs.

Isoquinoline alkaloid supplementation had no effect on ruminal digestion of organic matter, neutral detergent fiber, or starch (Aguilar-Hernandez et al., 2016), and there was no influence of IQ supplementation of diets fed to pigs during the nursery and grower phases on ileal digestibility of ether extract (Goodarzi Boroojeni et al., 2018). Isoquinoline alkaloids may increase ruminal efficiency of steers as observed by the increase in flow rate of non-ammonia N and decrease in flow of ammonia N (Aguilar-Hernandez et al., 2016). Postruminal total tract digestion of N increased linearly with the level of IQ in the diets (Aguilar-Hernandez et al., 2016). In contrast, there was no effect of IQ on protein efficiency ratio or apparent protein utilization when added to diets fed to red tilapia (Rawling et al., 2009).

Ileal digestibility of Asp, GLu, His, Leu, Met, Val, and total AA was greater in a diet supplemented with 120 mg/kg IQ than in a diet without IQ when fed to pigs, and there was also a

trend for 120 mg/kg IQ to increase the ileal digestibility of CP, Ala, Cys, Ile, Phe and Thr (Goodarzi Boroojeni et al., 2018). Blood concentrations of Gly, Ile, Leu, Lys, and Met were greater in growing pigs fed diets supplemented with IQ when compared to growing pigs fed the control diet without IQ (Liu et al., 2016).

GROWTH PERFORMANCE

Isoquinoline alkaloids are thought to improve growth performance through reduced inflammation and improved nutrient digestion and absorption (Zdarilova et al., 2006). Growth performance studies investigating the effects of IQ have been conducted in swine, poultry, fish, steers, and ewes.

Weanling pigs fed a diet containing 120 mg/kg IQ had a greater average daily gain (ADG) compared with control pigs fed a diet without IQ during the nursery period and entire 42 d experimental periods (Goodarzi Boroojeni et al., 2018). Supplementing nursery diets for pigs with 50 g/t IQ resulted in an increase in BW and an increase in ADG when compared with pigs fed the control diet without IQ (Kantas et al., 2015). Supplementing diets for growing pigs with 40 mg/kg IQ resulted in an increase in final BW at the end of the 14 d experimental period and in ADG from d 1 to 14 (Liu et al., 2016). Broiler chickens fed diets supplemented with 20 or 50 mg/kg IQ had an increased final BW as well as greater ADG from d 22 to 35 compared with chicks fed an unsupplemented control diet (Lee et al., 2015). *Macleaya cordata* extract, containing IQ, fed to Ross male broiler chickens enhanced BW on d 21 and 35 (Khadem et al., 2014). When combined with organic acids, 50 mg/kg IQ supplementation in broiler diets increased BW on d 7 and 14 of a 42 d growth performance experiment, whereas broilers fed the

diet containing only IQ had greater BW compared with control birds on d 21 (Vieira et al., 2008b). Broilers fed a diet containing 50 mg/kg IQ had a greater BW on d 21 compared with broilers fed a control diet without IQ (Vieira et al., 2008a). Feeding diets supplemented with IQ to red tilapia resulted in an increase in weight gain and final BW with the greatest gain and final BW observed in fish fed a diet containing the 25 mg/kg IQ (Rawling et al., 2009). In contrast, there was no influence of IQ supplementation on BW or ADG of finishing ewes under heat stress (Estrada-Angulo et al., 2016). There were also no differences in final BW or ADG of pigs fed diets supplemented with 1.5 g/kg or 0.75 g/kg IQ when compared with pigs fed the control diet without IQ in a 40 d experiment with an average initial BW of 8.96 kg for all pigs (Robbins et al., 2013).

Results of a few studies have indicated that adding IQ to nursery and growing pig diets did not affect feed intake (Robbins 2013; Khadem et al., 2014; Goodarzi Boroojeni et al., 2018). In contrast, average daily feed intake (ADFI) increased in growing pigs fed diets supplemented with 40 mg/kg IQ (Liu et al., 2016) and nursery pigs fed diets that included 50 g/t IQ had a greater ADFI compared with pigs fed the control diet (Kantas et al., 2015). Supplementing diets fed to red tilapia with IQ resulted in an increase in ADFI with the greatest ADFI observed in the 50 mg/kg IQ treatment (Rawling et al., 2009). Broiler chickens had an increased feed intake from d 22 to 35 if fed a diet supplemented with 50 g/t IQ compared with control fed chickens (Vieira et al., 2008a; Lee et al., 2015).

Pigs fed 120 mg/kg IQ had better gain:feed ratio (G:F) compared with pigs fed no IQ during the nursery and grower periods (Goodarzi Boroojeni et al., 2018). Supplementing nursery diets with 50 g/t IQ resulted in an increase in G:F of pigs when compared with pigs fed control

diets (Kantas et al., 2015), and G:F improved with 40mg/kg IQ supplementation in diets fed to growing pigs (Khadem et al., 2014; Liu et al., 2016). Broiler chickens had an increased G:F from d 22 to 35 if fed diets containing 50 g/t IQ compared with control fed chickens (Lee et al., 2015). Including 50 mg/kg IQ in diets fed to broilers resulted in an increase in G:F from d 8 to 14 and over the entire 42 d experimental period compared with broilers fed the control diet (Vieira et al., 2008b), and G:F was also improved in broilers fed 37.5 mg/kg IQ over a 35 d experimental period compared with broilers fed a control diet (Vieira et al., 2008a). Supplementing diets fed to finishing ewes with 0.5 g/d IQ resulted in improved G:F (Estrada-Angulo et al., 2016).

Chickens fed the 20 mg/kg IQ or 50 mg/kg IQ diets had lower jejunal weight, whereas jejunal and ileal length was increased in broilers fed both IQ diets, but there was no effect on organ weight, abdominal fat, or leg and breast muscles from any of the diets (Lee et al., 2015). Total cholesterol levels in plasma were also lower in broilers fed the IQ diets compared with broilers fed the control diet (Lee et al., 2015). Diet supplemented with *Macleaya cordata* extract reduced the pH in the proventriculus, but not in the rest of the gut of Ross broiler chickens (Jankowski et al., 2009). The group fed the alkaloid diet had reduced asparagine aminotransferase activity in the serum, along with decreased glucose and increased HDL-cholesterol indicating that IQ may directly influence metabolic processes (Jankowski et al., 2009).

CONCLUSIONS

Isoquinoline alkaloid supplementation to diets may have many beneficial effects on livestock production because growth performance, nutrient digestibility, and gastrointestinal

health may be improved in ruminants, swine, poultry, and fish. However, more research is needed to determine effects of isoquinoline alkaloids on the apparent ileal digestibility of nutrients including CP, acid hydrolyzed ether extract, starch, and individual AA, as well as effects on growth performance and gastrointestinal health in pigs. There is also a need to determine the impact of IQ on total tract digestibility of energy, and digestible and metabolizable energy concentrations of diets fed to pigs.

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CHAPTER 3: ISOQUINOLINE ALKALOIDS IMRPOVE APPARENT ILEAL DIGESTIBILITY OF NUTRIENTS AND STARCH BY YOUNG GROWING PIGS FED CORN-SOYBEAN MEAL DIETS

ABSTRACT

An experiment was conducted to test the hypothesis that a preparation of isoquinoline alkaloids (IQ) obtained from *Macleaya cordata* and added to corn-soybean meal diets increases the apparent ileal digestibility (AID) of amino acids (AA), crude protein (CP), starch, and acid hydrolyzed ether extract (AEE) when fed to young growing pigs. Thirty-two ileal cannulated barrows (initial body weight = 12.19 ± 1.38 kg) were allotted to a randomized complete block design with 4 diets and 8 replicate pigs per diet. Diets were supplemented with 0, 90, 180, or 360 mg/kg IQ and with 0.40% chromic oxide. Diets were fed for 27 d and ileal digesta were collected on d 13 and 14 (period 1) and on d 26 and 27 (period 2). Effects of IQ inclusions were analyzed using contrast statements, and differences between periods were analyzed using a repeated measures statement. A quadratic increase ($P < 0.05$) in the AID of Thr, Trp, Val, Pro, and Tyr was observed in period 1 as IQ was included in the diets, and AID of CP, Arg, His, Ile, Leu, Met, Phe, Thr, Trp, Val, Pro, and Tyr was greater in period 2 than in period 1 ($P < 0.05$). In period 1, a quadratic increase ($P < 0.05$) was observed for the AID of starch as IQ increased in the diet, but the AID of starch was less ($P < 0.05$) in period 2 than in period 1. No differences among treatments or periods were observed for AID of AEE. Results indicate that inclusion of approximately 90 mg/kg of IQ in diets for weanling pigs increases the AID of starch and some AA.

Key words: amino acids, apparent ileal digestibility, crude protein, isoquinoline alkaloids, pigs, starch

INTRODUCTION

Weaning poses a challenge for pigs as it commonly results in decreased growth performance and poor gastrointestinal health (Lalles et al., 2004). Consequently, antibiotic growth promoters are often included in post-weaning diets at sub-therapeutic levels to control diarrhea and increase growth performance. Recently, the use of antibiotics as growth promoters in diets fed to livestock has been discontinued or limited, and there is an increased interest in using feed additives, such as plant extracts, as an alternative strategy to improve growth and gut health (Gallois et al., 2009; de Lange et al., 2010; Thacker, 2013).

Sangrovit® Extra (Phytobiotics Futterzusatzstoffe GmbH, Eltville, GE) is a phytogenic feed additive derived from *Macleaya cordata* (plume poppy) composed of isoquinoline alkaloids (IQ). These IQ have anti-inflammatory (Agarwal et al., 1991), immuno-modulatory (Chaturvedi et al., 1997), and antimicrobial effects (Walker, 1990). Consequently, it has been shown that supplementing diets for pigs with IQ reduces intestinal inflammation and improves the intestinal barrier function (Robbins et al., 2013; Liu et al., 2016b) and thereby may increase absorption of essential nutrients. However, limited data have been published for effects of IQ on the digestibility of nutrients in pigs (Goodarzi Boroojeni et al., 2018). Therefore, it was the objective of this experiment to test the hypothesis that inclusion of IQ in a corn-soybean meal diet will increase the apparent ileal digestibility (AID) of starch, amino acids (AA), crude protein (CP), and acid hydrolyzed ether extract (AEE) if fed to young growing pigs.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment. Pigs used in the experiment were the offspring of Line 359 boars mated to Camborough sows (Pig Improvement Company, Hendersonville, TN). The main sources of CP and AA in the experimental diets were corn, soybean meal, and fish meal, which were obtained from the University of Illinois feed mill (Champaign, IL) and the same batches of these ingredients were used to produce all 4 diets.

Diets, Animals, and Experimental Design

A basal diet that primarily contained corn, soybean meal, fish meal, and lactose was formulated (Tables 3.1 and 3.2) to meet requirement estimates for pigs from 11 to 25 kg (NRC, 2012). Three additional diets were formulated by adding 90, 180, or 360 ppm of IQ to the basal diet. Thus, all diets were identical except for the inclusion of IQ, which resulted in different concentrations of alkaloids in the diets. Vitamins and minerals were included to meet or exceed current requirement estimates (NRC, 2012). All diets also contained 0.40% chromic oxide as an indigestible marker.

Thirty-two young growing barrows (initial body weight: 12.19 ± 1.38 kg) were equipped with a T-cannula in the distal ileum (Stein et al., 1998) and allotted to a randomized complete block design with 4 diets and 8 replicate pigs per diet. Pigs were housed in individual pens (1.2 × 1.5 m) with smooth sides and fully slatted tribar floors in an environmentally controlled room. A feeder and a nipple drinker were installed in each pen.

Feeding and Sample Collection

All pigs were provided feed on an ad libitum basis and water was available at all times. Pig weights were recorded at the beginning of the experiment. The initial 12 d of the experiment was considered an adaptation period to the diet. On d 13 and 14, ileal digesta were collected for 8 h using standard procedures (Stein et al., 1998). A 225-mL plastic bag was attached to the cannula barrel by a zip tie, and digesta that flowed into the bag were collected. Bags were replaced whenever they were filled with digesta or at least once every 30 min and stored at -20˚C to prevent bacterial degradation of AA in the digesta. At the end of the collection period on d 14, pig weights were recorded. No samples were collected from d 15 to 25, but ileal digesta were again collected on d 26 and 27. The final weight was recorded on d 27.

At the conclusion of the animal work, ileal digesta samples were thawed, mixed within animal, diet, and collection period, and a sub-sample was collected for chemical analysis. A sample of each diet was collected at the time of diet mixing, as was a sample of corn, fish meal, and soybean meal.

Chemical Analyses

Digesta samples were lyophilized and finely ground prior to chemical analysis. Corn, soybean meal, fish meal, and all samples of digesta and diets were analyzed in duplicate for dry matter (method 930.15; AOAC Int., 2007) and for CP using the Kjeldahl method by quantifying N and calculating CP via a conversion factor of 6.25 (method 984.13; AOAC Int., 2007) using a Kjeltec™ 8400 apparatus (FOSS Inc., Eden Prairie, MN). Samples were analyzed for AA on an AA analyzer (model L8800 Hitachi Amino Acid Analyzer, Hitachi High Technologies America Inc., Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the

internal standard. Before AA analysis, samples were hydrolyzed with 6N HCl for 24 h at 110˚C (method 982.30 E(a); AOAC Int., 2007). Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis (method 982.30 E(b); AOAC Int., 2007). Tryptophan was determined after NaOH hydrolysis for 22 h at 110 ˚C (method 982.30 E(c); AOAC Int., 2007). The chromium concentration of the diets and digesta was determined using an Inductive Coupled Plasma Atomic Emission Spectrometric method (method 990.08; AOAC Int., 2007). Samples were prepared using nitric acid-perchloric acid (method 968.08 D(b); AOAC Int., 2007). Total starch was determined in all samples using the glucoamylase procedure (method 979.10; AOAC Int., 2007), and total AEE was analyzed by acid hydrolysis using 3N HCl (AnkomHCl, Ankom Technology, Macedon, NY) followed by crude fat extraction using petroleum ether (AnkomXT15, Ankom Technology, Macedon, NY).

Corn, soybean meal, and diets were also analyzed for acid detergent fiber and neutral detergent fiber using Ankom Technology method 12 and 13, respectively (Ankom 2000 Fiber Analyzer, Ankom Technology, Macedon, NY), and for acid detergent lignin using Ankom Technology method 9 (Ankom DaisyII Incubator, Ankom Technology, Macedon, NY). Additionally, these samples were analyzed for dry ash (method 942.05; AOAC Int., 2007).

Calculations and Statistical Analysis

Values for the AID of AA in all diets were calculated using the following equation (Stein et al., 2007):

$$AID\_AA,\% = 100 - [(AA\_digesta / AA\_diet) \times (Cr\_diet / Cr\_digesta)] \times 100$$

where AIDAA is the apparent ileal digestibility of an AA (%), AAdigesta is the concentration of that AA in the ileal digesta, AAdiet is the AA concentration of that AA in the diet, Crdiet is the

chromium concentration in the diet, and Crdigesta is the chromium concentration in the ileal digesta. The AID for CP, starch, and AEE was also calculated using this equation.

Data were analyzed using the Proc MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Contrast statements were used with coefficients for unequally spaced treatments being generated using the Proc IML statement in SAS to determine linear and quadratic effects of IQ on the AID of CP, AA, AEE, and starch within each collection period. Results obtained in collection period 1 were also compared with results from collection period 2 using repeated measures analysis. Results were considered significant at $P \leq 0.05$ and a trend at $0.05 \leq P \leq 0.10$. The pig was the experimental unit for all analyses.

RESULTS

There was no effect of diet or period on the AID of AEE (Table 3.3). In period 1, the AID of starch increased and then decreased as IQ was added to the diet with the greatest response observed with the addition of 90 mg/kg of IQ (quadratic, $P < 0.05$). In period 2, starch tended to show a quadratic increase ($P < 0.10$) with the greatest response at 90 mg/kg of IQ as well. In addition, AID of starch was greater ($P < 0.05$) for all diets in period 1 than in period 2. A quadratic increase ($P < 0.05$) was observed in period 1 for the AID of Thr, Trp, Val, Pro, and Tyr, with the greatest values generally observed in the diet containing 90 mg/kg of IQ. There was also a trend (linear and quadratic, $P < 0.10$) for the AID of Ile and Met to increase as IQ was added to the diets. A period effect ($P < 0.05$) was observed for CP and all indispensable AA except Lys and also for Pro and Tyr with AID values in period 2 being greater than in period 1. In contrast, the AID of Cys was greater ($P < 0.05$) in period 1 than in period 2. A period effect (P

<0.05) in the average AID for all AA was also observed, with values in period 2 generally being greater than in period 1.

DISCUSSION

Nutritional Characteristics of Ingredients and Diets

The nutritional composition of corn was generally in agreement with expected values (NRC, 2012; Rojas and Stein, 2013) and the CP, AA, AEE, and ash values for soybean meal were in agreement with expected values (Cervantes-Pahm and Stein, 2010; Goebel and Stein, 2011; NRC, 2012). Concentrations of CP, AA, and ash in fish meal were as reported by NRC (2012), but values for dry matter and AEE were lower than reported. The composition of fish meal was in agreement with values reported by Sulabo et al. (2013) and Cervantes-Pahm and Stein (2010), but the concentration of AEE was lower and the ash was greater than reported by Rojas and Stein (2013).

The nutrient composition of all diets was in agreement with formulated values. Pigs readily consumed their provided feed throughout the experiment, indicating no issues with palatability of the diets; however, one pig on the 360 mg/kg diet was excluded from the study before digesta collection due to health complications unrelated to the experiment.

The isoquinoline alkaloid preparation used in this experiment is derived from *Macleaya cordata*, an herbaceous perennial plant native to China and Japan and also grown in North America and Europe. Specifically, these alkaloids include sanguinarine, chelerythrine, protopine, and allocryptopine. The analyzed concentrations of the individual alkaloids were as expected in all diets.

Ileal Digestibility of Starch

The quadratic increase in the AID of starch in both periods indicated that 90 mg/kg IQ is optimum to maximize starch digestion under the conditions of this experiment. To the best of our knowledge, there are no previous data on the effects of IQ on the AID of starch in pigs; however, IQ supplementation of diets fed to steers resulted in no effect on total tract digestibility of starch (Aguilar-Hernandez et al., 2016). Starch that was not fermented in the rumen or digested in the small intestine was likely fermented in the large intestine, thus preventing possible differences in the small intestinal digestion of starch from being identified. The decrease in the AID of starch from period 1 to period 2 may be a result of partial inhibition of α-amylase by isoquinoline alkaloids such as sanguinarine and chelerythrine, two components of the IQ preparation (Zajoncová et al., 2005). Clearly, this area needs more research.

Ileal Digestibility of AEE

Similar to the observations of this study, when digesta was collected from an excised ileum post-mortem, it was revealed that IQ supplementation had no effect on the ileal digestibility of ether extract in diets fed to post-weaning pigs (Goodarzi Boroojeni et al., 2018). Sanguinarine and chelerythrine, along with other alkaloids, may inhibit Candida rugosa lipase (Grippa et al., 1999), with sanguinarine having the strongest effect and chelerythrine being significantly less inhibitory. However, the lack of an effect of IQ on the AID of AEE indicates that greater concentrations of IQ than used in this experiment are needed to exhibit such an effect in pigs.

Ileal Digestibility of CP and AA

The quadratic increase in the AID of AA indicated that 90 mg/kg is the optimum dosage of IQ under the conditions of this experiment. The observation that the AID of many indispensable AA was greater ($P < 0.05$) in period 2 than in period 1 may indicate that longer exposure to IQ supplementation increases the AID of AA. However, the AID of some AA in the control diet also appeared to slightly increase, which may be a result of pigs increasing the AID of AA as they become older (Pedersen et al., 2016). Thus, the increase in AID of AA from period 1 to period 2 is likely partly due to the normal increase in AID as pigs become older and partly due to an increased effect of IQ. Dietary supplementation with sanguinarine enhanced serum AA levels in growing pigs compared with pigs fed a control diet (Liu et al., 2016a), which may be a result of greater absorption from the small intestine. Similarly, steers fed IQ supplemented diets had an increase in postruminal and total tract digestibility of N (Aguilar-Hernandez et al., 2016). Additionally, pre-caecal digestibility of Asp, Glu, His, Leu, Met, Val, and total AA was greater in post-weaning pigs fed a diet supplemented with 120 mg/kg IQ when compared to the control diet (Goodarzi Boroojeni et al., 2018). The increased digestibility of AA and N and increased serum AA levels may be due to the anti-inflammatory properties of IQ. Potential mechanisms of action include inhibition of neutrophil phagocytosis and degranulation (Agarwal et al., 1991), inhibition of nuclear factor kappa-light-chain enhancer of activated B cells activation (Chaturvedi et al., 1997), inhibition of tumor necrosis factor-α and nitric oxide production, and suppression of p38 MAPK and ERK1/2 phosphorylation in peritoneal macrophages (Niu et al., 2012). Isoquinoline alkaloids also increased expression of tight junction proteins (Robbins et al., 2013; Liu et al., 2016b) and thus, enhanced intestinal barrier function, which may contribute to the observed improvement in nutrient absorption in this study.

However, the current experiment was not designed to determine effects of IQ on immune response of pigs and additional research is, therefore, needed to address this hypothesis.

Conclusions

Results from this experiment indicate that 90 mg/kg IQ in corn-soybean meal diets fed to young growing pigs is optimum to maximize the AID of starch and AA and prolonged exposure to IQ increased the AID of CP and AA. Therefore, IQ may be used as a phytobiotic alternative to improve nutrient digestibility of diets fed to weanling pigs; however, further research is needed to determine the effects of IQ on growth performance parameters and on possible immunoprotective properties of IQ.

TABLES

Table 3.1. Composition (as-is basis) of experimental diets

Item, %	Isoquinoline alkaloids, mg/kg			
	-	90	180	360
Corn	42.70	42.30	41.90	41.10
Soybean meal	36.00	36.00	36.00	36.00
Soybean oil	4.00	4.00	4.00	4.00
Dicalcium phosphate	0.30	0.30	0.30	0.30
Limestone	0.80	0.80	0.80	0.80
Lactose	10.00	10.00	10.00	10.00
Fish meal	5.00	5.00	5.00	5.00
Sangrovit® Extra[1]	-	0.40	0.80	1.60
Chromic oxide	0.40	0.40	0.40	0.40
Sodium chloride	0.50	0.50	0.50	0.50
Vitamin-mineral premix[2]	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00

[1]Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany.

[2]Provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,128 IU; vitamin D_3 as cholecalciferol, 2,204 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.58 mg; pyridoxine as pyridoxine hydrocloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin as nicotinamide and niacotinic acid, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

Table 3.2. Analyzed nutrient composition of experimental diets and ingredients (as-fed basis)

Item, %	Isoquinoline alkaloids, mg/kg						
	-	90	180	360	Corn	SBM	Fish meal
Dry matter	87.46	88.75	88.85	87.75	86.12	85.93	90.50
AEE[1]	4.51	4.22	5.00	5.25	3.36	1.30	7.50
Ash	6.07	6.12	6.25	5.89	1.24	6.26	19.04
Crude protein	22.93	23.23	22.85	22.98	7.50	46.36	64.84
Starch	27.63	28.73	30.10	27.62	59.33	2.85	-
Neutral detergent fiber	7.29	7.16	6.36	7.25	10.75	7.71	-
Acid detergent fiber	3.17	3.82	3.38	2.97	4.02	6.22	-
Acid detergent lignin	0.89	0.89	0.87	0.88	1.12	0.63	-
Indispensable AA							
Arg	1.44	4.51	1.49	1.49	0.35	3.30	3.66
His	0.57	0.59	0.58	0.58	0.21	1.19	1.25
Ile	1.04	1.08	1.05	1.06	0.29	2.24	2.62
Leu	1.83	1.88	1.82	1.86	0.87	3.55	4.25
Lys	1.36	1.41	1.37	1.38	0.27	2.87	4.88
Met	0.35	0.37	0.36	0.36	0.14	0.64	1.66
Phe	1.11	1.16	1.12	1.13	0.37	2.38	2.40
Thr	0.83	0.86	0.84	0.85	0.26	1.76	2.39
Trp	0.32	0.31	0.32	0.31	0.07	0.72	0.68
Val	1.10	1.16	1.11	1.12	0.36	2.31	2.99
Dispensable AA							
Ala	1.13	1.15	1.13	1.15	0.54	1.98	4.00
Asp	2.27	2.37	2.31	2.33	0.51	5.16	5.42
Cys	0.31	0.32	0.33	0.32	0.16	0.65	0.50
Glu	3.86	3.99	3.88	3.92	1.35	8.24	7.96
Gly	1.08	1.10	1.08	1.10	0.32	2.01	4.91
Pro	1.29	1.31	1.25	1.29	0.66	2.51	3.16
Ser	0.90	0.93	0.91	0.92	0.33	1.96	2.09
Tyr	0.71	0.74	0.73	0.73	0.20	1.70	1.83

[1]AEE = Acid hydrolyzed ether extract.

Table 3.3. Effects of isoquinoline alkaloids on apparent ileal digestibility of acid hydrolyzed ether extract (AEE), starch and amino acids (AA) in diets fed to weanling pigs[1]

Item, %	Period 1[2]				SEM	*P*-value		Period 2[2]				SEM	*P*-value		*P*-value
	-	90	180	360		Linear	Quadratic	-	90	180	360		Linear	Quadratic	Period
AEE[3]	73.5	72.7	72.5	73.9	2.345	0.824	0.533	69.4	73.1	72.9	74.3	2.35	0.277	0.588	0.638
Crude protein	76.0	78.2	75.9	74.0	1.102	0.074	0.186	77.2	80.2	76.1	79.9	1.10	0.250	0.457	0.003
Starch	92.9	94.7	93.8	91.6	0.593	0.025	0.004	90.8	93.2	91.3	90.9	0.59	0.455	0.076	0.001
Indispensable AA															
Arg	89.1	90.4	89.1	88.5	0.460	0.128	0.165	89.1	91.0	90.1	90.5	0.46	0.119	0.123	0.010
His	80.8	82.6	82.0	81.1	1.008	0.865	0.198	82.9	84.7	80.6	84.2	1.01	0.714	0.159	0.016
Ile	82.5	84.7	82.8	81.4	0.726	0.062	0.063	83.4	85.5	82.3	84.7	0.73	0.564	0.453	0.019
Leu	81.4	83.3	81.9	81.0	0.799	0.383	0.189	82.9	84.9	81.5	84.1	0.80	0.640	0.297	0.004
Lys	80.0	81.0	80.6	79.6	1.030	0.616	0.407	79.3	81.7	80.3	82.4	1.03	0.078	0.858	0.335
Met	85.1	86.6	85.6	83.4	0.771	0.077	0.082	85.7	87.6	85.1	86.3	0.77	0.901	0.996	0.043
Phe	82.2	83.8	82.5	81.3	0.752	0.187	0.180	83.0	85.4	82.0	84.4	0.75	0.558	0.531	0.015
Thr	71.2	75.0	72.4	69.7	1.096	0.050	0.012	73.1	76.3	71.0	75.1	1.10	0.680	0.319	0.008
Trp	80.7	81.2	81.8	78.4	0.844	0.050	0.042	82.1	83.6	81.4	83.6	0.84	0.395	0.462	0.001
Val	76.8	79.5	78.1	76.2	0.919	0.273	0.034	78.4	81.3	77.0	80.1	0.92	0.627	0.436	0.008

Table 3.3. (cont.)

Mean	80.8	82.6	81.7	80.0	0.840	0.251	0.087	82.0	84.2	80.7	83.5	0.84	0.530	0.371	0.023
Dispensable AA															
Ala	76.8	79.0	77.7	78.1	1.336	0.674	0.421	78.4	80.6	76.8	79.9	1.01	0.679	0.294	0.135
Asp	73.5	77.3	74.1	73.4	0.956	0.264	0.058	74.4	78.0	72.7	77.3	0.96	0.247	0.268	0.069
Cys	57.0	59.6	58.8	54.7	2.914	0.393	0.267	59.5	64.1	54.4	66.5	2.91	0.227	0.127	0.044
Glu	82.6	83.5	80.4	80.2	1.460	0.106	0.919	81.3	85.2	79.6	85.4	1.46	0.194	0.312	0.129
Gly	67.4	71.1	66.9	67.4	1.888	0.517	0.584	67.8	71.9	65.4	73.9	1.89	0.113	0.188	0.157
Pro	78.8	81.4	79.2	76.1	0.971	0.009	0.027	81.6	83.4	78.3	82.4	0.97	0.925	0.058	0.001
Ser	76.8	79.4	76.9	75.9	1.103	0.193	0.179	77.2	80.5	75.1	79.5	1.10	0.521	0.299	0.210
Tyr	81.4	84.1	82.3	80.6	0.832	0.132	0.034	82.7	84.9	81.9	84.6	0.83	0.332	0.503	0.009
Mean	74.3	76.5	74.4	72.9	1.337	0.195	0.274	75.4	78.6	73.0	78.7	1.34	0.276	0.183	0.038

[1]Data are least squares means of 8 observations per treatment except for the 360 mg/kg diet where only 7 observations were used.

[2] The 4 diets contained 0, 90, 180 or 360 mg/kg Sangrovit® Extra (Phytobiotics Futterzusatzstoffe GmbH, Eltville, GE).

[3]AEE = Acid hydrolyzed ether extract.

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CHAPTER 4: EFFECTS OF ISOQUINOLINE ALKALOIDS ON APPARENT TOTAL TRACT DIGESTIBILITY OF ENERGY AND ON CONCENTRATION OF DIGESTIBLE AND METABOLIZABLE ENERGY IN DIETS FED TO YOUNG GROWING PIGS

ABSTRACT

An experiment was conducted to test the hypothesis that including isoquinoline alkaloids (IQ) in diets containing corn and soybean meal (SBM), or corn, SBM, and distiller's dried grains with solubles (DDGS) fed to young growing pigs increases the apparent total tract digestibility (ATTD) of gross energy (GE) and concentrations of digestible energy (DE) and metabolizable energy (ME) of the diets. Twenty-four gilts and 24 barrows (initial body weight: 13.67 ± 1.35 kg) were allotted to a randomized complete block design with 8 diets and 6 replicate pigs per diet. A basal diet consisting of corn and SBM and a second basal diet consisting of corn, SBM, and DDGS were prepared. Six additional diets were prepared by adding 90, 180, or 360 mg/kg IQ to each of the 2 basal diets. Pigs were housed individually in metabolism crates for the 12 d experimental period and were fed 3 times the estimated energy requirement in 2 equal meals provided at 0800 and 1600 h. The first 5 d were considered an adaptation period to the diets and fecal materials and urine were collected from d 6 to 11 using standard procedures. With the MIXED procedure of SAS, contrast statements were used to determine the linear and quadratic effects of IQ and to compare the 2 diets. Isoquinoline alkaloids quadratically increased ($P < 0.05$) average daily feed intake (ADFI), dry feces output, and fecal GE output of pigs fed corn-SBM-DDGS diets with the highest values observed in the 90 and 180 mg/kg IQ diets. Additionally, IQ linearly decreased ($P < 0.05$) the ME of the corn-SBM diets, with the highest ME value in the 90 mg/kg diet. Pigs fed the corn-SBM-DDGS diets tended to have a greater ($P <$

0.10) ADFI and had increased ($P < 0.05$) dry feces output, fecal GE output, and ATTD of GE compared with pigs fed corn-SBM diets. To conclude, IQ supplementation does not result in increased ATTD of energy, or increased DE or ME of diets fed to young growing pigs.

Key words: apparent total tract digestibility, digestible energy, gross energy, isoquinoline alkaloids, metabolizable energy, pigs

INTRODUCTION

Antibiotic growth promoters have been used in post-weaning diets at sub-therapeutic levels to minimize the reduction in growth performance and gut health that are commonly observed in newly weaned piglets. Due to increased limitations on the use of antibiotics in livestock feed, alternative feed additives are being investigated (Gallois et al., 2009; de Lange et al., 2010; Vondruskova et al., 2010; Thacker, 2013). Sangrovit® Extra is a preparation of isoquinoline alkaloids (IQ) obtained from *Macleaya cordata* (plume poppy). Isoquinoline alkaloids express anti-inflammatory (Agarwal et al., 1991), immuno-modulatory (Chaturvedi et al., 1997), and antimicrobial effects (Walker, 1990), and may improve intestinal barrier function (Robbins et al., 2013; Liu et al., 2016). Because of these properties, feeding diets containing IQ is being investigated as a potential strategy to improve gut health and growth performance.

A few experiments have been conducted to evaluate the efficacy of IQ supplementation on the digestibility of energy by livestock (Aguilar-Hernandez et al., 2016), but there are no data to demonstrate effects of IQ on energy digestibility or on concentrations of digestible energy (DE) and metabolizable energy (ME) in diets fed to swine. It was, therefore, the objective of this experiment to test the hypothesis that inclusion of IQ improves the apparent total tract

digestibility (ATTD) of gross energy (GE) and concentrations of DE and ME of diets fed to young growing pigs.

MATERIALS AND METHODS

The protocol for the experiment was submitted to the Institutional Animal Care and Use Committee at the University of Illinois and approved prior to initiation of the experiment. Pigs used in the experiment were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN). The main ingredients used in this experiment included corn, soybean meal (SBM), and dried distillers grains with solubles (DDGS), which were obtained from the University of Illinois feed mill (Champaign, IL) and the same batches of corn, SBM, and DDGS were used to produce all 8 diets (Table 4.1).

Diets, Animals, and Experimental Design

A basal diet consisting primarily of corn and SBM and a second basal diet based on corn, SBM, and DDGS were prepared (Table 4.2). Six additional diets were prepared by adding 90, 180, or 360 mg/kg of IQ to each of the 2 basal diets. All diets were provided to pigs in meal form and vitamins and minerals were included to meet requirements (NRC, 2012).

A total of 24 gilts and 24 barrows (initial body weight: 13.67 ± 1.35 kg) were allotted to a randomized complete block design with 8 treatments and 6 replicate pigs per treatment. Pigs were housed individually in metabolism crates that were equipped with a feeder and a nipple drinker, fully slatted floors, a screen floor and a urine tray, which allowed for the total, but separate, collection of fecal materials and urine from each pig.

150 ***Feeding and Sample Collection***

151 All pigs were fed at 3 times the estimated daily energy requirement for maintenance (i.e.,
152 197 kcal/kg × BW0.60; NRC, 2012) in 2 equal meals that were provided at 0800 and 1600 h.
153 Water was available at all times during the experiment. Pigs were housed in the metabolism
154 crates for 12 d. The initial 5 d were considered an adaptation period to the crates and the feed
155 that was provided. Fecal materials and urine were collected from the feed that was fed from d 6
156 to 11 according to standard procedures using the marker to marker approach (Adeola, 2001).
157 Urine was collected in urine buckets over a preservative of 50 mL HCl. Fecal samples and 10%
158 of the collected urine were stored at -20˚C immediately after collection. At the conclusion of the
159 experiment, urine samples were thawed and mixed and 2 subsamples were collected. One of the
160 sub-samples was lyophilized before analysis (Kim et al., 2009).

161 ***Analyses***

162 Fecal samples were dried in a 65˚C forced air drying oven and finely ground prior to
163 analysis. The lyophilized urine sample, fecal samples, and all diet and ingredient samples were
164 analyzed in duplicate for concentrations of gross energy (GE) using an isoperibol bomb
165 calorimeter (Model 6400, Parr Instruments, Moline, IL). In addition, fecal, diet, and ingredient
166 samples were analyzed in duplicate for dry matter (method 930.15; AOAC Int., 2007).Diet and
167 ingredient samples were also analyzed in duplicate for crude protein by measuring N (method
168 990.03; AOAC Int., 2007) using a Leco Nitrogen Determinator (model FP628, Leco Corp., St.
169 Joseph, MI) and for dry ash (method 942.05; AOAC Int., 2007). Additionally, ingredient
170 samples were analyzed for insoluble dietary fiber and soluble dietary fiber (method 991.43;
171 AOAC Int., 2007) using the AnkomTDF Dietary Fiber Analyzer (Ankom Technology, Macedon,
172 NY).

Calculations and Statistical Analysis

The ATTD of GE was calculated using the following equation (Almeida and Stein, 2010; NRC, 2012):

$$\text{ATTD \%} = [(\text{GE intake} - \text{GE in feces}) / \text{GE intake}] \times 100$$

The DE and ME in diets was calculated by subtracting the GE in feces and the GE in feces and urine, respectively, from GE in the diet (NRC, 2012).

Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with the pig as the experimental unit. Contrast statements were used with coefficients for unequally spaced treatments being generated using the Proc IML statement in SAS to determine linear and quadratic effects of IQ within each type of diet and to compare the 2 types of diets. Results were considered significant at $P \leq 0.05$ and considered a trend at $0.05 < P \leq 0.10$.

RESULTS

Isoquinoline alkaloid supplementation quadratically increased ($P < 0.05$) average daily feed intake (**ADFI**), dry feces output, and fecal GE output of pigs fed corn-SBM-DDGS diets, with the highest values observed in the 180 mg/kg IQ diet for ADFI, and 90 mg/kg IQ diet for dry feces output and fecal GE output (Table 4.3). When IQ was included in the corn-SBM diets, there was a tendency (quadratic; $P < 0.10$) for a reduction in the GE of dry feces and a decrease (quadratic; $P < 0.05$) in the GE of urine with the lowest values observed in the 90 mg/kg diet. Additionally, IQ inclusion linearly decreased ($P < 0.05$) the ME of the corn-SBM diets, with the highest ME value in the 90 mg/kg diet. When comparing the corn-SBM and corn-SBM-DDGS diets, ADFI of pigs fed the corn-SBM-DDGS diets tended to be greater ($P < 0.10$). Pigs fed

corn-SBM-DDGS diets also had increased ($P < 0.05$) dry feces output and fecal GE output, and reduced ($P < 0.05$) ATTD of GE compared with pigs fed corn-SBM diets.

DISCUSSION

Nutritional Characteristics of Ingredients and Diets

The proximate analysis values for SBM were generally in agreement with previous values (Cervantes-Pahm and Stein, 2010; Goebel and Stein, 2011; NRC, 2012; Rojas and Stein, 2013; Espinosa and Stein, 2018). Analysis confirmed expected values for the dry matter and ash of corn, but values for crude protein and GE were lower than reported (Pedersen et al., 2007; NRC, 2012; Curry et al., 2016). Values for dry matter and GE in DDGS were lower than reported; however, crude protein and ash values were within the range of reported values (Pedersen et al., 2007; Curry et al., 2016 Espinosa and Stein, 2018). Concentrations of insoluble dietary fiber and soluble dietary fiber in the corn, SBM, and DDGS used in this experiment were in accordance with previously reported values (Stein and Shurson, 2009; Espinosa and Stein, 2018; Navarro et al., 2018a), but greater than others (Navarro et al., 2018b). Total dietary fiber concentration in corn, SBM, and DDGS were within the range of reported values (Stein and Shurson, 2009; Anderson et al., 2012; NRC, 2012; Huang et al., 2017).

Nutrient composition of the experimental diets was in agreement with formulated values. The IQ preparation includes sanguinarine, chelerythrine, protopine, and allocryptopine. The analyzed concentrations of the individual alkaloids were as expected in all diets when compared with the formulated inclusion rates. Pigs readily consumed their provided feed throughout the experiment.

Average Daily Feed Intake

The tendency for the ADFI of the corn-SBM-DDGS diets to be greater than for the corn-SBM diets was expected because the amount of feed provided was calculated as 3 times the estimated metabolizable energy requirement for maintenance, and because the corn-SBM-DDGS diets had a lower ME value than the corn-SBM diets. The corn-SBM-DDGS diets was, therefore, provided to the pigs allotted to those diets in larger quantities compared with pigs allotted to the corn-SBM diets, but because feed intake was restricted this is not a reflection on voluntary feed intake. However, the quadratic increase in ADFI that was observed for pigs fed corn-SBM-DDGS diets as IQ was added to the diets was in agreement with the observations by Kantas et al. (2015), who reported that 50 g/t IQ in pig nursery diets increased ADFI when compared with the control diet. In contrast, Goodarzi Boroojeni et al. (2018) reported no impact of 60 mg/kg or 120 mg/kg IQ on feed intake of starter and grower pigs fed diets containing corn, soybean meal, barley, and wheat. In studies conducted with broilers, IQ supplementation had variable effects on ADFI (Vieira et al., 2008a; 2008b; Juskiewicz et al., 2011; Khadem et al., 2014; Xue et al., 2017). It is not known why IQ influenced the ADFI of corn-SBM-DDGS diets, but had no effect on the intake of corn-SBM diets under the conditions of this experiment.

Effect of Isoquinoline Alkaloids on Apparent Total Tract Digestibility of Energy

The observation that IQ had no effect on the ATTD of GE in corn-SBM or corn-SBM-DDGS diets was surprising because AID of starch and some AA is increased by IQ (Rundle and Stein, 2018). It is possible that because high-health status pigs were used in this experiment, the anti-inflammatory and immunomodulatory effects of IQ were not as apparent as they would have been if pigs were kept under commercial conditions with a greater disease pressure. The reduction in ATTD of GE when DDGS is added to corn-SBM diets that was observed in this

experiment is in agreement with previous data (Urriola and Stein, 2010). The ATTD of dietary fiber is greater in DDGS than in corn (Urriola et al., 2010) and if added to a corn-SBM diet, DDGS decreased the ATTD of dietary fiber (Stein and Shurson, 2009; Urriola and Stein, 2010), which is likely due to the greater concentration of insoluble fiber in DDGS than in SBM (Urriola et al., 2010). The dietary fiber in DDGS increases the flow of energy through the intestinal tract, resulting in a reduction in the digestibility of energy (Stein and Shurson, 2009; Urriola and Stein, 2010).

Digestible and Metabolizable Energy

The quadratic increase in dry feces output and fecal GE output that was observed as IQ was added to the corn-SBM-DDGS diet can be attributed to the quadratic increase in average daily feed intake that was observed. The increased fecal output and fecal GE output observed in the corn-SBM-DDGS diets compared with the corn-SBM diets is in agreement with reported values (Stein and Shurson, 2009; Kerr et al., 2018).

There are limited data regarding the influence of IQ on DE, but the DE of steam-flaked corn-DDGS diets fed to steers tended to increase as IQ was added (Aguilar-Hernandez et al., 2016). The reason for a lack of increase in DE when IQ was added to the diets in this experiment may be a result of the reduced fermentation in pigs compared with ruminants. The observation that DE in the corn-SBM diet was not different from the corn-SBM-DDGS diet is in agreement with studies in which DE in DDGS was not different from the DE in corn (Pedersen et al., 2007; Rojas et al., 2013; Corassa et al., 2017).

The tendency for reduced ME in the corn-SBM-DDGS diets when compared with the corn-SBM diets was in contrast with data indicating that there was no influence of DDGS on the ME of diets fed to pigs (Pedersen et al., 2007; Stein and Shurson, 2009; Rojas et al., 2013;

Corassa et al., 2017). It is not known why IQ resulted in a linear decrease of ME in the corn-SBM diets, but had no effect on the corn-SBM-DDGS diets. However, as indicated above, IQ appears to increase the ME during fermentation and it is likely that pigs fed the DDGS diets had more hindgut fermentation than pigs fed the corn-SBM diets due to the greater concentration of fiber in DDGS. More research is, therefore, needed to investigate the effects of IQ on ME in diets fed to pigs.

Conclusion

Isoquinoline alkaloids appear to have no effect on the ATTD of GE or the DE of diets containing corn and SBM or corn, SBM, and DDGS when fed to pigs. Results derived from this experiment indicate that whereas IQ may increase average daily feed intake, IQ supplementation does not result in increased energy digestibility. Therefore, the current data indicate that the immunomodulating effect of IQ is not associated with increased energy utilization and it appears that IQ does not have an energy sparing effect when included in diets fed to pigs.

TABLES

Table 4.1. Analyzed composition of ingredients (as-fed basis)

Item	Corn	Soybean meal	DDGS[1]
Gross energy, kcal/kg	3,754	4,101	4,478
Crude protein, %	6.88	47.28	27.58
Dry matter, %	86.02	88.70	82.61
Ash, %	1.04	6.90	5.15
Insoluble dietary fiber, %	10.30	16.00	33.80
Soluble dietary fiber, %	1.60	1.00	0.10
Total dietary fiber, %	12.00	17.00	33.90

[1]DDGS = Distiller's dried grains with solubles.

Table 4.2. Composition (as is basis) of experimental diets

Item	Corn-soybean meal diets				DDGS[1] diets			
IQ[2], mg/kg:	-	90	180	360	-	90	180	360
Ingredient, %								
Corn	49.35	48.95	48.55	47.75	36.45	36.05	35.65	34.85
Soybean meal	35.00	35.00	35.00	35.00	33.00	33.00	33.00	33.00
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
DDGS	-	-	-	-	15.00	15.00	15.00	15.00
Dicalcium phosphate	1.55	1.55	1.55	1.55	1.30	1.30	1.30	1.30
Limestone	0.80	0.80	0.80	0.80	0.95	0.95	0.95	0.95
L-Lys HCl	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-Thr	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
DL-Met	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Lactose	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
IQ premix[3]	-	0.40	0.80	1.60	-	0.40	0.80	1.60
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vit-mineral premix[4]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Analyzed values								
Gross energy, kcal/kg	3,892	3,879	3,881	3,923	3,988	3,982	3,969	4,092
Dry matter, %	87.95	88.33	88.14	89.05	87.91	87.77	87.88	88.04
Ash, %	5.25	5.27	5.61	5.69	5.55	5.82	5.84	5.73
Crude protein, %	20.41	19.47	20.71	19.74	21.04	20.42	20.08	21.17

[1]DDGS = Distiller's dried grains with solubles.

Table 4.2. (cont.)

[2] IQ = Isoquinoline alkaloids.

[3] Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany. Premix was prepared by mixing the feed additive, Sangrovit® Extra, with corn.

[4] The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,128 IU; vitamin D_3 as cholecalciferol, 2,204 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.58 mg; pyridoxine as pyridoxine hydrocloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin as nicotinamide and niacotinic acid, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

Table 4.3. Apparent total tract digestibility (ATTD) of gross energy (GE) and concentrations of digestible energy (DE) and metabolizable energy (ME) in diets containing corn and soybean meal (SBM) or corn, soybean meal, and DDGS[1] (as-fed basis)[2]

Item	Corn-SBM				Corn-SBM-DDGS				SEM	Corn-SBM *P*-value		Corn-SBM-DDGS *P*-value		Corn-SBM vs. Corn-SBM-DDGS
IQ, mg/kg	-	90	180	360	-	90	180	360		Linear	Quadratic	Linear	Quadratic	*P* -value
ADFI[3], kg	0.81	0.80	0.77	0.76	0.79	0.83	0.87	0.80	0.04	0.191	0.662	0.900	0.035	0.085
Dry feces output, kg/d	0.07	0.06	0.07	0.07	0.09	0.11	0.10	0.09	0.01	0.457	0.442	0.812	0.020	0.001
GE in dry feces, kcal/kg	4,592	4,539	4,554	4,650	4,616	4,584	4,585	4,661	51.23	0.114	0.064	0.250	0.162	0.251
Fecal GE output, kcal/d	315	294	301	342	404	499	466	425	43.58	0.400	0.357	0.935	0.031	0.001
ATTD, GE %	90.0	90.6	90.0	88.6	86.8	85.7	86.8	86.8	1.12	0.109	0.324	0.766	0.616	0.001
DE in diet, kcal/kg	3,506	3,531	3,505	3,450	3,484	3,440	3,482	3,482	44.26	0.115	0.332	0.763	0.612	0.223
Urine output, kg/d	3.33	3.88	3.48	3.55	4.50	3.69	3.69	3.72	0.70	0.932	0.746	0.363	0.352	0.356
GE in urine, kcal/kg	43	27	32	38	34	37	41	36	7.25	0.939	0.033	0.759	0.362	0.624
Urinary GE output, kcal/d	118	95	142	112	116	124	138	116	18.95	0.811	0.432	0.998	0.228	0.473
ME in diet, kcal/kg	3,367	3,398	3,317	3,294	3,300	3,281	3,321	3,331	51.61	0.031	0.796	0.315	0.863	0.092

[1] Distiller's dried grains with solubles.

Table 4.3. (cont.)

[2] Data are least squares means of 6 observations per treatment except for the corn-SBM diet without IQ where only 5 observations were used.

[3]ADFI = Average daily feed intake.

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CHAPTER 5: ISOQUINOLINE ALKALOIDS IN DIETS FED TO YOUNG GROWING PIGS IMPROVE GUT HEALTH AND AMINO ACID ABSORPTION

ABSTRACT

An experiment was conducted to test the hypothesis that dietary inclusion of isoquinoline alkaloids (IQ) to corn-soybean meal diets improves growth performance, blood characteristics, intestinal morphology, and indicators of intestinal health of weanling pigs. A total of 160 pigs (6.33 ± 0.61 kg) were allotted to 4 corn-soybean meal based treatments with 4 pigs per pen and 10 replicate pens per treatment. A 3-phase feeding program was used with d 0 to 8 as phase 1, d 8 to 21 as phase 2, and d 21 to 34 as phase 3. Within each phase, the 4 diets were identical except for the inclusion of IQ in the feed: 0, 90, 180, or 360 mg/kg IQ. Pig weights and the feed left in the feeders were recorded on the last d of each phase to calculate average daily feed intake (ADFI), average daily gain (ADG), and average gain:feed ratio (G:F). A blood sample was collected from 1 pig per pen on d 8, 21, and 34. Tissue samples from the ileum and jejunum were collected on d 34 to evaluate intestinal histology and ileal mucosa samples were collected to determine concentrations of secretory immunoglobulin A (SIgA). Data were analyzed by linear and quadratic contrasts. Results indicated that there were no effects of IQ on the overall growth performance; however, ADFI quadratically ($P < 0.05$) decreased in phase 1 and linearly decreased ($P < 0.05$) in phase 2. Additionally, ADG quadratically decreased during phase 1 and G:F quadratically increased ($P < 0.05$) in phase 3. Plasma urea nitrogen tended to increase in phases 2 and 3 (linear; $P < 0.10$) and total protein quadratically increased ($P < 0.05$) in phase 1 if IQ was added to the diet. In the ileum, lamina propia thickness and crypt depth quadratically decreased ($P < 0.05$) with the greatest response in the tissues of pigs fed the 180 mg/kg IQ diet.

There was also a tendency for the villus height:crypt depth ratio to increase (linear; $P < 0.10$) if IQ was added to the diet. As increasing concentrations of IQ were added to the diets, neutrophil infiltration tended to increase in the jejunum and decrease in the ileum (quadratic; $P < 0.10$) with the greatest response in the tissues of pigs fed diets containing 180 mg/kg IQ. Results indicate that IQ inclusion in diets fed to weanling pigs may improve intestinal health and AA absorption, with the greatest effect observed in the pigs fed the 180 mg/kg IQ diet.

Key words: growth performance, intestinal morphology, isoquinoline alkaloids, neutrophils, pigs

INTRODUCTION

Sangrovit® Extra, derived from *Macleaya cordata*, is a phytobiotic feed additive composed of isoquinoline alkaloids (IQ; Phytobiotics Futterzusatzstoffe GmbH, Eltville, GE). Isoquinoline alkaloids may improve gain:feed ratio (G:F; Vieira et al., 2008b; Khadem et al., 2014; Lee et al., 2015), increase body weight (BW; Vieira et al., 2008b; Khadem et al., 2014; Lee et al., 2015), and enhance overall gain (Lee et al., 2015) of broiler chickens. In weaned pigs, inclusion of IQ in the diet led to increased BW, increased average daily feed intake (ADFI), and improved G:F (Robbins et al., 2013; Kantas et al., 2015). Sanguinarine addition to diets for growing pigs increased average daily gain (ADG), ADFI, and G:F, and resulted in a greater concentration of serum amino acids such as Ile, Leu, and Met (Liu et al., 2016a). Proposed mechanisms of action include inhibiting nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) activation (Chaturvedi et al., 1997), reducing neutrophil viability (Agarwal et al., 1991), and inhibiting cell division of gram positive and gram negative bacteria (Walker, 1990), as well as improving intestinal barrier function (Robbins et al., 2013; Liu et al., 2016b).

Whereas a number of growth performance experiments with broiler chickens have been conducted, there are limited data on effects of IQ on growth performance parameters of weanling pigs. Therefore, it was the objective of this experiment to test the hypothesis that inclusion of IQ to corn-soybean meal diets improves growth performance and health status of weanling pigs.

MATERIALS AND METHODS

The protocol for the experiment was submitted to the Institutional Animal Care and Use Committee at the University of Illinois and approved prior to initiation of the experiment. Pigs that were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN) were used in this experiment.

Diets, Animals, and Experimental Design

The experiment was conducted for 5 wk, starting at weaning. A 3-phase feeding program was used with d 0 to 8 as phase 1, d 8 to 21 as phase 2, and d 21 to 34 as phase 3. Pigs were fed one of 4 diets during all phases; therefore, a total of 12 diets were formulated (Tables 5.1 and 5.2). Within each phase, there were 4 corn-soybean meal based diets with 0, 90, 180, or 360 mg/kg IQ included. Vitamins and minerals were included in all diets to meet requirement estimates and diets were formulated to meet nutrient requirements (NRC, 2012).

A total of 160 pigs were used (initial BW: 6.33 ± 0.61 kg). There were 4 treatments and 10 replicate pens per treatment for a total of 40 pens and 4 pigs per pen. Pigs were blocked by weight, and sex was balanced within pen. Individual pig weights were recorded at the beginning of the experiment and at the conclusion of each phase. Daily feed allotments were recorded and feed left in the feeders was weighed at the end of each phase to calculate feed consumption. Pigs

were checked daily for general condition, and the room temperature, lighting, water, and feeders were checked as well. Unanticipated events were recorded. If a pig was removed from a pen during the experiment, the feed left in the feeder and individual pig weights were recorded on the day the pig was removed and data for feed intake and G:F for the remaining pigs in the pen were adjusted for the feed consumed by the pig that was removed as described by Lindemann and Kim (2007). Data were summarized to calculate ADG, ADFI, and G:F for each treatment group.

Sample Collection

At the beginning of phase 1, the pig in each pen with the BW closest to the pen average was identified, with sex balanced within treatment. At the end of phases 1, 2, and 3, one blood sample was collected from the jugular vein of this pig in vacutainers containing lithium heparin and centrifuged at 4,000 × g for 13 min to recover the plasma. Plasma samples were stored at -20˚C until analysis.

On the last day of phase 3, the pig selected at the beginning of phase 1 was euthanized via captive bolt stunning. The pH of the stomach, ileum, and cecum was recorded using the in situ method described by Morgan et al. (2014). Immediately postmortem, a pH electrode was inserted into the digesta in the lumen of the stomach, ileum, and cecum while ensuring that the pH electrode did not touch the wall of the organs and the pH value was recorded. Each measurement was conducted in duplicate.

Chemical Analyses

Diets were analyzed in duplicate for dry matter (method 930.15; AOAC Int., 2007) and ash (method 942.05; AOAC Int., 2007); for crude protein by measuring N (method 990.03; AOAC Int., 2007) using a Leco Nitrogen Determinator (model FP628, Leco Corp., St. Joseph, MI), and for gross energy using an isoperibol bomb calorimeter (Model 6400, Parr Instruments,

Moline, IL). Acid detergent fiber and neutral detergent fiber components were analyzed using Ankom Technology method 12 and 13, respectively (Ankom 2000 Fiber Analyzer, Ankom Technology, Macedon, NY). Total acid hydrolyzed ether extract was analyzed by acid hydrolysis using 3N HCl (AnkomHCl, Ankom Technology, Macedon, NY) followed by crude fat extraction using petroleum ether (AnkomXT15, Ankom Technology, Macedon, NY). The above analyses were conducted at the University of Illinois. Diets were also analyzed for amino acids on an amino acid analyzer (model L8800 Hitachi Amino Acid Analyzer, Hitachi High Technologies America Inc., Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard at the University of Missouri Agricultural Experiment Station Chemical Laboratories. Before analysis, samples were hydrolyzed with 6N HCl for 24 h at 110˚C (method 982.30 E(a); AOAC Int., 2007). Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis (method 982.30 E(b); AOAC Int., 2007). Tryptophan was determined after NaOH hydrolysis for 22 h at 110 ˚C (method 982.30 E(c); AOAC Int., 2007).

The plasma samples collected at the end of each phase were analyzed at the University of Illinois for plasma urea nitrogen (PUN), albumin, and total protein using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA).

Intestinal Morphology

Jejunum and ileum samples between 2 and 3 cm long were collected approximately 2 m from the pylorus and 80 cm from the ileal-cecal junction, respectively. Samples were cut at the mesenteric side and pinned with the serosa side down on a piece of cardboard (Nabuurs et al., 1993). Samples were then fixed in 10% neutral buffered formalin until processing. After fixation, each sample was cut in 2-3 mm thick cross-sections and embedded in paraffin for slide

preparation. From each sample, 3 to 4 transverse sections were selected and stained with hematoxylin and eosin. Slides were then scanned using a 2.0-HT NanoZoomer (Hammatsu, Bridgewater, NJ). Ten villi and the associated crypts were measured using NDP.View2 (Hammatsu, Bridgewater, NJ). Villus height was measured from the villus tip to the crypt mouth and the crypts were measured from the crypt mouth to the top of the crypt valley. Villus width was measured at the third top of the villus, and villus width at the bottom was measured at the level of the crypt mouth. The width of the lamina propia was measured at the middle of the villus. Neutrophils were counted on 6 sections per slide and 10 slides per treatment, and expressed as neutrophils/mm2 for each pig (Li et al., 2016; Zhu et al., 2017).

Secretory Immunoglobulin A

Ileal mucosa samples were washed with phosphate-buffered saline (PBS), snap frozen in liquid N, and stored at -80˚C until sample analysis. Mucosa samples were homogenized in a PBS buffer solution containing a protease inhibitor cocktail (SKU, P8340; Sigma-Aldrich, St. Louis, MO) and triton (0.1%) using the Tissue-TearorTM (model 985370; BioSpec Products Inc., Bartlesville, OK). Subsequently, the homogenate was centrifuged at 10,000 × g at 4 ˚C for 15 min and the supernatant was stored in 2 aliquots (200 µl/tube). This supernatant was then analyzed for secretory immunoglobulin A (SIgA) using an ELISA kit according to recommendations from the manufacturer (Bethyl Laboratories Inc., Montgomery, TX). These values were normalized with total protein concentration quantified by a Pierce bicinchoninic acid (BCA) Protein Assay Kit (Thermo Scientific, Woltham, MA).

Calculations and Statistical Analyses

Data were analyzed by ANOVA using the PROC MIXED procedure of SAS in a randomized complete block design with the pen as the experimental unit. The statistical model

included the fixed effect of dietary treatment and the random effect of block. Least square means were calculated for each independent variable. Contrast statements were used with coefficients for unequally spaced treatments being generated using the Proc Interactive Matrix Language (IML) statement in SAS to determine linear and quadratic effects of IQ inclusion in the diets. Statistical significance and tendencies were considered at $P \leq 0.05$ and $0.05 < P \leq 0.10$, respectively.

RESULTS

A quadratic increase ($P < 0.05$) of total protein in plasma was observed in phase 1, with the highest value in the plasma of pigs fed the 180 mg/kg diet (Table 5.3). A tendency for PUN to increase linearly in phases 2 and 3 ($P < 0.10$) if IQ was added to the diet was observed. There was no effect of IQ on the concentration of albumin in the plasma.

There were no differences among treatments in ADG, BW, or G:F over the entire experimental period; however, in phase 1, ADFI, ADG, and G:F quadratically decreased ($P < 0.05$) with IQ inclusion with the least values observed for the 180 mg/kg IQ supplemented diet (Table 5.4). In phase 2, ADFI linearly decreased ($P < 0.05$), whereas G:F increased (quadratic, $P < 0.05$) in phase 3 as IQ was added to the diet with the greatest G:F obtained in diets containing 90 or 180 mg/kg IQ. There was a tendency for ADFI to decrease quadratically over the entire experimental period ($P < 0.10$) as IQ was added to the diets.

There was no influence of IQ inclusion on the villus height or villus width in the jejunum and ileum (Table 5.5). A quadratic decrease ($P < 0.05$) in the crypt depth of the ileum was observed, but there were no differences observed in the crypt depth of the jejunum. There was a tendency for the villus height:crypt depth ratio in the ileum to increase quadratically ($P < 0.10$)

as IQ was added to the diets with the greatest ratio observed in the ileum of pigs fed the 180 mg/kg IQ diet; however, there was no effect of IQ on the villus height:crypt depth ratio in the jejunum. The lamina propia of the ileum decreased in thickness (quadratic; $P < 0.05$) as IQ was added to the diets, with the least thickness observed in the ileum of pigs fed the 180 mg/kg IQ diet. There was no influence of addition of IQ to the diet on the lamina propia thickness of the jejunum. A tendency for a quadratic decrease ($P < 0.10$) in the number of neutrophils/mm2 was observed in the ileum, and there was a tendency for a quadratic increase ($P < 0.10$) in the number of neutrophils/mm2 in the jejunum. No effect of IQ was observed in the pH levels of the stomach, ileum, or colon of pigs fed the 4 experimental diets, nor was there an effect of IQ on the SIgA concentration in the ileal mucosa (Table 5.6).

DISCUSSION

The overall lack of effects of IQ on growth performance parameters in this experiment is in agreement with some previous data (Robbins et al., 2013; Estrada-Angulo et al., 2016). In contrast, IQ supplementation has improved ADG, ADFI, and G:F in other experiments with pigs (Kantas et al., 2015; Liu et al., 2016a; Chen et al., 2018; Goodarzi Boroojeni et al., 2018) and poultry (Khadem et al., 2014; Lee et al., 2015; Vieira et al., 2008a; Vieira et al., 2008b). It is not known why there was no effect on growth performance in this experiment; however, the pigs used in this experiment were of high health status, which may be the reason IQ had less impact on growth performance.

The increased total protein in plasma during phase 1 is consistent with the increased AA digestibility and absorption observed with addition of IQ to diets fed to pigs in previous experiments (Liu et al., 2016a; Goodarzi Boroojeni et al., 2018; Rundle and Stein, 2018). This

increase in total protein in plasma may also be the reason for the increased G:F in phase 3. Inclusion of IQ at 180 mg/kg increased AA absorption, and thereby reducing the need for feed intake with a subsequent improvement in G:F.

The tendency for PUN to increase in phases 2 and 3, even with reduced ADFI, may indicate that the increased AA absorption may not be the same for all amino acids - thus leading to an imbalance of AA utilization and efficiency. Alternatively, it is possible that IQ increases absorption of non-amino N as indicated by the tendency for increased apparent ileal digestibility of crude protein of diets containing IQ fed to young growing pigs (Goodarzi Boroojeni et al., 2018; Rundle and Stein, 2018). Likewise, ruminal N efficiency and postruminal total tract digestion of N was improved in steers fed diets supplemented with IQ through increased flow of non-amino N (Aguilar-Hernandez et al., 2016).

The lack of an effect on plasma albumin concentration is in accordance with results of previous studies (Kosina et al., 2003; Rawling et al., 2009; Abudabos et al., 2016) where it was observed that IQ supplementation did not influence serum albumin, total protein, and glucose. In contrast, Bavarsadi et al. (2017) observed that IQ in the diet reduced the serum albumin concentration in laying hens.

The reduction in crypt depth and increase in villus height to crypt ratio that was observed if IQ was added to the diet indicates that there is less tissue damage and possibly less inflammation in the intestine. The villi that cover the enterocytes in the small intestine are the primary absorptive structures in the small intestine. In contrast, the crypt primarily serves as the location for stem cell proliferation and differentiation into secretory, immune, and absorptive cells that migrate up the villus. Because reduction in crypt depth was only observed in the ileum, it is possible that there is an effect of IQ on the microbial population, which is more abundant in

the distal section of the small intestine when compared with the jejunum. This possible influence of IQ on the microbial population may indirectly cause the reduction in crypt depth. A decrease in crypt depth in both the jejunum and ileum and an increase in the villus height: crypt depth ratio in the ileum of broilers challenged with *Salmonella enteritidis* and supplied drinking water containing IQ was also reported (Pickler et al., 2013) and the present results are in agreement with these observations. Dietary IQ supplementation also decreased crypt depth and increased the villus height: crypt depth ratio in the jejunum of laying hens (Bavarsadi et al., 2017).

The reduction in lamina propia thickness in the ileum observed in this experiment further supports the hypothesis that IQ may reduce or modulate microbes in the ileum. The lamina propia, the supportive structure within the mucosa layer of the intestine, contains lymphocytes that release immune cells upon exposure to pathogens. Lymphocyte hyperplasia is common in cases of inflammation and infection (Yantiss and Antonioli, 2009), and the lamina propia thickens as a result of inflammation. The observed reduction in lamina propia thickness may, therefore, be an indication of reduced inflammation in the intestinal tract of pigs fed diets containing IQ. A reduced lamina propia may also result in increased amino acid and glucose absorption, which was previously observed (Rundle and Stein, 2018). A thinner lamina propia is easier for nutrients to pass through and also reduces the need for maintenance of nutrients, which may also explain the increase in PUN observed in phases 2 and 3.

Neutrophils contain proteases and reactive oxygen species as a first line of defense for the body against pathogens (Wright et al., 2010). The tendency for a reduction in neutrophil infiltration in the ileum of pigs fed diets containing IQ further indicates that inflammation is reduced if IQ is added to diets fed to weanling pigs. Previous data indicate that IQ reduced heterophil percentage in circulating blood serum of laying hens (Bavarsadi et al., 2017), reduced

neutrophil infiltration in the ruminal epithelium of ewes (Estrada-Angulo et al., 2016), and had no influence on neutrophil infiltration in the ileum of pigs (Robbins et al., 2013).

To the best of our knowledge, there are no published data indicating the effects of IQ on the SIgA concentration in ileal mucosa. However, the present findings indicate that there is no impact of IQ on SIgA in the ileum. The lack of effects of IQ on the pH of the stomach, ileum, and cecum indicate that IQ does not improve intestinal health through a change in pH; rather, it may influence the microbiota with antibacterial properties (Walker, 1990; Kosina et al., 2010), or it may reduce inflammation through disruption of a number of immuno-modulatory processes (Agarwal et al., 1991; Chaturvedi et al., 1997) and improvement of intestinal barrier function (Robbins et al., 2013). However, because these parameters were not measured in the present experiment, we are unable to confirm the exact mechanisms by which IQ improves intestinal health.

Conclusions

Supplementation of IQ to corn-soybean meal diets improves intestinal health of weanling pigs as evidenced by the reduction in crypt depth and lamina propia thickness, increase in villus height: crypt depth ratio, and the decrease in neutrophil infiltration in the ileum with the greatest response observed in pigs fed the 180 mg/kg IQ diet. As a result, AA absorption may increase, as indicated by the increase in total protein in plasma and plasma urea nitrogen. Because this improved AA absorption had no effect on overall growth performance under the conditions of this experiment, further research needs to be conducted to investigate the effects of IQ with health challenged pigs or in a more commercial environment.

TABLES

Table 5.1. Ingredient composition (as-is basis) of experimental diets

Item, %	Isoquinoline alkaloids, mg/kg											
	Phase 1				Phase 2				Phase 3			
	-	90	180	360	-	90	180	360	-	90	180	360
Corn	38.75	38.35	37.95	37.15	47.05	46.65	46.25	45.45	49.05	48.65	48.25	47.45
Soybean meal, 48% CP[1]	25.00	25.00	25.00	25.00	22.00	22.00	22.00	22.00	35.00	35.00	35.00	35.00
HP 300[2]	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	-	-	-	-
Soybean oil	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	2.50	2.50	2.50	2.50
Dicalcium phosphate	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55
Limestone	0.90	0.90	0.90	0.90	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
L-Lysine HCl	0.60	0.60	0.60	0.60	0.50	0.50	0.50	0.50	0.20	0.20	0.20	0.20
L-Threonine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	-	-	-	-
DL-Methionine	0.20	0.20	0.20	0.20	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Whey powder	20.00	20.00	20.00	20.00	15.00	15.00	15.00	15.00	10.00	10.00	10.00	10.00
IQ[3] premix	-	0.40	0.80	1.60	-	0.40	0.80	1.60	-	0.40	0.80	1.60
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

Table 5.1. (cont.)

Vit-mineral premix[4]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30

[1] CP = Crude protein.

[2] Hamlet Protein, Denmark.

[3] IQ = Isoquinoline alkaloids, Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany.

[4] The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2,210 IU; vitamin E as selenium yeast, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin,6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; $_{D\text{-}}$pantothenic acid as $_{D\text{-}}$calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

Table 5.2. Analyzed nutrient composition (as is basis) of experimental diets

Item	Isoquinoline alkaloids, mg/kg											
	Phase 1				Phase 2				Phase 3			
	-	90	180	360	-	90	180	360	-	90	180	360
Dry matter, %	90.08	90.06	90.55	89.95	90.19	90.15	89.73	89.95	88.72	88.85	88.65	88.67
Ash, %	6.78	7.21	6.69	6.79	6.07	6.13	6.10	6.16	5.96	6.07	6.08	6.23
Crude protein, %	22.02	23.43	21.77	21.33	19.42	20.47	20.71	20.07	20.65	21.00	22.12	21.95
Gross energy, kcal/kg	4,060	4,071	4,085	4,053	4,052	4,095	4,073	4,089	3,991	3,996	3,999	3,950
Neutral detergent fiber, %	5.53	5.76	5.25	5.90	6.19	5.21	6.13	5.69	6.09	6.82	6.54	6.18
Acid detergent fiber, %	2.13	2.31	2.17	2.49	2.23	2.03	2.36	2.34	2.23	2.52	2.74	2.46
AEE[1], %	5.60	5.17	4.37	4.24	4.55	4.51	4.91	4.81	4.73	4.93	5.04	5.12
Indispensable AA[2], %												
Arg	1.32	1.39	1.31	1.24	1.30	1.24	1.30	1.28	1.47	1.28	1.23	1.29
His	0.53	0.56	0.53	0.50	0.52	0.52	0.54	0.54	0.59	0.52	0.52	0.53
Ile	0.99	1.03	0.97	0.91	0.89	0.94	0.97	0.96	1.05	0.92	0.92	0.95
Leu	1.75	1.81	1.74	1.66	1.69	1.71	1.76	1.75	1.90	1.67	1.70	1.72
Lys	1.68	1.81	1.67	1.71	1.58	1.53	1.62	1.62	1.55	1.41	1.38	1.38
Met	0.47	0.49	0.48	0.43	0.36	0.36	0.38	0.39	0.39	0.38	0.36	0.42

Table 5.2. (cont.)

Phe	1.01	1.06	1.01	0.95	0.97	0.99	1.02	1.01	1.12	0.97	0.98	1.00
Thr	1.02	1.09	1.02	0.97	0.96	0.96	0.96	0.95	0.87	0.78	0.77	0.79
Trp	0.27	0.23	0.26	0.23	0.21	0.24	0.27	0.22	0.24	0.20	0.22	0.21
Val	1.04	1.08	1.02	0.96	0.96	0.99	1.03	1.02	1.11	0.98	0.98	1.01
Dispensable AA[2], %												
Ala	0.99	1.02	0.99	0.95	0.98	0.97	1.01	1.00	1.08	0.96	0.97	0.98
Asp	2.23	2.35	2.22	2.10	2.09	2.11	2.17	2.16	2.39	2.09	2.07	2.14
Cys	0.33	0.36	0.35	0.33	0.32	0.33	0.33	0.36	0.36	0.32	0.31	0.33
Glu	3.70	3.85	3.68	3.49	3.53	3.56	3.72	3.68	4.06	3.53	3.54	3.62
Pro	1.18	1.21	1.16	1.11	1.16	1.16	1.20	1.19	1.29	1.13	1.15	1.16
Ser	0.89	0.92	0.89	0.86	0.88	0.83	0.86	0.87	0.94	0.83	0.82	0.82
Tyr	0.72	0.76	0.72	0.69	0.71	0.65	0.67	0.67	0.77	0.69	0.62	0.68

[1] AEE = Acid hydrolyzed ether extract.

[2] AA = Amino acids.

Table 5.3. Effects of isoquinoline alkaloids on blood parameters of young growing pigs

Item	Isoquinoline alkaloids, mg/kg					*P*-value	
	0	90	180	360	SEM	Linear	Quadratic
PUN[1], mg/dL							
d 8	12.10	12.25	11.40	10.20	1.21	0.158	0.736
d 21	5.56	4.40	6.70	6.60	0.65	0.066	0.927
d 34	8.80	9.70	10.40	11.30	1.22	0.066	0.744
Total protein, g/dL							
d 8	4.75	4.78	4.98	4.57	0.11	0.227	0.030
d 21	4.65	4.50	4.56	4.59	0.75	0.840	0.280
d 34	4.93	4.92	4.87	4.97	0.18	0.767	0.565
Albumin, g/dL							
d 8	3.11	3.16	3.13	2.91	0.14	0.146	0.324
d 21	2.54	2.49	2.46	2.51	0.06	0.791	0.386
d 34	2.69	2.86	2.60	2.79	0.08	0.764	0.606

[1] PUN = Plasma urea nitrogen.

Table 5.4. Growth performance of pigs fed isoquinoline alkaloid supplemented diets

Item	Isoquinoline alkaloids, mg/kg				SEM	*P*-value	
	0	90	180	360		Linear	Quadratic
ADFI[1], g							
d 0 to 8	134	114	107	124	0.02	0.584	0.029
d 8 to 21	457	416	405	404	0.02	0.034	0.099
d 21 to 34	839	873	848	869	0.03	0.575	0.861
d 0 to 34	526	520	483	511	0.02	0.317	0.099
ADG[2], g							
d 0 to 8	74	46	29	40	0.01	0.037	0.020
d 8 to 21	318	322	305	302	0.01	0.314	0.964
d 21 to 34	567	599	563	585	0.02	0.749	0.992
d 0 to 34	355	363	338	351	0.01	0.577	0.562
G:F[3]							
d 0 to 8	0.53	0.42	0.34	0.41	0.06	0.138	0.049
d 8 to 21	0.71	0.78	0.76	0.76	0.50	0.405	0.156
d 21 to 34	0.65	0.69	0.69	0.67	0.01	0.323	0.029
d 0 to 34	0.66	0.69	0.67	0.68	0.02	0.748	0.413
BW[4], kg							
d 0	6.32	6.32	6.34	6.33	0.39	0.988	0.933
d 8	6.85	6.68	6.58	6.74	0.47	0.647	0.181
d 21	11.00	10.88	10.53	10.97	0.40	0.262	0.362
d 34	18.39	18.66	17.83	17.82	0.55	0.230	0.915

[1] ADFI = Average daily feed intake.

[2] ADG = Average daily gain.

[3] G:F = Gain:feed ratio.

[4] BW = Body weight.

Table 5. Effects of isoquinoline alkaloids on the morphology of the jejunum and ileum of young growing pigs at d 34

Item	Isoquinoline alkaloids, mg/kg					*P*-value	
	0	90	180	360	SEM	Linear	Quadratic
Ileum							
Villus height, μm	303.18	325.73	341.46	324.77	20.86	0.461	0.244
Villus width top, μm	78.40	76.69	77.63	79.06	3.80	0.800	0.703
Villus width bottom, μm	158.36	160.94	154.41	154.11	6.57	0.524	0.999
Crypt depth, μm	197.37	170.09	162.20	187.92	12.93	0.713	0.003
Villus height:crypt depth ratio	1.88	2.09	2.29	2.02	0.20	0.561	0.085
Lamina propia thickness, μm	61.89	59.80	51.45	60.01	3.24	0.535	0.033
Neutrophils, cells/mm^2	459.83	407.17	387.83	508.83	43.26	0.310	0.063
Jejunum							
Villus height, μm	407.97	400.68	396.67	372.01	30.61	0.393	0.875
Villus width top, μm	72.711	75.46	71.71	74.52	2.45	0.798	0.832
Villus width bottom, μm	152.02	158.50	141.02	146.62	5.10	0.198	0.583
Crypt depth, μm	181.32	191.09	180.18	191.30	11.23	0.633	0.878

Table 5.5. (cont.)

Villus height:crypt depth ratio	2.53	2.46	2.35	2.36	0.17	0.460	0.655
Lamina propia thickness, μm	47.71	52.26	45.35	47.95	2.46	0.637	0.951
Neutrophils, cells/mm^2	212.95	234.67	289.17	248.32	24.60	0.264	0.089

Table 5.6. Concentration of secretory immunoglobulin A (SIgA) in ileal tissues and pH levels of the stomach, ileum, and colon of pigs fed diets supplemented with isoquinoline alkaloids

Item	Isoquinoline alkaloids, mg/kg					*P*-value	
	0	90	180	360	SEM	Linear	Quadratic
SIgA, ug/mg of protein	3.74	3.32	3.84	4.19	0.5441	0.354	0.615
pH							
Stomach	2.98	2.79	2.68	2.64	0.33	0.419	0.685
Ileum	6.80	6.88	6.84	6.56	0.14	0.147	0.292
Cecum	5.74	5.75	5.86	5.71	0.09	0.860	0.315

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CHAPTER 6: CONCLUSIONS

Results from this work indicate that supplementing corn-soybean meal diets fed to young growing pigs with 90 mg/kg isoquinoline alkaloids (**IQ**) maximized the apparent ileal digestibility (**AID**) of starch and amino acids under the conditions of this experiment.

Dietary inclusion of IQ in corn-soybean meal or corn-soybean meal-distillers dried grains with solubles diets for young growing pigs appeared to have no effect on the apparent total tract digestibility of gross energy or digestible energy concentration of the diets. As a result, it appears that IQ does not improve energy utilization due to its immunomodulating effects; nor does it have an energy sparing effect.

Intestinal health of weanling pigs was improved by dietary supplementation with IQ to corn-soybean meal diets as evidenced by the reduction in crypt depth and lamina propia thickness, increase in villus height: crypt depth ratio, and the reduction in neutrophil infiltration in the ileum. The greatest response was observed in pigs fed the 180 mg/kg IQ diet. There were no effects of IQ on growth performance; however, amino acid absorption was likely increased through improved intestinal health and function as a result of IQ supplementation.

Overall, IQ may be included in diets at concentrations between 90 and 180 mg/kg as a phytobiotic alternative to antibiotic growth promoters to improve nutrient digestibility of diets fed to weanling pigs as well as to reduce inflammation and improve intestinal health and nutrient absorption in young growing pigs.