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Effects of isoquinoline alkaloids on apparent ileal digestibility of amino acids, crude protein, starch, and acid hydrolyzed ether extract and apparent total tract digestibility of energy and crude protein by growing and finishing pigs fed corn-soybean meal diets

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ABSTRACT

Two experiments were conducted to test the hypotheses 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 or finishing pigs. Experiment 1 utilized 12 barrows (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 of IQ to the basal diet. In experiment 2, twelve T-cannulated barrows (BW: 77.2 ± 6.23 kg) were allotted to the same treatments as in experiment 1, but diets were fed for 3 periods for a total of 9 replicate pigs per treatment. Each period had a 10-day adaptation period, followed by fecal sample collection on day 11 and 12, and ileal digesta collection on day 13 and 14. Results indicated that AID of some AA increased when IQ was included at 40 or 160 mg/kg in experiment 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 experiment 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 experiment 2. Albumin was reduced ($P < 0.05$) in plasma of finishing pigs fed 40 mg/kg IQ in experiment 2. Including 80 mg/kg IQ in a diet for growing pigs increased ($P < 0.05$) plasma Lys, and adding 80 or 160 mg/kg increased ($P < 0.05$) plasma 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.

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

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1. Introduction

Isoquinoline alkaloids (**IQ**) are commonly derived from the *Macleaya cordata* plant, or the plume poppy, and primarily include sanguinarine, chelerythrine, protopine, and allocryptopine (Kosina et al., 2010). These alkaloids have anti-inflammatory, antimicrobial, and immunomodulatory effects (Walker, 1990; Agarwal et al., 1991; Chaturvedi et al., 1997), and have been included in livestock diets to improve growth performance. The individual alkaloids of IQ have all shown anti-inflammatory and antimicrobial properties. Specifically, sanguinarine may inhibit nuclear factor kappa-light-chain enhancer of activated B cells, which is a nuclear transcription factor that regulates cytokine expression and, subsequently, inflammation (Chaturvedi et al., 1997). Sanguinarine reduce expression of cytokines involved in the inflammatory response in in vitro studies on the effects of enterotoxigenic *Escherichia coli* (Soler et al., 2016). Chelerythrine inhibits protein kinase C (Herbert et al., 1990), which is a signaling protein for tight junction regulation and is involved in the Toll-like receptor 2 pathway that identifies microbes and modulates immune responses (Cario et al., 2004; Farhadi et al., 2006). Protopine and allocryptopine reduce inflammation and blood clotting (Teng et al., 1991) and act as antimicrobial agents (Kosina et al., 2010). In this way, the effects of all four alkaloids in IQ combined may result in improvements in the intestinal environment conducive to increased nutrient utilization. *In vivo*, isoquinoline alkaloid supplementation to pig diets improves intestinal barrier function and reduces intestinal inflammation (Robbins et al., 2013; Liu et al., 2016a), which supports the proposed mechanism of action of IQ. Indeed, when fed to nursery pigs, IQ improve the apparent ileal digestibility (**AID**) of amino acids (**AA**) and crude protein (**CP**; Goodarzi Boroojeni et al., 2018; Rundle et al., 2020). Isoquinoline alkaloids are effective at improving nutrient utilization of young growing pigs when intestinal health is challenged, but questions remain regarding the influence of IQ in later stages of growth, particularly the growing-finishing phase of production. To the best of our knowledge, 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 CP by pigs.

As growing pigs from 25 to 50 kg utilize nutrients in a different manner than finishing pigs from 50 kg to market (i.e., maximum growth of lean tissue and minimal fat accretion vs. minimal lean tissue deposition and maximum fat accretion), two separate groups of animals were used to examine effects of IQ in diets for animals for these different life stages. Therefore, the first objective was to test the hypothesis that inclusion of IQ in corn-SBM diets fed to growing or finishing pigs will increase the AID of AA, CP, acid hydrolyzed ether extract (**AEE**), and starch and increase the ATTD of gross energy (**GE**) and CP. The second objective was to test the hypothesis that inclusion of IQ in corn-SBM diets fed to growing or finishing pigs will increase concentrations of plasma-free AA and change concentrations of plasma urea nitrogen (**PUN**), total protein, and albumin in plasma, indicators of systemic protein utilization.

2. 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 the 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.

2.1. Experimental diets and designs

In experiment 1, a corn-SBM basal diet was formulated to meet current requirement estimates for pigs from 25 to 50 kg (Table 1;

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

Item, g/kg	Experiment 1				Experiment 2			
	Isoquinoline alkaloids, mg/kg				Isoquinoline alkaloids, mg/kg			
	0	40	80	160	0	40	80	160
Ground corn	591.5	587.5	583.5	575.5	633.5	629.5	625.5	617.5
Soybean meal	340.0	340.0	340.0	340.0	300.0	300.0	300.0	300.0
Soybean oil	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Ground limestone	9.00	9.00	9.00	9.00	8.00	8.00	8.00	8.00
Dicalcium phosphate	9.00	9.00	9.00	9.00	8.00	8.00	8.00	8.00
Sodium chloride	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Titanium dioxide	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin-mineral mix[a]	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
IQ premix[b]	-	4.00	8.00	16.00	-	4.00	8.00	16.00

[a] 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, 2210 IU; vitamin E as DL alpha tocopheryl, 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, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

[b] IQ = Isoquinoline alkaloids. Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany. Premix was prepared by adding 1% of Sangrovit G Premix with ground corn.

NRC, 2012). Three additional diets were formulated by adding 40, 80, or 160 mg/kg IQ to the control diet for a total of four experimental diets. 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 experiment 2, a corn-SBM basal diet was formulated to meet current requirement estimates for pigs from 50 to 75 kg (Table 2; NRC, 2012). Three additional diets were formulated by adding 40, 80, or 160 mg/kg IQ to the control diet for a total of four experimental diets. 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. Before diet mixing, IQ was included in a corn premix that was subtracted from the total amount of corn in the diet and then added to the remaining ingredients, thereby ensuring that IQ was mixed thoroughly and evenly distributed in the diet. All pigs were fed their respective diets on an ad libitum basis and water was also available at all times. Functional feed additives such as enzymes, crystalline amino acids, or organic acids were not utilized in these diets.

In experiment 1, twelve 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 (Kim and Stein, 2009) with 4 dietary treatments in each square and 4 periods. Pigs were cannulated 7 d before the start of the experiment (Stein et al., 1998). In experiment 2, twelve ileal cannulated finishing barrows that were different from the pigs used in experiment 1 were used (initial body weight: 77.2 ± 6.23 kg). Pigs were cannulated when they were 20–25 kg and had been used in a different experiment before being allotted to the present experiment. They were fed a standard corn-SBM-based diet for 2 weeks between the 2 experiments. Pigs were allotted to a triplicated 4 × 3 incomplete Latin square design with 4 dietary treatments in each square and 3 periods. Each period lasted 14 days in both experiments. 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 experiment 1 and 9 replicate pigs per treatment in experiment 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.

2.2. Sampling procedures

Pig weights were recorded at the beginning of the experiment and at the end of each period. The initial 10 day of each period were considered an adaptation period to the diet. Fecal samples were collected on day 11 and 12 of each period using the grab sampling technique. These samples were immediately frozen at − 20 °C. Ileal digesta samples were collected for 8 h on day 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 filled or at least once every 30 min and immediately stored at − 20 °C to prevent bacterial degradation of AA in the digesta. On day 1 and at the conclusion of each collection period, a blood sample was collected from the jugular vein of each pig in vacutainers

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

Item	Experiment 1						Experiment 2					
	Isoquinoline alkaloids, mg/kg						Isoquinoline alkaloids, mg/kg					
	-	40	80	160	Corn	SBM[a]	-	40	80	160	Corn	SBM
Dry matter, g/kg	879.8	879.5	882.2	875.8	868.4	887.0	866.4	866.7	866.8	865.8	849.4	876.7
Ash, g/kg	53.9	56.2	55.3	52.9	11.6	68.8	49.9	45.0	51.0	45.6	10.6	65.2
AEE[a], g/kg	51.9	52.6	46.3	45.5	29.4	26.6	64.6	59.6	58.9	73.0	35.9	25.8
Starch, g/kg	419.6	427.7	426.9	424.0	672.2	40.7	436.2	433.9	420.6	445.9	697.4	ND
Gross energy, MJ/kg	16.88	16.86	16.89	16.75	15.95	17.44	16.74	16.91	16.84	17.00	15.80	17.44
Crude protein, g/kg	192.3	196.0	190.4	186.8	76.8	476.6	175.2	178.0	190.9	167.2	69.6	460.0
Indispensable amino acids, g/kg												
Arg	13.0	13.5	13.2	13.2	3.70	33.80	12.3	12.8	11.6	10.5	3.10	33.1
His	5.10	5.30	5.20	5.20	2.10	12.20	5.00	5.30	4.80	4.50	2.00	12.20
Ile	8.90	9.30	9.00	9.00	2.70	22.80	8.60	9.00	8.30	7.40	2.50	23.10
Leu	16.10	17.00	16.60	16.50	8.10	36.10	16.10	16.90	15.50	14.00	7.60	35.80
Lys	11.10	11.50	11.20	11.30	2.80	29.30	10.70	11.20	10.30	9.40	2.60	29.40
Met	2.80	2.90	2.80	2.90	1.40	6.40	2.80	3.10	2.70	2.50	1.40	6.60
Phe	9.60	10.10	9.80	9.80	3.50	23.80	9.70	10.20	9.30	8.30	3.20	24.40
Thr	7.20	7.60	7.40	7.50	2.60	17.80	6.90	7.20	6.60	5.90	2.30	17.50
Trp	2.30	2.50	2.40	2.40	0.60	6.60	2.00	2.00	1.90	1.60	0.40	5.50
Val	9.60	9.90	9.70	9.70	3.50	23.20	9.50	9.90	9.10	8.40	3.30	23.90
Dispensable amino acids, g/kg												
Ala	9.40	9.80	9.60	9.60	5.10	20.10	9.50	9.80	8.90	8.50	5.00	20.00
Asp	19.90	20.80	20.20	20.70	5.10	52.30	19.40	20.20	18.60	16.40	4.60	52.60
Cys	3.00	3.10	2.90	3.10	1.60	6.20	2.90	3.10	2.80	2.60	1.50	6.50
Glu	34.50	36.40	35.40	35.30	12.80	84.10	33.10	34.10	31.20	28.50	11.80	81.90
Gly	8.10	8.40	8.20	8.20	3.00	19.50	8.20	8.20	7.30	7.50	2.90	19.70
Pro	11.10	11.60	11.50	11.40	6.20	23.70	11.30	11.80	10.80	10.00	5.80	23.00
Ser	8.40	8.80	8.70	8.60	3.40	20.20	7.40	7.60	6.90	6.30	2.90	18.50
Tyr	6.80	7.20	7.00	7.00	2.40	17.30	5.80	6.20	5.50	4.80	1.60	15.30

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

containing lithium heparin. After collection, all blood samples were centrifuged at 4,000g 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 and 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.

2.3. Chemical analyses

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, USA) 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, USA). 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, USA) 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, USA) followed by crude fat extraction using petroleum ether (Ankom^{XT15}, Ankom Technology, Macedon, NY, USA). Corn, SBM, and diets were analyzed for ash (method 942.05; AOAC Int, 2007) and for GE using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA).

Plasma samples collected on day 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, USA) 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, USA).

Table 3
Apparent ileal digestibility of crude protein (CP), amino acids (AA), acid-hydrolyzed ether extract (AEE) and starch by growing pigs fed corn-soybean meal diets supplemented with isoquinoline alkaloids (IQ), experiment 1[a].

Item	Isoquinoline alkaloids, mg/kg					*P*-Value
	-	40	80	160	SEM	Diet
Starch	0.951	0.944	0.936	0.942	0.0079	0.106
AEE	0.716[a]	0.709[a]	0.647[b]	0.656[b]	0.0155	0.002
CP	0.763	0.771	0.754	0.758	0.0063	0.116
Indispensable AA						
Arg[b]	0.890[ab]	0.897[a]	0.886[b]	0.896[a]	0.0029	0.012
His[b],[c]	0.824	0.835	0.825	0.836	0.0050	0.104
Ile	0.814[bc]	0.827[a]	0.808[c]	0.822[ab]	0.0049	0.027
Leu	0.810[b]	0.826[a]	0.809[b]	0.823[a]	0.0051	0.021
Lys	0.820[b]	0.838[a]	0.819[b]	0.830[ab]	0.0065	0.019
Met	0.838[b]	0.853[a]	0.832[b]	0.851[a]	0.0050	0.004
Phe[c]	0.820[bc]	0.834[a]	0.818[c]	0.830[ab]	0.0048	0.023
Thr	0.721[b]	0.748[a]	0.721[b]	0.743[a]	0.0096	0.009
Trp	0.808	0.827	0.804	0.820	0.0089	0.071
Val	0.765[bc]	0.784[a]	0.761[c]	0.778[ab]	0.0066	0.020
Mean[c]	0.814[bc]	0.831[a]	0.812[c]	0.825[ab]	0.0050	0.008
Dispensable AA						
Ala	0.761[b]	0.783[a]	0.756[b]	0.773[ab]	0.0071	0.014
Asp[b]	0.774[ab]	0.787[a]	0.769[b]	0.786[a]	0.0067	0.039
Cys	0.654[a]	0.668[a]	0.623[b]	0.673[a]	0.0147	0.007
Glu	0.831	0.831	0.830	0.827	0.0065	0.633
Gly	0.673	0.683	0.662	0.669	0.0125	0.567
Pro	0.799	0.813	0.802	0.813	0.0083	0.072
Ser	0.789	0.805	0.792	0.803	0.0074	0.053
Tyr	0.831[b]	0.848[a]	0.831[b]	0.842[a]	0.0054	0.008
Mean[b]	0.788[ab]	0.801[a]	0.785[b]	0.794[ab]	0.0063	0.177
Total AA	0.800[b]	0.815[a]	0.797[b]	0.808[ab]	0.0054	0.048

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

[a] There were 12 observations used in the control treatment group fed 0 mg/kg IQ and 11 observations used in the treatment groups fed 40, 80, or 160 mg/kg IQ.

[b] 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.

[c] 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.

2.4. Calculations

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

$$AID_{AA} = 100 - \left[\left(\frac{AA_{digesta}}{AA_{feed}}\right) \times \left(\frac{Ti_{feed}}{Ti_{digesta}}\right)\right]$$

where AID_{AA} is the apparent ileal digestibility of an AA, $AA_{digesta}$ 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 average analyzed titanium concentration in diet DM, and $Ti_{digesta}$ 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 energy 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.

2.5. Statistical analyses

Data were analyzed using SAS (SAS Institute Inc, 2016). Normality of residuals was tested using Proc UNIVARIATE of SAS. Data for digestibility and plasma analyses from both experiments were analyzed using the Proc MIXED of SAS with pig as the experimental unit. 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. Pairwise comparisons were also performed using contrast statements to compare the control diet with the IQ diets. Results were considered significant at $P \leq 0.10$.

3. Results

Addition of 40 mg/kg IQ to corn-SBM diets fed to growing pigs resulted in an increase ($P < 0.10$) in the AID of Ile, Leu, Lys, Met, Phe, Thr, Val, Ala, and Tyr compared with the control diet (Table 3). Likewise, inclusion of 160 mg/kg IQ increased ($P < 0.05$) the AID of Leu, Met, Thr, and Tyr compared with the control diet. The AID of AEE was reduced ($P < 0.05$) as IQ was included at 80 mg/kg or 160 mg/kg IQ compared with the control diet.

Inclusion of 80 mg/kg IQ in the diet resulted in an increase ($P = 0.008$) in the AID of CP when fed to finishing pigs (Table 4). At an

Table 4
Apparent ileal digestibility of crude protein (CP), amino acids (AA), acid-hydrolyzed ether extract (AEE), and starch by finishing pigs fed corn-soybean meal diets supplemented with isoquinoline alkaloids (IQ), experiment 2[a].

Item	Isoquinoline alkaloids, mg/kg					*P*-Value
	-	40	80	160	SEM	Diet
Starch	0.917	0.913	0.920	0.903	0.0088	0.092
AEE	0.773	0.765	0.745	0.783	0.0218	0.352
CP	0.746^{bc}	0.754^{ab}	0.775^{a}	0.731^{c}	0.0107	0.008
Indispensable AA						
Arg	0.874^{a}	0.882^{a}	0.872^{a}	0.853^{b}	0.0046	< 0.001
His	0.824^{b}	0.844^{a}	0.827^{ab}	0.807^{c}	0.0073	0.002
Ile[b]	0.800^{a}	0.815^{a}	0.798^{a}	0.765^{b}	0.0088	< 0.001
Leu[b]	0.807^{a}	0.823^{a}	0.806^{a}	0.777^{b}	0.0093	< 0.001
Lys[b]	0.801^{ab}	0.814^{a}	0.793^{b}	0.771^{c}	0.0065	< 0.001
Met	0.830^{b}	0.848^{a}	0.825^{b}	0.801^{c}	0.0073	< 0.001
Phe[2]	0.820^{ab}	0.834^{a}	0.817^{b}	0.788^{c}	0.0080	< 0.001
Thr[b]	0.717^{a}	0.741^{a}	0.717^{a}	0.666^{b}	0.0102	< 0.001
Trp	0.816^{a}	0.824^{a}	0.824^{a}	0.764^{b}	0.0094	< 0.001
Val[b]	0.756^{ab}	0.777^{a}	0.753^{b}	0.724^{c}	0.0096	< 0.001
Mean[b]	0.806^{ab}	0.821^{a}	0.803^{b}	0.774^{c}	0.0078	< 0.001
Dispensable AA						
Ala	0.762^{ab}	0.777^{a}	0.753^{b}	0.729^{c}	0.0113	0.002
Asp	0.774^{ab}	0.787^{a}	0.766^{a}	0.72^{b}	0.0106	< 0.001
Cys[b]	0.68^{ab}	0.715^{a}	0.680^{b}	0.636^{c}	0.0125	< 0.001
Glu	0.831^{a}	0.837^{a}	0.821^{a}	0.793^{b}	0.0103	0.006
Gly[b]	0.646	0.695	0.660	0.633	0.0192	0.105
Pro	0.803^{b}	0.821^{a}	0.805^{ab}	0.776^{c}	0.0082	< 0.001
Ser	0.766^{a}	0.781^{a}	0.761^{a}	0.726^{b}	0.0085	< 0.001
Tyr[b]	0.806^{ab}	0.824^{a}	0.802^{b}	0.761^{c}	0.0091	< 0.001
Mean	0.783^{a}	0.800^{a}	0.778^{a}	0.746^{b}	0.0101	0.002
Total AA	0.791^{a}	0.807^{a}	0.788^{a}	0.756^{b}	0.0090	< 0.001

[a-c]Means in a row without a common superscript differ ($P < 0.05$).
[a] There were 9 observations used per treatment with the exception of the 40 and 80 mg/kg treatment groups which had 7 observations each.
[b] 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.

inclusion of 40 mg/kg IQ, there was an increase ($P < 0.10$) in the AID of His, Met, and Pro compared with the control diet. However, 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. The AID of starch reduced ($P < 0.10$) 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 5). Inclusion of 80 mg/kg IQ resulted in an increase ($P < 0.10$) in plasma concentrations of Arg, Lys, and Asp compared with pigs fed the control diet. When 80 or 160 mg/kg IQ was included, an increase ($P < 0.05$) in plasma His compared with pigs fed the diet with 40 mg/kg IQ was observed.

A reduction ($P < 0.10$) was observed in plasma albumin concentration of finishing pigs fed the 40 mg/kg IQ diet compared with the control diet (Table 6). There was no influence of IQ on the ATTD of GE or CP by growing pigs (Table 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.

4. Discussion

Effects of IQ on nutrient digestibility in diets fed to weanling pigs have been reported (Goodarzi Boroojeni et al., 2018; Rundle et al., 2020) and it was concluded that improved digestibility in pigs fed IQ was a result of the anti-inflammatory and antimicrobial effects of IQ. As the health of young growing pigs is challenged in the post-weaning environment (Pluske et al., 1997), the influence of IQ in improving intestinal integrity and immunity is likely the reason for the improvement in digestibility (Robbins et al., 2013; Liu et al., 2016a). The current experiments were conducted to determine if IQ also influence digestibility of nutrients in the growing and finishing phases of production, where health is less challenged and intestinal integrity is relatively stable.

Instead of continuing to use pigs from experiment 1 to perform experiment 2, different pigs were used to decrease the likelihood of a carryover effect of feeding IQ in previous weeks. As growing and finishing pigs deposit lean muscle and adipose tissue at different rates, it was important to study these different physiological stages separately to establish possible differences in effects of IQ when metabolism of nutrients changes. The IQ dosages were at or slightly above the amount of IQ recommended for growing-finishing pigs according to the manufacturer.

An incomplete Latin square design was used in experiment 2 due to the nature of finishing pigs in a cannulation experiment. Starting at a body weight of approximately 77 kg, the finishing pigs were provided the corn-SBM-based diets on an ad libitum basis and rapidly increased body weight. Pigs remained on trial only until they reached market weight. As a consequence, the experiment ended with 3 replicates of squares, and data were analyzed as an incomplete Latin square design while retaining enough replications to be

Table 5
Blood characteristics of growing pigs fed diets supplemented with isoquinoline alkaloids (IQ), experiment 1[a].

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 amino acids, μg/dL						
Arg	47.7[b]	46.9[b]	54.7[a]	51.5[ab]	3.55	0.016
His[2,3]	19.5[ab]	18.2[b]	20.7[a]	19.7[a]	0.97	0.016
Ile[b]	24.8	24.6	26.5	25.6	0.77	0.137
Leu[b]	37.4	37.1	39.6	37.9	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[c]	14.4	14.2	14.9	15.5	0.71	0.187
Val	44.0	43.3	46.4	44.8	1.85	0.280
Dispensable amino acids, μg/dL						
Ala	44.1	40.6	47.4	42.9	4.16	0.056
Asp	1.7[b]	1.9[ab]	2.0[a]	2.0[a]	0.24	0.024
Glu[b]	18.5	19.1	22.0	19.8	2.79	0.326
Gly	83.1	81.5	84.8	82.9	4.04	0.587
Pro[d]	48.2	44.3	49.1	49.2	1.51	0.100
Ser	21.7	20.4	23.0	22.0	0.91	0.095
Tyr	33.4	32.3	34.4	34.2	1.52	0.598

[a-c]Means in a row without a common superscript differ ($P < 0.05$).
[a] There were 10 observations used in the 0, 40, and 80 mg/kg IQ treatment and 11 observations used in the 160 mg/kg IQ treatment.
[b] 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.
[c] 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.
[d] 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.

Table 6
Blood characteristics of finishing pigs fed diets supplemented with isoquinoline alkaloids (IQ), experiment 2[a].

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	6.8	6.8	7.1	0.16	0.069
Indispensable amino acids μg/dL						
Arg	53.3	49.1	47.2	42.2	3.14	0.064
His[b]	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[b]	5.9	5.6	5.6	5.1	0.54	0.375
Phe[b]	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	12.9	12.8	12.0	1.05	0.181
Val	46.0	44.3	44.1	42.9	2.83	0.670
Dispensable amino acids, μg/dL						
Ala	46.3	41.3	45.7	37.9	4.76	0.051
Asp	2.2	1.8	2.1	1.9	0.29	0.185
Glu[2]	23.2	18.4	20.4	19.4	3.29	0.153
Gly	81.4	81.2	86.9	80.7	5.16	0.274
Pro	49.9	48.1	48.8	41.8	2.24	0.070
Ser	21.1	19.3	20.4	17.6	1.10	0.101
Tyr	27.5	26.3	26.8	22.3	1.63	0.123

[a-c]Means in a row without a common superscript differ ($P < 0.05$).
[a] There were 9 observations used for the 40 and 80 mg/kg IQ treatment, and 8 observations each used for the 0 and 160 mg/kg IQ treatment.
[b] 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.

Table 7
Apparent total tract digestibility (ATTD) of crude protein (CP), and gross energy (GE) by pigs fed isoquinoline alkaloid (IQ) supplemented corn-soybean meal diets[a].

Item	Isoquinoline alkaloids, mg/kg					*P*-Value
	-	40	80	160	SEM	Diet
Growing pigs, experiment 1						
ATTD of CP	0.836	0.836	0.832	0.854	0.0085	0.247
ATTD of GE	0.852	0.845	0.845	0.852	0.0052	0.493
Finishing pigs, experiment 2						
ATTD of CP	0.856[b]	0.860[ab]	0.868[a]	0.848[b]	0.0092	0.034
ATTD of GE	0.873	0.878	0.876	0.872	0.0055	0.460

[a-c]Means in a row without a common superscript differ ($P < 0.05$).
[a] There were 12 observations used for each treatment group in experiment 1 with the exception of the 0 mg/kg IQ treatment group, which used 11 observations. In experiment 2, 9 observations were used for the 0 mg/kg treatment, 8 observations were used for the 40 and 80 mg/kg IQ treatments, and 7 observations used for the 160 mg/kg IQ treatment.

considered statistically significant.

Previous data to demonstrate effects of IQ have been compared using linear and quadratic contrasts to elucidate the most effective inclusion level of IQ in diets for growing pigs (Rundle et al., 2020). However, to effectively determine the optimal inclusion rate of IQ, least square means were separated and effects were, therefore, not established as "linear" or "quadratic", but rather, the greatest digestibility of each nutrient was reported as statistically different from the control diet. Pairwise comparisons were also used to determine if the treatment groups varied significantly from the control group in terms of digestibility and plasma concentrations of PUN, total protein, free AA, and albumin.

4.1. Apparent ileal digestibility

The increase in the AID of some AA as IQ was added to the diets in both experiment 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 (Goodarzi Boroojeni et al., 2018; Rundle et al., 2020). However, the increase in AID of total AA in experiment 1 was observed when pigs were fed 40 mg/kg of IQ, whereas an increase in AID of total AA occurred at 120 mg/kg IQ supplementation in corn-SBM-barley-wheat diets fed to nursery pigs (Goodarzi Boroojeni et al., 2018). It is possible that the different diet type or the different age of the pigs is the reason for the different level of IQ that optimized AID of AA. It is not clear why the AID of AA decreased in experiment 2 at the inclusion level of 160 mg/kg IQ, but 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 experiment 1 indicating that AID of CP did not increase as IQ was added to the diet 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 experiment 2 agreed with results that IQ supplementation to corn-SBM-barley-wheat diets tended to increase the AID of CP by nursery pigs (Goodarzi Boroojeni et al., 2018). Further research may be needed to elucidate the effects of IQ to establish a clear response in terms of digestibility of CP.

Increases in the AID of AA and CP observed in these experiments at lower inclusion levels may potentially 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., 2016a). The alkaloids in the IQ preparation used in these experiments may 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. In addition, IQ has been shown to inhibit NF-kB activation, which plays a critical role in the expression of pro-inflammatory cytokines (Chaturvedi et al., 1997). Therefore, a reduction in inflammation along with an improvement in the intestinal barrier function may result in the enhanced absorption of essential nutrients in the small intestine observed in the present experiments. However, as the results from these experiments are variable in terms of digestibility of AA and CP, further work is needed to determine the effects of IQ on nitrogen efficiency and utilization.

The reduction in AID of starch in pigs fed the 80 mg/kg IQ diet in experiment 1 and the 160 mg/kg IQ diet in experiment 2 contrasts with 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). Specifically, 250 μM sanguinarine and chelerythrine cause complete inhibition of 1.9 nkat α-amylase from porcine pancreas and those same concentrations only caused 23.9% and 7.5% inhibition of 1.9 nkat α-amylase from human saliva, respectively, indicating that effects of IQ on α-amylase is variable. Therefore, it is possible that IQ only had an inhibitory effect on the endogenous α-amylase of pigs at the highest level of IQ needed to produce an inhibitory effect under in vivo conditions, however, the effects observed in the present experiments were minimal.

The lack of an effect in experiment 2 on the AID of AEE is in accordance with data indicating that IQ does not influence the digestibility of AEE in nursery pigs (Goodarzi Boroojeni et al., 2018; Rundle et al., 2020). It is not clear why IQ supplementation reduced the AID of AEE in experiment 1; however, it may have been related to modulation of the intestinal microbiome as microbial concentrations may influence lipid digestibility (Espinosa et al., 2019; Schoeler and Caesar, 2019).

4.2. 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 experiment 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 experiment 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 experiment 1 and 2 were in agreement with data demonstrating that IQ supplementation to swine diets resulted in greater serum AA concentrations (Liu et al., 2016b). 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., 2016b; Goodarzi Boroojeni et al., 2018).

4.3. Apparent total tract digestibility

The lack of an influence of IQ on the ATTD of GE in both experiment 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). The previously mentioned increase in AID of AA and starch in the present experiments seemingly contradicts the observations of ATTD of GE as both AA and starch are substrates contributing to energy provided by the diet, however it is likely that the difference in AID of AA and starch was not great enough to significantly impact the ATTD of GE by the pigs under the conditions of this experiment. In contrast to these observations, 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, although vastly different in terms of metabolism, 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 may also have influenced results.

Increased ATTD of CP at 80 mg/kg IQ inclusion in experiment 2 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). However, the differences between ruminant and non-ruminant metabolism of N and most other nutrients are significant and likely influence results. As there was no effect of IQ on the AID of CP by growing pigs in experiment 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.

4.4. Limitations

It is not clear why digestibility of AA was improved in the 40 and 160 mg/kg treatment groups, but not in the 80 mg/kg IQ treatment group in experiment 1. Samples of each diet from both experiments were analyzed for IQ, and results were as expected demonstrating that there was no problem with dosage at diet mixing. The IQ premix was added to a small batch of corn and mixed thoroughly into the entire diet to minimize separation of the diet.

Weanling 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. The lower and somewhat variable responses to IQ that were observed in the present experiments compared with experiments with weanling pigs is likely a result of the high health status of the pigs used in this experiment and the overall improved health of growing and finishing pigs compared with weanling pigs. It is possible that pigs of a lower health status or pigs in a health-challenged environment are better experimental models for the effects of IQ on nutrient digestibility.

5. Conclusions

Including isoquinoline alkaloids at around 40 mg/kg to corn-soybean meal diets fed to growing or finishing pigs generally results in the greatest digestibility of amino acids and 80 mg/kg isoquinoline alkaloids in corn-soybean meal diets fed to finishing pigs resulted in the greatest apparent total tract digestibility of crude protein. However, further research is needed to investigate the mechanism of action behind the reduction of apparent ileal digestibility of acid hydrolyzed ether extract and amino acids by finishing pigs fed diets supplemented with isoquinoline alkaloids at 160 mg/kg. Likewise, research needs to be conducted to determine if isoquinoline alkaloids has a greater effect on growth performance and nutrient digestibility under conditions where pig growth performance and health is challenged.

CRediT authorship contribution statement

H.H. Stein and V. Artuso-Ponte conceptualized the experiments. C.M. Rundle conducted the experiment and summarized data. H.H. Stein contributed with data interpretation. C.M. Rundle wrote the first draft of the manuscript. H.H. Stein and V. Artuso-Ponte edited the final version of the manuscript. H.H. Stein supervised the project.

Conflict of interest

VA-P is an employee of Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany, a global supplier of feed additives to animals. CMR and HHS have no conflicts of interest.

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