ISOQUINOLINE ALKALOIDS IMPROVE AMINO ACID DIGESTIBILITY AND INTESTINAL FUNCTION OF PIGS

BY

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DISSERTATION

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Abstract

Isoquinoline alkaloids (IQ) have been used as phytogenic feed additives to improve growth performance and intestinal health of growing pigs. Therefore, 7 experiments were conducted to test the hypothesis that inclusion of IQ to corn-soybean meal diets improve growth performance and nutrient digestibility by growing and finishing pigs. The second hypothesis was that if amino acids (AA) are included in corn-soybean meal diets at concentrations that do not meet requirements, IQ may ameliorate the negative effects of formulating diets below AA requirements by increasing digestibility of nutrients and by improving intestinal health, which may result in growth performance not being different between pigs fed diets with AA below or at requirements. In experiments 1 and 2, 12 ileal T-cannulated growing and finishing barrows were fed diets that included either 0, 40, 80, or 160 mg IQ/kg feed to determine effects of IQ on apparent ileal digestibility (AID) of nutrients. Results indicated that IQ supplementation increased ($P < 0.05$) AA digestibility by both growing and finishing pigs, with the greatest digestibility values observed in the 40 and 80 mg/kg treatment groups. In addition, an increase ($P < 0.05$) in the AID of CP was observed at an IQ inclusion rate of 40 mg/kg fed to finishing pigs. Experiment 3 utilized 40 growing barrows in metabolism crates that were fed corn-soybean meal diets with either 0, 40, 80, or 160 mg IQ/kg feed to determine effects of IQ on apparent total tract digestibility (ATTD) of energy, fiber, and N and concentrations of digestible energy (DE) and metabolizable energy (ME) in the diets. Results indicated that ME tended to decrease ($P < 0.10$) as IQ was included in the diets. Inclusion of IQ also resulted in a decrease (quadratic, $P < 0.05$) in N retention by pigs, but there was no effect of IQ on the biological value of N. Due to the increase in AA digestibility observed in previous experiments, experiments 4 and 5 were designed to test the hypothesis that inclusion of IQ in diets formulated below AA requirements

may result in increased growth performance, systemic health, and total tract digestibility of energy and N. Experiment 4 was conducted for 56 d and 192 growing pigs were allotted to a 2 × 2 factorial with diets formulated at requirements for indispensable AA or 10% below AA requirements and with either 0 or 90 mg IQ/kg feed. If AA were reduced in the diet, a reduction ($P < 0.05$) in average daily gain (ADG) and gain:feed (G:F) was observed. On d 14, gilts had greater interleukin- (IL-)1β and IL-18 than barrows if no IQ was used, but if IQ was added to the diets, IL-1β and IL-18 were reduced in plasma of gilts compared with barrows regardless of dietary AA concentration (interaction, $P < 0.05$). In experiment 5, 40 growing barrows were housed in metabolism crates and allotted to a 2 × 2 factorial with diets formulated at 5% below requirements for indispensable AA or 8% below AA requirements and with either 0 or 90 mg IQ/kg feed. Intake and retention of N decreased ($P < 0.05$) for pigs fed the diet with AA 8% below requirements compared with pigs fed the diet with AA 5% below requirements. The biological value of N was not affected by IQ inclusion or AA level. The ATTD of gross energy and DE and ME of the diet were unaffected by treatment, but ATTD of P decreased if diets contained IQ ($P < 0.05$). Experiment 6 was conducted to determine effects of dietary IQ and narasin, alone or in combination, when fed to finishing pigs on growth performance and carcass characteristics. Results indicated that ADG and G:F tended to decrease ($P < 0.10$) if IQ was added to the diet. Loin muscle area tended to be reduced ($P < 0.10$) if both IQ and narasin were added to the diet. Minolta L* increased ($P < 0.05$) with inclusion of both narasin and IQ in the diet, indicating a lighter chop compared with the chop of pigs fed the control diet or a diet in which only one of the additives was included. Because IQ increased digestibility of AA in previous experiments, experiment 7 was conducted to test the hypothesis that IQ inclusion in diets formulated below AA requirements for weanling pigs subjected to a health challenge would

improve growth performance and intestinal health. Results indicated that if dietary AA were at the requirement, IQ increased lamina propria thickness, but if dietary AA were below the requirement, IQ inclusion decreased lamina propria thickness (interaction, $P < 0.05$), indicating greater absorptive capacity of the jejunal villi. Inclusion of IQ to diets with AA at requirements decreased IL-4 and IL-10, but if AA were below the requirement, IL- 4 and IL-10 increased if IQ was also included in the diet (interaction, $P < 0.05$). If dietary AA were at requirements, IQ tended to increase occludin abundance in the jejunal mucosa; however if AA were at requirements, IQ reduced occludin abundance (interaction, $P < 0.10$). In conclusion, IQ increase the AID of AA and CP by growing and finishing pigs. Isoquinoline alkaloid inclusion does not affect the ATTD of energy, nor does it affect the DE and ME in diets. Retention of N was reduced by IQ if diets were formulated at AA requirements, indicating that IQ influences N metabolism in addition to increasing digestibility of N-containing compounds by pigs. If AA are reduced in the diet, IQ changes cytokine expression in plasma of weanling and growing pigs, and modulates markers of intestinal function in weanling pigs, indicating that IQ reduces inflammation and improves intestinal function if included in corn-soybean meal diets. Further research is needed to elucidate the effects of IQ on systemic cytokine production and to determine the effects of IQ on function and secretion of digestive enzymes in the small intestine.
Key words: digestibility, growth performance, intestinal health, isoquinoline alkaloids, pig

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CHAPTER 1: Introduction

Antibiotics have been used at sub-therapeutic levels as growth promoters in livestock production since the 1950's due to their ability to improve animal health and growth performance indices (van den Bogaard and Sobberingh, 1999; Gaskins et al., 2002; Dibner and Richards, 2005; Thacker et al., 2013). However, due to concerns over antibiotic resistance in livestock and humans, restrictions and regulations have been put into place to limit the use of sub-therapeutic antibiotic supplementation in the European Union, South Korea, China, and the United States (Maron et al., 2013). Proposed alternatives to antibiotic growth promoters include feed additives such as acidifiers, essential oils, plant extracts, herbs, minerals, enzymes, pre- and probiotics, and yeast (Close, 2000; Thacker, 2013; Gleeson and Collins, 2015). Optimized gastrointestinal health is important for effective nutrient digestibility and subsequent use of nutrients for growth and maintenance of the animal. Consequently, it is of great importance to improve gastrointestinal health of livestock to keep production costs low and increase rate of growth. Plant derived feed additives such as isoquinoline alkaloids (**IQ**) may be used for this purpose.

Isoquinoline alkaloids are derived from plant extracts and have antimicrobial and immunomodulatory effects. Specifically, IQ inhibit NF-kB (Chaturvedi et al., 1997), which is a transcription factor for pro-inflammatory cytokines (Neutra et al., 2003), indicating that IQ reduces an inflammatory response. Additionally, IQ may have antimicrobial effects (Kosina et al., 2010). When added to diets fed to pigs, IQ increased transepithelial resistance (Robbins et al. 2013), reduced expression of inflammatory cytokines *in vitro* (Soler et al., 2016), and decreased expression of inducible NO synthase genes (Khadem et al., 2014).

When used as a feed additive in livestock production, isoquinoline alkaloids improve apparent ileal digestibility of nutrients in weanling pigs (Boroojeni et al., 2018; Rundle et al.,

2020). Isoquinoline alkaloids also increase the digestible energy and net energy of diets fed to ruminants (Aguilar-Hernandez et al., 2016; Estrada-Angulo et al., 2016). Growth performance parameters including average daily gain, average daily feed intake, and gain:feed ratio of growing pigs are improved when IQ is added to the diet (Robbins et al., 2013; Kantas et al., 2015; Liu et al., 2016). However, there are no published data to indicate the effects of IQ on nutrient digestibility in growing and finishing pigs, nor are there data reflecting effects of IQ when added to diets formulated below nutrient requirements. Therefore, it was the objective of this dissertation to test 4 hypotheses 1) IQ increases the apparent ileal and apparent total tract digestibility of nutrients and energy when supplemented to corn-soybean meal diets formulated at or below amino acid requirements for growing and finishing pigs; 2) growth performance of growing and finishing pigs is improved by supplementing IQ to corn-soybean meal diets formulated at or below amino acid requirements; 3) carcass characteristics of finishing pigs are improved when pigs are fed corn-soybean meal diets supplemented with IQ, alone or in combination with another feed additive; 4) intestinal health and growth performance of nursery pigs fed corn-soybean meal diets below amino acid requirements in challenging environmental conditions is improved when IQ is added to the diet.

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CHAPTER 2: Use of non-antibiotic growth promoters in swine nutrition: A review of the literature

Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
AGP	antibiotic growth promoters
AID	apparent ileal digestibility
BW	body weight
DE	digestible energy
GE	gross energy
G:F	gain:feed
IL-	interleukin-
INF-	interferon-
IQ	isoquinoline alkaloids
ME	metabolizable energy
TNF	tumor necrosis factor-

Antibiotic Usage in Swine Production

Antimicrobial growth promoters (**AGP**) have been used in livestock production to promote growth and improve feed efficiency since the 1950's (Dibner and Richards, 2005). Used at subtherapeutic levels, AGP maximize growth potential of the animal and improve feed efficiency (van den Bogaard and Sobberingh, 1999; Gaskins et al., 2002; Thacker, 2013). These AGP are needed, often during the post-weaning period in swine production, to offset the negative effects

that weaning has on the young pig. There are a variety of stressors during the weaning period, including environmental and dietary changes, which often results in sub-optimal growth for the pig (Pluske et al., 1997; Williams, 2003; Lalles et al., 2004). During the growing and finishing phases of swine production, AGP are also used, mostly to improve feed efficiency through increased nutrient absorption and utilization.

The main mechanism of action for AGP is to regulate intestinal microbial populations and thereby maximize nutrient absorption in the intestine because of reduced use of nutrients by the microbiota, reduced infections caused by microbial dysbiosis, reduced concentration of metabolites produced by the microbiota, and enhanced uptake of nutrients due to improved gut function (Visek, 1978; Anderson et al., 1999; Gaskins et al., 2002). Antibiotics used as growth promotants have physiological, nutritional, and metabolic effects (Commission on Antimicrobial Feed Additives, 1997; Gaskins et al., 2002). Effects include increases in feed intake and nutrient absorption, energy and nutrient retention, mineral absorption, liver protein synthesis, and synthesis of gut alkaline phosphatase. Reductions observed as a result of antibiotic supplementation include decreased food transit time, ammonia production, fatty acid oxidation, and energy loss (Commission on Antimicrobial Feed Additives, 1997; Gaskins et al., 2002).

According to the World Health Organization, there is a concern for a negative impact of AGP use in animal production on human health (1997) and legislation has been passed to restrict the use of AGP in livestock production to minimize the resistance of bacteria to antibiotics in the future. In the European Union, use of AGP in livestock production has been banned since 2006. The United States Food and Drug Administration initiated a Veterinary Feed Directive indicating that drugs critical for maintaining human health may only be allowed in animal production under the supervision of a licensed veterinarian (FDA, 2013). Consequently, it is imperative that

researchers investigate the use of alternative methods to improve feed efficiency and intestinal health in livestock.

Alternatives to AGP in livestock feed include acidifiers, enzymes, herbs, essential oils, plant extracts, minerals, prebiotics, probiotics, yeast, carbohydrates including oligosaccharides and non-starch polysaccharides, bacteriophage therapy, and immunopotentiators (Close, 2000; Thacker, 2013; Gleeson and Collins, 2015). Management and husbandry techniques may also be improved through changes in biosecurity protocols, air quality, temperature, and production flow to enhance growth potential and performance in swine (Kil and Stein, 2010; Gleeson and Collins, 2015). All these potential alternatives need to be investigated, singularly and combined, to determine the appropriate methods to promote growth and increase intestinal and overall health in swine to the full extent.

Isoquinoline Alkaloids

Mechanisms of action

Isoquinoline alkaloids (**IQ**) are derived from *Macleaya cordata*, or the plume poppy, and primarily include sanguinarine, chelerythrine, protopine, and allocryptopine (Figure 2.1; Kosina et al., 2010). These alkaloids act as anti-inflammatory and immunomodulatory agents and have some antimicrobial actions. Isoquinoline alkaloids may also be derived from other plants including *Argemone mexicana*, another type of poppy plant (Capasso, et al., 1997), or from *Sanguinara canadensis*, otherwise known as bloodroot. Sanguinarine is primarily metabolized in the cytosol of gastrointestinal cells or in the liver into dihydrosanguinarine to reduce the iminium bond of the structure (Wu et al., 2013; Wu et al., 2020).

A proposed mechanism of action for sanguinarine is to inhibit nuclear factor kappa-light -chain-enhancer of activated B cell activation (Chaturvedi et al., 1997), which is a nuclear transcription factor that primarily regulates expression of cytokines, major histocompatibility complex genes, and genes involved in inflammation, cell proliferation, and viral replication (Van Antwerp et al., 1996; Chaturvedi et al., 1997). When activated, the release of cytokines and chemokines results in systemic inflammation, including an increase in acute-phase proteins in the liver and the activation of nitric oxide synthase (Artuso-Ponte et al., 2020). Additionally, *Macleaya cordata* extract has been shown to reduce inflammatory response, specifically reducing IL-1β and TNFα, to adherent enterotoxigenic *E. coli* and non-adherent *E. coli* in an *in vitro* study (Soler et al., 2016), whereas results of other experiments have indicated an increase in IL-1β and a decrease in inducible NO synthase gene expression in the jejunum of chickens as *Macleaya cordata* extract was added to the diet (Khadem et al., 2014). When supplemented in sow diets, IQ increased the immunoglobulin G concentration in colostrum, a vital component to transfer passive immunity from the sow to the pig (Sureda et al., 2021). In human hepatocytes, sanguinarine increases heme-oxygenase-1(Vrba et al., 2012), which is an enzyme involved in heme catabolism to form carbon monoxide and as a result, IL-10 and IL-1RA expression is upregulated (Piantadosi et al., 2011). Sanguinarine also inhibits NADPH oxidase activity (Vrba et al., 2004), inhibits amino acid decarboxylase (Drsata et al., 1996), and induces cell apoptosis (Malikova et al., 2006).

The other alkaloids included in IQ are chelerythrine, protopine, and allocryptopine. Protopine and allocryptopine reduce inflammation and blood clotting (Teng et al., 1991), which have antimicrobial effects (Kosina et al., 2010). Allocryptopine may inhibit K current in myocardium (Fu et al., 2016) and protopine may inhibit K(ATP) channels (Jiang et al., 2004).

Chelerythrine inhibits protein kinase C (Herbert et al., 1990), a protein involved in the expression of tight junction proteins and Toll-like receptor 2 pathway (Cario et al., 2004; Farhadi et al., 2006). The anti-inflammatory, immunomodulatory, and antimicrobial actions of these alkaloids all have the potential to improve gastrointestinal health and subsequently enhance growth performance of livestock species.

Digestibility

Published data on the impact of IQ on nutrient digestibility in livestock are limited, but IQ does not affect the digestibility of organic matter, neutral detergent fiber, or starch when supplemented to diets for steers (Aguilar-Hernandez, 2016). In contrast, IQ supplementation increased the flow rate of non-ammonia N, decreased the flow of ammonia N, and linearly increased postruminal digestion of N as IQ increased in the diet, indicating improved N utilization (Aguilar-Hernandez, 2016). Isoquinoline alkaloids did not influence digestibility of ether extract when supplemented to diets for weanling and growing pigs; however, the digestibility of individual amino acids including Asp, Glu, His, Leu, Met, Val, and total AA increased and a trend for an increase in the pre-cecal digestibility of crude protein, Ala, Cys, Ile, Phe, and Thr was observed when IQ was supplemented at 120 mg/kg feed (Boroojeni et al., 2018). Similarly, IQ increased the apparent ileal digestibility (**AID**) of Thr, Trp, Val, Pro, Tyr, and starch in weanling pigs fed corn-soybean meal diets with the greatest digestibility observed if 90 or 180 mg/kg feed IQ was included in diets (Rundle et al., 2020).

Digestible energy (**DE**) tended to increase as IQ increased in high-energy diets fed to steers (Aguilar-Hernandez, 2016), and ewes fed diets containing 0.50 g/d IQ had increased concentrations of dietary net energy (Estrada-Angulo et al., 2016). When fed to young growing pigs, IQ supplementation to corn-soybean meal or corn-soybean meal-DDGS diets had no impact

on apparent total tract digestibility of gross energy (**GE**), or the concentration of DE; however, a linear decrease in metabolizable energy (**ME**) of corn-soybean meal diets was reported with the greatest ME value in the 90 mg/kg feed IQ supplemented diet (Rundle, 2018).

Growth performance

Isoquinoline alkaloids may improve growth performance through an improvement in intestinal health and subsequent increase in nutrient digestion and absorption. Studies have been conducted to determine the effects of IQ on growth performance of swine, poultry, fish, steers, and ewes.

Inclusion of 50 mg/kg feedIQ resulted in an increase in the body weight (**BW**) and average daily gain (**ADG**) and a greater average daily feed intake (**ADFI**) of pigs compared with pigs fed a control diet, and IQ also improved gain:feed (**G:F**; Kantas et al., 2015). Including IQ at either 1.5 or 0.75 mg/kg feed did not result in differences in feed intake, final BW, or ADG of pigs when compared with a control diet during a 40 d experiment starting at 8.96 kg BW (Robbins et al., 2013). When included at 120 mg/kg feed, IQ supplementation resulted in an increase in ADG during the nursery period, but did not affect feed intake, and therefore, G:F was improved (Boroojeni et al., 2018). Supplementing diets for growing pigs with 40 mg/kg feed IQ resulted in an increase in final BW and ADG during a 14 d experimental period, with concurrent increases in ADFI and G:F, indicating a greater increase in ADG than in ADFI (Liu et al., 2016a).

Broilers fed diets supplemented with either 20 or 50 mg/kg feed IQ had increased final BW and ADG (Lee et al., 2015), and broilers fed the 50 mg/kg diet had increased feed intake and improved G:F from d 22 to 35 compared with broilers fed the control diet (Vieira et al., 2008a; Lee et al., 2015). Combining IQ with organic acids resulted in an increase in BW on d 7 and 14 of a 42 d experiment, whereas BW was improved on d 21 and G:F was improved from d 8 to 14

and for the overall 42 d experimental period in broilers fed diets supplemented with 50 mg/kg feed IQ compared with broilers fed a control diet (Vieira et al., 2008b). Additionally, supplementing broiler diets with *Macleaya cordata* extract, which contains IQ, increased BW on d 21 and 35 compared with birds fed the control diet (Khadem et al., 2014). Broilers under heat stress conditions had greater BW gain and feed intake when supplemented with 100 mg IQ/kg feed compared with broilers that did not receive IQ (Kikusato et al., 2021). Broiler chickens fed 20 or 50 mg IQ/kg feed had lower jejunal weight, increased jejunal and ileal length, but not different organ, abdominal fat, or leg and breast muscle weight compared with broilers fed a control diet (Lee et al., 2015).

Supplementing IQ to diets fed to red tilapia resulted in an increase in weight gain, final BW, and ADFI with the greatest weight gain and final BW observed in the group fed a diet containing 25 mg/kg feed IQ and the greatest ADFI in the 50 mg/kg IQ treatment group (Rawling et al., 2009). When supplemented to Pacific white shrimp infected with *V. parahaemolyticus*, IQ provided in either powdered form at 200 or 300 mg/kg feed or a granular, water-soluble form at either 100 or 150 mg/kg feed increased BW compared with shrimp in the control groups that were not given IQ (Bussabong et al., 2021). Whereas supplementing IQ to diets fed to finishing ewes had no effect on BW or ADG, including 0.5 g/d IQ to the diet resulted in improved G:F, indicating a lower feed intake of ewes under heat stress with no subsequent reduction in gain (Estrada-Angulo et al., 2016).

Intestinal and systemic health

Isoquinoline alkaloids reduce inflammation, regulate the immune system, and are thought to influence the morphology of the gastrointestinal tract in a way that improves digestion and absorption of nutrients. Indeed, pigs supplemented with 1.5 g/kg feed IQ had greater

transepithelial resistance of the ileum, indicating improved intestinal health compared with pigs fed a control diet (Robbins et al., 2013). Similarly, colonic permeability was lower in pigs fed 150 mg/kg IQ in heat stress conditions compared with pigs fed a control diet (Le et al., 2020). In addition, expression of tight junction proteins, specifically zonula occludens-1 and claudin-1, which are essential for maintaining a healthy gut barrier, increased in pigs fed diets supplemented with 40 mg IQ/kg feed compared with pigs fed a control diet (Liu et al., 2016b). Whereas serum fluorescein isothiocyanate-dextran, a marker of intestinal barrier function, increased under heat stress conditions in broilers fed control diets, broilers fed diets containing 100 mg IQ/kg feed did not have increased serum fluorescein isothiocyanate-dextran, further indicating that IQ improves intestinal barrier function (Kikusato et al., 2021).

Broilers challenged with *Salmonella enteritidis* supplemented with 100g/1,000 L sanguinarine in distilled water had reduced villus height in the duodenum, jejunum, ileum, and cecum, and a greater crypt depth in the jejunum and ileum compared with broilers fed a control diet (Pickler et al., 2013). Supplementing diets with 20 mg/kg IQ led to a decrease in villus height of the duodenal tissue and a reduction in small intestinal mass (Jankowski et al., 2009). In contrast, broiler chickens fed diets supplemented with 50 mg/kg feed IQ for 21 d and 35 mg/kg feed IQ for a subsequent 21 days did not have differences in intestinal morphology when compared with birds fed a control diet (Vieira et al., 2008b). Intestinal morphology of pigs fed diets supplemented with 60 or 120 mg/kg feed IQ was also not different compared with pigs fed a control diet (Boroojeni et al., 2018). However, nursery pigs fed a corn-SBM diet supplemented with 180 mg/kg IQ had an increased villus height: crypt depth ratio in ileal tissue (Rundle, 2018).

Alterations in microbiota of the intestine may result in changes to the overall health of the animal (Fouhse et al., 2016). When fed at an inclusion rate of 15 mg/kg feed to male broilers, IQ

supplementation resulted in an increase in ammonia concentration in cecal digesta, along with increased concentrations of propionate, iso-valerate, and valerate in the cecum compared with control fed broilers (Juskiewicz et al., 2011). In contrast, IQ inclusion at 20 mg/kg in broiler diets resulted in decreased SCFA concentrations of cecal contents, indicating that IQ inhibited the fermentation process in the ceca of broiler chickens (Jankowski et al., 2009). In addition, Lee et al. (2015) did not observe differences in total microbes or coli forms in cecal contents of broilers fed IQ compared with control fed broilers, but lactic acid bacteria were increased as a result of 20 mg IQ/kg feed in the diet.

Porcine Gastrointestinal Physiology and Immunology

Gastrointestinal health has many working definitions, but has been described as the 'absence/prevention/avoidance of disease so that the animal is able to perform its physiological functions to withstand exogenous and endogenous stressors' (Kogut and Arsenault, 2016), whereas others define it as a generalized homeostasis of the gastrointestinal tract in both structure and function (Pluske et al., 2017). Indeed, a healthy gastrointestinal tract is ideal for optimal digestion and absorption of nutrients, immune function, and growth performance of livestock animals. Gastrointestinal health is of prime importance when it comes to newly weaned pigs due to the dietary and environmental changes that the pig goes through (Pluske et al., 1997; Pickler et al., 2013; Jayaraman and Nyachoti, 2017), but gut health should be considered through all stages of development.

Immunology

The first line of defense against pathogens involves anatomical barriers including the skin, respiratory epithelium, and intestinal epithelium, and chemical barriers including mucus in the

digestive tract and respiratory system (Murphy and Weaver, 2016). The immune system can be divided into two categories: innate and adaptive. The innate immune system involves immediate responses to pathogens including antigens, bacteria, fungi, and parasites. Innate immune cells respond to pathogens without previous exposure and include macrophages, granulocytes, and natural killer cells (Murphy and Weaver, 2016). The adaptive immune system develops a long term highly specific response after exposure to a pathogen with the development of B cells, or antibodies, and T cells, which function to recognize infected cells and respond accordingly (Murphy and Weaver, 2016).

When innate immune cells detect a pathogen, they will initiate the expression of cytokines and chemokines, which activate and attract other immune cells to the site of infection to destroy the pathogen. Additionally, some pathogens including bacteria and viruses, antioxidant stress, as well as cytokines can activate expression of NF-kB, a pro-inflammatory transcription factor, which upregulates pro-inflammatory cytokines (Neutra et al., 2003). Cytokines can be divided into three main categories: interleukins (**IL**-), interferons (**INF**-), and the tumor necrosis factor superfamily (**TNF**), and can be either pro-inflammatory (Il-6, IL-1β, TNFα) or anti-inflammatory (TGFβ, IL-10; Murphy and Weaver, 2016). Chemokines are named and grouped by their cysteine residues on the amino terminus, CC, CXC, CX3C, and are able to bind multiple ligands per receptor (Murphy and Weaver, 2016). This inflammation results in increased blood flow and loosening of tight junctions to allow immune factors to enter infected tissue. Additionally, phagocytes may engulf and lyse, or kill, the pathogen if it is a bacteria, fungi, or parasite. Granulocytes, which include neutrophils, eosinophils, basophils, and mast cells, are polymorphonuclear cells that infiltrate tissues from systemic circulation and work to destroy pathogens through antimicrobial and toxic functions (Murphy and Weaver, 2016).

The adaptive immune system involves lymphocytes including T cells and B cells, which originate in the thymus and the bone marrow, respectively. T cells are divided into 2 categories: CD4+ T helper cells, and CD8+ cytotoxic T cells (Murphy and Weaver, 2016). The cytotoxic CD8+ cells will lyse or kill infected cells that have a specific antigen presentation, whereas CD4+ T cells license other immune cells to destroy the cell that is presenting a specific antigen (Murphy and Weaver, 2016). T cells also may bring the antigen presenting cells to a lymph node for recognition by B cells. Also referred to as plasma cells, B cells that have the highly specific receptor for that pathogen will clonally expand, creating a plethora of antibodies that are able to mount a defense against the pathogen (Murphy and Weaver, 2016).

Intestinal structure and function

The small intestine is divided into 3 regions: the duodenum, jejunum, and ileum. Enzymes secreted from the brush border membrane are most important in the first two regions, as digestion and absorption mainly occur there (Turner, 2003). The jejunum is the primary location of absorption of monosaccharides, amino acids, and fatty acids, whereas vitamins and minerals are absorbed in the duodenum and early jejunum (Turner, 2003; Kong et al., 2018). Vitamin B_{12}, bile acids, and remaining nutrients are absorbed in the ileum (Kong et al., 2018).

The intestinal mucosa is comprised of a layer of epithelium, a basal membrane, a layer of stroma, the lamina propria, and a layer of smooth muscle (Turner, 2003). Within the mucosa, the lamina propria supports villi, which increase the surface area of the intestinal epithelium 10 times for maximized absorptive potential (Turner, 2003). Nutrients are absorbed through these villi into either capillaries that lead to the hepatic portal vein or lacteals that lead to the thoracic duct (Turner, 2003). In addition to villi, crypts are present within the mucosa, and contain stem

cells that differentiate and proliferate into enterocytes, goblet cells that secrete mucin, and Paneth cells that secrete antimicrobial peptides and proteins (Kong et al., 2018).

Mucosal immunology

Lymphoid tissues are present in the sub-mucosa, and are commonly referred to as mucosa-associated lymphoid tissue or, more specific to the gastrointestinal tract, gut-associated lymphoid tissue (Murphy and Weaver, 2016). These tissues include Peyer's patches and microfold cells along with B and T cells, macrophages, and dendritic cells (Neutra et al., 2003). Peyer's patches are large groups of lymphoid follicles that are the site of B cell differentiation and precursor cells for immunoglobulin A in the intestine (Henry et al., 1970; Craig and Cebra, 1971). Microfold cells are located within Peyer's patches and are commonly used as a site of entry into the mucosa by bacteria and viruses, which results in T-cell independent immunoglobulin A responses that eliminate the pathogens before entering systemic circulation (Neutra et al., 2003).

Immunoglobulin A is a stable antibody that retains activity after passage into the gut (Murphy and Weaver, 2016). Functionally, immunoglobulin A has broad specificity, and can effectively neutralize and clump together pathogens for proper elimination (Murphy and Weaver, 2016). Immunoglobulin A, which is secreted in the mucosa of the intestine, is also referred to as secretory immunoglobulin A, and has mucophilic properties, working to trap pathogens within the mucus layer (Murphy and Weaver, 2016).

Tight junctions and intestinal permeability

The single layer epithelium acts as a selectively permeable barrier and can be infiltrated in two ways: transcellularly and paracellularly. The transcellular pathway of absorption is primarily regulated by selective transporters for nutrients including amino acids, fatty acids, electrolytes, and carbohydrates (Groschwitz and Hogan, 2009). This allows for absorption of nutrients, water,

and electrolytes while also preventing pathogens, antigens, and toxins from infiltrating the host system (Groschwitz and Hogan, 2009).

Paracellular transport is primarily regulated by tight junction proteins and adherens junctions proteins including zonula occludens, tricellulin, occludin, junctional adhesion molecule, cadherins, catenins, and claudins that link adjacent cells (Edelblum and Turner, 2009; Groschwitz and Hogan, 2009). Concentration and regulation of these proteins depend on intestinal compartment, location on the cellular membrane, and location on villi or crypts (Groschwitz and Hogan, 2009). Tight junctions have 2 main functions: to modulate ion selectivity and pore size of the epithelium and to polarize the cells to maintain a Na^+ gradient for nutrient transport (Moeser et al., 2017). Adherens junctions are also crucial for regulation of paracellular transport and form between two connecting cells. The intestinal epithelium will also recognize antigens and secrete cytokines and chemokines, which act to recruit other immune cells for elimination of the foreign pathogen (Moeser et al., 2017).

Digestibility

Nutrient digestibility experiments indicate the availability of a nutrient for the animal to use for growth and maintenance by calculating the amount of a nutrient that is absorbed in sections of the gastrointestinal tract of the animal. Depending on how endogenous losses of nutrients are accounted for, digestibility can be expressed as apparent, standardized, or true digestibility (Stein et al., 2007). Ileal digestibility experiments are conducted by analyzing nutrient concentration in the diet and in the ileal digesta and calculating the difference, whereas total tract digestibility considers dietary intake and fecal output (Adeola, 2001; Stein, 2017).

Ileal digestibility

Amino acids are digested and absorbed in the small intestine of pigs, and as such, determination of digestibility of amino acids and crude protein is most accurate from ileal digestibility trials (Sauer and Ozimek, 1986). These experiments can be performed in a multitude of ways, including insertion of a T-cannula in the distal ileum of the pig (Laplace et al., 1994; Sauer et al., 2000). The T-cannula procedure allows for routine sampling of digesta that is representative of overall ileal digestibility with inclusion of an indigestible marker in the diet (Yin and McCracken, 1996).

Apparent ileal digestibility is the net disappearance of consumed dietary nutrients from the digestive tract proximal to the distal ileum (Stein et al., 2007), and is calculated by comparing the concentration of a nutrient consumed in the feed to the concentration of that same nutrient in the ileal digesta. Apparent ileal digestibility may be used to determine digestibility of nutrients in a mixed diet; however, concerns arise with using AID because AID values obtained in individual ingredients are not always additive in a mixed diet (Stein et al., 2007), as they do not account for ileal endogenous losses of the nutrient. Ileal endogenous losses may be significant factors when considering protein and AA digestibility, as these endogenous losses are synthesized proteins secreted into the intestinal lumen that are not digested and reabsorbed by the animal before reaching the end of the small intestine (Tamminga et al., 1995; Hodgkinson and Moughan, 2000). Ileal endogenous losses are divided into basal and specific losses. Basal losses represent the minimum of a nutrient lost by the animal regardless of diet, whereas specific losses are losses that are specific to the diet being consumed by the animal (Stein et al., 2007). To accommodate for these losses, true ileal digestibility is calculated. True ileal digestibility considers the disappearance of dietary nutrients from the digestive tract proximal to the distal

ileum and is calculated in the same way as AID except that the total ileal endogenous losses are subtracted from the ileal outflow of nutrients (Stein et al., 2007). Standardized ileal digestibility may also be calculated if basal ileal endogenous losses rather than total ileal endogenous losses are used to correct AID values (Stein et al., 2007). Because specific endogenous losses are unique to the diet, standardized ileal digestibility reduces variation in digestibility amongst samples of the same ingredient and is more likely to be additive in a mixed diet (Stein et al., 2007).

Total tract digestibility

Total tract digestibility experiments are used to determine the digestibility of energy, fiber, and minerals such as Ca and P due to the nature of microbial fermentation of nutrients in the large intestine. These values can be influenced by chemical composition of the feed, body weight or physiological stage of the animal, and by method of evaluation (Le Goff and Noblet, 2001). The total collection method involves placement of pigs into individual metabolism crates and an adaptation period to the diet of 4 to 8 days is followed by 4 to 6 days of total fecal collection (Zhang and Adeola, 2017). This is commonly done using the marker to marker approach (Adeola, 2001); however, a time-based approach may also be used in which pigs develop constant fecal output over an extended adaptation period (Zhang and Adeola, 2017). Considering the concentration of a nutrient in the feed as well as in the feces, total tract digestibility of a nutrient is calculated. The index method may also be used to evaluate digestibility of a nutrient by adding a specific concentration of an indigestible compound and a test ingredient to the diet (Zhang and Adeola, 2017). Chromic oxide and titanium dioxide are commonly used indigestible markers in this method. Considering both the concentration of the indigestible marker in the diet and in the feces will result in determination of digestibility of energy or specific nutrients in the

diet (Zhang and Adeola, 2017). However, this approach may need a longer adaptation period to establish a consistent passage rate of both the indigestible compound and the test ingredient (Zhang and Adeola, 2017).

To determine digestibility of energy, as well as DE and ME concentrations, the direct procedure, in which the test ingredient is the sole source of a component in the test diet (Adeola, 2001), or the difference procedure, in which the test ingredient is combined with other ingredients to a test diet, can be used (Zhang and Adeola, 2017). The total collection method is preferred over the index method in experiments determining energy digestibility due to variability in passage rate among animals (Jang et al., 2014; Zhang and Adeola, 2017). Digestible energy and ME concentrations in the diet are calculated by subtracting GE in feces and GE in feces and urine, respectively, from GE in the diet (NRC, 2012).

Ionophores

Ionophores are molecules that have the ability to render cations lipid-soluble to allow them to pass through membranes such as the phospholipid bilayer (Occolowitz et al., 1976). Specifically, narasin is a polyether ionophore that is closely related to, and shares properties with, salinomycin, lasalocid, and monensin (Weppelman et al., 1977; Berg and Hamill, 1978). *In vitro*, narasin reduces gram-positive bacteria, anaerobic bacteria, and fungi cell growth (Berg and Hamill, 1978). Thus, narasin has potential to be included in diets fed to livestock to replace conventional AGP for growth promoting effects. Ionophores have been used to improve feed efficiency and gain in ruminants, and as an anti-coccidial agent in poultry (Ruff et al., 1980; Spears, 1990). Ionophores alter microbial metabolism in the gastrointestinal tract, which may result in increased digestion and absorption of nutrients (Spears, 1990).

Growth performance

When fed to chickens, narasin is effective in reducing coccidial infections and has a suggested dosage of 100 mg/kg feed (Weppelman et al., 1977). Alone and in combination with nicarbazin, 70 mg/kg feed narasin improved growth performance of broilers challenged with *Eimeria* (Long et al., 1988). Broilers challenged with *Clostridium perfringens* fed diets supplemented with 70 mg/kg feed narasin, alone or in combination with bacitracin methylene dialicylate, had reduced mortality and reduced lesion scores due to necrotic enteritis and increased ADG and improved G:F compared with birds fed a control diet (Brennan et al., 2003). Therefore, narasin may be included in diets fed to broilers to improve growth performance and ameliorate negative effects of bacterial infection and necrotic enteritis.

In a 3-phase marketing system, where the first 1 to 4 pigs per pen were sold on d 64, the second group of 11 pigs were sold on d 78, and the remaining pigs were sold on d 85, supplementing finishing pig diets with 15 mg/kg feed narasin for up to 85 d resulted in improved feed efficiency, but did not influence carcass characteristics (Arkfeld et al., 2015). In contrast, finishing pigs in a similar 3-phase marketing system fed 15 mg/kg feed narasin had increased final live BW, overall ADG, hot carcass weight and carcass yield compared with pigs fed a control diet and a zinc bacitracin diet (Rickard et al., 2017). When narasin was supplemented at 15 mg/kg feed to diets fed to finishing pigs for 35 d, an improvement in ADG and G:F was observed compared with pigs fed the control diet; however, there was also a tendency for a reduction in market weight for pigs fed narasin compared with pigs fed the control diet (Knauer and Artenson, 2017). Similarly, supplementing diets fed to growing pigs with either 15 or 30 mg/kg feed narasin increased the ADG and ADFI compared with pigs fed the control diet, but only pigs fed diets supplemented with 30 mg/kg feed narasin had an overall improvement in G:F

(Artenson et al., 2016). Therefore, narasin may be included in diets fed to pigs to improve growth performance and carcass characteristics during the growing and finishing phases of production.

Digestibility

Supplementation with narasin at either 15 or 30 mg/kg feed to diets for finishing pigs resulted in an increase in the apparent total tract digestibility of N and a subsequent reduction in the concentration of fecal N and increase in the concentration of propionic acid in the large intestine (Wuethrich et al., 1998). Inclusion of 30 mg/kg feed narasin to diets containing corn, soybean meal, and distillers dried grains with solubles resulted in increased apparent total tract digestibility of gross energy, dry matter, C, S, P, NDF, and ADF by young growing pigs (Kerr et al., 2017). Hence, narasin may improve digestibility of nutrients and energy and influence concentrations of short chain fatty acids in the large intestine of pigs, which may be correlated with the improvement in growth performance observed in other studies.

Conclusions

Improving intestinal health is an imperative goal to maximize production efficiency in livestock production. Due to increasing regulations and restrictions on subtherapeutic dosages of in-feed antibiotics, alternative feed additives that may improve intestinal health need to be identified. Isoquinoline alkaloids and ionophores may be used in swine production to improve intestinal health, increase growth performance and increase digestibility of nutrients to maximize production potential. However, more research is needed to determine effects of IQ in diets fed to growing and finishing pigs on nutrient digestibility and growth performance as well as effects of IQ on immune parameters in weanling pigs.

Figure

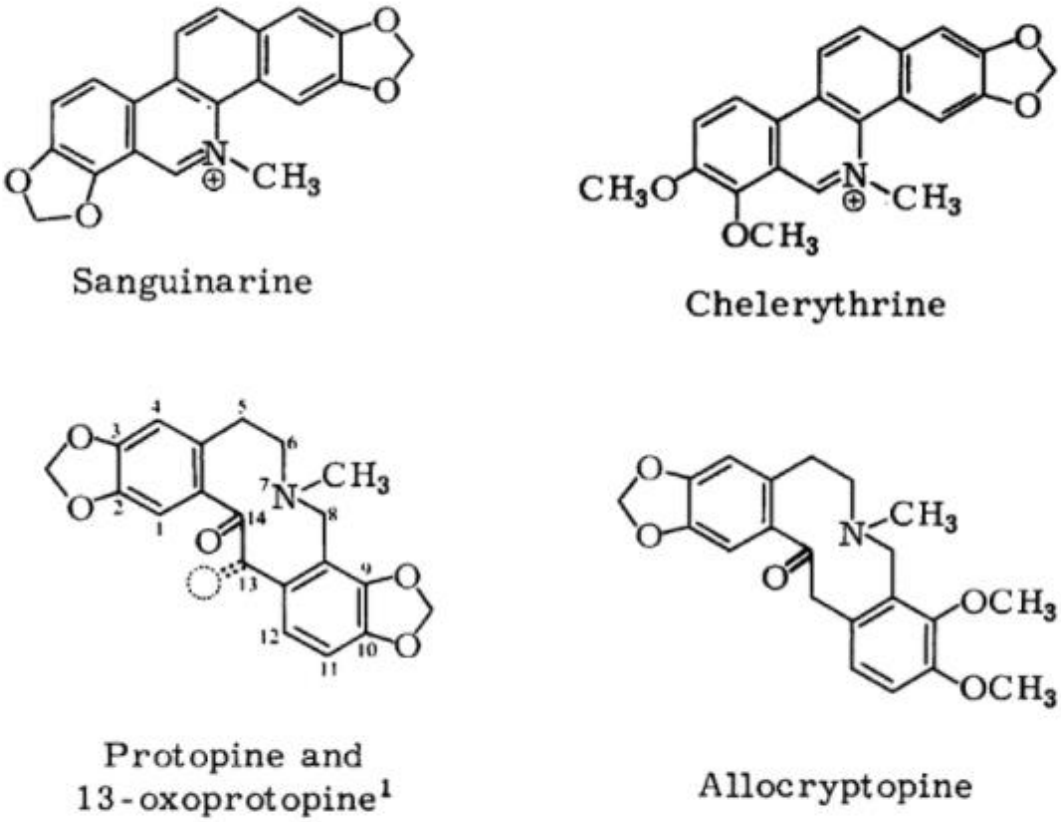

Figure 2.1. Structure of the isoquinoline alkaloids (Image adapted from Shamma, 1972).

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CHAPTER 3: Isoquinoline alkaloids increase apparent ileal digestibility of amino acids and crude protein by growing and finishing pigs fed corn-soybean meal diets

Abstract

Two experiments were conducted to test the hypothesis that a preparation of isoquinoline alkaloids (IQ) obtained from *Macleaya cordata* included in corn-soybean meal (SBM) diets increases apparent ileal digestibility (AID) of nutrients, increases apparent total tract digestibility (ATTD) of gross energy (GE) and crude protein (CP) and increases plasma amino acids (AA) and protein when fed to growing and finishing pigs. Experiment 1 utilized 12 barrows (initial body weight: 27.1 ± 2.10 kg) equipped with a T-cannula in the distal ileum, which were allotted to 4 treatments and 4 periods for 12 replicates per treatment. Treatments included a corn-SBM basal diet and three diets formulated by adding 40, 80, or 160 mg/kg feed of IQ to the basal diet. In Exp. 2, twelve T-cannulated barrows (initial body weight: 77.2 ± 6.23 kg) were allotted to the same treatments as Exp. 1, but diets were fed for 3 periods for a total of 9 replicate pigs per treatment. Each period had a 10 d adaptation period, followed by fecal sample collection on d 11 and 12, and ileal digesta collection on d 13 and 14. Results indicated that AID of some AA increased when IQ was included at 40 or 160 mg/kg in Exp. 1, but a reduction ($P < 0.05$) in the AID of acid hydrolyzed ether extract (AEE) and starch was observed at 80 mg/kg IQ. Including 80 mg/kg IQ to diets in Exp. 2 increased the AID of CP, whereas 40 mg/kg IQ resulted in an increased ($P < 0.05$) AID of His, Met, and Pro, and a tendency ($P < 0.10$) for an increase in AID of Ile, Leu, Lys, Cys, Gly, and Tyr. The ATTD of CP was also increased ($P < 0.05$) if the diet

containing 80 mg/kg IQ was fed in Exp. 1. Albumin was reduced ($P < 0.05$) in plasma of finishing pigs fed 40 mg/kg IQ in Exp. 2. Including 40 mg/kg IQ in a diet for growing pigs increased ($P < 0.05$) plasma Ala, Lys, and Asp, but a reduction ($P < 0.05$) in some plasma AA as 160 mg/kg IQ was added to diets for finishing pigs was observed. In conclusion, IQ may be added to diets for growing and finishing pigs to increase the AID of AA and CP, with optimal results observed at inclusion levels of between 40 and 80 mg/kg IQ.

Key words: amino acids, apparent ileal digestibility, apparent total tract digestibility, isoquinoline alkaloids, pigs

Abbreviations

AA	amino acids
AID	apparent ileal digestibility
AEE	acid hydrolyzed ether extract
ATTD	apparent total tract digestibility
CP	crude protein
GE	gross energy
IQ	isoquinoline alkaloids
PUN	plasma urea nitrogen
SBM	soybean meal

Introduction

Isoquinoline alkaloids (**IQ**) have anti-inflammatory, antimicrobial, and immunomodulatory effects (Walker, 1990; Agarwal et al., 1991; Chaturvedi, 1997), and they have been included in livestock diets to improve growth performance. Isoquinoline alkaloid supplementation to pig diets also increases intestinal barrier function and reduces inflammation in the intestines

(Robbins et al., 2013; Liu et al., 2016b), which may result in enhanced absorption of essential nutrients. When fed to nursery pigs, IQ improve the apparent ileal digestibility (**AID**) of nutrients (Boroojeni et al., 2018; Rundle et al., 2020). However, there are no data for effects of IQ on the AID of nutrients in corn-soybean meal (**SBM**) diets fed to pigs during the growing-finishing phase of production; nor are there data for effects of IQ on the apparent total tract digestibility (**ATTD**) of energy or crude protein (**CP**). Therefore, the first objective of these experiments was to test the hypothesis that inclusion of IQ in corn-SBM diets fed to growing or finishing pigs will increase the AID of amino acids (**AA**), CP, acid hydrolyzed ether extract (**AEE**), and starch and increase the ATTD of gross energy (**GE**) and CP. The second hypothesis was that inclusion of IQ in corn-SBM diets fed to growing and finishing pigs will increase concentrations of plasma free AA and change concentrations of plasma urea nitrogen (**PUN**), total protein, and albumin in plasma

Materials and Methods

Two experiments were conducted, and the protocols for both experiments were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois before animal work was initiated. In both experiments, barrows that were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN) were used.

Experimental diets and designs

Two corn-SBM basal diets were formulated to meet current requirement estimates for pigs from 25 to 50 kg or 50 to 75 kg for Exp. 1 and Exp. 2, respectively (Tables 3.1 and 3.2; NRC, 2012). Six additional diets were formulated by adding 40, 80, or 160 mgIQ/kg feed to each corn-SBM

basal diet. Vitamins and minerals were included in all diets to meet or exceed current requirement estimates (NRC, 2012). All diets also contained 0.40% titanium dioxide as an indigestible marker.

In Exp. 1, 12 growing barrows (initial body weight: 27.1 ± 2.10 kg) were equipped with a T-cannula in the distal ileum and allotted to a triplicated 4 × 4 Latin square design with 4 dietary treatments in each square and 4 periods. In Exp. 2, 12 ileal cannulated finishing barrows (initial body weight: 77.2 ± 6.23 kg) were allotted to a triplicated 4 × 3 incomplete Latin square design with 4 dietary treatments in each square and 3 periods. Pigs were allotted to diets in such a way that no pig received the same diet more than once during the experiment and there were, therefore, 12 replicate pigs per treatment in Exp. 1 and 9 replicate pigs per treatment in Exp. 2. Pigs were housed individually in pens (1.2 × 1.5 m) in an environmentally controlled room. Pens had smooth sides and fully slatted tribar floors. A feeder and a nipple drinker were installed in each pen.

Sampling procedures

All pigs were fed their respective diets on an *ad libitum* basis and water was also available at all times. Pig weights were recorded at the beginning of the experiment and at the end of each period. The initial 10 d of each period were considered an adaptation period to the diet. Fecal samples were collected on d 11 and 12 of each period using the grab sampling technique. These samples were immediately frozen at -20^{o}C. Ileal digesta were collected for 8 h on d 13 and 14 by attaching a plastic bag to the cannula barrel and digesta flowing into the bag were collected (Stein et al., 1998). Bags were removed whenever they were filled with digesta - or at least once every 30 minutes - and immediately stored at -20^{o}C to prevent bacterial degradation of AA in the digesta. At the conclusion of each collection period, a blood sample was collected from the

jugular vein of each pig in vacutainers containing lithium heparin. After collection, all blood samples were centrifuged at 4,000 × g for 13 min to recover the plasma. Plasma samples were stored at -20 ˚C until analysis.

At the conclusion of each period, ileal digesta samples were thawed, mixed within animal and diet, and a sub-sample was collected for analysis. A sample of each diet and of corn and SBM was collected at the time of diet mixing. Digesta and fecal samples were lyophilized and finely ground prior to analysis.

Titanium was analyzed in diets, ileal digesta, and fecal samples 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). Corn, SBM, diets, and ileal digesta 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. Starch was analyzed in corn, SBM, diets, and ileal digesta using the glucoamylase procedure (method 979.10; AOAC Int., 2007). Titanium, starch, and AA analyses were conducted at the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO). At the University of Illinois (Urbana, IL), corn and SBM, diets, ileal digesta, and fecal samples were analyzed for DM (method 930.15; AOAC Int., 2007). All samples were analyzed for N using a Leco Nitrogen Determinator (model FP628, Leco Corp., St. Joseph, MI) and CP was calculated as N × 6.25. Corn, SBM, diets, and ileal digesta samples were analyzed for AEE by acid hydrolysis using 3*N* HCl ($Ankom^{HCl}$, Ankom Technology, Macedon, NY) followed by crude fat extraction using petroleum ether ($Ankom^{XT15}$, Ankom Technology, Macedon, NY). Corn, SBM, diets, and fecal samples were analyzed for ash (method 942.05;

AOAC Int., 2007) and for GE using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL).

Apparent ileal digestibility values for AA in each diet were calculated using the following equation (NRC, 2012):

$$\text{AID}_{\text{AA}}, \% = 100 - [\left(\frac{\text{AA}_{\text{digesta}}}{\text{AA}_{\text{feed}}}\right) \times \left(\frac{\text{Ti}_{\text{feed}}}{\text{Ti}_{\text{digesta}}}\right)] \times 100$$

where AID_{AA} is the apparent ileal digestibility of an AA (%), $\text{AA}_{\text{digesta}}$ is the concentration of that AA in the ileal digesta DM, AA_{feed} is the AA concentration of that AA in the feed DM, Ti_{feed} is the titanium concentration in the feed DM, and $\text{Ti}_{\text{digesta}}$ is the titanium concentration in the ileal digesta DM. The AID for CP, starch, and AEE was also calculated using this equation. The ATTD of GE and CP was also calculated using this equation, with the exception that energy and nutrients in feces rather than in ileal digesta were used to calculate ATTD values.

Plasma samples collected on d 1 and on the last day of each period were analyzed for total protein, albumin, and PUN using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA) at the University of Illinois. Plasma samples were also analyzed for free AA using cation-exchange liquid chromatography with post column ninhydrin reaction and detection at the University of Missouri (Columbia, MO).

Statistical analyses

Data from both experiments were analyzed using the Proc MIXED of SAS (SAS Institute Inc., Cary, NC) with pig as the experimental unit. Normality of residuals was tested using Proc UNIVARIATE of SAS. The model included diet as the fixed effect and random effects included period, pig, and square. Least squares means were estimated and separated using the LSMEANS statement with the PDIFF (*P*-values for differences of least squares means) option. Contrast statements were used to determine differences between effects of the control diet and each

experimental diet on the AID of AA, CP, AEE, and starch, as well as the ATTD of GE and CP and the plasma AA, PUN, TP, and albumin. Results were considered significant at $P \leq 0.05$ and considered a trend at $0.05 < P \leq 0.10$.

Results

Addition of 40 mg/kg IQ to corn-SBM diets fed to growing pigs resulted in an increase ($P < 0.05$) in the AID of Ile, Leu, Lys, Met, Phe, Thr, Trp, Val, Ala, Pro, Ser, Tyr, and total AA compared with the control diet (Table 3.3). There was also a tendency for the AID of Arg, His, Asp, and the mean of dispensable AA to increase ($P < 0.10$) at 40 mg/kg IQ compared with the control diet. Likewise, inclusion of 160 mg/kg IQ increased ($P < 0.05$) the AID of Leu, Met, Thr, Pro, Ser, and Tyr compared with the control diet. A tendency was also observed for the AID of His and Phe to increase ($P < 0.10$) in the 160 mg/kg IQ diet compared with the control diet. The AID of starch and AEE was reduced ($P < 0.05$) as IQ was included at 80 mg/kg compared with the control diet and the AID of AEE was also reduced ($P < 0.05$) if 160 mg/kg of IQ was used.

Inclusion of 80 mg/kg IQ in the diet resulted in an increase ($P < 0.05$) in the AID of CP when fed to finishing pigs (Table 3.4). At an inclusion of 40 mg/kg IQ, there was an increase ($P < 0.05$) in the AID of His, Met, and Pro compared with the control diet. There was also a tendency for the AID of Ile, Leu, Lys, Cys, Gly, Tyr, and the mean AID of indispensable AA to increase ($P < 0.10$) in the 40 mg/kg IQ diet compared with the control diet. Addition of 160 mg/kg IQ to finishing pig diets resulted in a decrease ($P < 0.05$) in the AID of most AA, with the exception of Asp and Gly. There was a tendency ($P < 0.10$) for a reduction in the AID of starch at an inclusion rate of 160 mg/kg IQ; however, the AID of starch was not different among the

control, 40, or 80 mg/kg IQ diets. There was no influence of IQ supplementation in diets for finishing pigs on the AID of AEE.

There was no influence of IQ inclusion in diets fed to growing pigs on PUN, albumin, or total protein (Table 3.5). Inclusion of 80 mg/kg IQ resulted in an increase ($P < 0.05$) in plasma concentrations of Ala, Lys, and Asp compared with pigs fed the control diet. When 40 mg/kg IQ was included, there was a tendency for a reduction ($P < 0.10$) in plasma concentrations of His and Pro. In contrast, including 80 mg/kg IQ tended to increase ($P < 0.10$) concentrations of His, Ile, Leu, and Glu compared with the control diet, and including 160 mg/kg IQ tended to increase ($P < 0.10$) the concentration of Trp in plasma of pigs compared with pigs fed the control diet.

A reduction ($P < 0.05$) was observed in plasma albumin and Glu concentrations of finishing pigs fed the 40 mg/kg IQ diet compared with the control diet (Table 3.6). There was also a reduction ($P < 0.05$) in Arg, Trp, Ala, Pro, Ser, and Tyr in plasma of finishing pigs fed the 160 mg/kg IQ diet compared with the control diet, and there was a tendency for a reduction ($P < 0.10$) in plasma concentrations of His, Phe, Met, and Glu of pigs fed the 160 mg/kg diet compared with pigs fed the control diet.

There was no influence of IQ on the ATTD of GE or CP by growing pigs (Table 3.7), and there was also no effect of IQ on the ATTD of GE by finishing pigs. However, inclusion of 80 mg/kg IQ resulted in an increase ($P < 0.05$) in the ATTD of CP compared with the control diet when fed to finishing pigs.

Discussion

Apparent ileal digestibility

The increase in the AID of some AA as IQ was added to the diets in both Exp. 1 and 2 is in agreement with reports indicating the AID of AA in diets fed to nursery pigs is increased when IQ is included in the diet (Boroojeni et al., 2018; Rundle et al., 2020). Increases in the AID of total AA in Exp. 1 concur with the observation that an increase in AID of total AA occurs at 120 mg/kg IQ supplementation in corn-SBM-barley-wheat diets fed to nursery pigs (Boroojeni et al., 2018). It is not known why the AID of AA decreased in Exp. 2 at the inclusion level of 160 mg/kg IQ; however, a decrease in the AID of AA was also observed at the highest inclusion level of IQ when included in diets fed to nursery pigs (Rundle et al., 2020). It is possible that high concentrations of IQ resulted in reduced efficiency of digestive enzymes necessary for AA digestion (Drsata et al., 1996). Results from Exp. 1 for the AID of CP are in agreement with the observation that there was no effect of IQ on AID of CP in nursery pigs (Rundle et al., 2020). However, the increase in the AID of CP at 80 mg/kg IQ in Exp. 2 agreed with results that IQ supplementation to corn-SBM-barley-wheat diets tended to increase the AID of CP by nursery pigs (Boroojeni et al., 2018).

Increases in the AID of AA and CP observed in these experiments may be attributed to the immunomodulatory and anti-inflammatory effects of IQ. Isoquinoline alkaloid supplementation to diets for pigs improves intestinal barrier function (Robbins et al., 2013), and reduces inflammation in the intestines (Liu et al., 2016b). The alkaloids in the IQ preparation used in these experiments also inhibit gram negative and gram positive bacterial cell proliferation (Walker, 1990), which may result in reduced concentrations of potentially harmful bacteria in the gastrointestinal tract, further improving intestinal health. Additionally, IQ has

been shown to inhibit NF-kB, which plays a critical role in the expression of pro-inflammatory cytokines (Chaturvedi et al., 1997). Therefore, a reduction in inflammation along with an increase in intestinal barrier function may result in the improved absorption of essential nutrients in the small intestine observed in the present experiments.

The reduction in AID of starch in pigs fed the 80 mg/kg IQ diet in Exp. 1 and the 160 mg/kg IQ diet in Exp. 2 contrasts the observation that AID of starch increases as IQ is supplemented to nursery pig diets with the greatest digestibility observed by pigs fed 90 mg/kg IQ (Rundle et al., 2020). Isoquinoline alkaloids may have inhibitory effects on α-amylase, the digestive enzyme necessary to digest starch; however, inhibition of both pancreatic and salivary amylase by IQ depends on the incubation time and concentration of the alkaloids *in vivo* (Zajoncova et al., 2005). Therefore, it is not likely that IQ has an inhibitory effect on the endogenous α-amylase of pigs in these experiments due to the high level of IQ needed to produce an inhibitory effect under *in vivo* conditions (Zajoncova et al., 2005). Nursery pigs are often stressed during the weaning period, and consequently, may have a greater use for immunomodulatory supplements such as IQ in terms of impacting intestinal health and digestibility of nutrients than growing and finishing pigs. When IQ was supplemented to high-energy diets fed to steers, there were no differences in the digestibility of starch among treatments (Aguilar-Hernandez, 2016).

The lack of an effect in Exp. 2 on the AID of AEE is in accordance with data indicating that IQ does not influence the digestibility of AEE in nursery pigs (Boroojeni et al., 2018; Rundle et al., 2020). It is not clear why IQ supplementation reduced the AID of AEE in Exp. 1; however, as components of IQ may inhibit amylase activity (Zajoncova et al., 2005), it may be

possible that IQ could inhibit activity of other pancreatic enzymes. Further research is needed to elucidate these effects.

Blood characteristics

The observation that IQ did not influence the albumin concentration of the growing pigs is in agreement with published data (Kosina et al., 2003; Abudabos et al., 2016). The reduction in albumin concentrations in the plasma of finishing pigs in Exp. 2 as IQ was added to the diets is in agreement with results from laying hens indicating that serum albumin concentrations were reduced as IQ was added to the diet (Bavarsadi et al., 2017). As the primary functions of albumin are modulation of plasma oncotic pressure and transportation of nutrients and hormones through the bloodstream (Bern et al., 2015), the reduction in albumin concentration observed under the conditions of Exp. 2 may be a result of the lower concentration of plasma AA observed in the 40 and 160 mg/kg IQ treatments.

The observation that total protein in plasma was not impacted by IQ supplementation is in agreement with results from research with broilers (Kosina et al., 2003; Abudabos et al., 2016). Increases in individual plasma AA concentrations observed in both Exp. 1 and 2 were in agreement with data demonstrating that IQ supplementation to swine diets resulted in greater serum AA concentrations (Liu et al., 2016a). As AA may be transported as free AA in plasma, the decrease in albumin is not a direct contradiction to the increase in individual plasma AA observed under the conditions of this experiment. The increase in plasma AA may be an indicator of a potential increase in protein synthesis by pigs fed diets supplemented with IQ because systemically circulating AA are an indication of AA utilization by the animal for growth and maintenance of body tissues and IQ improves growth performance of growing pigs (Kantas et al., 2015; Liu et al., 2016a; Boroojeni et al., 2018).

Apparent total tract digestibility

The lack of an influence of IQ on the ATTD of GE in both Exp. 1 and 2 is in agreement with data for nursery pigs fed corn-SBM or corn-SBM-distillers dried grains with solubles diets supplemented with IQ (Rundle, 2018). In contrast, supplementation with IQ to diets fed to ruminants resulted in increases in digestible energy of high-energy diets fed to steers (Aguilar-Hernandez et al., 2016) and dietary net energy of diets fed to ewes (Estrada-Angulo et al., 2016). This variability in digestibility among species may be a result of physiological differences between ruminants and non-ruminants and the degree to which microbial fermentation plays a role in utilization of nutrients.

Increased ATTD of CP at 80 mg/kg inclusion in Exp. 1 was in agreement with results from an experiment conducted with steers fed diets supplemented with IQ, in which there was an increase in the postruminal digestion of N as dietary IQ increased (Aguilar-Hernandez et al., 2016). As there was no effect of IQ on the AID of CP by growing pigs in Exp. 1, it may be speculated that IQ increased hindgut disappearance of CP, resulting in increased ATTD of CP that was observed in pigs fed the 80 mg/kg IQ diet. However, it is also possible that reduced microbial activity due to IQ supplementation resulted in a decrease in microbial protein synthesis, therefore, resulting in the increase in the ATTD of CP that was observed.

Conclusions

Including IQ at either 40 or 80 mg IQ/kg feed to corn-SBM diets fed to growing or finishing pigs results in the greatest digestibility of AA, and 80 mg IQ/kg feed in corn-SBM diets fed to finishing pigs resulted in the greatest ATTD of CP. However, further research is needed to demonstrate if IQ influences growth performance and intestinal health of pigs, specifically under conditions where pig growth performance and health is challenged.

Tables

Table 3.1. Composition (as-is basis) of experimental diets for growing and finishing pigs, Exp. 1 and Exp. 2, respectively

Item, %	Experiment 1				Experiment 2			
	Isoquinoline alkaloids, mg/kg				Isoquinoline alkaloids, mg/kg			
	0	40	80	160	0	40	80	160
Ground corn	59.15	58.75	58.35	57.55	63.35	62.95	62.55	61.75
Soybean meal	34.00	34.00	34.00	34.00	30.00	30.00	30.00	30.00
Soybean oil	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Limestone	0.90	0.90	0.90	0.90	0.80	0.80	0.80	0.80
Dicalcium phosphate	0.90	0.90	0.90	0.90	0.80	0.80	0.80	0.80
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Titanium dioxide	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin-mineral mix[1]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
IQ premix[2]	-	0.40	0.80	1.60	-	0.40	0.80	1.60

[1] 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; $_{D}$-pantothenic acid as $_{D}$-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,

Table 3.1 (cont.)

60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

[2]Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany.

Table 3.2. Analyzed composition of experimental diets and ingredients from Exp. 1 (growing pigs) and Exp. 2 (finishing pigs)

Item	Experiment 1						Experiment 2					
	Isoquinoline alkaloids, mg/kg						Isoquinoline alkaloids, mg/kg					
	-	40	80	160	Corn	SBM[1]	-	40	80	160	Corn	SBM
Dry matter, %	87.98	97.95	88.22	87.58	86.84	88.70	86.64	86.67	86.68	86.58	84.94	87.67
Ash, %	5.39	5.62	5.53	5.29	1.16	6.88	4.99	4.50	5.10	4.56	1.06	6.52
AEE[1], %	5.19	5.26	4.63	4.55	2.94	2.66	6.46	5.96	5.89	7.30	3.59	2.58
Ca, %	0.80	0.73	0.75	0.75	0.02	0.31	0.66	0.44	0.60	0.47	<0.10	0.34
P, %	0.60	0.57	0.59	0.58	0.26	0.69	0.44	0.45	0.44	0.41	0.19	0.56
Starch, %	41.96	42.77	42.69	42.40	67.22	4.07	36.66	35.81	38.00	37.49	60.78	ND
GE, kcal/kg	4,035	4,032	4,038	4,002	3,813	4,170	4,003	4,042	4,025	4,062	3,777	4,168
CP, %	19.23	19.60	19.04	18.68	7.68	47.66	17.52	17.80	19.09	16.72	6.96	46.00
Indispensable AA, %												
Arg	1.30	1.35	1.32	1.32	0.37	3.38	1.23	1.28	1.16	1.05	0.31	3.31
His	0.51	0.53	0.52	0.52	0.21	1.22	0.50	0.53	0.48	0.45	0.20	1.22
Ile	0.89	0.93	0.90	0.90	0.27	2.28	0.86	0.90	0.83	0.74	0.25	2.31

Table 3.2 (cont.)

Item	Experiment 1						Experiment 2					
	Isoquinoline alkaloids, mg/kg						Isoquinoline alkaloids, mg/kg					
	-	40	80	160	Corn	SBM[1]	-	40	80	160	Corn	SBM
Leu	1.61	1.70	1.66	1.65	0.81	3.61	1.61	1.69	1.55	1.40	0.76	3.58
Lys	1.11	1.15	1.12	1.13	0.28	2.93	1.07	1.12	1.03	0.94	0.26	2.94
Met	0.28	0.29	0.28	0.29	0.14	0.64	0.28	0.31	0.27	0.25	0.14	0.66
Phe	0.96	1.01	0.98	0.98	0.35	2.38	0.97	1.02	0.93	0.83	0.32	2.44
Thr	0.72	0.76	074	0.75	0.26	1.78	0.69	0.72	0.66	0.59	0.23	1.75
Trp	0.23	0.25	0.24	0.24	0.06	0.66	0.20	0.20	0.19	0.16	0.04	0.55
Val	0.96	0.99	0.97	0.97	0.35	2.32	0.95	0.99	0.91	0.84	0.33	2.39
Dispensable AA, %												
Ala	0.94	0.98	0.96	0.96	0.51	2.01	0.95	0.98	0.89	0.85	0.50	2.00
Asp	1.99	2.08	2.02	2.07	0.51	5.23	1.94	2.02	1.86	1.64	0.46	5.26
Cys	0.30	0.31	0.29	0.31	0.16	0.62	0.29	0.31	0.28	0.26	0.15	0.65
Glu	3.45	3.64	3.54	3.53	1.28	8.41	3.31	3.41	3.12	2.85	1.18	8.19

Table 3.2 (cont.)

Item	Experiment 1						Experiment 2					
	Isoquinoline alkaloids, mg/kg						Isoquinoline alkaloids, mg/kg					
	-	40	80	160	Corn	SBM[1]	-	40	80	160	Corn	SBM
Gly	0.81	0.84	0.82	0.82	0.30	1.95	0.82	0.82	0.73	0.75	0.29	1.97
Pro	1.11	1.16	1.15	1.14	0.62	2.37	1.13	1.18	1.08	1.00	0.58	2.30
Ser	0.84	0.88	0.87	0.86	0.34	2.02	0.74	0.76	0.69	0.63	0.29	1.85
Tyr	0.68	0.72	0.70	0.70	0.24	1.73	0.58	0.62	0.55	0.48	0.16	1.53

[1] SBM= soybean meal; AEE= acid hydrolyzed ether extract.

Table 3.3. Apparent ileal digestibility of CP, AA, AEE and starch by growing pigs fed corn-soybean meal diets supplemented with isoquinoline alkaloids, Exp. 1

Item, %	Isoquinoline alkaloids, mg/kg					*P*-Value
	-	40	80	160	SEM	Diet
Starch	95.1[a]	94.4[ab]	93.6[b]	94.2[ab]	0.79	0.106
AEE	71.6[a]	70.9[a]	64.7[b]	65.6[b]	1.55	0.002
CP	76.3[ab]	77.1[a]	75.4[b]	75.8[ab]	0.63	0.116
Indispensable AA						
Arg[1]	89.0[ab]	89.7[a]	88.6[b]	89.6[a]	0.29	0.012
His[1,2]	82.4	83.5	82.5	83.6	0.50	0.104
Ile	81.4[bc]	82.7[a]	80.8[c]	82.2[ab]	0.49	0.027
Leu	81.0[b]	82.6[a]	80.9[b]	82.3[a]	0.51	0.021
Lys	82.0[b]	83.8[a]	81.9[b]	83.0[ab]	0.65	0.019
Met	83.8[b]	85.3[a]	83.2[b]	85.1[a]	0.50	0.004
Phe[2]	82.0[bc]	83.4[a]	81.8[c]	83.0[ab]	0.48	0.023
Thr	72.1[b]	74.8[a]	72.1[b]	74.3[a]	0.96	0.009
Trp	80.8[b]	82.7[a]	80.4[b]	82.0[ab]	0.89	0.071
Val	76.5[bc]	78.4[a]	76.1[c]	77.8[ab]	0.66	0.020
Mean[2]	81.4[bc]	83.1[a]	81.2[c]	82.5[ab]	0.50	0.008
Dispensable AA						
Ala	76.1[b]	78.3[a]	75.6[b]	77.3[ab]	0.71	0.014

Table 3.3 (cont.)

Item, %	Isoquinoline alkaloids, mg/kg					*P*-Value
	-	40	80	160	SEM	Diet
Asp[1]	77.4[ab]	78.7[a]	76.9[b]	78.6[a]	0.67	0.039
Cys	65.4[a]	66.8[a]	62.3[b]	67.3[a]	1.47	0.007
Glu	83.1	83.7	83.0	82.7	0.65	0.633
Gly	67.3	68.3	66.2	66.9	1.25	0.567
Pro	79.9[b]	81.3[a]	80.2[ab]	81.3[a]	0.83	0.072
Ser	78.9[b]	80.5[a]	79.2[ab]	80.3[a]	0.74	0.053
Tyr	83.1[b]	84.8[a]	83.1[b]	84.2[a]	0.54	0.008
Mean[1]	78.8[ab]	80.1[a]	78.5[b]	79.4[ab]	0.63	0.177
Total AA	80.0[b]	81.5[a]	79.7[b]	80.8[ab]	0.54	0.048

[1] Using Proc MIXED of SAS, contrast statements indicated a tendency ($0.05 < P \leq 0.10$) for the digestibility of the nutrient to increase in the 40 mg/kg IQ diet compared with the 0 mg/kg IQ diet.

[2] Using Proc MIXED of SAS, contrast statements indicated a tendency ($0.05 < P \leq 0.10$) for the digestibility of the nutrient to increase in the 160 mg/kg IQ diet compared with the 0 mg/kg IQ diet.

[a-c] Means in a row without a common superscript differ ($P < 0.05$).

Table 3.4. Apparent ileal digestibility of CP, AA, AEE and starch by finishing pigs fed corn-soybean meal diets supplemented with isoquinoline alkaloids, Exp. 2

Item, %	Isoquinoline alkaloids, mg/kg					*P*-Value
	-	40	80	160	SEM	Diet
Starch	91.7[a]	91.3[ab]	92.0[a]	90.3[b]	0.88	0.092
AEE	77.3	76.5	74.5	78.3	2.18	0.352
CP	74.6[bc]	75.4[ab]	77.5[a]	73.1[c]	1.07	0.008
Indispensable AA						
Arg	87.4[a]	88.2[a]	87.2[a]	85.3[b]	0.46	<0.001
His	82.4[b]	84.4[a]	82.7[ab]	80.7[c]	0.73	0.002
Ile[1]	80.0[a]	81.5[a]	79.8[a]	76.5[b]	0.88	<0.001
Leu[1]	80.7[a]	82.3[a]	80.6[a]	77.7[b]	0.93	<0.001
Lys[1]	80.1[ab]	81.4[a]	79.3[b]	77.1[c]	0.65	<0.001
Met	83.0[b]	84.8[a]	82.5[b]	80.1[c]	0.73	<0.001
Phe[1]	82.0[ab]	83.4[a]	81.7[b]	78.8[c]	0.80	<0.001
Thr[1]	71.7[a]	74.1[a]	71.7[a]	66.6[b]	1.02	<0.001
Trp	81.6[a]	82.4[a]	82.4[a]	76.4[b]	0.94	<0.001
Val[1]	75.6[ab]	77.7[a]	75.3[b]	72.4[c]	0.96	<0.001
Mean[1]	80.6[ab]	82.1[a]	80.3[b]	77.4[c]	0.78	<0.001
Dispensable AA						
Ala	76.2[ab]	77.7[a]	75.3[b]	72.9[c]	1.13	0.002
Asp	77.4[ab]	78.7[a]	76.6[a]	72.5[b]	1.06	<0.001

Table 3.4 (cont.)

Item, %	Isoquinoline alkaloids, mg/kg					*P*-Value
	-	40	80	160	SEM	Diet
Cys[1]	68.5[ab]	71.5[a]	68.0[b]	63.6[c]	1.25	<0.001
Glu	83.1[a]	83.7[a]	82.1[a]	79.3[b]	1.03	0.006
Gly[1]	64.6[ab]	69.5[a]	66.0[ab]	63.3[b]	1.92	0.105
Pro	80.3[b]	82.1[a]	80.5[ab]	77.6[c]	0.82	<0.001
Ser	76.6[a]	78.1[a]	76.1[a]	72.6[b]	0.85	<0.001
Tyr[1]	80.6[ab]	82.4[a]	80.2[b]	76.1[c]	0.91	<0.001
Mean	78.3[a]	80.0[a]	77.8[a]	74.6[b]	1.01	0.002
Total AA	79.1[a]	80.7[a]	78.8[a]	75.6[b]	0.90	<0.001

[1] Using Proc MIXED of SAS, contrast statements indicated a tendency ($0.05 < P \leq 0.10$) for the digestibility of the nutrient to increase in the 40 mg/kg IQ diet compared with the 0 mg/kg IQ diet.

[a-c] Means in a row without a common superscript differ ($P < 0.05$).

Table 3.5. Blood characteristics of growing pigs fed diets supplemented with isoquinoline alkaloids, Exp. 1

Item	Isoquinoline alkaloids, mg/kg					*P*-Value
	-	40	80	160	SEM	Diet
Plasma urea nitrogen, mg/dL	15.6	15.7	15.5	16.2	1.02	0.869
Albumin, g/dL	3.7	3.7	3.7	3.7	0.11	0.762
Total protein, g/dL	6.4	6.3	6.5	6.4	0.20	0.305
Indispensable AA, µg/dL						
Arg	47.7[b]	46.9[b]	54.7[a]	51.5[ab]	3.55	0.016
His[1,2]	19.5[ab]	18.2[b]	20.7[a]	19.7[a]	0.97	0.016
Ile[2]	24.8[ab]	24.6[b]	26.5[a]	25.6[ab]	0.77	0.137
Leu[2]	37.4[ab]	37.1[b]	39.6[a]	37.9[ab]	1.24	0.185
Lys	31.6[b]	32.4[b]	37.6[a]	34.8[ab]	2.71	0.023
Met	5.6	5.7	6.0	5.8	0.40	0.555
Phe	20.4	20.5	21.3	21.3	1.06	0.615
Thr	33.0	31.1	33.8	33.0	1.44	0.375
Trp[3]	14.4[ab]	14.2[b]	14.9[ab]	15.5[a]	0.71	0.187
Val	44.0	43.3	46.4	44.8	1.85	0.280
Dispensable AA, µg/dL						
Ala	44.1[ab]	40.6[b]	47.4[a]	42.9[ab]	4.16	0.056
Asp	1.7[b]	1.93[ab]	2.0[a]	2.0[a]	0.24	0.024
Glu[2]	18.5[ab]	19.1[a]	22.0[a]	19.8[a]	2.79	0.326

Table 3.5 (cont.)

Item	Isoquinoline alkaloids, mg/kg					*P*-Value
	-	40	80	160	SEM	Diet
Gly	83.1	81.5	84.8	82.9	4.04	0.587
Pro[1]	48.2[ab]	44.3[b]	49.1[a]	49.2[a]	1.51	0.100
Ser	21.7[ab]	20.4[b]	23.0[a]	22.0[ab]	0.91	0.095
Tyr	33.4	32.3	34.4	34.2	1.52	0.598

[1] Using Proc MIXED of SAS, contrast statements indicated a tendency ($0.05 < P \leq 0.10$) for the digestibility of the nutrient to decrease in the 40 mg/kg IQ diet compared with the 0 mg/kg IQ diet.

[2] Using Proc MIXED of SAS, contrast statements indicated a tendency ($0.05 < P \leq 0.10$) for the digestibility of the nutrient to increase in the 80 mg/kg IQ diet compared with the 0 mg/kg IQ diet.

[3] Using Proc MIXED of SAS, contrast statements indicated a tendency ($0.05 < P \leq 0.10$) for the digestibility of the nutrient to increase in the 160 mg/kg IQ diet compared with the 0 mg/kg IQ diet.

[a,b] Means in a row without a common superscript differ ($P < 0.05$).

Table 3.6. Blood characteristics of finishing pigs fed diets supplemented with isoquinoline alkaloids, Exp. 2

Item	Isoquinoline alkaloids, mg/kg					*P*-Value
	-	40	80	160	SEM	Diet
Plasma urea nitrogen, mg/dL	15.9	15.6	16.2	16.3	1.27	0.834
Albumin, g/dL	3.7[a]	3.5[b]	3.6[ab]	3.7[a]	0.12	0.019
Total protein, g/dL	6.9[ab]	6.8[b]	6.8[b]	7.1[a]	0.16	0.069
Indispensable AA, µg/dL						
Arg	53.3[a]	49.1[ab]	47.2[ab]	42.2[b]	3.14	0.064
His[1]	18.6	17.5	18.0	16.5	0.86	0.244
Ile	25.1	24.0	23.9	22.6	1.15	0.470
Leu	38.4	36.9	37.2	34.7	1.63	0.451
Lys	39.7	37.7	34.9	35.9	4.38	0.539
Met[1]	5.9	5.6	5.6	5.1	0.54	0.375
Phe[1]	18.9	18.2	18.1	16.6	0.92	0.291
Thr	31.5	30.4	30.2	28.3	1.54	0.498
Trp	14.1[a]	12.9[ab]	12.8[ab]	12.0[b]	1.05	0.181
Val	46.0	44.3	44.1	42.9	2.83	0.670
Dispensable AA, µg/dL						
Ala	46.3[a]	41.3[ab]	45.7[a]	37.9[b]	4.76	0.051
Asp	2.2	1.8	2.1	1.9	0.29	0.185
Glu[1]	23.2[a]	18.4[b]	20.4[ab]	19.4[ab]	3.29	0.153

Table 3.6 (cont.)

Item	Isoquinoline alkaloids, mg/kg					*P*-Value
	-	40	80	160	SEM	Diet
Gly	81.4	81.2	86.9	80.7	5.16	0.274
Pro	49.9[a]	48.1[a]	48.8[a]	41.8[b]	2.24	0.070
Ser	21.1[a]	19.3[ab]	20.4[ab]	17.6[b]	1.10	0.101
Tyr	27.5[a]	26.3[ab]	26.8[ab]	22.3[b]	1.63	0.123

[1] Using Proc MIXED of SAS, contrast statements indicated a tendency ($0.05 < P \leq 0.10$) for the digestibility of the nutrient to decrease in the 160 mg/kg IQ diet compared with the 0 mg/kg IQ diet.

[a,b] Means in a row without a common superscript differ ($P < 0.05$).

Table 3.7. Apparent total tract digestibility (ATTD) of crude protein (CP) and gross energy (GE) by pigs fed isoquinoline alkaloid supplemented corn-soybean meal diets

Item, %	Isoquinoline alkaloids, mg/kg					*P*-Value
	-	40	80	160	SEM	Diet
Growing pigs, Exp. 1						
ATTD of CP	83.6	83.6	83.2	85.4	0.85	0.247
ATTD of GE	85.2	84.5	84.5	85.2	0.52	0.493
Finishing pigs, Exp. 2						
ATTD of CP	85.6[b]	86.0[ab]	86.8[a]	84.8[b]	0.92	0.034
ATTD of GE	87.3	87.8	87.6	87.2	0.55	0.460

[a,b] Means in a row without a common superscript differ ($P < 0.05$).

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CHAPTER 4: Effects of isoquinoline alkaloids on apparent total tract digestibility of energy, fiber, and N, on concentration of digestible and metabolizable energy in diets fed to growing pigs, and on N balance of pigs

Abstract

An experiment was conducted to test the hypothesis that including isoquinoline alkaloids (IQ) in diets containing corn, soybean meal, and distiller's dried grains with solubles fed to growing pigs improves the apparent total tract digestibility (ATTD) of gross energy (GE), fiber, and N, as well as concentrations of digestible energy (DE) and metabolizable energy (ME) in diets and N balance of pigs. Forty barrows (initial body weight: 57.18 ± 7.41 kg) were allotted to a randomized complete block design with 4 diets and 10 replicate pigs per diet. A basal diet consisting of corn and soybean meal was prepared. Three additional diets were formulated by adding either 40, 80, or 160 mg IQ/kg feed to the basal diet. Pigs were housed individually in metabolism crates for the 12 d experimental period and were fed 3.2 times the estimated energy requirement in two equal meals provided at 0700 and 1600 h. The first 5 d were considered an adaptation period to the diets and feces and urine were collected from d 6 to 10 using standard procedures. With the MIXED procedure of SAS, contrast statements were used to determine the linear and quadratic effects of IQ. There was a tendency for a quadratic decrease ($P < 0.10$) in the ME as IQ was added to diets; which was a result of quadratically increased ($P < 0.05$) urine GE output. There was a quadratic decrease ($P < 0.05$) in N retention by pigs as IQ was added to diets; however, this did not influence the overall biological value of N. To conclude, IQ did not

influence the ATTD of GE or fiber, or concentration of DE, and did not influence the biological value of N by growing pigs fed corn, soybean meal, distiller's dried grains with solubles diets.

Key words: apparent total tract digestibility, energy, fiber, isoquinoline alkaloids, nitrogen balance, pig

Abbreviations

ATTD	apparent total tract digestibility
BV	biological value
DDGS	distiller's dried grains with solubles
DE	digestible energy
GE	gross energy
IQ	isoquinoline alkaloids
ME	metabolizable energy
SBM	soybean meal
TDF	total dietary fiber

Introduction

Isoquinoline alkaloids (**IQ**) have been used in livestock feed to promote growth due to their anti-inflammatory (Agarwal et al., 1991), antimicrobial (Walker, 1990), and immunomodulatory effects (Chaturvedi et al., 1997). When fed to young growing pigs, IQ did not improve the apparent total tract digestibility (**ATTD**) of energy or digestible energy (**DE**) and metabolizable energy (**ME**) concentrations of corn-soybean meal (**SBM**) diets, but did improve the apparent ileal digestibility of amino acids and starch (Rundle et al., 2020), which may indicate improved N retention by the pig. However, there are no data for effects of IQ on the ATTD of energy or

fiber, or DE and ME concentrations of corn-SBM-distiller's dried grains with solubles (**DDGS**) diets fed to pigs during the growing-finishing phase of production; nor are there data regarding the effect of IQ on nitrogen balance of pigs fed a corn-SBM-DDGS diet during the growing-finishing phase. Therefore, it was the objective of this experiment to test the hypothesis that IQ inclusion in corn-SBM-DDGS diets improves the ATTD of gross energy (**GE**), fiber, and N, and the concentrations of DE and ME in diets fed to growing pigs as well as N balance of 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. Growing barrows that were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN) were used in this experiment.

A total of 40 barrows (initial body weight: 57.18 ± 7.41 kg) were used in two blocks and fed 4 diets in a randomized complete block design with 5 pigs per diet in each block. There were, therefore, 10 replicate pigs per diet. Pigs were housed individually in metabolism crates that were equipped with a feeder and a nipple drinker, fully slatted floors and a screen floor and a urine tray, which allowed for the total, but separate, collection of fecal materials and urine from each pig.

A diet based primarily on corn, SBM, and DDGS was formulated to meet current requirement estimates for pigs from 50 to 75 kg (Tables 4.1 and 4.2; NRC 2012). Three additional diets were prepared by adding 40, 80, or 160 mg IQ/kg feed (Phytobiotics

Futterzusatzstoffe GmbH, Eltville, GE) to the basal diet. All diets were provided in meal form and vitamins and minerals were included to meet current requirements (NRC, 2012).

Feed was provided at 3.2 times the estimated daily energy requirement for maintenance (i.e., 197 kcal/kg ME × $BW^{0.60}$; NRC, 2012) and provided every day in two equal meals at 0700 and 1600 h. During the experiment, pigs had free access to water. Pigs stayed in the metabolism crates for 12 d. The initial 5 d were considered an adaptation period to the crates and diets, whereas feces and urine were collected from the feed provided from d 6 to d 10 according to standard procedures using the marker to marker approach (Adeola, 2001). Urine was collected in urine buckets over a preservative of 50 mL HCl. Fecal samples and 20% of the collected urine were stored at -20°C immediately after collection. At the conclusion of the experiment, urine samples were thawed and mixed and 2 subsamples were collected. One of these sub-samples was lyophilized before analysis (Kim et al., 2009).

Fecal samples were dried in a 65°C forced air-drying oven and finely ground prior to analysis. The lyophilized urine sample, fecal samples, and diet and ingredient samples were analyzed in duplicate for concentrations of GE using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL). The urine sample that was not lyophilized was analyzed for N using the Kjeldahl method (method 976.05; AOAC Int., 2007). Diets, ingredients, and fecal samples were also analyzed for DM (method 930.15; AOAC Int., 2007), and N using a Leco Nitrogen Determinator (model FP628, Leco Corp., St. Joseph, MI). Additionally, all diets, ingredients, and fecal samples were analyzed for insoluble dietary fiber and soluble dietary fiber using the Ankom TDF Dietary Fiber Analyzer (method 991.43;AOAC Int., 2007; Ankom Technology, Macedon, NY). Total dietary fiber (**TDF**) in each sample was calculated as the sum of insoluble dietary fiber and soluble dietary fiber. Diets and ingredients were 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 (Columbia, MO). Before analysis, samples were hydrolyzed with 6*N* 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). Starch was determined in diet and ingredient samples using the glucoamylase procedure (method 979.10; AOAC Int., 2007). Amino acids and starch were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO) and all other analyses were conducted at the University of Illinois (Urbana, IL).

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 ATTD of N and TDF was also calculated using this equation. The DE and ME in diets were calculated by subtracting the GE in feces and the GE in feces and urine, respectively, from GE in the diet (NRC, 2012).

The retention of N (**Nr**) for each pig was calculated using the following equation (Pedersen et al., 2007):

$$Nr = \{[Ni - (Nf + Nu)]/Ni\} \times 100\%,$$

where Nr is the retention of N (%), Ni is the N intake (g), Nf and Nu are N output (g) in feces and urine, respectively. The biological value (**BV**) of the protein in the diets was also calculated

by expressing the retention of N as a percentage of the difference between N intake and N output in feces (Rojas and Stein, 2013).

Data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with the pig as the experimental unit. The fixed effect was diet. Random effects included pig, block, and replicate within block. Normality of residuals was tested using the Proc UNIVARIATE of SAS. Least square means were calculated for each dependent variable. Contrast statements were used to determine linear and quadratic effects of IQ on the ATTD of GE and TDF and on DE and ME, as well as N retention and BV. Results were considered significant at $P \leq 0.05$ and considered a trend at $0.05 < P \leq 0.10$.

Results

There was no effect of IQ inclusion on ADFI, ATTD of GE, or ATTD of TDF; nor was there an effect of IQ inclusion on the DE of the diets (Table 4.3). However, a quadratic increase and then decrease in the urinary GE output ($P < 0.05$) was observed as IQ was added to the diet with the greatest GE output coming from the pigs fed diets with 40 or 80 mg/kg IQ. This resulted in a tendency (quadratic; $P < 0.10$) for a reduced ME of the diet as IQ was added with the least ME for the diets with 40 or 80 mg/kg of IQ.

Isoquinoline alkaloid inclusion in the diets did not affect N intake or N output in feces and urine. There was a tendency for a reduction (quadratic; $P < 0.10$) in the ATTD of N as IQ was added to the diets (Table 4.4) with the least ATTD for diets with 40 or 80 mg/kg IQ. Likewise, there was a decrease in N retention when calculated as grams per day or as a percent of intake (quadratic; $P < 0.05$) with the least retention for diets with 40 or 80 mg/kg IQ. However, there was no impact of IQ inclusion on the BV of N in the diets.

Discussion

Effects of isoquinoline alkaloids on ATTD of energy, and on DE and ME

The lack of an effect of IQ on the ATTD of GE and on DE is in accordance with research conducted with weanling pigs (Rundle, 2018). However, there has been evidence that IQ may increase the apparent ileal digestibility of starch (Rundle et al., 2020), indicating that IQ may improve absorption of carbohydrates and, therefore, increase energy digestibility by the pig. However, such an effect was not demonstrated, which may be because older pigs with presumably greater ability to digest starch were used in this experiment. The tendency for ME in the diet to decrease quadratically as IQ was added to the diet was a result of the increase in urinary GE output; however, it is not known why this occurred. The proposed mechanism of action for IQ is that it acts as an anti-inflammatory (Agarwal et al., 1991; Chaturvedi et al., 1997), and antimicrobial agent (Walker et al., 1990). Pigs used in this experiment were from a high health status herd and there may, therefore, not have been a need for modulating the immune status of these pigs.

Effects of isoquinoline alkaloids on ATTD of fiber

The absence of an influence of IQ on the ATTD of TDF was unexpected due to the proposed antimicrobial effects of IQ (Walker, 1990; Newton et al., 2002). When included in swine diets, IQ may promote colonization of beneficial bacteria and shift the microbial composition of the gut (Liu et al., 2016). Likewise, changes in fecal SCFA concentrations indicated that IQ may change the activity of microbes in the gastrointestinal tract of canines (Faehnrich et al., 2019). Isoquinoline alkaloids also decrease incidence of diarrhea in pigs (Liu et al., 2016), which is commonly associated with rapid growth of pathogenic bacteria in the intestine. Therefore, it was

expected that IQ would increase the ATTD of TDF; however, that was not the case, which may be a result of the high health status of the herd.

Effects of isoquinoline alkaloids on N balance

The lack of differences among treatments in N intake, N output in the feces and urine, and BV of diets in this experiment is in agreement with data indicating that IQ does not impact N retention in young pigs (Tschirner et al., 2003) or in red tilapia (Rawling et al., 2009). In contrast, when IQ was added to corn-DDGS diets fed to steers, an increase in non-ammonia N and a decrease in ammonia N was observed, indicating an improvement in N efficiency with IQ supplementation (Aguilar-Hernandez et al., 2016). The steers fed the diets supplemented with IQ also had an improved post-ruminal total tract digestion of N as IQ was increased in the diet (Aguilar-Hernandez et al., 2016). Likewise, inclusion of 50 mg IQ/kg feed in diets for early weaned pigs resulted in a decrease in ammonia concentration in the cecum compared with pigs fed the control diet without IQ (Chen et al., 2018), indicating either improved N efficiency or reduced N fermentation in the large intestine of those pigs. It is possible that the lack of a response under the conditions of this experiment may be a result of the diets being at or above individual amino acid requirements. As IQ may improve the apparent ileal digestibility of AA (Boroojeni et al., 2018; Rundle et al., 2020), further research is needed to elucidate the effects of IQ on the ATTD of N and N balance by pigs fed diets that are below the AA requirements.

Conclusions

Isoquinoline alkaloids did not influence the ATTD of GE, fiber, or N, and IQ did not impact DE, or the biological value of N under the conditions of this experiment. It is possible that a different outcome may be observed in immune challenged pigs or in pigs that are fed diets containing AA below requirements. The high health status of the pigs used in this experiment may be the reason

for the lack of differences among treatments. Therefore, further research is needed to elucidate effects of IQ on total tract digestibility of nutrients in pigs when nutrients are fed below the requirements.

Tables

Table 4.1. Composition (as-is basis) of experimental diets

Item	Isoquinoline alkaloids, mg/kg			
	0	40	80	160
Ground corn	65.32	64.92	64.52	63.72
DDGS[1]	15.00	15.00	15.00	15.00
Soybean meal	16.00	16.00	16.00	16.00
Soybean oil	1.00	1.00	1.00	1.00
Ground limestone	0.80	0.80	0.80	0.80
DCP[1]	0.90	0.90	0.90	0.90
L-Lys HCl	0.30	0.30	0.30	0.30
L-Thr	0.03	0.03	0.03	0.03
Salt	0.50	0.50	0.50	0.50
IQ premix[2]	-	0.40	0.80	1.60
Vit-mineral premix[3]	0.15	0.15	0.15	0.15

[1] DDGS = Distiller's dried grains with solubles; DCP= dicalcium phosphate.

[2] Phytobiotics Futterzusatzstoffe GmbH, Eltville, GE. Premix was prepared by mixing 10 g IQ with 990 g ground corn.

[3] 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-pantothenic acid as D-

Table 4.1 (cont.)

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 4.2. Proximate analysis of experimental diets and ingredients (as-is basis)

Item	Isoquinoline alkaloids, mg/kg						
	0	40	80	160	Corn	SBM[1]	DDGS[1]
GE, kcal/kg	3,966	3,929	3,924	3,919	3,802	4,137	4,454
DM, %	87.11	86.78	86.77	86.79	86.16	88.22	84.54
N, %	2.57	2.40	2.55	2.50	1.09	7.40	4.26
IDF[2], %	13.30	13.10	12.70	12.80	10.10	16.10	28.50
SDF[2], %	1.10	1.50	1.00	0.80	0.20	0.90	0.50
TDF[2], %	14.40	14.60	13.70	13.60	10.30	17.00	29.00
Starch, %	42.61	39.84	41.48	41.84	62.08	2.17	4.54
Indispensable AA							
Arg	0.90	0.94	0.87	0.99	0.30	3.39	1.19
His	0.43	0.43	0.42	0.45	0.19	1.22	0.72
Ile	0.68	0.69	0.64	0.73	0.24	2.28	1.08
Leu	1.53	1.54	1.45	1.61	0.75	3.60	3.02
Lys	1.01	0.94	0.94	1.04	0.22	2.97	0.78
Met	0.26	0.27	0.24	0.30	0.13	0.64	0.53
Phe	0.77	0.79	0.73	0.83	0.31	2.38	1.22
Thr	0.62	0.61	0.59	0.64	0.23	1.80	0.99
Trp	0.18	0.20	0.18	0.17	0.06	0.65	0.23
Val	0.79	0.79	0.75	0.84	0.31	2.34	1.36

[1]SBM = soybean meal; DDGS = Distiller's dried grains with solubles.

[2]IDF = insoluble dietary fiber; SDF = soluble dietary fiber; TDF = total dietary fiber.

Table 4.3. Apparent total tract digestibility (ATTD) of gross energy (GE) and total dietary fiber (TDF), and digestible energy (DE) and metabolizable energy (ME) concentrations of diets supplemented with isoquinoline alkaloids fed to growing pigs (as-fed basis)[1]

Item	Isoquinoline alkaloids, mg/kg					*P*-Value	
	-	40	80	160	SEM	Linear	Quadratic
Initial BW, kg	56.80	57.34	57.46	56.92	6.285	0.959	0.065
ADFI, kg	2.18	2.18	2.16	2.14	0.152	0.317	0.847
Dry feces output, kg/d	0.26	0.26	0.27	0.25	0.010	0.523	0.285
GE in dry feces, kcal/kg	4,741	4,721	4,734	4,761	22.9	0.404	0.401
Fecal GE output, kcal/d	1,219	1,251	1,266	1,198	45.2	0.675	0.306
TDF in dry feces, %	40.86	41.40	39.92	40.60	1.060	0.563	0.632
ATTD of GE, %	85.75	85.40	84.94	85.72	0.995	0.989	0.242
ATTD of TDF, %	65.28	64.28	64.31	65.81	1.75	0.697	0.411
DE in diet, kcal/kg	3,374	3,360	3,342	3,373	39.1	0.988	0.242
Urine output, kg/d	7.40	8.02	10.28	8.29	1.18	0.538	0.135
GE in urine, kcal/kg	30.25	32.76	27.74	28.16	3.8	0.532	0.994
Urinary GE output, kcal/d	203.38	240.91	266.00	208.75	31.334	0.995	0.031
ME in diet, kcal/kg	3,278	3,250	3,220	3,275	35.6	0.985	0.062

[1]Data are least square means of 10 observations per treatment, except for the 0 mg/kg treatment which used 9 observations and 160 mg/kg treatment which used 8 observations.

Table 4.4. Apparent total tract digestibility (ATTD) of N and N balance of growing pigs fed diets supplemented with isoquinoline alkaloids during a 4-d collection period (as-fed basis)[1]

Item	Isoquinoline alkaloids, mg/kg					*P*-Value	
	-	40	80	160	SEM	Linear	Quadratic
Feed intake, g/4 d	8,713	8,716	8,647	8,592	606.32	0.369	0.946
N intake, g/4 d	223.54	209.31	220.37	214.37	15.22	0.248	0.270
N output in feces , g/4 d	37.33	38.30	40.19	37.31	1.70	0.990	0.188
N output in urine, g/4 d	35.01	37.57	37.56	35.33	5.58	0.941	0.374
ATTD of N, %	83.26	81.67	81.51	82.52	1.77	0.636	0.061
N retention, g/4 d	151.33	133.30	142.63	142.20	11.26	0.370	0.039
N retention, %	67.75	63.77	64.55	66.13	1.13	0.672	0.034
Biological value[2], %	81.43	78.24	79.15	80.11	1.58	0.779	0.173

[1] Data are least square means of 10 observations per treatment.

[2]Biological value was calculated as (N retained/ [N intake – N output in feces]) × 100 (Rojas and Stein, 2013).

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CHAPTER 5: Effects of isoquinoline alkaloids on growth performance, blood characteristics, N balance and total tract digestibility of energy, Ca, and P by pigs fed corn-soybean meal diets formulated below amino acid requirements

Abstract

Two experiments were conducted to test the hypothesis that isoquinoline alkaloids (IQ) may be included in a diet formulated below amino acid (AA) requirements to improve AA utilization and subsequently maintain growth performance and nutrient and energy digestibility compared with a diet formulated at AA requirements. Experiment 1 was conducted for 56 d in which 192 pigs (initial body weight (BW): 24.74 ± 1.98 kg) were allotted to a 2 × 2 factorial that included two levels of AA (at requirements for indispensable AA or 10% below requirements) and two levels of IQ (0 or 90 mg IQ/kg feed). Average daily gain (ADG), average daily feed intake (ADFI) and gain:feed (G:F) were calculated. Blood samples were collected, and plasma was analyzed for indicators of protein metabolism and acute phase proteins. Experiment 2 utilized 40 barrows (initial BW: 23.28 ± 1.91 kg) housed in metabolism crates to determine the effects of IQ in diets with reduced AA concentrations on the apparent total tract digestibility (ATTD) of energy and N, and on the biological value of N. Pigs were allotted to a 2 × 2 factorial in which there were two levels of AA (5 or 8% below indispensable AA requirements) and two levels of IQ (0 or 90 mg IQ/kg feed). Following a 7 d adaptation to the treatments, feces and urine were collected using standard procedures. Results of Exp. 1 indicated that if AA were reduced in the diet, there was a reduction ($P < 0.05$) in ADG and G:F from d 1 to 28, d 29 to 56, and for the

overall experiment. On d 14, IQ increased ($P < 0.05$) concentration of plasma IL-1β and IL-18, whereas on d 42, IQ inclusion resulted in an increase of IL-8 and TNFα in the plasma of gilts regardless of AA concentration (interaction, $P < 0.05$) and there was an increased concentration of IL-2 if AA were reduced compared with when AA were at requirements (interaction, $P < 0.05$). Results of Exp. 2 indicated that pigs fed diets with 8% reduced AA concentrations had decreased ($P < 0.05$) N intake and N retention compared with pigs fed 5% reduced AA diets; however, biological value of N was not affected by IQ or by AA level. The ATTD of GE and DE and ME of the diet were unaffected by treatment, but ATTD of P decreased ($P < 0.05$) if diets contained IQ. In conclusion, inclusion of IQ in diets formulated below AA requirements did not impact growth performance or biological value of N, but changed plasma concentrations of acute phase proteins and markers of N utilization in pigs.

Key words: digestibility, isoquinoline alkaloids, nitrogen balance, pig

Abbreviations

AA	amino acids
AEE	acid hydrolyzed ether extract
ADFI	average daily feed intake
ADG	average daily gain
AID	apparent ileal digestibility
ATTD	apparent total tract digestibility
BV	biological value
BW	body weight
DE	digestible energy
GE	gross energy

G:F	gain:feed
IL-	interleukin-
IQ	isoquinoline alkaloids
ME	metabolizable energy
PUN	plasma urea nitrogen
TNFα	tumor necrosis factor-α

Introduction

Isoquinoline alkaloids (**IQ**) have anti-inflammatory and immunomodulatory effects (Agarwal et al., 1991; Chaturvedi, 1997), and may be included in diets fed to swine to improve growth performance (Robbins et al., 2013; Kantas et al., 2015; Liu et al., 2016a). Isoquinoline alkaloids also increase apparent ileal digestibility (**AID**) of nutrients (Boroojeni et al., 2018; Rundle et al., 2020), specifically crude protein and amino acids (**AA**), and improve intestinal health (Robbins et al., 2013; Liu et al., 2016b) of pigs. However, there are minimal data regarding the effects of IQ in diets formulated below AA requirements and the effects of IQ in diets for growing pigs beyond the nursery period.

Due to the demonstrated increase in digestibility of AA of diets containing IQ, it is possible that diets with less AA can be fed if IQ is used without a consequent reduction in growth performance or nutrient digestion and absorption, but data to demonstrate this have not been reported. Therefore, the objective of this experiment was to test the hypothesis that when IQ is added to diets formulated below AA requirements, growth performance and N utilization of growing pigs will be increased.

Materials and Methods

Protocols for two experiments were submitted to the Institutional Animal Care and Use Committee at the University of Illinois and approved prior to initiation of the experiments. Growing pigs that were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN) were used in both experiments.

Experiment 1: Effects of IQ on growth performance and blood characteristics of growing pigs

The experiment was conducted for 56 days. There were 2 phases: phase 1 diets were formulated for pigs weighing 25 to 50 kg and fed from d 1 to 28, and phase 2 diets were formulated for pigs weighing 50 to 75 kg and fed from d 29 to 56 (NRC, 2012; Table 5.1). Within each phase, dietary treatments were organized as a 2 × 2 factorial in which there were two levels of AA (at indispensable AA requirements or 10% below requirements) and two inclusion levels of IQ (0 or 90 mg IQ/kg feed; Phytobiotics Futterzusatzstoffe GmbH, Eltville, GE). Therefore, a total of 8 experimental diets were formulated.

A total of 192 pigs were used (initial body weight (**BW**): 24.74 ± 1.98 kg). There were 4 treatments and 12 replicate pens per treatment for a total of 48 pens and 4 pigs per pen. Pens had partially slatted flooring and were equipped with a nipple drinker and a feeder that allowed for *ad libitum* access to feed. For each diet, there were six replicate pens housing barrows and six replicate pens housing gilts. Individual pig weights were recorded at the beginning of the experiment (d 1), d 14, 28, 42, and at the conclusion of the experiment (d 56). Daily feed allotments were recorded, and feed left in the feeders were weighed on d 28 and 56 to calculate feed consumption. Pigs were checked daily for general physical condition and feeders and water nipples were checked as well to ensure pigs had free access to feed and water. If a pig was

removed from a pen, the feed left in the feeder of the pen the pig was removed from and individual pig weights of the remaining pigs in the pen were recorded on the day the pig was removed and data for feed intake and gain:feed (**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 average daily gain (**ADG**), average daily feed intake (**ADFI**), and G:F for d 1 to 28, d 29 to 56, and for the entire experiment.

On d 1, the pig in each pen with the BW closest to the pen average was identified. Two blood samples were collected on d 1, 14, 28, 42, and 56 from the jugular vein of this pig in vacutainers containing either lithium heparin or ethylenediaminetetraacetic acid. Blood samples were centrifuged at 2,000 × g at 4˚C for 15 min to recover the plasma. The heparinized sample was analyzed for plasma urea nitrogen (**PUN**), albumin, and total protein using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA). The heparinized sample was also analyzed for free AA using cation-exchange liquid chromatography with post column ninhydrin reaction and detection at the University of Missouri (Columbia, MO). The blood sample collected in ethylenediaminetetraacetic acid was analyzed for acute phase proteins including tumor necrosis factor-α (**TNFα**) and interleukins (**IL-)** 1α, 1β, 1RA, 2, 4, 6, 8, 10, 12, 18 using a cytokine/chemokine magnetic bead panel according to the manufacturer's specifications (MILLIPLEX Porcine Cytokine/Chemokine Magnetic Bead Panel; EMD Millipore, Darmstadt, Germany).

Diets were analyzed for dry matter (method 930.15; AOAC Int, 2019), dry ash (method 942.05; AOAC Int, 2019), and gross energy (**GE**) was determined using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL) with benzoic acid as the standard for calibration. Acid hydrolyzed ether extract (**AEE**) was determined by acid hydrolysis using 3*N* HCl

(Ankom^{HCl}, Ankom Technology, Macedon, NY) followed by fat extraction (Ankom $^{XT-15}$, Ankom Technology, Macedon, NY). Crude protein was calculated as N × 6.25, and N was analyzed using the combustion procedure (method 990.03; AOAC Int, 2019) on the LECO FP628 Nitrogen Analyzer (Leco Corp., St. Joseph, MI). Diets were also analyzed for AA 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. Prior to analysis, samples were hydrolyzed with 6*N* HCl for 24 h at 110°C [method 982.30 E(a); AOAC Int., 2019]. 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., 2019]. Tryptophan was determined after NaOH hydrolysis for 22 h at 110°C [method 982.30 E(c); AOAC Int., 2019]. Amino acids were analyzed at the University of Missouri Experiment Station Chemical Laboratory (Columbia, MO). All other analyses were conducted at the University of Illinois (Urbana, IL.).

Data were analyzed as a 2 × 2 × 2 factorial using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with a randomized complete block design. The model included the fixed effects of AA level, IQ inclusion, sex, and all possible interactions and the random effect of group. Least square means were calculated for each independent variable and means were separated using the PDIFF option. For blood characteristics, data from d 1 was used as a covariate for data from d 14, 28, 42, and 56. Normality of residuals was tested using the Proc UNIVARIATE of SAS and outliers were identified and removed using the BOXPLOT procedure. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Experiment 2: Effects of IQ on total tract digestibility of energy, Ca, and P, and N balance of growing pigs

A total of 40 barrows (initial BW: 23.28 ± 1.91 kg) were allotted to a completely randomized design with 4 dietary treatments and 2 blocks of 20 pigs for a total of 10 replicate pigs per dietary treatment. Pigs were housed individually in metabolism crates that were equipped with a feeder and a nipple drinker, fully slatted floors and a screen floor and a urine tray, which allowed for the total, but separate, collection of fecal materials and urine from each pig.

The four diets were arranged in a 2 × 2 factorial with two levels of AA (5% or 8% below indispensable AA requirements) and two levels of IQ inclusion (0 or 90 mg IQ/kg feed). All diets met nutrient requirements for 25 to 50 kg pigs, with the exception that AA were provided below requirements (NRC, 2012; Tables 5.2 and 5.3). Feed was provided at 3.4 times the estimated daily energy requirement for maintenance (i.e., 197 kcal/kg ME × $BW^{0.60}$; NRC, 2012) and provided every day in 2 equal meals at 0700 and 1600 h. During the experiment, pigs had free access to water. Pigs stayed in the metabolism crates for 14 d. The initial 7 d were considered the adaptation period to the crates and diets, whereas fecal and urine samples were collected from the feed provided from d 8 to d 12 according to standard procedures using the marker to marker approach (Adeola, 2001). Urine was collected in urine buckets over a preservative of 50 mL HCl. Fecal samples and 20% of the collected urine were stored at -20°C immediately after collection. At the conclusion of the experiment, urine samples were thawed and mixed and a subsample was collected.

Fecal samples were dried in a 65°C forced air-drying oven and finely ground prior to analysis. Diets, ingredients, and fecal samples were analyzed for dry matter, dry ash, and N as explained in Exp. 1. Crude protein was calculated as N × 6.25. Gross energy in diets, ingredients,

and fecal and urine samples was determined as mentioned in Exp. 1. Diets and ingredients were also analyzed for AEE and AA as described in Exp. 1. Urine samples were analyzed for N using the Kjeldahl method (method 984.13; AOAC Int, 2019) on a Kjeltec^{TM} 8400 apparatus (FOSS, Eden Prairie, MN). Diets, ingredients, and fecal samples were analyzed for Ca and P by inductively coupled plasma spectroscopy (ICP-OES; Avio 200, PerkinElmer, Waltham, MA, USA; method 985.01 A, B, and C; AOAC Int., 2019). Sample preparation included dry ashing for 600°C for 4 h (method 942.05; AOAC Int., 2019) and wet digestion with nitric acid (method 3050 B; U.S. Environmental Protection Agency, 2000).

The apparent total tract digestibility (**ATTD**) of N was calculated based on the method described by Pedersen et al. (2007) using the following equation:

$$\text{ATTD of N} = [(\text{Ni} - \text{Nf})/\text{Ni}] \times 100\%,$$

where ATTD of N is the apparent total tract digestibility of N (%); Ni is the N intake (g); and Nf is the N output (g) in feces. The ATTD of Ca, P, and GE was also calculated using this equation. Digestible energy (**DE**) and metabolizable energy (**ME**) in diets were calculated by subtracting GE in feces and GE in feces and urine, respectively, from GE in the diet (NRC, 2012).

Retention of N (Nr) was calculated using the following equation (Rojas and Stein, 2013):

$$\text{Nr} = \{[\text{Ni} - (\text{Nf} + \text{Nu})]/\text{Ni}\} \times 100\%,$$

where Nr is the retention of N (%), Ni is the N intake (g), Nf and Nu are N output (g) in feces and urine, respectively. The biological value (**BV**) of protein was calculated by expressing retention of N as a percentage of the difference between N intake and N output in feces (Rojas and Stein, 2013).

Data were analyzed as a 2 × 2 factorial using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with the pig as the experimental unit. The model included the fixed

effects of AA level, IQ inclusion, and the interaction between AA level and IQ inclusion. The interaction between AA level and IQ inclusion was not significant and, therefore, removed from the final model. The random effect was block. Least square means were calculated for each independent variable and means were separated using the PDIFF option. Normality of residuals was tested using the Proc UNIVARIATE of SAS and outliers were identified and excluded using the BOXPLOT procedure. Results were considered significant at $P < 0.05$ and considered a trend at $0.05 \leq P < 0.10$.

Results

Experiment 1

There were no 2- or 3-way interactions between the main effects of AA level, IQ inclusion, and sex on growth performance parameters. A reduction in ADG was observed ($P < 0.05$) from d 1 to 28, d 29 to 56, and during the overall experimental period if dietary AA were below requirements and ADFI was reduced ($P < 0.05$) from d 29 to 56 and during the overall experimental period if AA were below requirements (Table 4). The G:F was reduced ($P < 0.05$) from d 1 to 28, d 29 to 56, and during the overall experimental period if AA were below requirements, and BW of pigs was also reduced ($P < 0.05$) on d 28, d 42, and d 56. Average daily gain and ADFI were reduced ($P < 0.05$) in gilts compared with barrows from d 1 to 28, d 29 to 56, and for the overall experimental period; however, there was no effect of sex on G:F from d 1 to 28, d 29 to 56, or overall. Body weight was reduced ($P < 0.05$) in gilts compared with barrows on d 28, d 42, and d 56. There was no effect of IQ on growth performance parameters including ADFI, ADG, or G:F, nor was there an effect of IQ on BW of pigs.

On d 14, gilts had greater IL-1β and IL-18 than barrows if no IQ was used, but if IQ was added to the diets, IL-1β and IL-18 were reduced in plasma of gilts compared with barrows (interaction, $P < 0.05$; Table 5). Plasma concentrations of IL-1α, IL-1RA, IL-2, IL-6, and IL-10 also tended to be greater in gilts than in barrows on d 14 if no IQ was used, whereas the opposite was observed if IQ was included in the diets (interaction, $P < 0.10$). On d 28, plasma concentrations of IL-1β, IL-1RA, IL-6, and IL-10 tended to be greater in gilts than in barrows if no IQ was included; however, when IQ was supplemented in the diets, these cytokines were greater in the plasma of barrows than in gilts (interaction, $P < 0.10$). If IQ was not included in the diet on d 42, barrows had greater concentration of plasma IL-8 and TNFα compared with gilts, whereas when IQ was added to the diet, gilts had greater concentration of these cytokines in plasma than barrows regardless of dietary AA concentration (interaction, $P < 0.05$). If AA were at requirements, IQ reduced plasma concentration of IL-2 on d 42, whereas if AA were below requirements, IQ supplementation increased the concentration of plasma IL-2 (interaction, $P < 0.05$). Plasma IL-6 also tended to be reduced on d 42 if IQ was added to the diet at AA requirements, but increased if IQ was added to the diet with AA below requirements (interaction, $P < 0.10$). On d 56, TNFα was greater in gilts than barrows when fed the diet with reduced AA and 90 mg/kg IQ, whereas if AA were included at requirements, TNFα was not affected by IQ in the diet (interaction, $P < 0.05$). Regardless of dietary AA concentration, if IQ was not included, gilts and barrows had concentrations of plasma IL-8 that were not different, but if IQ was added to the diet, gilts had greater concentrations of plasma IL-8 than barrows (interaction, $P < 0.05$).

For plasma AA on d 14, the only 3-way interactions ($P < 0.05$) were for Arg and Gly (Table 6). Gilts had lower plasma Arg than barrows if diet AA were at requirements and no IQ was used, but if IQ was used or if diet AA were below requirements, plasma Arg in gilts was not

different from barrows. Barrows fed the diet with IQ also had lower plasma Arg if AA were at the requirement, but if AA were below requirements, no effect of IQ was observed. If diet AA were at requirements, there was no difference in plasma Arg between barrows fed IQ and gilts fed no IQ, but if diet AA was below the requirement, barrows fed IQ had lower plasma Arg than gilts fed no IQ. Plasma Gly was greater in barrows than gilts if IQ were included in the diet formulated at requirements; however, if IQ was not included or if AA were 10% below requirements, plasma Gly was not different between barrows and gilts. If IQ were included in the diet, barrows fed the diet at requirements had greater plasma Gly than barrows fed the diet 10% below requirements. There was also a 2-way interaction ($P < 0.05$) between IQ and AA concentration with IQ reducing plasma Cys if dietary AA were at requirements, but if diet AA were below requirements, there was no impact of IQ on plasma Cys. Gilts had reduced plasma concentration of Met and Tyr compared with barrows if there was no IQ in the diet, but if IQ was added to the diet, there was no difference between gilts and barrows for plasma Met and Tyr (interaction, $P < 0.05$). For most AA, plasma concentrations were reduced ($P < 0.05$) if dietary AA were reduced, but there were no consistent effects of sex or IQ on plasma AA.

On d 28, no 3-way interactions were observed. However, inclusion of IQ resulted in an increase in plasma concentrations of Gln, Lys, and Phe if diet AA were below requirements, but if diet AA were at requirements, no impact of IQ was observed (interaction, $P < 0.05$; Table 7). Plasma urea nitrogen, total protein, and albumin concentrations were also reduced to a lesser extent in pigs fed the diet formulated below AA requirements compared with pigs fed the diets formulated at AA requirements when IQ was included (interaction, $P < 0.05$). Reducing AA in the diet resulted in reduced ($P < 0.05$) plasma concentrations of Arg, Asn, Cys, His, Ile, Leu,

Phe, Trp, Tyr, and Val, and increased ($P < 0.05$) concentrations of Ala, Asp, Gln, Glu, Lys, Thr, in the plasma regardless of IQ inclusion or sex.

On d 42, there were no 3-way interactions. Pigs fed the diet formulated at AA requirements had increased PUN on d 42 when IQ was included compared with pigs fed diets without IQ, whereas if the diet contained AA below requirements, PUN was reduced when IQ was included in the diet (interaction, $P < 0.05$; Table 8). Concentration of albumin in the plasma was reduced if IQ was included in diets formulated at AA requirements, but there was no influence of IQ on plasma albumin if pigs were fed the diet with AA below requirements (interaction, $P < 0.05$). Total protein was reduced in the plasma of gilts compared with barrows if diets with AA at requirements were fed, but that was not the case if diets with AA below requirements were fed (interaction, $P < 0.05$). Plasma Arg was reduced in gilts compared with barrows if diets with AA at requirements were fed, but if AA were below requirements, no differences between gilts and barrows were observed for plasma Arg (interaction, $P < 0.05$). Adding IQ to the diet with AA below requirements reduced plasma Asn, Ile, and Val, but that was not the case if dietary AA were at the requirements (interaction, $P < 0.05$).

The only 3-way interaction ($P < 0.05$) for plasma AA on d 56 was for Gly (Table 9). If AA were at requirements and no IQ was used, plasma Gly in gilts was greater than in barrows, but if IQ was used, plasma Gly was not different between barrows and gilts. If dietary AA were below requirements, barrows had greater plasma Gly than gilts if no IQ was used and barrows fed diets without IQ had greater plasma Gly than barrows fed with IQ. Barrows fed the diet without IQ had greater plasma Gly if AA were below requirements than at AA requirements. Plasma Ala of barrows was greater than of gilts if the diet with IQ was fed, whereas if IQ was not included in the diet, there was no difference in plasma Ala between barrows and gilts

regardless of dietary AA concentration (interaction, $P < 0.05$). Plasma Arg, Phe, and Pro was less in barrows when IQ was included in the diet compared with barrows fed the diet without IQ whereas Arg, Phe, and Pro increased in the plasma of gilts when IQ was included in the diet compared with gilts fed the diet without IQ (interaction, $P < 0.05$). Total protein in plasma of barrows fed diets without IQ was greater than for barrows fed diets with IQ, but there was no effect of IQ on total protein in plasma of gilts (interaction, $P < 0.05$). On d 56, PUN was reduced ($P < 0.05$) in diets formulated below AA requirements compared with pigs fed diets with adequate AA regardless of IQ inclusion or sex of pigs. For most AA, plasma concentrations were reduced ($P < 0.05$) if dietary AA were reduced, but effects of sex or IQ on plasma AA were not consistent.

Experiment 2

Inclusion of IQ in diets resulted in decreased Ca and P intake of pigs compared with pigs fed diets without IQ ($P < 0.05$; Table 10). Additionally, Ca and P intake were reduced ($P < 0.05$) if diets were formulated with AA 8% below requirements compared with diets that were 5% below AA requirements. Isoquinoline alkaloid inclusion in the diet reduced ATTD of P ($P < 0.05$) compared with diets without IQ. A reduction in dietary AA from 5% to 8% below requirements tended to decrease ($P < 0.10$) fecal P output.

Formulating diets 8% below AA requirements resulted in a decrease ($P < 0.05$) in N intake, urinary N output, and N retention (g) and tended to reduce ($P < 0.10$) the ATTD of N compared with diets that were 5% below AA requirements (Table 11). Addition of IQ to diets tended to decrease ($P < 0.10$) ADFI compared with diets that did not contain IQ. There was no effect of IQ on the ATTD of N, BV, or N retention. Reducing AA to 8% below requirements

tended to decrease ($P < 0.10$) urinary GE output, but there was no effect of IQ or dietary AA concentration on the ATTD of GE or on DE and ME in diets (Table 12).

Discussion

Excess N in diets for pigs often results in excessive fermentation in the large intestine, resulting in bacterial dysbiosis and gastrointestinal distress for the animal (Stein and Kil, 2006). Therefore, the optimal crude protein and AA level for each production stage to maximize feed efficiency and health has been determined (NRC, 2012). Benefits of low crude protein diets include decreased feed cost and reduced N excretion, improvements in N efficiency, and improved intestinal health (Wang et al., 2018). However, reduction of crude protein in the diet commonly results in diminished growth performance (Liu et al., 1999; Nyachoti et al., 2006; Opapeju et al., 2008). When IQ is fed to growing pigs, AA digestibility is increased (Boroojeni et al., 2018; Rundle et al., 2018). Therefore, the objective of this study was to test the hypothesis that IQ will increase growth performance and N utilization if added to diets with reduced AA to negate the potential negative effects of formulating diets below AA requirements.

Reductions in growth performance parameters including ADFI, ADG, and G:F when crude protein is reduced in the diet are well documented (Liu et al., 1999; Nyachoti et al., 2006; Opapeju et al., 2008; Limbach et al., 2021; Lynegaard et al., 2021). Therefore, the decrease in growth performance when AA was reduced under the conditions of Exp. 1 were as expected. The observed differences in growth performance between barrows and gilts were also as expected (Friesen et al., 1994; Madrid et al., 2012). The lack of an effect of IQ on growth performance parameters was in accordance with previous work (Zhao et al., 2017; Rundle, 2018), but an improvement in growth performance of young growing pigs fed diets supplemented with IQ has

also been reported (Robbins et al., 2013; Kantas et al., 2015; Liu et al., 2016a; Boroojeni et al., 2018). A potential reason for the different results in the current experiment and previous experiments may be the difference in initial BW of the animals because older pigs were used in this experiment compared with previous experiments.

Protein utilization and efficiency is often indicated by concentrations of PUN in plasma (Coma et al., 1995), and plasma of young growing pigs fed diets with reduced crude protein contains less PUN than pigs fed diets at standard levels of crude protein (Limbach et al., 2021). The results of Exp. 1 were, therefore, in agreement with previous data. In contrast, PUN may also increase when excess AA are included in a diet, as protein synthesis is limited by the amount of indispensable AA that are provided by the diet and N from AA above that limit are excreted. It is possible that the increase in PUN as IQ was added to the diet that contained AA at requirements is a result of increased AA digestibility due to IQ inclusion (Boroojeni et al., 2018; Rundle et al., 2020). In the diet with AA below requirements, an increased AA digestibility caused by IQ may have reduced excess N from dispensable AA because protein synthesis was increased. However, this observation was not the same for PUN at all collection time points and it is, therefore, difficult to make firm conclusions about the effect of IQ on PUN.

The primary functions of albumin is to bind and transport nutrients and hormones in the bloodstream (Bern et al., 2015). Therefore, the observation that albumin decreased in the plasma collected on d 28, 42, and 56 when pigs were fed diets below AA requirements is likely a result of a reduction of AA in the bloodstream, because absorption of AA was reduced. The observations from Exp. 1 are in agreement with previous results demonstrating that IQ does not influence serum or plasma albumin (Kosina et al., 2003; Rawling et al., 2009; Abudabos et al., 2016; Rundle, 2018). In contrast, serum albumin concentration increased as IQ was added to a

diet for laying hens (Bavarsadi et al., 2017). The increases in plasma concentrations of some AA in Exp. 1 for pigs fed IQ are in agreement with the observation that concentrations of plasma AA were greater in pigs fed diets supplemented with IQ compared with control fed pigs (Liu et al., 2016a). The observation that most AA decreased in the plasma of pigs fed the diet formulated below AA requirements is likely a result of reduced AA absorption. The increases in plasma concentrations of some AA as dietary AA was reduced may be a result of reduced protein synthesis caused by one or more AA being limiting in the diet. The differences in plasma total protein and amino acids among barrows and gilts if IQ was included in the diet indicates that IQ may influence AA utilization in gilts and barrows to a different extent. It is possible this is related to differences in efficiency of protein deposition between gilts and barrows, but more research is needed to fully elucidate the interactive effects of IQ and sex on AA digestibility and utilization.

Cytokines are small proteins secreted from immune cells that are responsible for signaling to other immune cells. Cytokines may be pro- or anti-inflammatory in nature, they are present in innate and adaptive immune responses, and are indicative of the overall inflammatory response (Murphy and Weaver, 2016). There is variation among pigs in terms of immune response and cytokine synthesis; however, sex differences are likely negligible (De Groot et al., 2005). The interaction between inclusion of IQ and sex for plasma concentrations of pro-inflammatory cytokines indicates that IQ may have a greater effect on the immune system of gilts than of barrows. Further research is needed to fully elucidate these effects. Previous data indicate that reducing crude protein in diets for pigs increase the concentration of circulating IL-12, but does not influence concentrations of TNFα and IL-6 (Spring et al., 2020). However, the main mechanism of action for reducing crude protein in diets for pigs to improve health is to

reduce excess N fermentation, and therefore, the lack of differences in cytokine concentrations as a result of reducing AA was expected.

Results of previous studies with steers indicated that IQ increases post-ruminal total tract digestion of N and decreases ammonia N, indicating improved N utilization (Aguilar-Hernandez et al., 2016). A decrease in ammonia in the cecum of pigs fed diets with IQ compared with pigs fed diets without IQ may be attributed to an improvement in N efficiency or a decrease in N fermentation in the large intestine of those pigs (Chen et al., 2018) and may be a result of improved apparent ileal digestibility of AA as IQ were added to the diet (Boroojeni et al., 2018; Rundle et al., 2020). However, the lack of an effect of IQ on N retention and BV is in agreement with observations demonstrating that IQ does not influence N retention (Tschirner et al., 2003; Rawling et al., 2009). The differences in N intake that were observed are a consequence of reducing AA in the diet. When crude protein is reduced in the diet, retention of N (g/d) decreased whereas the BV of N increased (Otto et al., 2003). Indeed, the decrease in N retention (g) for pigs fed diets with AA below requirements reflects the decrease in N intake, whereas N retention (%) and BV of N was unaffected by differences in AA concentration in the diet.

Results of previous research have indicated that IQ may increase the digestibility of starch (Rundle et al., 2020), and consequently, may increase energy digestibility by the pig; however, the observed lack of an effect of IQ on the ATTD of GE is also in agreement with previous work (Rundle, 2018). The lack of an effect of IQ on DE and ME in diets contrasts previous observations indicating that supplemental IQ decreased ME of corn-soybean meal diets when fed to young growing pigs (Rundle et al., 2018). In contrast, DE increased as IQ increased in high-energy diets fed to steers (Aguilar-Hernandez et al., 2016) and an increase in dietary net energy was observed in diets supplemented with IQ fed to finishing ewes (Estrada-Angulo et al.,

2016). There are limited data for effects of IQ on the ATTD of Ca and P by growing pigs. Inclusion of 120 mg IQ/kg feed to diets for young growing pigs resulted in an increase in pre-cecal digestibility of P, but there was no effect of IQ inclusion on digestibility of Ca (Boroojeni et al., 2018). The decrease in ATTD of P as IQ was included in the diet that was observed in this experiment was, therefore, unanticipated, and may potentially be due to IQ reducing bioavailability of P, but more research is needed to fully determine this effect.

Conclusions

Isoquinoline alkaloids did not influence growth performance of growing pigs fed diets formulated to contain AA below requirements, but changed concentrations of protein metabolism markers and cytokines in the plasma, indicating that IQ affects N utilization and systemic inflammation of growing pigs. Isoquinoline alkaloids did not influence the BV of nitrogen, ATTD of GE and N, or DE and ME in diets fed to growing pigs, but did decrease the ATTD of P. More research is needed to elucidate the mechanism of action of IQ on N efficiency as well as to determine the specific effects of IQ on the immune system and digestibility and utilization of N-containing compounds.

Tables

Table 5.1. Composition of experimental diets (as-is basis), Exp. 1[1]

Item	Phase 1				Phase 2			
	PC	NC	PC + IQ	NC + IQ	PC	NC	PC + IQ	NC + IQ
Ingredients, %								
Ground corn	69.47	78.95	68.57	78.05	74.58	84.04	73.68	83.14
Soybean meal, 44% CP	25.00	15.00	25.00	15.00	20.00	10.00	20.00	10.00
Soybean oil	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Dicalcium phosphate	1.10	1.25	1.10	1.25	1.10	1.25	1.10	1.25
Ground limestone	0.80	0.80	0.80	0.80	0.70	0.70	0.70	0.70
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin-mineral mix[2]	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-Lys HCl, 78% Lys	0.25	0.45	0.25	0.45	0.25	0.45	0.25	0.45
L-Thr	0.05	0.13	0.05	0.13	0.05	0.13	0.05	0.13
DL-Met	0.03	0.05	0.03	0.05	0.02	0.04	0.02	0.04
L-Val	-	0.04	-	0.04	-	0.05	-	0.05
L-Trp	-	0.03	-	0.03	-	0.04	-	0.04
IQ premix[3]	-	-	0.90	0.90	-	-	0.90	0.90
Analyzed composition								
GE, kcal/kg	3,992	3,955	3,959	3,954	3,957	3,949	3,947	3,940
Crude protein, %	15.87	12.13	15.52	12.05	14.20	10.72	13.81	10.23
Dry matter, %	87.25	87.08	87.03	86.99	87.07	87.01	86.93	86.92

Table 5.1 (cont.)

Item	Phase 1				Phase 2			
	PC	NC	PC + IQ	NC + IQ	PC	NC	PC + IQ	NC + IQ
Ash, %	4.45	3.95	4.60	3.86	4.18	3.51	4.02	3.51
AEE[4], %	4.65	5.22	4.84	5.42	5.54	5.67	5.62	5.25
Indispensable AA[4], %								
Arg	1.07	0.77	1.07	0.72	0.89	0.60	0.90	0.56
His	0.45	0.35	0.45	0.33	0.38	0.28	0.39	0.27
Ile	0.78	0.57	0.77	0.52	0.64	0.46	0.67	0.45
Leu	1.52	1.26	1.51	1.15	1.35	1.09	1.37	1.06
Lys	1.12	1.00	1.11	0.96	0.96	0.85	0.97	0.79
Met	0.29	0.25	0.28	0.23	0.25	0.22	0.26	0.21
Phe	0.9	0.70	0.89	0.63	0.77	0.58	0.78	0.57
Thr	0.66	0.57	0.68	0.56	0.60	0.52	0.57	0.47
Trp	0.22	0.19	0.22	0.19	0.20	0.18	0.20	0.17
Val	0.85	0.68	0.84	0.62	0.72	0.59	0.74	0.58

[1]PC = positive control; NC = negative control, formulated at 10% below amino acid requirement; PC + IQ = positive control + 90 mg/kg IQ; NC + IQ = negative control + 90 mg/kg IQ.

[2]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;

Table 5.1 (cont.)

pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-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, 1.26mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30mg as sodium selenite and selenium yeast; and Zn, 125.1mg as zinc hydroxychloride.

[3]Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany.

[4]AEE = acid hydrolyzed ether extract; AA = amino acids.

Table 5.2. Composition of experimental diets (as-is basis), Exp. 2[1]

Item, %	PC	PC + IQ	NC	NC + IQ
Ground corn	78.93	78.03	80.85	79.95
Soybean meal, 44% CP	15.00	15.00	13.00	13.00
Soybean oil	2.50	2.50	2.50	2.50
Dicalcium phosphate	1.25	1.25	1.30	1.30
Ground limestone	0.80	0.80	0.75	0.75
Salt	0.50	0.50	0.50	0.50
Vitamin-mineral mix[2]	0.15	0.15	0.15	0.15
L-Lys HCl, 78% Lys	0.50	0.50	0.55	0.55
L-Thr	0.16	0.16	0.17	0.17
L-Val	0.08	0.08	0.09	0.09
DL-Met	0.06	0.06	0.06	0.06
L-Trp	0.04	0.04	0.04	0.04
L-Ile	0.03	0.03	0.04	0.04
IQ Premix[3]	-	0.90	-	0.90

[1]PC = positive control, formulated at 5% below amino acid requirements; NC = negative control, formulated at 8% below amino acid requirements; PC + IQ = positive control + 90 mg/kg IQ; NC + IQ = negative control + 90 mg/kg IQ.

[2]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;

Table 5.2 (cont.)

pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-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, 1.26mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30mg as sodium selenite and selenium yeast; and Zn, 125.1mg as zinc hydroxychloride.

[3]Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany.

Table 5.3. Analyzed composition of experimental diets and ingredients, Exp. 2[1]

Item	PC	PC + IQ	NC	NC + IQ	Corn	SBM
Dry matter, %	86.12	86.86	86.13	86.23	84.90	88.92
Ash, %	4.12	4.22	3.85	3.84	1.08	6.85
Crude protein, %	11.17	11.27	10.07	10.15	5.75	44.31
Gross energy, kcal/kg	3,910	3,932	3,901	3,906	3,797	4,171
AEE[2], %	5.73	6.10	5.58	5.85	3.83	2.32
Ca, %	0.59	0.56	0.58	0.56	0.01	0.27
P, %	0.59	0.54	0.56	0.55	0.34	0.77
Indispensable AA, %						
Arg	0.73	0.73	0.74	0.74	0.33	3.31
His	0.33	0.33	0.33	0.34	0.19	1.23
Ile	0.55	0.56	0.58	0.57	0.25	2.31
Leu	1.15	1.18	1.17	1.17	0.79	3.57
Lys	1.17	1.02	1.13	1.05	0.24	2.93
Met	0.23	0.24	0.25	0.25	0.12	0.6
Phe	0.62	0.62	0.63	0.63	0.33	2.4
Thr	0.58	0.63	0.7	0.58	0.25	1.75
Trp	0.15	0.16	0.15	0.16	0.05	0.65
Val	0.67	0.69	0.7	0.69	0.33	2.36

[1] PC = positive control; NC = negative control; PC + IQ = positive control + 90 mg/kg IQ; NC + IQ = negative control + 90 mg/kg IQ; SBM = soybean meal; AEE = acid hydrolyzed ether extract; AA = amino acids.

Table 5.4. Growth performance of growing pigs fed corn-soybean meal diets formulated below amino acid (AA) requirements and supplemented without or with isoquinoline alkaloids (IQ), Exp. 1[1]

Item	AA[2]			IQ[3]			Sex[4]			*P*-value		
	-	10%	SEM	0 mg/kg	90 mg/kg	SEM	Barrow	Gilt	SEM	AA	IQ	Sex
D 1 to 28												
ADG[5], kg	0.87	0.80	0.037	0.84	0.84	0.037	0.87	0.81	0.037	0.001	0.828	0.006
ADFI[5], kg	1.77	1.71	0.054	1.73	1.74	0.053	1.79	1.68	0.053	0.123	0.848	0.005
G:F[5]	0.49	0.47	0.008	0.49	0.48	0.008	0.48	0.48	0.008	0.002	0.319	0.684
D 29 to 56												
ADG, kg	1.12	0.98	0.015	1.04	1.05	0.015	1.12	0.97	0.015	<0.001	0.417	<0.001
ADFI, kg	2.72	2.54	0.062	2.63	2.63	0.063	2.83	2.42	0.063	0.001	0.947	<0.001
G:F	0.41	0.38	0.010	0.40	0.40	0.010	0.40	0.40	0.010	<0.001	0.378	0.596
D 1 to 56												
ADG, kg	0.99	0.89	0.020	0.94	0.94	0.020	1.00	0.89	0.020	<0.001	0.792	<0.001
ADFI, kg	2.24	2.12	0.059	2.18	2.19	0.059	2.31	2.05	0.059	0.006	0.906	<0.001
G:F	0.44	0.42	0.003	0.43	0.43	0.003	0.43	0.43	0.003	<0.001	0.892	0.507

Table 5.4 (cont.)

Item	AA[2]			IQ[3]			Sex[4]			*P*-value		
	-	10%	SEM	0 mg/kg	90 mg/kg	SEM	Barrow	Gilt	SEM	AA	IQ	Sex
BW[5], kg												
D 1	24.83	24.78	0.593	24.84	24.77	0.587	24.89	24.73	0.587	0.923	0.889	0.770
D 14	35.64	34.89	0.956	35.25	35.28	0.950	35.65	34.89	0.950	0.299	0.972	0.283
D 28	49.30	47.26	1.612	48.37	48.18	1.606	49.17	47.38	1.606	0.028	0.832	0.050
D 42	63.73	59.92	1.454	61.87	61.78	1.446	63.59	60.06	1.446	<0.001	0.924	0.001
D 56	80.51	74.58	1.662	77.41	77.68	1.650	80.57	74.52	1.650	<0.001	0.827	<0.001

[1]There was no interaction of main effects; therefore, the interaction terms were not included in the final analysis and only main effects are shown.

[2] Main effect of AA; AA were formulated at AA requirements or 10% below requirements.

[3]Main effect of IQ; IQ were included at either 0 or 90 mg/kg diet.

[4]Main effect of sex.

[5]ADG = average daily gain; ADFI = average daily feed intake; G:F = gain to feed ratio; BW = body weight.

Table 5.5. Plasma cytokine expression of growing pigs fed corn-soybean meal diets formulated at or below amino acid (AA) requirements and without or with isoquinoline alkaloids (IQ)[1], Exp. 1

Item	AA at requirements				AA 10 % below requirements											
IQ, mg/kg	0	0	90	90	0	0	90	90		*P*-value						
Sex	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	SE	AA	Sex	IQ	AA × IQ	AA × Sex	IQ × Sex	AA × IQ × Sex
Day 14, ng/mL																
IL-1α	0.021	0.026	0.031	0.014	0.010	0.028	0.024	0.017	0.006	0.619	0.862	0.695	0.918	0.365	0.091	0.789
IL-1β	0.111^{abc}	0.208^{ab}	0.255^{a}	0.076^{c}	0.123^{bc}	0.187^{abc}	0.132^{abc}	0.096^{bc}	0.074	0.650	0.414	0.652	0.612	0.476	0.009	0.248
IL-1RA	0.234	0.235	0.393	0.251	0.234	0.337	0.299	0.240	0.063	0.934	0.411	0.257	0.620	0.321	0.088	0.834
IL-2	0.125	0.164	0.238	0.095	0.130	0.200	0.129	0.112	0.050	0.834	0.617	0.477	0.715	0.339	0.077	0.529
IL-4	0.177	0.299	0.336	0.107	0.277	0.293	0.238	0.160	0.166	0.706	0.376	0.776	0.457	0.825	0.106	0.350
IL-6	0.041	0.045	0.064	0.021	0.028	0.057	0.035	0.021	0.014	0.583	0.410	0.738	0.529	0.342	0.069	0.992
IL-8	0.023	0.039	0.019	0.018	0.020	0.023	0.026	0.019	0.007	0.757	0.306	0.194	0.710	0.449	0.209	0.849
IL-10	0.178	0.196	0.396	0.140	0.189	0.259	0.210	0.157	0.071	0.838	0.935	0.340	0.312	0.288	0.059	0.558
IL-12	1.441	1.471	1.312	1.372	1.187	1.636	1.585	1.416	0.157	0.693	0.956	0.371	0.421	0.674	0.239	0.187
IL-18	0.573^{ab}	0.678^{ab}	0.939^{a}	0.521^{b}	0.684^{ab}	0.835^{ab}	0.732^{ab}	0.482^{b}	0.172	0.919	0.665	0.226	0.273	0.725	0.021	0.809
TNFα	0.028	0.026	0.025	0.019	0.030	0.028	0.027	0.033	0.009	0.335	0.604	0.539	0.777	0.528	0.924	0.521
Day 28, ng/mL																
IL-1α	0.017	0.021	0.024	0.012	0.015	0.013	0.022	0.015	0.007	0.679	0.800	0.547	0.448	0.993	0.349	0.624
IL-1β	0.161	0.197	0.187	0.087	0.119	0.131	0.266	0.098	0.048	0.817	0.880	0.265	0.166	0.751	0.055	0.903

Table 5.5 (cont.)

Item	AA at requirements				AA 10 % below requirements											
IQ, mg/kg	0	0	90	90	0	0	90	90		*P*-value						
Sex	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	SE	AA	Sex	IQ	AA × IQ	AA × Sex	IQ × Sex	AA × IQ × Sex
IL-1RA	0.249	0.386	0.375	0.249	0.227	0.301	0.230	0.244	0.055	0.185	0.719	0.793	0.559	0.618	0.098	0.339
IL-2	0.114	0.168	0.175	0.085	0.116	0.106	0.130	0.082	0.051	0.474	0.725	0.921	0.423	0.838	0.182	0.504
IL-4	0.154	0.295	0.311	0.100	0.144	0.133	0.229	0.200	0.097	0.752	0.744	0.404	0.643	0.852	0.223	0.253
IL-6	0.023	0.039	0.024	0.021	0.020	0.035	0.041	0.019	0.014	0.863	0.704	0.600	0.902	0.638	0.099	0.593
IL-8	0.022	0.030	0.021	0.030	0.028	0.016	0.019	0.033	0.012	0.698	0.832	0.662	0.477	0.492	0.223	0.263
IL-10	0.160	0.249	0.262	0.125	0.158	0.155	0.245	0.113	0.066	0.518	0.936	0.747	0.272	0.618	0.054	0.671
IL-12	1.559	1.273	1.498	1.372	1.265	1.557	1.397	1.283	0.169	0.642	0.847	0.680	0.586	0.186	0.572	0.198
IL-18	0.641	0.910	0.557	0.604	0.403	0.731	0.538	0.578	0.143	0.417	0.596	0.519	0.242	0.800	0.394	0.786
TNFα	0.028	0.019	0.022	0.021	0.044	0.029	0.033	0.043	0.013	0.022	0.964	0.773	0.471	0.745	0.232	0.599
Day 42, ng/mL																
IL-1α	0.016	0.020	0.016	0.007	0.016	0.011	0.024	0.011	0.006	0.757	0.509	0.183	0.108	0.663	0.137	0.527
IL-1β	0.166	0.167	0.162	0.083	0.132	0.179	0.227	0.142	0.046	0.432	0.645	0.249	0.353	0.572	0.110	0.911
IL-1RA	0.192	0.236	0.141	0.154	0.169	0.212	0.199	0.196	0.039	0.551	0.282	0.178	0.395	0.875	0.554	0.832
IL-2	0.113^{a}	0.078^{ab}	0.084^{ab}	0.054^{b}	0.108^{ab}	0.090^{ab}	0.133^{a}	0.078^{ab}	0.030	0.227	0.428	0.328	0.047	0.896	0.570	0.728
IL-4	0.183	0.212	0.237	0.054	0.147	0.095	0.228	0.156	0.075	0.998	0.899	0.134	0.115	0.699	0.242	0.214
IL-6	0.038	0.045	0.035	0.017	0.045	0.023	0.053	0.024	0.017	0.812	0.398	0.217	0.056	0.341	0.330	0.453
IL-8	0.024^{b}	0.016^{b}	0.014^{b}	0.022^{b}	0.026^{ab}	0.019^{b}	0.020^{b}	0.046^{a}	0.006	0.059	0.563	0.159	0.372	0.434	0.003	0.625

Table 5.5 (cont.)

Item	AA at requirements				AA 10 % below requirements											
IQ, mg/kg	0	0	90	90	0	0	90	90		*P*-value						
Sex	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	SE	AA	Sex	IQ	AA × IQ	AA × Sex	IQ × Sex	AA × IQ × Sex
IL-10	0.148	0.135	0.114	0.081	0.138	0.202	0.202	0.101	0.047	0.211	0.233	0.614	0.402	0.894	0.139	0.364
IL-12	1.225	1.227	1.210	1.158	0.955	1.172	1.321	1.144	0.112	0.464	0.438	0.222	0.948	0.725	0.198	0.316
IL-18	0.589	0.860	0.557	0.339	0.525	0.655	0.588	0.658	0.175	0.710	0.324	0.211	0.805	0.605	0.263	0.386
TNFα	0.033^{a}	0.028^{ab}	0.016^{b}	0.029^{ab}	0.038^{a}	0.021^{ab}	0.027^{ab}	0.031^{a}	0.010	0.460	0.326	0.254	0.984	0.148	0.026	0.924
Day 56, ng/mL																
IL-1α	0.017	0.010	0.016	0.010	0.008	0.011	0.019	0.009	0.003	0.549	0.590	0.537	0.163	0.609	0.306	0.228
IL-1β	0.112^{ab}	0.084^{b}	0.151^{ab}	0.110^{ab}	0.072^{b}	0.199^{a}	0.119^{ab}	0.115^{ab}	0.021	0.753	0.500	0.421	0.631	0.046	0.171	0.203
IL-1RA	0.192	0.183	0.191	0.171	0.132	0.265	0.175	0.204	0.026	0.878	0.907	0.849	0.185	0.057	0.249	0.372
IL-2	0.098	0.058	0.077	0.060	0.047	0.065	0.103	0.089	0.017	0.941	0.356	0.179	0.535	0.309	0.834	0.433
IL-4	0.163	0.088	0.143	0.068	0.046	0.064	0.143	0.082	0.027	0.366	0.512	0.251	0.300	0.461	0.513	0.623
IL-6	0.030	0.044	0.018	0.016	0.015	0.018	0.036	0.017	0.007	0.524	0.523	0.060	0.806	0.468	0.224	0.703
IL-8	0.021^{ab}	0.014^{b}	0.018^{ab}	0.023^{ab}	0.032^{a}	0.014^{b}	0.022^{ab}	0.033^{a}	0.008	0.169	0.293	0.799	0.438	0.742	0.010	0.440
IL-10	0.109	0.094	0.097	0.081	0.067	0.138	0.128	0.087	0.033	0.848	0.941	0.719	0.992	0.604	0.363	0.401
IL-12	1.501	1.516	0.997	1.063	1.134	1.313	1.402	1.211	0.150	0.885	0.056	0.009	0.819	0.818	0.471	0.292
IL-18	0.582	0.574	0.568	0.490	0.398	0.765	0.399	0.397	0.166	0.552	0.446	0.668	0.655	0.458	0.466	0.632
TNFα	0.031^{ab}	0.030^{abc}	0.030^{ab}	0.020^{bc}	0.032^{ab}	0.014^{c}	0.027^{abc}	0.038^{a}	0.010	0.860	0.582	0.047	0.149	0.978	0.216	0.018

[1]Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany.

Table 5.6. Blood characteristics of gilts and barrows fed diets formulated at or below amino acid (AA) requirements and without or with isoquinoline alkaloids (IQ)[1], d 14, Exp. 1

Item	AA at requirement				AA 10 % below requirement					*P*-value						
IQ, mg/kg	0	0	90	90	0	0	90	90								
Sex	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	SE	AA	IQ	Sex	AA × IQ	AA × Sex	IQ × Sex	AA × IQ × Sex
PUN[2]	9.62	8.02	8.26	9.97	4.57	3.79	3.67	4.06	1.153	<0.001	0.986	0.599	0.905	0.837	0.059	0.368
TP[2]	6.06	5.82	5.93	5.98	5.76	5.93	5.59	5.79	0.194	0.070	0.444	0.385	0.639	0.142	0.395	0.492
AL[2]	3.14	3.23	3.17	3.12	2.96	2.95	3.06	3.02	0.103	0.017	0.736	0.360	0.958	0.732	0.465	0.670
AA, %																
Ala,	2,017	1,544	1,659	1,674	2,405	2,282	1,887	1,999	221.990	0.002	0.041	0.284	0.406	0.383	0.147	0.624
Arg	192.75^{a}	140.66^{cd}	161.34^{bc}	169.15^{ab}	113.12^{ef}	131.72^{de}	96.94^{f}	105.37^{ef}	10.458	<0.001	0.068	0.138	0.586	0.009	0.060	0.011
Asn	79.17	68.17	73.66	72.00	61.58	55.65	67.86	58.00	5.674	0.001	0.619	0.512	0.097	0.829	0.706	0.370
Asp	23.84	20.62	20.88	20.07	21.67	21.48	20.68	22.65	1.252	0.726	0.282	0.233	0.469	0.064	0.140	0.937
Cys	5.39^{ab}	3.71^{c}	5.59^{a}	4.31^{bc}	4.11^{c}	3.55^{c}	4.15^{c}	3.39^{c}	0.660	0.002	0.546	0.436	0.004	0.164	0.850	0.623
Gln	367.33	377.56	371.82	361.55	435.96	462.58	381.70	414.71	24.459	<0.001	0.058	0.113	0.288	0.290	0.804	0.625
Glu	153.23	131.26	126.68	142.54	158.14	164.45	156.84	177.06	19.707	0.031	0.930	0.576	0.651	0.471	0.275	0.599
Gly	1,746^{ab}	1,815^{ab}	1,940^{a}	1,497^{b}	1,807^{ab}	1,802^{ab}	1,596^{b}	1,777^{ab}	196.960	0.957	0.279	0.736	0.560	0.102	0.329	0.044
His	37.14	35.52	33.36	39.89	21.73	20.61	16.48	20.76	2.807	<0.001	0.543	0.442	0.276	0.811	0.071	0.709
Ile	213.79	205.65	217.72	210.00	143.60	131.06	130.97	121.20	13.403	<0.001	0.649	0.327	0.244	0.836	0.919	0.940
Leu	337.16	311.54	323.10	313.22	297.51	279.07	253.33	260.46	20.513	<0.001	0.123	0.312	0.392	0.621	0.395	0.843

Table 5.6 (cont.)

Item	AA at requirement				AA 10 % below requirement											
IQ, mg/kg	0	0	90	90	0	0	90	90		*P*-value						
Sex	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	SE	AA	IQ	Sex	AA × IQ	AA × Sex	IQ × Sex	AA × IQ × Sex
Lys	533.21	566.90	547.00	538.74	639.42	698.17	574.38	625.96	36.057	<0.001	0.086	0.133	0.096	0.306	0.552	0.668
Met	51.38[a]	34.46[b]	42.43[ab]	49.32[a]	48.36[ab]	43.07[ab]	38.40[ab]	39.87[ab]	5.553	0.60	0.617	0.194	0.333	0.670	0.040	0.245
Phe	140.30	131.54	144.41	154.20	124.13	122.77	111.75	111.36	8.705	<0.001	0.890	0.025	0.974	0.898	0.390	0.421
Pro	456.56	444.57	430.91	418.63	443.40	444.17	389.57	405.93	23.947	0.287	0.025	0.530	0.916	0.514	0.803	0.807
Ser	114.55	86.37	94.79	89.08	89.72	84.52	83.86	87.78	10.156	0.118	0.418	0.555	0.158	0.180	0.207	0.579
Thr	261.75	235.48	214.67	202.06	270.37	255.95	217.56	228.40	41.324	0.523	0.087	0.999	0.650	0.699	0.674	0.899
Trp	70.52	61.32	71.12	61.57	48.15	46.56	40.23	47.34	5.549	<0.001	0.665	0.584	0.369	0.100	0.571	0.533
Tyr	243.93[a]	191.34[b]	208.80[ab]	207.64[b]	150.65[c]	148.98[c]	123.47[c]	144.69[c]	13.763	<0.001	0.168	0.726	0.346	0.047	0.049	0.433
Val	357.79	320.84	356.50	339.76	261.89	261.97	225.59	224.83	21.291	<0.001	0.279	0.082	0.285	0.308	0.709	0.682

[1]Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany.

[2]PUN = plasma urea nitrogen, mg/dL; TP = total protein, g/dL; AL = albumin, g/dL.

Table 5.7. Blood characteristics of gilts and barrows fed diets formulated at or below amino acid (AA) requirements and without or with isoquinoline alkaloids (IQ)[1], d 28, Exp. 1

Item	AA at requirement				AA 10 % below requirement					*P*-value						
IQ, mg/kg	0	0	90	90	0	0	90	90								
Sex	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	SE	AA	IQ	Sex	AA × IQ	AA × Sex	IQ × Sex	AA × IQ × Sex
PUN[2]	10.82[a]	8.00[b]	9.86[ab]	9.37[ab]	5.47[c]	3.47[c]	4.17[c]	4.58[c]	0.788	<0.001	0.916	0.754	0.016	0.392	0.019	0.968
TP[2]	6.81[a]	6.16[b]	6.34[b]	6.18[b]	6.12[b]	5.90[b]	6.21[b]	6.04[b]	0.165	0.009	0.581	0.131	0.014	0.317	0.197	0.312
AL[2]	3.65[a]	3.20[bc]	3.48[ab]	3.09[c]	3.10[c]	2.98[c]	3.19[bc]	3.02[c]	0.115	0.001	0.615	0.189	0.002	0.081	0.975	0.697
AA, %																
Ala	1191.48	1476.00	1395.37	1428.67	1732.29	1790.34	1974.77	1792.85	179.820	0.001	0.396	0.856	0.673	0.354	0.305	0.981
Arg	152.93	149.97	133.09	163.61	99.01	104.13	121.59	135.71	11.010	<0.001	0.087	0.034	0.101	0.761	0.144	0.374
Asn	68.09	69.65	58.74	68.47	57.14	58.06	72.63	67.28	6.669	0.527	0.361	0.035	0.670	0.312	0.904	0.353
Asp	18.74	20.98	19.11	18.74	23.04	22.33	21.99	22.40	1.140	0.000	0.332	0.774	0.657	0.468	0.606	0.240
Cys	5.57	4.32	5.06	4.30	4.52	4.36	3.75	3.89	0.411	0.015	0.106	0.512	0.073	0.069	0.454	0.871
Gln	331.09[de]	373.11[bcd]	308.76[e]	360.00[cde]	380.22[abcd]	422.70[ab]	409.76[abc]	423.06[a]	22.341	<0.001	0.916	0.202	0.005	0.459	0.697	0.440
Glu	107.19	122.40	106.03	110.53	155.38	123.09	168.99	156.89	17.741	0.000	0.344	0.118	0.497	0.083	0.802	0.399
Gly	1806.91	1790.76	1884.76	1588.26	2018.21	1979.96	1947.01	1828.91	215.890	0.074	0.397	0.801	0.239	0.685	0.355	0.611
His	38.91	40.40	30.98	40.73	20.28	19.26	20.45	24.34	3.587	<0.001	0.789	0.143	0.111	0.337	0.134	0.699
Ile	204.51	196.46	198.66	217.97	126.75	110.01	138.28	131.72	16.938	<0.001	0.235	0.679	0.797	0.410	0.367	0.686

Table 5.7 (cont.)

Item	AA at requirement				AA 10 % below requirement											
IQ, mg/kg	0	0	90	90	0	0	90	90		*P*-value						
Sex	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	SE	AA	IQ	Sex	AA × IQ	AA × Sex	IQ × Sex	AA × IQ × Sex
Leu	310.76	329.52	305.88	342.72	259.36	264.40	295.19	286.42	19.534	0.001	0.174	0.328	0.364	0.235	0.930	0.526
Lys	464.72^{c}	545.54^{b}	450.38^{c}	520.85^{bc}	559.59^{ab}	625.95^{a}	590.87^{ab}	616.44^{a}	34.125	<0.001	0.816	0.385	0.001	0.404	0.473	0.664
Met	49.42	39.68	43.29	52.45	46.99	41.01	43.04	53.47	5.735	0.983	0.313	0.901	0.796	0.736	0.220	0.868
Phe	129.44^{ab}	135.31^{ab}	126.66^{ab}	147.79^{a}	100.52^{c}	118.57^{bc}	118.96^{bc}	122.29^{bc}	8.451	0.001	0.158	0.581	0.041	0.803	0.981	0.190
Pro	389.12	419.53	400.33	424.69	356.53	395.75	391.33	411.59	26.928	0.251	0.291	0.624	0.166	0.945	0.689	0.858
Ser	76.45	87.54	75.74	86.55	82.68	83.98	100.45	102.10	12.044	0.140	0.227	0.188	0.384	0.499	0.998	0.982
Thr	195.77	196.08	172.41	207.68	279.57	282.46	249.26	299.27	52.609	0.002	0.805	0.986	0.364	0.864	0.428	0.904
Trp	70.79	63.65	68.74	72.42	45.19	56.29	58.20	61.40	6.324	0.002	0.138	0.492	0.517	0.286	0.862	0.260
Tyr	206.38	208.87	186.34	216.07	136.62	147.78	149.29	167.57	20.252	<0.001	0.610	0.243	0.120	0.942	0.386	0.602
Val	369.96	327.69	334.03	362.57	254.12	257.07	265.80	260.37	27.458	<0.001	0.834	0.810	0.803	0.867	0.357	0.240

[1]Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany.

[2]PUN = plasma urea nitrogen, mg/dL; TP = total protein, g/dL; AL = albumin, g/dL.

Table 5.8. Blood characteristics of gilts and barrows fed diets formulated at or below amino acid (AA) requirements and without or with isoquinoline alkaloids (IQ)[1], d 42, Exp. 1

Item	AA at requirement				AA 10 % below requirement											
IQ, mg/kg	0	0	90	90	0	0	90	90		*P*-value						
Sex	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	SE	AA	IQ	Sex	AA × IQ	AA × Sex	IQ × Sex	AA × IQ × Sex
PUN[2]	8.98[a]	5.87[b]	8.87[a]	8.22[a]	4.47[bc]	3.45[c]	2.83[c]	3.24[c]	0.818	<0.001	0.852	0.050	0.036	0.132	0.061	0.610
TP[2]	6.98[a]	6.40[b]	6.37[b]	6.46[b]	6.43[b]	6.25[b]	6.09[b]	6.33[b]	0.179	0.020	0.073	0.525	0.346	0.204	0.015	0.597
AL[2]	3.60[a]	3.36[abc]	3.55[ab]	3.27[bc]	3.31[abc]	3.21[c]	3.41[abc]	3.13[c]	0.121	0.029	0.747	0.633	0.011	0.652	0.485	0.650
AA, %																
Ala	1,361	1,471	1,516	1,473	2,212	2,050	1,729	1,998	212.970	<0.001	0.446	0.226	0.815	0.940	0.576	0.296
Arg	187.17	134.20	154.68	151.80	99.17	104.23	90.98	93.02	12.058	<0.001	0.253	0.880	0.113	0.039	0.135	0.079
Asn	74.13[a]	60.36[abc]	68.83[ab]	63.23[abc]	62.02[abc]	53.91[bc]	58.58[abc]	50.51[c]	6.516	0.011	0.546	0.789	0.043	0.838	0.601	0.606
Asp	18.53	18.02	19.11	18.62	22.76	22.48	21.23	21.67	1.453	0.001	0.762	0.367	0.834	0.761	0.848	0.859
Cys	5.68	5.62	5.54	5.48	4.87	5.71	4.31	4.83	0.913	0.066	0.218	0.393	0.389	0.287	0.816	0.814
Gln	370.02	354.07	373.54	345.77	414.53	470.08	417.49	427.01	34.907	<0.001	0.510	0.591	0.744	0.103	0.385	0.597
Glu	99.13	120.61	99.87	101.40	126.66	107.26	145.36	167.92	13.456	0.001	0.083	0.010	0.451	0.567	0.542	0.081
Gly	1,917	1,760	2,017	1,844	2,159	2,062	2,143	1,900	143.660	0.060	0.985	0.347	0.092	0.979	0.667	0.735
His	45.34	34.76	43.02	39.97	19.91	16.28	15.06	17.35	3.401	<0.001	0.920	0.444	0.095	0.164	0.128	0.854
Ile	213.97[a]	169.49[b]	222.29[a]	188.99[ab]	109.84[c]	83.62[c]	90.55[c]	74.04[c]	23.938	<0.001	0.979	0.174	0.009	0.393	0.614	0.972
Leu	336.59	298.38	356.19	319.71	292.04	276.48	254.98	250.02	26.961	<0.001	0.684	0.075	0.141	0.342	0.826	0.877

Table 5.8 (cont.)

Item	AA at requirement				AA 10 % below requirement											
IQ, mg/kg	0	0	90	90	0	0	90	90		*P*-value						
Sex	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	SE	AA	IQ	Sex	AA × IQ	AA × Sex	IQ × Sex	AA × IQ × Sex
Lys	521.12	523.76	532.49	506.15	588.63	678.48	606.39	632.32	53.560	<0.001	0.712	0.803	0.301	0.127	0.307	0.695
Met	55.80	39.61	49.26	50.15	57.39	35.33	39.74	43.44	13.923	0.375	0.775	0.515	0.163	0.880	0.033	0.678
Phe	133.32	126.21	156.58	138.59	108.08	93.57	97.81	98.74	14.177	<0.001	0.313	0.185	0.211	0.705	0.885	0.388
Pro	424.11	398.03	474.64	384.45	426.98	426.82	384.75	391.09	36.439	0.589	0.666	0.235	0.258	0.204	0.545	0.466
Ser	110.71	82.80	104.41	91.27	111.49	81.42	83.06	91.55	14.431	0.538	0.634	0.563	0.102	0.572	0.123	0.493
Thr	221.96	126.08	198.39	187.89	232.87	336.70	173.35	221.70	65.713	0.081	0.302	0.105	0.724	0.052	0.821	0.280
Trp	89.53	65.81	76.49	69.23	70.40	71.53	61.93	62.54	8.894	0.143	0.246	0.738	0.222	0.166	0.499	0.469
Tyr	237.72	183.30	219.22	201.82	143.96	140.34	122.51	118.15	18.949	<0.001	0.336	0.337	0.089	0.158	0.439	0.408
Val	378.38^{a}	299.39^{cd}	364.99^{ab}	328.07^{abc}	302.25^{bcd}	267.22^{cde}	263.83^{de}	228.78^{e}	35.278	<0.001	0.330	0.146	0.005	0.469	0.510	0.502

[1]Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany.

[2]PUN = plasma urea nitrogen, mg/dL; TP = total protein, g/dL; AL = albumin, g/dL.

Table 5.9. Blood characteristics of gilts and barrows fed diets formulated at or below amino acid (AA) requirements without or with isoquinoline alkaloids (IQ)[1], d 56, Exp. 1

Item	AA at requirement				AA 10 % below requirement											
IQ, mg/kg	0	0	90	90	0	0	90	90		*P*-value						
Sex	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	SE	AA	IQ	Sex	AA × IQ	AA × Sex	IQ × Sex	AA × IQ × Sex
PUN[2]	9.26	7.12	9.09	8.50	4.20	3.59	4.65	4.04	0.842	<0.001	0.330	0.884	0.068	0.487	0.467	0.465
TP[2]	7.16^{a}	6.44^{bcd}	6.60^{bc}	6.71^{b}	6.69^{b}	6.24^{d}	6.36^{cd}	6.35^{cd}	0.113	<0.001	0.095	0.816	0.001	0.598	<0.001	0.176
AL[2]	3.88^{a}	3.55^{c}	3.82^{ab}	3.52^{cd}	3.56^{bc}	3.25^{de}	3.50^{cd}	3.16^{e}	0.108	<0.001	0.439	0.829	<0.001	0.942	0.989	0.826
AA, %																
Ala	1,393^{d}	1,512^{d}	1,254^{d}	1,626^{bcd}	2,166^{a}	2,010^{abc}	1,536^{cd}	2,113^{ab}	180.300	<0.001	0.193	0.390	0.110	0.875	0.026	0.301
Arg	178.66^{a}	144.39^{c}	148.41^{bc}	176.53^{ab}	113.33^{d}	92.96^{d}	98.10^{d}	108.72^{d}	11.013	<0.001	0.932	0.962	0.585	0.899	0.003	0.274
Asn	67.31	66.17	60.92	72.08	63.83	52.27	64.25	60.22	5.007	0.052	0.545	0.511	0.680	0.054	0.139	0.714
Asp	17.68	18.37	17.16	17.73	22.34	22.29	20.04	22.12	1.039	<0.001	0.188	0.626	0.240	0.776	0.461	0.417
Cys	9.36	8.07	9.02	7.90	7.13	6.86	7.49	6.53	2.355	<0.001	0.764	0.744	0.052	0.466	0.740	0.613
Gln	356.83^{d}	393.85^{cd}	343.94^{d}	401.88^{bcd}	459.48^{ab}	445.37^{abc}	420.39^{bc}	490.07^{a}	35.838	<0.001	0.990	0.855	0.021	0.497	0.066	0.272
Glu	124.44	128.69	82.51	91.83	147.62	122.15	145.10	176.89	12.193	<0.001	0.397	0.000	0.526	0.817	0.062	0.103
Gly	1,817^{c}	2,174^{ab}	2,032^{bc}	2,094^{bc}	2,443^{a}	2,088^{bc}	2,116^{bc}	2,180^{ab}	124.320	0.036	0.758	0.263	0.703	0.035	0.707	0.038
His	48.99^{a}	46.42^{a}	45.78^{a}	51.80^{a}	20.02$^{\mathbf{b}}$	14.91^{b}	14.99^{b}	17.75^{b}	2.697	<0.001	0.997	0.480	0.859	0.347	0.011	0.907
Ile	206.23	196.00	208.63	210.76	133.67	111.57	118.31	111.03	15.780	<0.001	0.972	0.371	0.246	0.563	0.456	0.947
Leu	338.89	338.81	326.95	338.21	307.87	283.45	273.99	295.47	20.338	<0.001	0.362	0.812	0.874	0.725	0.132	0.378

Table 5.9 (cont.)

Item	AA at requirement				AA 10 % below requirement											
IQ, mg/kg	0	0	90	90	0	0	90	90		*P*-value						
Sex	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	SE	AA	IQ	Sex	AA × IQ	AA × Sex	IQ × Sex	AA × IQ × Sex
Lys	508.93^{cd}	576.64^{bc}	475.52^{d}	561.60^{bcd}	642.34^{ab}	658.86^{ab}	590.32^{bc}	699.09^{a}	60.010	<0.001	0.529	0.686	0.004	0.757	0.235	0.418
Met	62.69	51.47	57.30	65.25	63.50	49.70	65.01	66.84	11.589	0.670	0.151	0.593	0.446	0.648	0.068	0.855
Phe	146.82^{ab}	134.78^{bc}	135.03^{bc}	158.95^{a}	118.92^{cd}	113.07^{cd}	102.32^{d}	112.57^{d}	8.534	<0.001	0.824	0.178	0.457	0.729	0.025	0.361
Pro	431.54^{a}	451.32^{a}	421.08^{a}	467.29^{a}	441.69^{a}	414.69^{ab}	347.76^{b}	445.25^{a}	25.276	0.070	0.362	0.310	0.085	0.946	0.022	0.154
Ser	96.00	110.92	105.39	116.72	118.00	89.43	92.25	103.61	8.722	0.275	0.874	0.255	0.700	0.064	0.122	0.064
Thr	199.80^{bc}	203.14^{bc}	183.24^{c}	249.67^{bc}	276.19^{bc}	398.18^{a}	211.96^{bc}	293.09^{b}	54.199	0.001	0.164	0.046	0.008	0.178	0.823	0.290
Trp	83.24	74.45	88.70	91.57	80.19	66.03	70.12	72.94	7.821	0.021	0.344	0.214	0.405	0.791	0.172	0.794
Tyr	229.20	218.94	204.56	229.32	162.51	137.57	128.82	140.63	14.851	<0.001	0.203	0.637	0.969	0.424	0.054	0.960
Val	372.17	378.67	365.98	390.76	333.71	305.64	308.08	299.39	17.990	<0.001	0.593	0.434	0.910	0.166	0.442	0.982

[1]Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany.

[2]PUN = plasma urea nitrogen, mg/dL; TP = total protein, g/dL; AL = albumin, g/dL.

Table 5.10. Apparent total tract digestibility (ATTD) by growing pigs of Ca and P in corn-soybean meal diets formulated at 5 or 8% below amino acid (AA) requirements and without or with isoquinoline alkaloids (IQ; as-fed basis), Exp. 2[1]

Item	AA reduction[2]			IQ inclusion[2]			*P*-value	
	5%	8%	SE	0 mg/kg	90 mg/kg	SE	AA	IQ
Ca intake, g/d	7.27	7.00	0.123	7.37	6.89	0.121	0.046	<0.001
P intake, g/d	7.09	6.75	0.118	7.18	6.65	0.116	0.010	<0.001
Dry feces output, g/d	124.32	118.18	4.12	112.31	120.20	4.061	0.138	0.600
Ca in dry feces, %	1.34	1.29	0.090	1.33	1.30	1.30	0.449	0.596
Fecal Ca output, g/d	1.67	1.52	0.087	1.63	1.56	1.56	0.153	0.517
P in dry feces, %	2.00	1.96	0.057	1.98	1.98	0.057	0.427	0.935
Fecal P output, g/d	2.46	2.30	0.066	2.40	2.36	0.066	0.087	0.622
ATTD of Ca, %	77.18	78.54	1.251	77.96	77.47	1.237	0.391	0.687
ATTD of P, %	65.13	65.85	0.743	66.64	64.35	0.743	0.449	0.022

[1]There was no interaction of main effects; therefore, the interaction term was not included in the final analysis and only main effects are shown.

[2]Dietary AA were 5 or 8% below amino acid requirements; Isoquinoline alkaloids were included at 0 or 90 mg/kg diets.

Table 5.11. Apparent total tract digestibility (ATTD) of N by growing pigs and N balance of pigs fed diets formulated at 5 or 8% below amino acid (AA) requirements without or with isoquinoline alkaloids (IQ; as-fed basis), Exp. 2[1]

Item	AA reduction[2]			IQ inclusion[2]			*P*-value	
	5%	8%	SE	0 mg/kg	90 mg/kg	SE	AA	IQ
ADFI[3], g	1,247	1,236	30.98	1,263	1,214	30.98	0.850	0.079
N intake, g/d	22.28[a]	19.86[b]	0.514	21.44	20.70	0.514	<0.001	0.112
Fecal N output, g/d	4.05	3.89	0.161	3.95	4.00	0.158	0.359	0.768
Urine N output, g/d	2.31	1.96	0.136	2.24	2.03	0.134	0.021	0.145
ATTD of N, %	81.84	80.16	0.788	81.48	80.52	0.775	0.052	0.256
N retention, g/d	15.93	13.91	0.380	15.16	14.68	0.373	<0.001	0.268
N retention, %	71.50	70.19	1.002	71.00	70.69	0.985	0.224	0.771
Biological value[4], %	87.37	87.53	0.793	87.12	87.78	0.780	0.852	0.435

[1]There was no interaction of main effects; therefore, the interaction term was not included in the final analysis and only main effects are shown.

[2] Dietary AA were 5 or 8% below amino acid requirements; Isoquinoline alkaloids were included at 0 or 90 mg/kg diets.

[3]ADFI = average daily feed intake.

[4]Biological value was calculated as (N retained/ [N intake – N output in feces]) × 100 (Rojas and Stein, 2013).

Table 5.12. Apparent total tract digestibility (ATTD) of gross energy (GE) by growing pigs and concentrations of digestible energy (DE) and metabolizable energy (ME) in diets formulated either 5 or 8% below amino acid (AA) requirements without or with isoquinoline alkaloids (IQ), Exp. 2[1]

Item	AA reduction[2]			IQ inclusion[2]			*P*-value	
	5%	8%	SE	0 mg/kg	90 mg/kg	SE	AA	IQ
Dry feces output, kg/4d	0.50	0.47	0.017	0.49	0.48	0.016	0.138	0.600
Fecal GE, kcal/kg	4,711	4,728	20.24	4,705	4,734	19.85	0.490	0.247
Fecal GE output, kcal/4d	2,311	2,271	74.72	2,340	2,243	72.94	0.673	0.308
ATTD GE, %	88.21	88.19	0.286	88.17	88.22	0.279	0.965	0.893
DE, kcal/kg diet	3,458	3,444	11.40	3,443	3,459	11.13	0.367	0.331
Urine output, kg/4d	13.43	11.17	2.937	14.07	10.53	2.977	0.430	0.215
Urine GE, kcal/kg	32.91	31.50	8.025	34.46	29.95	7.938	0.838	0.509
Urinary GE output, kcal/4d	187.52	186.97	0.201	187.33	187.17	0.196	0.053	0.567
ME, kcal/kg diet	3,421	3,406	11.35	3,406	3,420	11.09	0.345	0.381

[1]There was no interaction of main effects; therefore, the interaction term was not included in the final analysis and only main effects are shown.

[2] Dietary AA were 5 or 8% below amino acid requirements; Isoquinoline alkaloids were included at 0 or 90 mg/kg diets.

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CHAPTER 6: Isoquinoline alkaloids and narasin (Skycis) may influence carcass characteristics but do not affect overall growth performance of finishing pigs fed corn-soybean meal diets

Abstract

An experiment was conducted to test the hypothesis that narasin, an ionophore, and isoquinoline alkaloids (IQ) improve feed efficiency and carcass characteristics either alone or in combination when included in corn-soybean meal diets fed to pigs during the finishing phase of production. A total of 192 pigs (body weight: 73.23 ± 6.11 kg) were allotted to 4 treatments and 12 replicate pens per treatment for a total of 48 pens with sex split evenly within pen. The negative control (NC) diet was formulated to meet nutrient requirements of pigs from 75 to 100 kg in phase 1 and 100 to 130 kg in phase 2 of the experimental period. A second diet was formulated as NC in phase 1, and the NC with the addition of 15 mg narasin/kg feed in phase 2. The third treatment group received a diet formulated as NC, but supplemented with 90 mg IQ/kg feed in both experimental phases. The fourth treatment group received the NC diet supplemented with 90 mg IQ/kg feed in phase 1 and a diet supplemented with both 90 mg IQ/kg feed and 15 mg narasin/kg feed in phase 2. The trial was conducted for 56 d with weights being recorded on d 1, 28 (end of phase 1), and 56 (end of phase 2). Daily feed allotments were also recorded and feed left in feeders on d 28 and 56 was weighed to calculate feed disappearance. Average daily gain (ADG), average daily feed intake (ADFI), and gain:feed (G:F) were calculated. On the last d of the experiment, 1 pig per pen was euthanized and carcass characteristics were measured. Results indicated that average daily gain and G:F tended to decrease ($P < 0.10$) when IQ was added to

the diet. As both IQ and narasin were included in the diet, there tended to be a reduction ($P < 0.10$) in loin muscle area; however, Minolta L* increased ($P < 0.05$) with inclusion of both additives compared with NC or IQ and narasin alone in the diet. Barrows had greater ($P < 0.05$) ADG, end live weight, hot carcass weight, and carcass yield compared with gilts fed the same diet. Overall, IQ and narasin in combination did not influence growth performance of finishing pigs, but IQ supplementation to corn-soybean meal diets may alter subjective evaluations of longissimus muscle quality.

Key words: carcass characteristics, growth performance, isoquinoline alkaloids, narasin, pig

Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
AEE	acid hydrolyzed ether extract
BW	body weight
ELW	end live weight
G:F	gain:feed
HCW	hot carcass weight
IQ	isoquinoline alkaloids
LM	longissimus muscle
NC	negative control

Introduction

Ractopamine hydrochloride has been used in finishing diets for pigs to maximize growth and increase lean deposition. However, the use of ractopamine hydrochloride as a growth promoter

for livestock has been restricted or prohibited in certain markets including the European Union, China, Russia, and Taiwan (Niño et al., 2015). Accordingly, there is a need to investigate alternatives to maximize protein deposition and growth performance in finishing pigs.

Ionophores may improve protein utilization by inhibiting gram-positive bacteria in the gastrointestinal tract of cattle and swine (Russell, 1987; Wuethrich et al., 1998). When included in diets for finishing pigs, ionophores increase growth performance parameters including ADG, ADFI, and G:F compared with pigs fed a control diet (Artenson et al., 2016; Knauer and Artenson, 2017; Rickard et al., 2017).

Isoquinoline alkaloids (**IQ**) is a phytogenic feed additive that improves the apparent ileal digestibility of amino acids and other nutrients by growing pigs (Boroojeni et al., 2018; Rundle et al., 2020), and therefore, may also increase protein utilization and improve feed efficiency when included in diets for finishing pigs. There is, however, limited information about the use of ionophores and IQ in diets for finishing pigs fed corn-soybean meal diets and there is no information about combined effects of the two additives.

Therefore, it was the objective of this experiment to test the hypothesis that the ionophore narasin and IQ improve feed efficiency and carcass characteristics when included, either separately or in combination, in corn-soybean meal diets fed to pigs during the finishing phase of production.

Materials and Methods

Experimental design

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.

Growing pigs that were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN) were used.

The experiment was conducted during the final 56 d of production. There were 2 phases, with each phase consisting of 28 d. A negative control (NC) group was fed a diet formulated to meet nutrient requirements of 75 to 100 kg and 100 to 130 kg pigs during phases 1 and 2, respectively (Table 6.1; NRC, 2012). A second treatment group (Skycis) was fed the NC diet during the first phase of the experiment, and then switched to a diet formulated in the same way as the NC diet, but supplemented with 15 mg narasin/kg feed (Skycis; Elanco Animal Health, Greenfield, IN) during phase 2. Diets fed to the third treatment (IQ) were also formulated as the NC diet during both phases, but supplemented with 90 mg IQ/kg feed (Sangrovit G Premix; Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany). A fourth treatment group (IQ + Skycis) received the IQ diet during phase 1, and the NC diet supplemented with both 90 mgIQ /kg feed and 15 mg narasin/kg feed during phase 2 of the experiment. Therefore, there were 4 dietary treatments in the experiment.

Growth performance

A total of 192 pigs were used (initial body weight (**BW**): 73.23 ± 6.11 kg). There were 4 treatments and 12 replicate pens per treatment for a total of 48 pens with 2 gilts and 2 barrows in each 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 on d 28 and d 56 to calculate feed disappearance. Pigs were checked daily for general condition and accessibility of water and feed, and the barn temperature was recorded. If a pig was removed from a pen during the experiment, the feed left in the feeder and individual pig weights of the remaining pigs in the pen were recorded on the day the pig was removed. 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 average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain:feed (**G:F**) for phases 1 and 2 and for the overall experimental period.

Carcass characteristics and fresh loin quality

On the last day of the experiment, the pig in each pen having a BW closest to the average for the pen was identified and equal number of barrows and gilts were selected for all treatments. These pigs were transported to the University of Illinois Meat Science Laboratory (Urbana, IL). Pigs were provided *ad libitum* access to water, but did not have access to feed, during the time between transportation and slaughter which was approximately 16 h. Pigs were weighed immediately before slaughter to determine end live weight (**ELW**). Pigs were immobilized using head-to-heart electrical stunning and slaughtered via exsanguination. Carcasses were weighed approximately 45 min postmortem to determine hot carcass weight (**HCW**) and then stored for 24 h at 4˚C. Carcass yield was calculated by dividing the HCW by ELW and expressed as a percentage. Carcasses were split down the midline and fresh longissimus muscle (**LM**) quality was determined on the left side of the carcass. Loin quality parameters were measured on the cut surface of LM posterior to the 10^{th} rib and oxygenation of myoglobin occurred at 4˚C for approximately 20 min before quality measurements were recorded. Fat depth was measured between the 10^{th} and 11^{th} ribs, at three-fourths distance of the LM from the dorsal side of the vertebral column. Instrumental color on the LM (L*, a*, and b*, CIE, 1978) was measured with one measurement in the center of the LM surface using a CR-400 Chroma meter (Minolta Camera Co., Ltd, Osaka, Japan) with a D65 light source and a 10˚ observer angle with an aperture size of 8 mm, using the procedure established by the National Pork Producers Council

(National Pork Producers Council, 1999). Ultimate pH was measured using a handheld pH meter fitted with a Hanna glass electrode calibrated at 4˚C (REED SD-230 Series pH/ORP Datalogger, 0.00 to 14.00 ph/0-199 mV; Hanna FC200B electrode). Subjective color and marbling scores (National Pork Producers Council, 1999) and firmness scores (National Pork Producers Council, 1991) were determined by a single technician. The LM area was measured by tracing the surface of the LM on a double-matted acetate paper. A chop of 1.27 cm was used to measure drip loss. Chops were weighed and suspended from a fishhook in a plastic bag for 24 h at 4 ˚C, and then weighed again and the difference was recorded (Boler et al., 2011).

Analyses

Diets were analyzed for dry matter (method 930.15; AOAC Int., 2007) and ash (method 942.05; AOAC Int., 2007), and gross energy was determined using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL). Acid hydrolyzed ether extract (**AEE**) was analyzed in diets by acid hydrolysis using 3*N* HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY), and crude protein was determined as 6.25 × N, which was analyzed on a LECO FP628 Nitrogen analyzer (method 990.03; AOAC Int., 2007; Leco Corp., St. Joseph, MI). 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 [method 982.30 (a, b, c); AOAC Int., 2007]. Amino acids were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO), but all other analyses were completed at the University of Illinois at Urbana-Champaign.

Statistical analyses

Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC), with the pen as the experimental unit. Normality of the residuals was determined using Proc Univariate of SAS (SAS Institute Inc., Cary, NC) and outliers, defined as data with an internalized student residual value over 3.0, were excluded from statistical analysis. The main effect of dietary treatment was included in the model for growth performance with group as a random effect. The model included dietary treatment and sex as the main effects as well as the interaction between the two terms for carcass characteristics. Data representing the main effect of dietary treatment on carcass characteristics was also presented. Least square means were calculated for each independent variable using the LS means statement in SAS and, if significant, means were separated using the PDIFF option in SAS. Significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

There was no effect of diet on ADFI, nor was there an effect of diet on BW at d 1 or 56 (Table 6.2). There were tendencies for both ADG and G:F to decrease ($P < 0.10$) when IQ was added to the diet. There was no effect of dietary treatment on ELW, HCW, carcass yield, fat depth, carcass lean, ultimate pH, or drip loss of the carcasses of pigs fed diets supplemented with narasin or IQ (Table 6.3). However, there was a tendency for a reduction ($P < 0.10$) in the LM area as IQ and narasin were added to the diet. Subjective evaluations including color, marbling, and firmness score were unaffected by dietary treatment. Instrumental colors Minolta a* and b* were not influenced by dietary treatment; however, Minolta L* increased ($P < 0.05$) with

inclusion of both IQ and narasin compared with NC or dietary treatments with either IQ or narasin.

Inclusion of IQ to the diet decreased ($P < 0.05$) BW on d 56 and ADG during the overall experimental period (Table 6.4). Barrows had greater ($P < 0.05$) BW on d 1 and 56, ADG, ELW, HCW, and carcass yield compared with gilts. Minolta L* was greater in gilts fed diets supplemented with narasin, IQ, or both IQ and narasin compared with the NC, whereas barrows had a decreased L* as IQ was added to the diet compared with NC, narasin, and IQ and narasin (interaction, $P < 0.05$).

Discussion

Isoquinoline alkaloids have been fed to young growing pigs to promote growth and intestinal health. Similarly, narasin has been included in diets for pigs to promote health and growth of growing and finishing pigs. In a previous experiment, there was no effect of narasin supplementation on the ADG of finishing gilts; however, ADG of barrows increased and G:F was improved in an 85 d feeding period (Arkfeld et al., 2015). In an 84 d growth assay, supplementing diets for growing-finishing pigs with 15 mg/kg narasin increased final body weight and ADG compared with pigs fed a control diet, whereas overall G:F was improved with supplementation of either 15 or 20 mg/kg narasin compared with pigs fed a control diet (Puls et al., 2021). Average daily gain and ADFI improved with 15 mg/kg narasin supplementation compared with pigs fed control diets (Rickard et al., 2017; Linneen et al., 2021). There was no effect of IQ on live weight, ADG, or feed intake of finishing pigs fed corn, wheat, and barley-based diets supplemented with 100 mg/kg IQ for 28 d (Zhao et al., 2017). However, IQ improved growth performance of nursery pigs fed corn-soybean meal diets (Robbins et al., 2013; Kantas et

al., 2015; Liu et al., 2016). Therefore, the observation that inclusion of IQ tended to reduce ADG and G:F compared with the control diet and diets supplemented with narasin and a combination of narasin and IQ in this experiment was unanticipated.

Sex-based differences were observed when pigs were fed narasin, with barrows having a greater amount of leaf fat that made up a greater percentage of HCW compared with gilts fed the same experimental diet; however, there were no differences at slaughter between pigs fed a diet supplemented with narasin and those fed a control diet (Shircliff et al., 2018). The sex differences observed in this experiment, including for HCW, ELW, and carcass yield, were therefore expected. The observation that LM area tended to decrease when IQ and narasin were included in the diet are in agreement with an experiment conducted in a 3-phase marketing system, in which narasin tended to reduce loin depth, but did not affect carcass lean percentage or fat depth (Arkfeld et al., 2015). However, pigs fed narasin had greater HCW and carcass yield in a similar 3-phase marketing system compared with pigs fed a control diet (Rickard et al., 2017). Pigs fed a narasin diet had greater carcass yield, HCW, and loin depth compared with pigs fed a corn-soybean meal control diet or a control diet supplemented with an antibiotic (Linneen et al., 2021). Additionally, after an 84 d feeding period, pigs fed diets supplemented with 15 mg/kg narasin had improved HCW and tended to improve carcass yield (Puls et al., 2021). Data for effects of IQ on carcass traits of finishing pigs are limited. However, sanguinarine extract from *Macleaya cordata*, from which IQ is derived, was not present in organs and tissues of finishing pigs after 28 days of feeding (Zhao et al., 2017).

Minolta L* is an instrumental color defined as the measure of lightness to darkness, with greater L* values indicating a lighter color. Preferences for instrumental color vary greatly among consumers. Consumer preference studies indicated that high quality chops, qualified as

having an L* of 43.00 to 46.00 as well as other qualifications including marbling, NPPC color score, and extractable lipid content, were not noticeably different from low or medium-quality chops in terms of tenderness or juiciness, but medium and low-quality chops were more flavorful (Wilson et al., 2017). Therefore, the observed increase in L* as IQ and narasin were added to the diet does not necessarily indicate any differences in consumer preference.

Conclusions

The combination of narasin and IQ had no effect on growth performance or on carcass traits, with the exception that inclusion of IQ in the diet resulted in an increase in L*, indicating that IQ act in some manner to change the LM tissue color of pig carcasses. Further research is needed to elucidate the exact mechanism of action of IQ on carcass characteristics of finishing pigs.

Tables

Table 6.1. Composition of experimental diets (as-is basis)

Item	Phase 1		Phase 2			
	PC	IQ[1]	PC	SKY[2]	IQ	IQ+SKY
Ingredients, %						
Ground corn	79.82	78.92	83.33	83.18	82.43	82.28
Soybean meal	15.25	15.25	12.00	12.00	12.00	12.00
Soybean oil	2.50	2.50	2.50	2.50	2.50	2.50
Dicalcium phosphate	0.80	0.80	0.65	0.65	0.65	0.65
Ground limestone	0.70	0.70	0.65	0.65	0.65	0.65
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Lys HCL	0.23	0.23	0.18	0.18	0.18	0.18
Vit-mineral mix[3]	0.15	0.15	0.15	0.15	0.15	0.15
L-Thr	0.05	0.05	0.04	0.04	0.04	0.04
Narasin[4]	-	-	-	0.15	-	0.15
IQ Premix[5]	-	0.90	-	-	0.90	0.90
Analyzed composition						
Dry matter, %	86.49	86.75	86.33	86.50	86.48	86.56
Ash, %	3.61	3.48	3.30	3.22	3.31	3.15
Crude protein, %	11.44	12.86	11.77	11.57	10.93	11.22
Gross energy, kcal/kg	3,942	3,943	3,914	3,942	3,926	3,901
AEE[6], %	5.74	5.68	5.59	5.73	6.11	5.79

Table 6.1 (cont.)

Item	Phase 1		Phase 2			
	PC	IQ[1]	PC	SKY[2]	IQ	IQ+SKY
Indispensable AA, %						
Arg	0.84	0.78	0.66	0.69	0.74	0.62
His	0.37	0.35	0.31	0.32	0.34	0.29
Ile	0.6	0.55	0.48	0.52	0.55	0.45
Leu	1.26	1.17	1.10	1.15	1.24	1.06
Lys	0.87	0.90	0.69	0.73	0.72	0.61
Met	0.20	0.18	0.16	0.19	0.20	0.16
Phe	0.69	0.63	0.57	0.61	0.65	0.55
Thr	0.54	0.53	0.44	0.47	0.51	0.46
Trp	0.16	0.14	0.12	0.14	0.11	0.11
Val	0.68	0.63	0.56	0.58	0.63	0.52
Total AA, %	13.66	12.72	11.31	12.13	12.78	10.97
Narasin, g/ton	-	-	< 1.98	14.00	< 1.98	16.09

[1]SE = PC + 90 mg/kg IQ.

[2]SKY = PC + 15 mg/kg Narasin.

Table 6.1 (cont.)

[3] 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; $_{D}$-pantothenic acid as $_{D}$-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, 1.26mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30mg as sodium selenite and selenium yeast; and Zn, 125.1mg as zinc hydroxychloride.

[4]Skycis; Elanco Animal Health, Greenfield, IN.

[5]Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany.

[6]AEE = acid hydrolyzed ether extract.

Table 6.2. Growth performance of finishing pigs fed corn-soybean meal diets supplemented with isoquinoline alkaloids (IQ) and narasin (SKY)[1]

Item	Dietary treatment					*P*-value
	PC	SKY	IQ	IQ + SKY	SEM	Diet
ADFI, kg	2.93	3.00	2.89	3.01	0.083	0.390
ADG, kg	1.01	1.01	0.95	1.02	0.022	0.093
G:F	0.34	0.34	0.33	0.34	0.005	0.074
BW, kg						
D 1	73.27	73.29	73.22	73.14	2.954	0.999
D 56	129.56	129.98	126.25	129.96	3.660	0.301

[1]ADFI = average daily feed intake; ADG = average daily gain; G:F = gain:feed; BW = body weight.

Table 6.3. Carcass characteristics and fresh loin quality of finishing pigs fed corn-soybean meal diets supplemented with isoquinoline alkaloids (IQ) and narasin (SKY)

Item	Dietary treatment					*P*-value
	PC	SKY	IQ	IQ + SKY	SEM	Diet
ELW[1], kg	125.47	124.13	123.49	125.61	3.899	0.820
HCW[1], kg	99.15	97.98	97.39	99.05	3.559	0.842
Carcass yield, %	79.00	78.90	78.81	78.85	0.453	0.967
Fat depth, cm	2.03	1.99	1.80	2.14	0.105	0.156
Carcass lean, %	54.40	53.98	54.52	52.90	0.606	0.232
LM[1] area, cm^2	56.05	52.94	51.59	50.95	3.091	0.075
Ultimate pH	5.47	5.50	5.46	5.47	0.036	0.374
Subjective evaluations[2]						
Color score	2.79	3.00	2.96	2.96	0.180	0.852
Marbling score	1.75	1.58	1.83	1.63	0.182	0.754
Firmness score	2.08	2.00	2.17	2.04	0.172	0.908
Instrumental color[3]						
Minolta L*	49.70[b]	50.02[b]	50.12[b]	52.26[a]	2.057	0.032
Minolta a*	7.86	7.68	8.26	7.04	0.347	0.106
Minolta b*	3.46	3.43	3.76	3.73	0.266	0.701
Drip loss, %	3.96	3.72	3.64	4.69	0.511	0.186

[1] ELW = end live weight; HCW = hot carcass weight; LM = longissimus muscle.

Table 6.3 (cont.)

[2] NPPC color (1 = pale pink to 6 = dark purplish red), NPPC marbling (1 = 1% intramuscular lipid to 10 = ≥ 10% intramuscular lipid), and NPPC firmness (1 = very soft to 5 = very firm) were based on NPPC (1991, 1999) standards.

[3] L* = measure of darkness to lightness (greater value indicates a lighter color); a* = measure of redness (greater value indicates a redder color); and b* = measure of yellowness (greater value indicates a more yellow color).

Table 6.4. Effects of diet and sex on average daily gain (ADG), body weight (BW), and carcass characteristics of pigs fed diets supplemented with isoquinoline alkaloids (IQ) and narasin (SKY)

Item	Dietary treatment					*P*-value	Sex			*P*-value
	PC	SKY	IQ	IQ × SKY	SEM	Diet	Barrow	Gilt	SEM	Sex
BW d 1, kg	73.25	73.35	73.23	73.14	2.796	0.998	75.94[a]	70.54[b]	2.751	<0.001
BW d 56, kg	129.83[a]	130.08[a]	126.26[b]	129.96[a]	3.541	0.036	134.34[a]	123.72[b]	3.454	<0.001
ADG, kg	1.02[a]	1.01[a]	0.95[b]	1.02[a]	0.018	0.007	1.05[a]	0.95[b]	0.013	<0.001
ELW[1], kg	125.47	124.13	123.49	125.61	3.842	0.776	127.32[a]	122.04[b]	3.649	0.003
HCW[1], kg	99.15	97.98	97.39	99.05	3.504	0.793	100.87[a]	95.92[b]	3.349	0.002
Carcass yield, %	79.00	78.90	78.81	78.85	0.446	0.962	79.21[a]	78.58[b]	0.403	0.025
Fat depth, cm	2.03	1.99	1.80	2.14	0.103	0.144	2.07	1.91	0.073	0.106
Carcass lean, %	54.40	53.98	54.52	52.90	0.606	0.233	53.66	54.24	0.429	0.342
LM[1] area, cm^2	56.05	52.94	51.59	50.95	3.083	0.069	53.99	51.77	2.915	0.126
Ultimate pH	5.48	5.50	5.56	5.47	0.036	0.369	5.49	5.47	0.034	0.230

Table 6.4 (cont.)

Item	Dietary treatment					*P*-value	Sex			*P*-value
	PC	SKY	IQ	IQ × SKY	SEM	Diet	Barrow	Gilt	SEM	Sex
Subjective evaluations[2]										
Color score	2.79	3.00	2.96	2.96	0.181	0.854	3.00	2.85	0.128	0.425
Marbling score	1.75	1.58	1.83	1.63	0.182	0.756	1.63	1.771	0.129	0.428
Firmness score	2.08	2.00	2.17	2.04	0.171	0.906	2.19	1.96	0.125	0.173
Instrumental color[3]										
Minolta L*[4]	49.70[b]	50.02[b]	50.12[b]	52.26[a]	2.057	0.032	50.15	50.90	2.004	0.260
Minolta a*	7.86	7.68	8.26	7.04	0.346	0.105	7.91	7.51	0.244	0.262
Minolta b*	3.44	3.43	3.76	3.72	0.264	0.700	3.72	3.45	0.190	0.299
Drip loss, %	3.96	3.73	3.64	4.69	0.514	0.191	3.81	4.20	0.433	0.297

[1] ELW = end live weight; HCW = hot carcass weight; LM = longissimus muscle.

[2] NPPC color (1 = pale pink to 6 = dark purplish red), NPPC marbling (1 = 1% intramuscular lipid to 10 = ≥ 10% intramuscular lipid), and NPPC firmness (1 = very soft to 5 = very firm) were based on NPPC (1991, 1999) standards.

Table 6.4 (cont.)

[3] L* = measure of darkness to lightness (greater value indicates a lighter color); a* = measure of redness (greater value indicates a redder color); and b* = measure of yellowness (greater value indicates a more yellow color).

[4]An interaction between effects of diet and sex for Minolta L* was observed ($P = 0.028$).

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CHAPTER 7: Isoquinoline alkaloids affect inflammation and intestinal function of weanling pigs fed corn-soybean meal diets formulated below amino acid requirements

Abstract

An experiment was conducted to test the hypothesis that isoquinoline alkaloids (IQ), when included in diets formulated below amino acid (AA) requirements, will improve intestinal and systemic health while maintaining growth performance of pigs compared with a diet formulated at AA requirements. A total of 200 weanling pigs (6.11 ± 0.61 kg) were allotted to 4 dietary treatments with 5 pigs per pen and 10 replicate pens per treatment. There were two phases with d 1 to 14 as phase 1 and d 15 to 27 as phase 2. Within each phase, diets were arranged as a 2 × 2 factorial with AA at or 10% below requirements and with IQ at 0 or 120 mg/kg diet. Average daily feed intake (ADFI), average daily gain (ADG), and gain: feed ratio (G:F) were calculated. Fecal and blood samples were collected to determine systemic and intestinal health. Tissue samples from the ileum and jejunum and jejunal mucosa samples were collected on d 27. Results indicated that overall, IQ tended to increase ($P < 0.10$) ADFI and tended to decrease ($P < 0.10$) G:F, whereas reducing dietary AA concentration reduced ($P < 0.05$) overall G:F. The average fecal score and diarrhea incidence were reduced ($P < 0.05$) in phase 2 and for the overall experimental period if AA were reduced in the diet irrespective of IQ inclusion in the diet. If AA were at the requirement, lamina propria thickness in the jejunum was not affected by IQ inclusion; however, if AA were below the requirement, IQ inclusion resulted in decreased lamina propria thickness (interaction, $P < 0.05$). When AA were at the requirement, IQ increased the

concentration of phenols in the feces on d 14, whereas if AA were below the requirement, IQ inclusion decreased phenols in the feces (interaction, $P < 0.05$). Adding IQ to the diet that met AA requirements resulted in decreased concentrations of plasma interleukin (IL-) 4 and IL-10 on d 14; however, if IQ was added to the reduced AA diet, the opposite was true (interaction, $P < 0.05$). If AA were below the requirement, IQ tended to increase mRNA abundance of occludin in the jejunal mucosa, whereas if AA were at requirements, IQ reduced occludin abundance (interaction, $P < 0.10$). On d 26, reducing AA in the diet reduced ($P < 0.05$) plasma albumin regardless of IQ inclusion, and IQ tended to increase ($P < 0.10$) tumor necrosis factor-α irrespective of dietary AA. In conclusion, whereas IQ did not affect growth performance of pigs, if AA were provided below requirements, inclusion of IQ improved systemic and intestinal health of weanling pigs.

Key words: amino acids, growth performance, intestinal health, isoquinoline alkaloids, pig

Abbreviations

AA	amino acids
ADFI	average daily feed intake
ADG	average daily gain
CLDN1	claudin-1
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
G:F	gain:feed
HPRT	hypoxanthine-guanine phosphoribosyl transferase
IFNγ	interferon-γ

IL-	interleukin-
IQ	isoquinoline alkaloids
MDA	malondialdehyde
MUC2	mucin-2
OCLN	occludin
PUN	plasma urea nitrogen
qRT-PCR	quantitative reverse-transcription polymerase chain reaction
RNA	ribonucleic acid
TNFα	tumor necrosis factor α
TP	total protein
VH:CD	villus height:crypt depth
ZO1	zonula occludens-1

Introduction

Weaning is a crucial time for young pigs when they are exposed to environmental and nutritional changes (Pluske et al., 1997). Consequently, numerous strategies have been proposed to mitigate the negative effects of this change to promote feed efficiency, growth performance, and health of pigs (Close et al., 2000). Isoquinoline alkaloids (**IQ**) have been used in swine diets to improve growth performance (Robbins et al., 2013; Kantas et al., 2015; Liu et al., 2016a) and intestinal health (Robbins et al., 2013; Liu et al., 2016b) of pigs due to their beneficial effects on immune factors (Agarwal et al., 1991; Chaturvedi et al., 1997). Additionally, IQ improve apparent ileal digestibility of starch and amino acids by weanling pigs (**AA**; Boroojeni et al., 2018; Rundle et al., 2020), indicating that IQ improve utilization and absorption of nutrients by the pig. Excess N

in the diet during the nursery phase of production commonly leads to fermentation in the large intestine, resulting in bacterial overabundance and increased gastrointestinal distress and diarrhea for the animal (Stein and Kil, 2006). Therefore, reducing dietary crude protein improves intestinal health and reduces diarrhea incidence of weanling pigs (Heo et al., 2008; Limbach et al., 2021). Subsequently, reducing post-weaning diarrhea is associated with increased growth performance and decreased mortality of pigs (Pluske et al., 1997).

Due to the demonstrated ability of IQ to improve AA utilization and absorption, diets with reduced AA may potentially be used during the nursery phase of production to improve intestinal health and maintain growth performance if IQ is supplemented to the diet. However, data are limited regarding the effects of IQ on growth performance and intestinal health of weanling pigs if dietary AA are provided below requirements. Therefore, it was the objective of this experiment to test the hypothesis that inclusion of IQ in diets for weanling pigs formulated below requirements for indispensable AA will improve intestinal and systemic health while maintaining growth performance of 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. Growing pigs that were the offspring of Line 359 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN) were used.

The experiment was conducted for 27 d, starting with pigs at weaning. There were 2 phases, with phase 1 (d 1 – 14) diets formulated for pigs weighing 5 to 7 kg and phase 2 (d 15 – 27) diets formulated for pigs weighing 7 to 11 kg (Table 7.1). The experimental design was a 2 ×

2 factorial with two levels of AA – either at requirements for indispensable AA or 10% below requirements – and two levels of IQ inclusion – either 0 or 90 mg IQ/kg feed (Phytobiotics Futterzusatzstoffe GmbH, Eltville, GE). Therefore, there were 4 phase 1 diets and 4 phase 2 diets and all diets met nutrient requirements except for AA (NRC, 2012).

A total of 200 newly weaned pigs (initial body weight: 6.11 ± 0.98 kg) were placed in uncleaned pens using a sanitation challenge model and pens remained uncleaned throughout the experiment (Adewole et al., 2016). There were 4 treatments and 10 replicate pens per treatment for a total of 40 pens and 5 pigs per pen. Individual pig weights were recorded at the beginning of the experiment, on d 14, and at the conclusion of the experiment (d 27). Daily feed allotments were recorded and feed left in the feeders was weighed on d 14 and 27 to calculate feed disappearance. Pigs were checked daily and temperature and lighting were checked as well. If a pig was removed from a pen during the experiment, the feed left in the feeder and individual pig weights of the remaining pigs were recorded on the day the pig was removed and data for feed intake and gain:feed (**G:F**) for the remaining pigs in the pen were adjusted for the feed consumed by the pig that was removed (Lindemann and Kim, 2007). Data were summarized to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**), and G:F for d 1 to 14, d 15 to 27, and for the entire experiment. Diarrhea scores were assessed visually per pen every other day using a score from 1 to 5 (1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea; and 5 = watery diarrhea). Diarrhea frequency was calculated by totaling the number of pen days with diarrhea scores ≥ 3 divided by the total number of pen days multiplied by 100.

On d 1, 14, and 26, fecal samples were collected from all pigs in the pen and subsampled for analysis. At the end of each phase, 2 blood samples were collected via vena puncture from 1 pig per pen, and the same pig was sampled both times. One sample was collected in a vacutainer

with heparin and the other sample was collected in a vacutainer with ethylenediaminetetraacetic acid (**EDTA**). Both samples were centrifuged at 2,000 × g at 4˚C for 15 min to yield blood plasma, which was stored at -20˚C until analyzed.

At the conclusion of the experiment, the pig in each pen that was used for blood collection was sacrificed via captive bolt stunning. Jejunal and ileal tissue 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 and pinned with the serosa side down on a piece of cardboard. Samples were then fixed in 10% neutral buffered formalin until processing for morphological evaluation and immunohistochemistry staining. Jejunal mucosa samples were washed with phosphate-buffered saline, snap frozen in liquid N, and stored at -80^{o}C until used for ribonucleic acid (**RNA**) extraction and quantitative reverse-transcription polymerase chain reaction (**qRT-PCR**).

Chemical Analyses

Diets were analyzed for dry matter (method 930.15; AOAC Int, 2019) and dry ash (method 942.05; AOAC Int, 2019; Table 7.2). Gross energy was determined with bomb calorimetry (Model 6400; Parr Instruments, Moline, IL) using benzoic acid as the standard for calibration. Acid hydrolyzed ether extract was analyzed by acid hydrolysis using 3*N* HCl (Ankom^{HCl} Hydrolysis System, Ankom Technology, Macedon, NY) followed by fat extraction using petroleum ether (Ankom^{XT-15} Extractor, Ankom Technology, Macedon, NY). Crude protein was calculated as 6.25 × N, and N was measured using the combustion procedure (method 990.03; AOAC Int, 2019) on the LECO FP628 Nitrogen Analyzer (Leco Corp., St. Joseph, MI). Diets were also analyzed for AA 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. Prior to analysis, samples were hydrolyzed with 6*N* HCl for 24 h at 110°C [method 982.30 E(a); AOAC Int., 2019]. 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., 2019]. Tryptophan was determined after NaOH hydrolysis for 22 h at 110°C [method 982.30 E(c); AOAC Int., 2019]. Amino acids were analyzed at the University of Missouri Experiment Station Chemical Laboratory (Columbia, MO). All other analyses were conducted at the University of Illinois (Urbana, IL.).

Fecal ammonia concentrations were determined according to the method by Chaney and Marbach (1962) and fecal short-chain fatty acid concentrations were determined using previously established procedures (Sunvold et al., 1995). Fecal phenol and indole concentrations were determined using gas chromatography according to methods described by Flickinger et al. (2003). Fecal samples were also analyzed for calprotectin concentrations using the Porcine Calprotectin ELISA kit (My BioSource, San Diego, CA) according to previously established procedures (Grzeskowiak et al., 2018).

Heparinized plasma samples were analyzed for plasma urea nitrogen (**PUN**), total protein (**TP**), and albumin using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA). Plasma EDTA samples were analyzed for malondialdehyde (**MDA**) as an indicator of oxidative stress status using an assay kit according to manufacturer specifications (My BioSource, San Diego, CA). The EDTA sample was also analyzed for acute phase proteins including tumor necrosis factor alpha (**TNFα**), interferon-γ (**IFNγ**), and interleukins (**IL-**) 1α, 1β, 1RA, 2, 4, 6, 8, 10, 12, and 18 using a cytokine/chemokine magnetic bead panel according to

manufacturer specifications (MILLIPLEX Porcine Cytokine/Chemokine Magnetic Bead Panel; EMD Millipore, Darmstadt, Germany).

Villus height, crypt depth, and lamina propria thickness of the jejunum and ileum were measured from 10 villi and their associated crypts of each sample using NDP.View2 (Hammatsu, Bridgewater, NJ). Villus height:crypt depth (**VH:CD**) was also calculated. The RNA was extracted from 40 mg of frozen jejunal mucosa using β-mercaptoethanol (Alfa Aesar, Tewksbury, MA) according to the RNeasy Mini Kit (QIAGEN, Germantown, MD) manufacturer's instructions. Total RNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). The RNA quality was determined using a Fragment Analyzer™ Automated CE System (Method DNF-471-33-SS Total RNA 15nt; Advanced Analytical, Ankeny, IA) and RNA samples with an RNA quality number greater than 7 were used for complementary deoxyribonucleic acid (**DNA**) synthesis. The complementary DNA was then diluted 1:4 with DNase/RNase-free water to conduct qRT-PCR analysis which was performed using 4 µL of diluted complementary DNA and 6 µL of a mixture including forward and reverse primers and DNase/RNase free water in a MicroAmp™ Optical 384-Well Reaction Plate (Applied Biosystems, Foster City, CA). Two internal control genes, glyceraldehyde 3-phosphate dehydrogenase (**GAPDH**; Gonzalez et al., 2013), and hypoxanthine-guanine phosphoribosyl transferase (**HPRT**; Nygard et al., 2007) were used to normalize the abundance of tested genes (Table 3). Tested genes included occludin (**OCLN**), zonula occludens-1 (**ZO1**) and claudin-1 (**CLDN1**), because these genes regulate intestinal permeability and paracellular absorption of nutrients (Hu et al., 2013), and mucin 2 (**MUC2**), which regulates mucin production in the intestine (Ferrandis Vila et al., 2018).

Statistical Analyses

Data were analyzed as a 2 × 2 factorial using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with the pen as the experimental unit. Normality of the residuals was verified and outliers were identified using UNIVARIATE and BOXPLOT procedures, respectively (SAS Institute Inc., Cary, NC). The model included dietary AA concentration and IQ inclusion and the interaction between AA concentration and IQ as fixed effects, and pen as the random effect. Least square means were calculated for each independent variable and means were separated using the PDIFF option. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

From d 1 to 14, pigs fed diets supplemented with IQ had greater ($P < 0.05$) ADFI than pigs fed diets without IQ regardless of AA concentration in the diet (Table 7.4). Pigs had greater ($P < 0.05$) G:F if fed the diet formulated at AA requirements compared with pigs fed a diet with AA below the requirements from d 15 to 27 and in the overall experimental period regardless of IQ inclusion in the diet. Body weight of pigs was not affected by treatments on d 1, 14, or 27. The average fecal score of pigs was reduced ($P < 0.05$) from d 1 to 14, d 15 to 27, and in the overall experimental period if AA were below requirements regardless of IQ inclusion (Table 7.5). From d 15 to 27 and in the overall experimental period, diarrhea frequency was reduced ($P < 0.05$) if AA were below the requirement irrespective of IQ inclusion in the diet.

When AA were at requirements on d 14, IQ inclusion increased the concentration of phenol in the feces, whereas when AA were below the requirements, IQ inclusion decreased phenol concentration in the feces (interaction, $P < 0.05$; Table 7.6). Isoquinoline alkaloid

inclusion in the diet tended to reduce ($P < 0.10$) isovalerate concentrations in the feces on d 14. Also on d 14, ammonia concentration in feces tended to increase ($P < 0.10$) if AA were included below the requirements instead of at requirements. There was no effect of IQ or dietary AA concentration on calprotectin concentration in the feces on d 14. When AA were at requirements, IQ tended to decrease concentrations of acetate and 4-ethylphenol in feces on d 26; however, when AA were below requirements, IQ inclusion tended to increase concentrations of acetate and 4-ethylphenol (interaction, $P < 0.10$; Table 7.7). On d 26, feeding AA below requirements rather than at requirements resulted in an increase ($P < 0.05$) in 7-methylindole and tended to reduce ($P < 0.10$) calprotectin, butyrate, and valerate. Inclusion of IQ in the diet tended to increase ($P < 0.10$) ammonia concentration in feces on d 26 regardless of AA concentration.

Including IQ in the diet formulated to contain AA at requirements resulted in a decrease in IL-4 and IL-10 on d 14; however, when IQ was added to the diet with AA below the requirement, IL-4 and IL-10 increased (interaction, $P < 0.05$; Table 7.8). Including IQ in the diet with AA at requirements tended to decrease IL-1β on d 14, whereas adding IQ to the diet with AA below requirements tended to increase IL-1β in plasma (interaction, $P < 0.10$). Including dietary AA below requirements decreased ($P < 0.05$) IL-18 and TNFα and tended to decrease ($P < 0.10$) IFNγ and IL-2 regardless of IQ inclusion in the diet on d 14. On d 27, including AA below requirements resulted in a decrease ($P < 0.05$) in albumin in plasma irrespective of IQ inclusion (Table 7.9). Inclusion of IQ in the diet tended to increase ($P < 0.10$) TNFα concentrations in plasma regardless of dietary AA concentration. There was no effect of IQ or AA concentration on MDA in plasma.

When the diet formulated at AA requirements was supplemented with IQ, lamina propria thickness in the jejunum of pigs was not affected, whereas when IQ was included in the diet with

AA below requirements, lamina propria thickness in the jejunum was reduced (interaction, $P < 0.05$; Table 7.10). Villus height tended to increase ($P < 0.10$) in the jejunum of pigs fed diets supplemented with IQ regardless of AA concentration. Villus height also tended to increase ($P < 0.10$) if dietary AA concentration was below the requirement regardless of IQ supplementation and VH:CD tended to increase ($P < 0.10$) if AA were provided below requirements regardless of IQ inclusion. In the ileum, villus height tended to be reduced ($P < 0.10$) and lamina propria thickness tended to increase ($P < 0.10$) when IQ was added to the diet irrespective of dietary AA concentration. When AA were at requirements, IQ tended to reduce mRNA abundance of OCLN, whereas when AA were below requirements, IQ inclusion tended to increase OCLN abundance in the jejunum (interaction, $P < 0.10$; Table 7.11). There was no effect of AA level or IQ supplementation on the mRNA abundance of MUC2, CLDN, or ZO1.

Discussion

Post-weaning diarrhea is common in swine production and results in decreased growth performance and increased mortality of weaned pigs (Pluske et al., 1997). Excess N in the diet may contribute to this disorder as undigested protein may be fermented in the large intestine, resulting in proliferation of pathogenic bacteria and consequently increase the incidence of diarrhea (Moeser et al., 2017). Thus, crude protein may be reduced in the diet to decrease excess fermentation and reduce post-weaning diarrhea (Stein and Kil, 2006). Indeed, results of experiments with low crude protein in diets have demonstrated an improvement in fecal score of weaned pigs (Opapeju et al., 2008; Wang et al., 2011; Limbach et al., 2021). Therefore, the observed reduction in diarrhea frequency and average fecal score of pigs fed diets with AA included below the requirements was anticipated. Previous work indicates that inclusion of IQ to

diets fed to pigs results in decreased diarrhea score compared with that of control fed pigs (Liu et al., 2016b; Chen et al., 2019), but a lack of differences in fecal score among treatment groups, as was observed in this experiment, has also been reported (Boroojeni et al., 2018).

Increased ADG and G:F have been observed for growing pigs fed diets containing IQ compared with control-fed pigs (Kantas et al., 2015; Liu et al., 2016a; Boroojeni et al., 2018; Chen et al., 2019), although no effect of IQ supplementation on growth performance has also been reported (Robbins et al., 2013). Sanguinarine, a component of IQ, may regulate serotonin synthesis (Ni et al., 2016), and may be the reason for the observed increase in feed intake by pigs fed IQ supplemented diets (Kantas et al., 2015; Chen et al., 2019). The observed decrease in G:F as dietary AA concentration was reduced is in agreement with previous data (Opapeju et al., 2008; Limbach et al., 2021; Lynegaard et al., 2021), and is likely a result of reduced AA availability, which may have limited growth potential.

The observation that ammonia tended to increase as dietary AA concentration was reduced is in contrast with previous observations (Opapeju et al., 2008; Wang et al., 2011), but the observed tendency for a reduction in fecal metabolites is in accordance with published data (Opapeju et al., 2008; Wang et al., 2011). The tendency for IQ to increase ammonia concentrations in the feces on d 26 is in agreement with reported data (Juskiewicz et al., 2011). As the conversion of N to bacterial protein is needed for bacterial proliferation in the large intestine, it is possible that IQ reduced bacterial cell growth (Herrera-Mata et al., 2002), resulting in an increased presence of ammonia due to diminished bacterial protein synthesis. A reduction in short chain fatty acid concentrations in the ceca of broiler chickens fed a diet containing IQ has also been reported, indicating reduced fermentation when IQ was included in the diet (Jankowski et al., 2009).

Malondialdehyde is an indicator of local oxidative stress, with greater MDA values indicating an increase in lipid peroxidation (Marnett, 1999). Data for effects of IQ on plasma MDA are limited; however, IQ decreased MDA in leg muscle tissue of broiler chickens (Lee et al., 2015), reduced serum MDA, and increased total antioxidant capacity of weaned pigs (Chen et al., 2019) indicating a reduction in oxidative stress as a result of IQ inclusion in the diet. The lack of an effect of IQ on plasma MDA concentration observed in this experiment may be due to the fact that plasma MDA reflects systemic oxidative stress and the challenge of uncleaned pens may have resulted in a more localized systemic immune response. More research should be conducted to determine the antioxidative effects of IQ both systemically and localized in tissues of pigs.

Because the primary function of albumin is to bind to nutrients and transport them through circulation (Bern et al., 2015), the observed decrease in albumin concentrations on d 26 as dietary AA concentration was reduced is reflective of a reduction in absorbed AA entering the bloodstream. The lack of an effect of IQ on plasma albumin is in agreement with previous observations (Kosina et al., 2003; Rawling et al., 2009; Abudabos et al., 2016; Rundle, 2018). However, the lack of an effect of dietary AA concentration and IQ supplementation on PUN and TP is in contrast with evidence that TP and PUN increase as a result of IQ inclusion in diets fed to pigs (Liu et al., 2016a; Rundle, 2018) and that PUN and TP are decreased when crude protein is reduced in the diet (Limbach et al., 2021). Because PUN is an indicator of the efficiency of AA utilization, it was expected that PUN would decrease as dietary AA concentrations were reduced; however, because all indispensable AA in the diet were reduced by 10% based on standardized ileal digestible AA ratios, it is possible that AA were utilized equally across experimental diets.

The immune system responds to pathogens in two ways: innate responses and adaptive responses. The innate response involves immediate action after pathogen exposure whereas the adaptive response is a long-term solution resulting in development of antibodies and T-helper cells that secrete small proteins responsible for cell signaling to other immune cells called cytokines (Murphy and Weaver, 2016). Cytokines can be either pro-inflammatory or anti-inflammatory, are involved in both the innate and adaptive immune systems, and are, therefore, indicative of the overall inflammatory response (Murphy and Weaver, 2016). The tendency for IQ to increase TNFα on d 26 is in contrast with observations of others that indicated a decrease in TNFα with supplementation of IQ (Liu et al., 2016b; Soler et al., 2016; Le et al., 2020). Whereas IL-10, an anti-inflammatory cytokine, was increased by IQ in this experiment, IQ also decreased expression of pro-inflammatory cytokines including IL-1β, IL-6, IL-8, and TL1A in previous work (Soler et al., 2016; Kikusato et al., 2021), indicating that IQ reduces inflammation and immune response in pigs. Other immune-regulating activities of IQ include increased phagocytic activity (Kantas et al., 2015; Bussabong et al., 2021) and inhibition of NF-κβ, a pro-inflammatory nuclear transcription factor of cytokine production in immune cells (Chaturvedi et al., 1997).

The lamina propria supports intestinal villi, which increases the surface area of the intestinal lumen to maximize absorptive potential and contains lymphocytes that release cytokines after pathogenic exposure (Turner, 2003). As a consequence, the lamina propria may thicken in response to inflammation due to increased immune cell infiltration in the area (Yantiss and Antonioli, 2009). Therefore, the observed reduction in lamina propria thickness when AA were below requirements if IQ were included in the diet indicates that either less absorptive capability was needed as AA were reduced in the diet or, more likely, that inflammation in the

lamina propria was reduced as a result of IQ supplementation, leading to faster absorption of nutrients into the capillaries, which in turn may explain previously observed increases in nutrient digestibility (Boroojeni et al., 2018; Rundle et al., 2020). Intestinal villi are the site for absorption of nutrients, with the jejunum being the primary site for absorption of AA, fatty acids, and monosaccharides (Turner, 2003). The tendency for villus height of the jejunum to increase as IQ was added to the diet is in agreement with previous observations (Chen et al., 2019), and indicates improved capacity for absorption of nutrients. However, others have reported a decrease in villus height as IQ was added to broiler or pig diets (Jankowski et al., 2009; Pickler et al., 2013), and no changes in intestinal morphology of broilers and pigs as a result of IQ in the diet have also been reported (Vieira et al., 2008; Boroojeni et al., 2018). Thus, the observation that IQ tended to decrease villus height in the ileum is in agreement with reported data, and may be a result of an increased presence of inflammatory cells in the intestine limiting absorptive capacity due to the environmental health challenge. Indeed, sanguinarine, a component of IQ, increases $CD3^+$ cells in intestinal lining of broilers during a *Salmonella* challenge, and may explain the effects observed in this experiment (Pickler et al., 2013).

The intestinal barrier is a highly regulated, selectively permeable barrier that prevents pathogens from entering the system while simultaneously allowing nutrients to pass through for crucial metabolic function. Dysfunction of the intestinal barrier results in gastrointestinal disorders and reduced health of the animal (Moeser et al., 2017). Tight junction proteins such as OCLN, ZO-1, and CLDN regulate paracellular transport and are essential for function and management of the tight junctions (Fanning et al., 1998; Edelblum and Turner et al., 2009; Groschwitz and Hogan, 2009). The observed increase in OCLN when dietary AA were reduced is in accordance with previous work, indicating that IQ supplementation to growing pig diets

results in increased ZO-1 and CLDN expression in jejunal mucosa (Liu et al., 2016b). Indeed, intestinal permeability indicated by fluorescein isothiocyanate-dextran quantification was reduced (Le et al., 2020; Kikusato et al., 2021) and transepithelial resistance in the ileum increased (Robbins et al., 2013) if IQ was supplemented to diets fed to growing pigs, further indicating that IQ increases intestinal health through improving intestinal barrier function.

Conclusions

Feeding diets with reduced dietary AA, but supplemented with IQ, to weanling pigs subjected to an environmental health challenge improves intestinal health as evidenced by improvements in intestinal morphology, increased abundance of the tight junction protein OCLN in the jejunum, and improved systemic health as evidenced by cytokine expression in the plasma. This improvement in intestinal function may result in increased absorption and utilization of nutrients, specifically N, although this improvement does not always result in an increase in growth performance of pigs.

Tables

Table 7.1. Ingredient composition of experimental diets (As-is basis)

Item, %	Phase 1				Phase 2			
	PC	PC + IQ	NC	NC + IQ	PC	PC + IQ	NC	NC + IQ
Ground corn	41.09	39.89	44.28	43.08	48.43	47.23	52.45	51.25
Soybean meal, 47% CP	22.00	22.00	19.00	19.00	20.00	20.00	16.00	16.00
Whey powder	20.00	20.00	20.00	20.00	15.00	15.00	15.00	15.00
HP 300[2]	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Soybean oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Dicalcium phosphate	1.20	1.20	1.20	1.20	1.00	1.00	1.10	1.10
Ground limestone	0.85	0.85	0.90	0.90	0.95	0.95	0.95	0.95
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-Lys HCl	0.50	0.50	0.40	0.40	0.40	0.40	0.35	0.35
Vit-mineral mix[3]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
DL-Met	0.20	0.20	0.15	0.15	0.14	0.14	0.10	0.10
L-Thr	0.12	0.12	0.07	0.07	0.08	0.08	0.05	0.05
L-Val	0.04	0.04	-	-	-	-	-	-
IQ Premix[4]	-	1.20	-	1.20	-	1.20	-	1.20

[1] PC = positive control; PC + IQ = positive control + 90 mg/kg IQ; NC = negative control, formulated 10% below amino acid requirements; NC + IQ = negative control + 90 mg/kg IQ.

[2]Hamlet Protein, Horsens, Denmark.

[3] The vitamin-micromineral premix provided the following quantities of vitamins and micro

Table 7.1 (cont.)

minerals per kg of complete diet: vitamin A as retinyl acetate, 10,622 IU; vitamin D_3 as cholecalciferol, 1,660 IU; vitamin E as selenium yeast, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.2 mg; niacin, 43.4 mg; folic acid, 1.56 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 123 mg as iron sulfate; I, 1.24 mg as ethylenediamine dihydriodide; Mn, 59.4 mg as manganese hydroxychloride; Se, 0.27 mg as sodium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

[4] Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany.

Table 7.2. Analyzed nutrient composition of experimental diets and ingredients[1]

Item	Phase 1				Phase 2					
	PC	PC + IQ	NC	NC + IQ	PC	PC + IQ	NC	NC + IQ	Corn	SBM
Dry matter, %	88.09	88.55	88.51	88.45	88.18	87.97	87.86	88.74	86.14	87.90
Ash, %	6.21	6.17	6.14	6.10	5.65	5.66	5.52	5.53	1.19	6.07
Crude protein, %	21.70	21.01	20.06	19.46	20.13	19.99	18.66	18.97	6.66	46.02
Gross energy, kcal/kg	3,969	4,004	3,991	3,955	3,982	3,989	3,978	3,980	3,830	4,125
AEE, %	4.28	4.58	4.73	4.69	5.19	5.09	5.29	5.23	3.21	1.11
IAA, %										
Arg	1.25	1.28	1.22	1.04	1.21	1.18	1.09	1.12	0.31	3.32
His	0.51	0.52	0.50	0.45	0.50	0.49	0.46	0.47	0.19	1.20
Ile	1.00	1.00	0.97	0.84	0.91	0.93	0.87	0.86	0.26	2.25
Leu	1.76	1.77	1.74	1.61	1.72	1.71	1.63	1.62	0.82	3.59
Lys	1.72	1.59	1.38	1.21	1.39	1.39	1.29	1.27	0.24	2.94
Met	0.51	0.48	0.43	0.39	0.42	0.40	0.36	0.36	0.15	0.65
Phe	1.02	1.03	1.01	0.90	1.00	0.98	0.93	0.93	0.36	2.46
Thr	0.97	0.94	0.87	0.78	0.89	0.84	0.78	0.79	0.24	1.79
Trp	0.30	0.30	0.28	0.28	0.27	0.26	0.25	0.24	0.06	0.72
Val	1.05	1.06	0.98	0.85	0.94	0.95	0.87	0.88	0.30	2.23

Table 7.2 (cont.)

[1]PC= positive control; PC + IQ= positive control + 90 mg/kg IQ; NC= negative control, formulated 10% below amino acid requirements; NC + IQ= negative control + 90 mg/kg IQ; SBM = soybean meal; IQ = isoquinoline alkaloids; AEE = acid hydrolyzed ether extract; IAA = indispensable amino acids.

Table 7.3. Forward and reverse primer sequences used for quantitative reverse transcription-polymerase chain reaction[1]

Item	Primer sequences (5′→3′)		Reference
	Forward	Reverse	
Internal control genes			
GAPDH	ATCCTGGGCTACACTGAGGAC	AAGTGGTCGTTGAGGGCAATG	Gonzalez et al., 2013
HPRT	GGACTTGAATCATGTTTGTG	CAGATGTTTCCAAACTCAAC	Nygard et al., 2007
Gut-protective target genes			
MUC2	GGCTGCTCATTGAGAGGAGT	ATGTTCCCGAACTCCAAGG	Ferrandis Vila et al., 2018
CLDN	AGAAGATGCGGATGGCTGTC	CCCAGAAGGCAGAGAGAAGC	Hu et al., 2013
OCLN	TCCTGGGTGTGATGGTGTTC	CGTAGAGTCCAGTCACCGCA	Hu et al., 2013
ZO1	AAGCCCTAAGTTCAATCACAATCT	ATCAAACTCAGGAGGCGGC	Hu et al., 2013

[1]GAPDH = glyceraldehyde 3-phosphate dehydrogenase; HPRT = hypoxanthine-guanine phosphoribosyl transferase; MUC2 = mucin 2; CLDN1 = claudin-1; OCLN = occludin; ZO1 = zonula occludens-1.

Table 7.4. Growth performance of pigs fed diets formulated at amino acid (AA) requirements or with reduced AA without or with isoquinoline alkaloids (IQ)[1]

Item	Experimental diet					*P*-value		
	PC	PC + IQ	NC	NC + IQ	SEM	AA	IQ	AA × IQ
D 1 - 14								
ADG, kg	0.70	0.79	0.70	0.77	0.054	0.823	0.060	0.834
ADFI, kg	0.86	0.96	0.88	0.99	0.078	0.677	0.032	0.915
G:F	0.81	0.82	0.80	0.78	0.029	0.351	0.852	0.508
D 15 - 27								
ADG, kg	2.13	2.25	2.00	2.05	0.235	0.067	0.361	0.681
ADFI, kg	2.93	3.20	2.97	3.08	0.291	0.730	0.095	0.476
G:F	0.73	0.70	0.67	0.66	0.017	0.001	0.115	0.558
Overall								
ADG, kg	2.90	3.09	2.75	2.88	0.288	0.126	0.172	0.758
ADFI, kg	3.88	4.25	3.92	4.15	0.378	0.836	0.063	0.665
G:F	0.75	0.73	0.70	0.69	0.009	<0.001	0.086	0.615
Body weight, kg								
D 1	6.12	6.11	6.10	6.08	0.828	0.902	0.919	0.986
D 14	8.08	8.32	8.09	8.24	0.939	0.887	0.410	0.847
D 27	13.66	14.09	13.38	13.56	1.507	0.324	0.454	0.759

Table 7.4 (cont.)

[1]PC = positive control; PC + IQ = positive control + 90 mg/kg IQ; NC = negative control, formulated 10% below amino acid requirements; NC + IQ = negative control + 90 mg/kg IQ; ADG = average daily gain; ADFI = average daily feed intake; G:F = gain:feed ratio.

Table 7.5. Average fecal score and diarrhea frequency of growing pigs fed diets formulated at or below amino acid (AA) requirements supplemented without or with isoquinoline alkaloids (IQ)[1]

Item	Experimental diet					*P*-value		
	PC	PC + IQ	NC	NC + IQ	SEM	AA	IQ	AA × IQ
Average fecal score[2]								
D 1 - 14	2.60	2.76	2.54	2.57	0.130	0.045	0.131	0.259
D 15 - 27	2.62	2.59	2.44	2.47	0.076	0.019	0.954	0.591
Overall	2.61	2.98	2.49	2.49	0.103	0.005	0.526	0.524
Diarrhea frequency[3]								
D 1 - 14	52.86	55.71	45.71	45.71	10.381	0.119	0.791	0.792
D 15 - 27	35.71	30.00	21.43	18.57	6.752	0.019	0.419	0.787
Overall	44.29	42.86	33.57	32.14	8.009	0.015	0.735	1.000

[1]PC = positive control; PC + IQ= positive control + 90 mg/kg IQ; NC = negative control, formulated 10% below amino acid requirements; NC + IQ = negative control + 90 mg/kg IQ.

[2]Fecal scores were assessed visually per pen every other day using a score from 1 to 5 (1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea; and 5 = watery diarrhea).

[3]Diarrhea frequency was calculated by totaling the number of pen days with diarrhea scores ≥ 3 divided by the total number of pen days multiplied by 100.

Table 7.6. Fecal metabolite, volatile fatty acid, ammonia, and calprotectin concentrations in the feces of growing pigs fed diets formulated at or below amino acid (AA) requirements supplemented without or with isoquinoline alkaloids (IQ), d 14[1]

Item	Experimental diet					*P*-value		
	PC	PC + IQ	NC	NC + IQ	SEM	AA	IQ	AA × IQ
Calprotectin, ng/ml	107.6	108.7	107.4	110.2	1.74	0.655	0.188	0.581
Metabolite, µmole/g dry matter								
Phenol	0.05[b]	0.05[ab]	0.06[a]	0.05[ab]	0.007	0.118	0.511	0.037
4-Methylphenol	0.84	0.70	0.88	0.87	0.113	0.180	0.331	0.358
4-Ethylphenol	0.06	0.07	0.06	0.08	0.016	0.546	0.460	0.831
Indole	0.14	0.17	0.15	0.17	0.019	0.708	0.139	0.957
7-Methylindole	0.20	0.20	0.21	0.23	0.031	0.393	0.794	0.682
3-Methylindole	0.40	0.42	0.39	0.40	0.089	0.801	0.834	0.880
2,3-Dimethylindole	0.96	0.89	0.99	0.94	0.129	0.756	0.659	0.930
VFA, µmole/g dry matter								
Acetate	65.92	64.22	66.89	72.06	5.655	0.263	0.656	0.380
Propionate	26.39	24.23	27.11	25.08	2.115	0.686	0.283	0.974

Table 7.6 (cont.)

Item	Experimental diet					*P*-value		
	PC	PC + IQ	NC	NC + IQ	SEM	AA	IQ	AA × IQ
Isobutyrate	2.49	2.77	3.42	2.60	0.570	0.262	0.421	0.110
Butyrate	12.12	12.15	13.03	12.20	1.735	0.710	0.755	0.741
Isovalerate	2.92	2.82	3.56	2.81	0.666	0.212	0.091	0.187
Valerate	3.16	2.67	3.08	2.47	0.586	0.717	0.155	0.871
Ammonia, mg/g dry matter	0.55	0.51	0.69	0.56	0.051	0.064	0.120	0.382

[1]PC = positive control; PC + IQ = positive control + 90 mg/kg IQ; NC = negative control, formulated 10% below amino acid requirements; NC + IQ = negative control + 90 mg/kg IQ; VFA = volatile fatty acid.

Table 7.7. Fecal metabolite, volatile fatty acid, ammonia, and calprotectin concentrations in the feces of growing pigs fed diets formulated at or below amino acid (AA) requirements supplemented without or with isoquinoline alkaloids (IQ), d 26[1]

Item	Experimental diet					*P*-value		
	PC	PC + IQ	NC	NC + IQ	SEM	AA	IQ	AA × IQ
Calprotectin ng/ml	114.6	115.7	113.4	112.4	1.19	0.063	0.931	0.363
Metabolite, µmole/g dry matter								
Phenol	0.05	0.05	0.05	0.05	0.005	0.780	0.451	0.926
4-Methylphenol	0.86	1.03	0.88	1.07	0.129	0.816	0.162	0.910
4-Ethylphenol	0.11	0.09	0.07	0.09	0.013	0.189	0.675	0.088
Indole	0.13	0.14	0.11	0.12	0.019	0.253	0.638	0.958
7-Methylindole	0.24	0.25	0.32	0.28	0.025	0.048	0.559	0.333
3-Methylindole	0.40	0.51	0.40	0.42	0.067	0.525	0.317	0.469
2,3-Dimethylindole	0.36	0.32	0.44	0.42	0.066	0.139	0.631	0.906
VFA, µmole/g dry matter								
Acetate	96.70	81.47	76.67	80.87	2.652	0.053	0.848	0.084
Propionate	39.90	37.76	35.62	36.79	2.348	0.163	0.796	0.377

Table 7.7 (cont.)

Item	Experimental diet					*P*-value		
	PC	PC + IQ	NC	NC + IQ	SEM	AA	IQ	AA × IQ
Isobutyrate	3.29	3.74	3.34	3.52	0.368	0.826	0.397	0.707
Butyrate	20.71	19.60	16.28	18.48	1.375	0.051	0.693	0.237
Isovalerate	3.29	3.89	3.29	3.54	0.448	0.699	0.349	0.692
Valerate	5.47	5.28	4.39	4.53	0.474	0.052	0.957	0.708
Ammonia, mg/g dry matter	0.71	0.90	0.73	0.87	0.093	0.962	0.083	0.805

[1]PC = positive control; PC + IQ = positive control + 90 mg/kg IQ; NC = negative control, formulated 10% below amino acid requirements; NC + IQ = negative control + 90 mg/kg IQ; VFA = volatile fatty acid.

Table 7.8. Plasma characteristics and markers of systemic health of pigs fed diets formulated at or below amino acid (AA) requirements without or with isoquinoline alkaloid (IQ) supplementation, d 14[1]

Item	Experimental diet					*P*-value		
	PC	PC + IQ	NC	NC + IQ	SEM	AA	IQ	AA × IQ
PUN, mg/dL	4.43	4.36	4.34	4.30	0.093	0.348	0.490	0.850
TP, g/dL	10.40	9.00	9.40	7.90	1.072	0.334	0.185	0.963
Albumin, g/dL	2.45	2.53	2.48	2.39	0.063	0.357	0.933	0.158
MDA, nmol/ml	2.95	2.92	3.11	3.07	0.172	0.380	0.824	0.983
Cytokine, ng/ml								
IFNγ	6.40	7.09	3.92	4.24	1.468	0.076	0.727	0.898
IL-1α	0.10	0.06	0.03	0.09	0.039	0.559	0.819	0.133
IL-1β	0.52	0.35	0.32	0.46	0.096	0.592	0.858	0.085
IL-1RA	0.99	1.06	1.16	1.25	0.190	0.344	0.688	0.954
IL-2	0.44	0.32	0.13	0.20	0.127	0.075	0.809	0.436
IL-4	1.04[a]	0.15[b]	0.23[b]	0.29[b]	0.225	0.108	0.049	0.028
IL-6	0.29	0.15	0.32	0.17	0.095	0.737	0.115	0.971
IL-8	0.08	0.03	0.02	0.04	0.029	0.382	0.517	0.295
IL-10	0.72[a]	0.25[b]	0.22[b]	0.28[b]	0.127	0.068	0.117	0.043
IL-12	1.16	1.16	1.01	1.04	0.126	0.225	0.938	0.897
IL-18	2.22[a]	1.46[ab]	0.77[b]	1.04[b]	0.401	0.023	0.539	0.197
TNFα	0.05	0.06	0.04	0.03	0.013	0.035	0.934	0.384

Table 7.8 (cont.)

[1]PC = positive control; PC + IQ = positive control + 90 mg/kg IQ; NC = negative control, formulated 10% below amino acid requirements; NC + IQ = negative control + 90 mg/kg IQ; PUN = plasma urea nitrogen; TP = total protein; MDA = malondialdehyde; IFNγ = interferon-γ; IL = interleukin; TNFα = tumor necrosis factor-α.

Table 7.9. Plasma characteristics and markers of systemic health of pigs fed diets formulated at or below amino acid (AA) requirements without or with isoquinoline alkaloid (IQ) supplementation, d 27[1]

Item	Experimental Diet					*P*-Value		
	PC	PC + IQ	NC	NC + IQ	SEM	AA	IQ	AA × IQ
PUN, mg/dL	4.73	4.70	4.65	4.63	0.099	0.452	0.802	0.960
TP, g/dL	7.70	6.90	7.00	7.30	0.709	0.834	0.727	0.443
Albumin, g/dL	2.68	2.61	2.52	2.38	0.108	0.043	0.265	0.708
MDA, nmol/ml	1.99	1.99	2.21	2.08	0.110	0.173	0.565	0.533
Cytokine, ng/ml								
IFNγ	6.35	4.60	3.32	3.45	1.550	0.118	0.533	0.472
IL-1α	0.07	0.03	0.05	0.06	0.019	0.807	0.233	0.154
IL-1β	0.22	0.13	0.25	0.18	0.074	0.539	0.242	0.846
IL-1RA	0.49	0.47	0.46	0.57	0.084	0.650	0.615	0.465
IL-2	0.23	0.10	0.19	0.19	0.087	0.709	0.336	0.339
IL-4	0.65	0.16	0.34	0.36	0.232	0.816	0.295	0.271
IL-6	0.09	0.04	0.12	0.08	0.043	0.392	0.216	0.864
IL-8	0.19	0.09	0.24	0.21	0.091	0.286	0.415	0.699
IL-10	0.31	0.16	0.30	0.32	0.101	0.402	0.507	0.344
IL-12	1.76	1.61	1.66	1.83	0.198	0.722	0.964	0.307
IL-18	1.77	1.48	1.34	1.60	0.231	0.477	0.945	0.233
TNFα	0.026	0.042	0.027	0.033	0.007	0.492	0.074	0.408

Table 7.9 (cont.)

[1]PC = positive control; PC + IQ = positive control + 90 mg/kg IQ; NC = negative control, formulated 10% below amino acid requirements; NC + IQ = negative control + 90 mg/kg IQ; PUN = plasma urea nitrogen; TP = total protein; MDA = malondialdehyde; IFNγ = interferon-γ; IL = interleukin; TNFα = tumor necrosis factor-α.

Table 7.10. Intestinal tissue morphology of pigs fed diets formulated at or below amino acid (AA) requirements without or with isoquinoline alkaloid (IQ) supplementation[1]

Item	Experimental diet					*P*-value		
	PC	PC + IQ	NC	NC + IQ	SEM	AA	IQ	AA × IQ
Jejunum, µm								
Villus height	463.90	473.88	475.19	528.98	17.128	0.052	0.061	0.192
Crypt depth	202.76	210.97	197.67	206.51	14.755	0.476	0.206	0.963
VH:CD	2.53	2.54	2.65	2.86	0.166	0.062	0.344	0.397
LPT	56.10^{ab}	59.34^{ab}	60.64^{a}	53.74^{b}	2.304	0.812	0.414	0.028
Ileum, µm								
Villus height	424.94	377.54	412.89	384.71	19.500	0.901	0.061	0.625
Crypt depth	214.10	216.15	205.48	196.05	12.513	0.259	0.770	0.649
VH:CD	2.17	2.09	2.24	2.14	0.140	0.670	0.504	0.912
LPT	63.02	73.33	68.27	70.36	3.219	0.725	0.062	0.210

[1]PC = positive control; PC + IQ = positive control + 90 mg/kg IQ; NC = negative control, formulated 10% below amino acid requirements; NC + IQ = negative control + 90 mg/kg IQ; VH:CD = villus height: crypt depth ratio; LPT = lamina propria thickness.

Table 7.11. Least squares means (log_2-backtransformed) for expression of genes in the jejunum of pigs fed diets formulated at or below amino acid (AA) requirements without or with isoquinoline alkaloid (IQ) supplementation[1]

Item	Experimental diet					*P*-value		
	PC	PC + IQ	NC	NC + IQ	SEM	AA	IQ	AA × IQ
MUC2	0.72	0.77	0.78	0.85	0.084	0.361	0.431	0.886
CLDN1	1.08	1.86	1.04	0.78	0.172	0.242	0.740	0.282
OCLN	1.34	1.25	1.13	1.45	0.273	0.876	0.249	0.054
ZO1	1.86	1.61	1.50	1.57	0.355	0.125	0.530	0.235

[1]PC = positive control; PC + IQ = positive control + 90 mg/kg IQ; NC = negative control, formulated 10% below amino acid requirements; NC + IQ = negative control + 90 mg/kg IQ; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; HPRT = hypoxanthine-guanine phosphoribosyl transferase; MUC2 = mucin 2; CLDN1 = claudin-1; OCLN = occludin; ZO1 = zonula occludens-1.

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CHAPTER 8: Conclusions

Isoquinoline alkaloids (**IQ**) have been included in livestock diets to improve growth performance and health. If included in weanling pig diets, IQ improve apparent ileal digestibility (**AID**) of nutrients. Therefore, it was hypothesized that IQ may be included in diets for growing and finishing pigs to improve digestibility of nutrients in a similar manner. Results indicated that IQ can be included in corn-soybean meal diets for growing and finishing pigs at 40 or 80 mg IQ/kg feed to improve the AID of amino acids (**AA**) and crude protein (**CP**) and the apparent total tract digestibility (**ATTD**) of CP by finishing pigs.

Because IQ influences the digestibility of AA and CP, 2 follow-up experiments were conducted to determine effects of IQ on total tract digestibility of energy and nutrients and on N balance of pigs. Isoquinoline alkaloids did not influence the ATTD of gross energy or fiber if included in corn-soybean meal diets formulated to meet all nutrient requirements for growing pigs, nor did it influence the ATTD of gross energy if included at 90 mg IQ/kg feed in diets formulated to be 5% or 8% below indispensable AA requirements for growing pigs. Nitrogen retention was reduced if IQ was included at 40 or 80 mg IQ/kg feed in diets that met nutrient requirements but had no effect on N retention if 90 mg IQ/kg feed was included in diets formulated with indispensable AA below requirements for growing pigs. There was no effect of IQ on biological value of N irrespective of dietary AA concentration, indicating that pigs fed diets containing IQ utilize absorbed N with the same efficiency as pigs fed diets without IQ.

There are, however, no data for effects of IQ on carcass composition of pigs, but due to the effects of IQ on AA digestibility, it was hypothesized that IQ, alone or in combination with narasin, an ionophore that increases feed efficiency and gain of finishing pigs, may improve growth performance and carcass characteristics of finishing pigs. However, this hypothesis was

rejected because results of an 8 wk experiment with finishing pigs indicated that IQ inclusion in corn-soybean meal diets did not influence growth performance, alone or in combination with narasin. Carcass characteristics were also not influenced by IQ with the exception that IQ did increase L* of loin chops, indicating that IQ changes the coloring of loin chops.

Due to the demonstrated ability of IQ to improve digestibility of N-containing compounds by pigs, it was hypothesized that IQ may improve intestinal health and increase digestibility of nutrients of pigs fed diets containing reduced dietary AA and that no differences in growth performance would be observed between pigs fed a control diet that met requirements for AA and pigs fed diets formulated below AA requirements and supplemented with IQ. However, results of an 8 wk experiment with growing pigs indicated that whereas reducing dietary AA concentration by 10% resulted in decreased gain:feed and final body weight of pigs, there was no effect of IQ on growth performance of pigs during the growing phase of production regardless of dietary AA concentration. Results of a 4 wk experiment with newly weaned pigs subjected to a sanitation challenge indicated that pigs fed diets with AA at requirements or 10% below requirements consumed more feed if IQ was included in the diet, which tended to reduce overall gain:feed, but there were no differences in final body weight among treatment groups. Intestinal function, indicated by intestinal morphology and tight junction protein gene abundance, was improved as a result of IQ inclusion if diets contained AA below requirements for weanling pigs. Plasma concentration of anti-inflammatory cytokines also increased due to IQ inclusion in diets for weanling or growing pigs, indicating that IQ reduces systemic inflammation. In conclusion, IQ may be included in diets for weanling and growing pigs to increase digestibility, improve utilization of N-containing compounds, improve intestinal function; however, these effects do not directly result in improved growth performance of pigs.