Effects of isoquinoline alkaloids on apparent ileal digestibility of amino acids, acid hydrolyzed ether extract, and starch by young growing pigs fed corn-soybean meal diets

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

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

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INTRODUCTION

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

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

MATERIALS AND METHODS

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

Diets, Animals, and Experimental Design

A basal diet that primarily contained corn, soybean meal, fish meal, and lactose was formulated (Tables 1 and 2) to meet requirement estimates for pigs from 11 to 25 kg (NRC, 2012). Three additional diets were formulated by adding 90, 180, or 360 mg/kg of IQ to the basal diet. Thus, all diets were identical except for the inclusion of IQ, which resulted in different concentrations of alkaloids in the diets. Vitamins and minerals were included to meet or exceed current requirement estimates (NRC, 2012). All diets also contained 0.40% chromic oxide as an indigestible marker.

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

Feeding and Sample Collection

All pigs were provided feed on an ad libitum basis and water was available at all times. Pig weights were recorded at the beginning of the experiment.

Table 1. Composition (as-is basis) of experimental diets

	Isoquinoline alkaloids, mg/kg			
Item, %	–	90	180	360
Ground corn	42.70	42.30	41.90	41.10
Soybean meal, 46% CP	36.00	36.00	36.00	36.00
Soybean oil	4.00	4.00	4.00	4.00
Dicalcium phosphate	0.30	0.30	0.30	0.30
Limestone	0.80	0.80	0.80	0.80
Lactose	10.00	10.00	10.00	10.00
Fish meal	5.00	5.00	5.00	5.00
Isoquinoline alkaloid premix[a]	–	0.40	0.80	1.60
Chromic oxide	0.40	0.40	0.40	0.40
Sodium chloride	0.50	0.50	0.50	0.50
Vitamin-mineral premix[b]	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00

[a]Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany.

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

Table 2. Analyzed nutrient composition of experimental diets and ingredients (as-fed basis)

	Isoquinoline alkaloids, mg/kg						
Item	–	90	180	360	Corn	SBM	Fish meal
Dry matter, %	87.46	88.75	88.85	87.75	86.12	85.93	90.50
AEE, %	4.51	4.22	5.00	5.25	3.36	1.30	7.50
Ash, %	6.07	6.12	6.25	5.89	1.24	6.26	19.04
CP, %	22.93	23.23	22.85	22.98	7.50	46.36	64.84
Starch, %	27.63	28.73	30.10	27.62	59.33	2.85	–
Neutral detergent fiber, %	7.29	7.16	6.36	7.25	10.75	7.71	–
Acid detergent fiber, %	3.17	3.82	3.38	2.97	4.02	6.22	–
Acid detergent lignin, %	0.89	0.89	0.87	0.88	1.12	0.63	–
Indispensable AA, %							
Arg	1.44	4.51	1.49	1.49	0.35	3.30	3.66
His	0.57	0.59	0.58	0.58	0.21	1.19	1.25
Ile	1.04	1.08	1.05	1.06	0.29	2.24	2.62
Leu	1.83	1.88	1.82	1.86	0.87	3.55	4.25
Lys	1.36	1.41	1.37	1.38	0.27	2.87	4.88
Met	0.35	0.37	0.36	0.36	0.14	0.64	1.66
Phe	1.11	1.16	1.12	1.13	0.37	2.38	2.40
Thr	0.83	0.86	0.84	0.85	0.26	1.76	2.39
Trp	0.32	0.31	0.32	0.31	0.07	0.72	0.68
Val	1.10	1.16	1.11	1.12	0.36	2.31	2.99
Dispensable AA, %							
Ala	1.13	1.15	1.13	1.15	0.54	1.98	4.00
Asp	2.27	2.37	2.31	2.33	0.51	5.16	5.42
Cys	0.31	0.32	0.33	0.32	0.16	0.65	0.50
Glu	3.86	3.99	3.88	3.92	1.35	8.24	7.96
Gly	1.08	1.10	1.08	1.10	0.32	2.01	4.91
Pro	1.29	1.31	1.25	1.29	0.66	2.51	3.16
Ser	0.90	0.93	0.91	0.92	0.33	1.96	2.09
Tyr	0.71	0.74	0.73	0.73	0.20	1.70	1.83
Isoquinoline alkaloids, mg/kg							
Sanguinarine	ND	0.44	0.82	1.8	–	–	–

ND, not detected; SBM, soybean meal.

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

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

Chemical Analyses

Digesta samples were lyophilized and finely ground prior to chemical analysis. Corn, soybean meal, fish meal, and all samples of digesta and diets were analyzed in duplicate for dry matter (method 930.15; AOAC International, 2007) and for CP using the Kjeldahl method (Kjeltec 8400; FOSS Inc., Eden Prairie, MN) by quantifying N and calculating CP via a conversion factor of 6.25 (method 984.13; AOAC International, 2007). Samples were analyzed for AA on an AA analyzer (model L8800 Hitachi Amino Acid Analyzer, Hitachi High Technologies America Inc., Pleasanton, CA) using ninhydrin for postcolumn derivatization and norleucine as the internal standard. Before AA analysis, samples were hydrolyzed with 6*N* HCl for 24 h at 110 °C [method 982.30 E(a); AOAC International, 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 International, 2007]. Tryptophan was determined after NaOH hydrolysis for 22 h at 110 °C [method 982.30 E(c); AOAC International, 2007]. The chromium concentration of the diets and digesta was determined using an Inductive Coupled Plasma Atomic Emission Spectrometric method (method 990.08; AOAC International, 2007). Samples were prepared using nitric acid-perchloric acid [method 968.08 D(b); AOAC International, 2007]. Total starch was determined in all samples using the glucoamylase procedure (method 979.10; AOAC International, 2007), and total AEE was analyzed 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, soybean meal, and diets were also analyzed for acid detergent fiber and neutral detergent fiber using Ankom Technology methods 12 and 13, respectively (Ankom 2000 Fiber Analyzer, Ankom Technology, Macedon, NY), and for acid detergent lignin using Ankom Technology method 9 (Ankom DaisyII Incubator, Ankom Technology, Macedon, NY). Additionally, these samples were analyzed for dry ash (method 942.05; AOAC International, 2007). Diets were also analyzed for sanguinarine concentration using a proprietary method, HPLC/LUFA SP 1012 (Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany).

Calculations and Statistical Analysis

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

$$AID_{AA},\ \% = 100 - [\left(\frac{AA_{digesta}}{AA_{diet}}\right) \times \left(\frac{Cr_{diet}}{Cr_{digesta}}\right)] \times 100$$

where AID_{AA} is the AID of an AA (%), $AA_{digesta}$ is the concentration of that AA in the ileal digesta (g/kg dry matter), AA_{diet} is the AA concentration of that AA in the diet (g/kg dry matter), Cr_{diet} is the chromium concentration in the diet (g/kg dry matter), and $Cr_{digesta}$ is the chromium concentration in the ileal digesta (g/kg dry matter). The AID for CP, starch, and AEE was also calculated using this equation.

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

RESULTS

There was no effect of diet or period on the AID of AEE (Table 3). In period 1, the AID of starch increased and then decreased as IQ was added to the diet with the greatest response observed with the addition of 90 mg/kg of IQ (quadratic, $P < 0.05$). In period 2, starch tended to show a quadratic increase ($P < 0.10$) with the greatest response at 90 mg/kg of IQ as well. In addition, AID of starch was greater ($P < 0.05$) for all diets in period 1 than in period 2. A quadratic increase ($P < 0.05$) was observed in period 1 for the AID of Thr, Trp, Val, Pro, and Tyr, with the greatest values generally observed in the diet containing 90 mg/kg of IQ. There was also a trend (linear and quadratic, $P < 0.10$) for the AID of Ile and Met to increase as IQ was added to the diets. A period effect ($P < 0.05$) was observed for CP and all indispensable AA except Lys, with AID values in period 2 being greater than in period 1. In contrast, the AID of Cys was greater ($P < 0.05$) in period 1 than in period 2. A period effect ($P < 0.05$) for the average AID for all AA was also observed, with values in period 2 being greater than in period 1.

DISCUSSION

Nutritional Characteristics of Ingredients and Diets

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

Table 3. Effects of isoquinoline alkaloids on AID of AEE, crude protein, starch, and AA in diets fed to weanling pigs[a]

	Period 1[b]					*P*-value		Period 2[b]					*P*-value		
Item, %	–	90	180	360	SEM	Linear	Quadratic	–	90	180	360	SEM	Linear	Quadratic	Period
AEE	73.5	72.7	72.5	73.9	2.345	0.824	0.533	69.4	73.1	72.9	74.3	2.35	0.277	0.588	0.638
CP	76.0	78.2	75.9	74.0	1.102	0.074	0.186	77.2	80.2	76.1	79.9	1.10	0.250	0.457	0.003
Starch	92.9	94.7	93.8	91.6	0.593	0.025	0.004	90.8	93.2	91.3	90.9	0.59	0.455	0.076	0.001
Indispensable AA															
Arg	89.1	90.4	89.1	88.5	0.460	0.128	0.165	89.1	91.0	90.1	90.5	0.46	0.119	0.123	0.010
His	80.8	82.6	82.0	81.1	1.008	0.865	0.198	82.9	84.7	80.6	84.2	1.01	0.714	0.159	0.016
Ile	82.5	84.7	82.8	81.4	0.726	0.062	0.063	83.4	85.5	82.3	84.7	0.73	0.564	0.453	0.019
Leu	81.4	83.3	81.9	81.0	0.799	0.383	0.189	82.9	84.9	81.5	84.1	0.80	0.640	0.297	0.004
Lys	80.0	81.0	80.6	79.6	1.030	0.616	0.407	79.3	81.7	80.3	82.4	1.03	0.078	0.858	0.335
Met	85.1	86.6	85.6	83.4	0.771	0.077	0.082	85.7	87.6	85.1	86.3	0.77	0.901	0.996	0.043
Phe	82.2	83.8	82.5	81.3	0.752	0.187	0.180	83.0	85.4	82.0	84.4	0.75	0.558	0.531	0.015
Thr	71.2	75.0	72.4	69.7	1.096	0.050	0.012	73.1	76.3	71.0	75.1	1.10	0.680	0.319	0.008
Trp	80.7	81.2	81.8	78.4	0.844	0.050	0.042	82.1	83.6	81.4	83.6	0.84	0.395	0.462	0.001
Val	76.8	79.5	78.1	76.2	0.919	0.273	0.034	78.4	81.3	77.0	80.1	0.92	0.627	0.436	0.008
Mean	80.8	82.6	81.7	80.0	0.840	0.251	0.087	82.0	84.2	80.7	83.5	0.840	0.530	0.371	0.023
Dispensable AA															
Ala	76.8	79.0	77.7	78.1	1.336	0.674	0.421	78.4	80.6	76.8	79.9	1.010	0.679	0.294	0.135
Asp	73.5	77.3	74.1	73.4	0.956	0.264	0.058	74.4	78.0	72.7	77.3	0.956	0.247	0.268	0.069
Cys	57.0	59.6	58.8	54.7	2.914	0.393	0.267	59.5	64.1	54.4	66.5	2.914	0.227	0.127	0.044
Glu	82.6	83.5	80.4	80.2	1.460	0.106	0.919	81.3	85.2	79.6	85.4	1.460	0.194	0.312	0.129
Gly	67.4	71.1	66.9	67.4	1.888	0.517	0.584	67.8	71.9	65.4	73.9	1.888	0.113	0.188	0.157
Pro	78.8	81.4	79.2	76.1	0.971	0.009	0.027	81.6	83.4	78.3	82.4	0.971	0.925	0.058	0.001
Ser	76.8	79.4	76.9	75.9	1.103	0.193	0.179	77.2	80.5	75.1	79.5	1.103	0.521	0.299	0.210
Tyr	81.4	84.1	82.3	80.6	0.832	0.132	0.034	82.7	84.9	81.9	84.6	0.832	0.332	0.503	0.009
Mean	74.3	76.5	74.4	72.9	1.337	0.195	0.274	75.4	78.6	73.0	78.7	1.337	0.276	0.183	0.038

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

[b]The four diets contained 0, 90, 180, or 360 mg/kg isoquinoline alkaloid premix (Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany).

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

The isoquinoline alkaloid preparation used in this experiment, of which sanguinarine is the main component, is derived from *M. cordata*, an herbaceous perennial plant native to China and Japan and also grown in North America and Europe. Concentrations of isoquinoline alkaloids in the experimental diets were as expected (Table 2).

Ileal Digestibility of Starch

The quadratic increase in the AID of starch in both periods indicated that 90 mg/kg IQ is optimum to maximize starch digestion under the conditions of this experiment. To the best of our knowledge, there are no previous data on the effects of IQ on the AID of starch in pigs; but, IQ supplementation of diets fed to steers resulted in no effect on apparent total tract digestibility of starch (Aguilar-Hernandez et al., 2016). However, starch that was not fermented in the rumen or digested in the small intestine was likely fermented in the large intestine, thus preventing possible differences in the small intestinal digestion of starch from being identified. Because α-amylase activity was not measured in this experiment, it is not known if IQ changed activity of this enzyme.

Ileal Digestibility of AEE

Similar to the observations of this study, when digesta were collected from an excised ileum postmortem, it was observed that IQ supplementation had no effect on the ileal digestibility of ether extract in diets fed to postweaning pigs (Goodarzi Boroojeni et al., 2018). Sanguinarine and chelerythrine, along with other alkaloids, may inhibit *Candida rugosa* lipase (Grippa et al., 1999), with sanguinarine having the strongest effect and chelerythrine being significantly less inhibitory.

However, the lack of an effect of IQ on the AID of AEE indicates that greater concentrations of IQ than used in this experiment are needed to exhibit such an effect in pigs.

Ileal Digestibility of CP and AA

The quadratic increase in the AID of AA indicated that 90 mg/kg is the optimum dosage of IQ under the conditions of this experiment. The observation that the AID of many indispensable AA was greater ($P < 0.05$) in period 2 than in period 1 may indicate that longer exposure to IQ supplementation increases the AID of AA. However, the AID of some AA in the control diet also appeared to slightly increase, which may be a result of pigs increasing the AID of AA as they become older (Pedersen et al., 2016). Thus, the increase in AID of AA from period 1 to period 2 is likely partly due to the normal increase in AID as pigs become older and partly due to an increased effect of IQ. Dietary supplementation with sanguinarine enhanced serum AA levels in growing pigs compared with pigs fed a control diet (Liu et al., 2016a), which may be a result of greater absorption from the small intestine. Similarly, steers fed IQ-supplemented diets had an increase in postruminal and total tract digestibility of N (Aguilar-Hernandez et al., 2016). Preceacal digestibility of Asp, Glu, His, Leu, Met, Val, and total AA was also greater in postweaning pigs fed a diet supplemented with 120 mg/kg IQ when compared to the control diet (Goodarzi Boroojeni et al., 2018). The increased digestibility of AA and N and increased serum AA levels may be due to the anti-inflammatory properties of IQ. Potential mechanisms of action include inhibition of neutrophil phagocytosis and degranulation (Agarwal et al., 1991), inhibition of nuclear factor kappa-light-chain enhancer of activated B cells activation (Chaturvedi et al., 1997), inhibition of tumor necrosis factor-α and nitric oxide production, and suppression of p38 mitogen-activated protein kinases and extracellular signal-regulated kinases/2 phosphorylation in peritoneal macrophages (Niu et al., 2012). Isoquinoline alkaloids also increased expression of tight junction proteins (Robbins et al., 2013; Liu et al., 2016b) and, thus, enhanced intestinal barrier function, which may contribute to the improvement in nutrient absorption that was observed in this experiment. However, the current experiment was not designed to determine effects of IQ on immune responses of pigs and additional research is, therefore, needed to address this hypothesis.

CONCLUSIONS

Results demonstrate that, among the inclusion rates used in this experiment, 90 mg/kg IQ in corn-soybean meal diets fed to young growing pigs is optimum to maximize the AID of starch and AA and prolonged exposure to IQ increased the AID of CP and AA. However, further research needs to be conducted to demonstrate if inclusion of different quantities of IQ will result in the same or better response as 90 mg/kg. Nevertheless, IQ may be used as a phytobiotic alternative to improve nutrient digestibility of diets fed to weanling pigs, but additional research is needed to determine the effects of IQ on growth performance parameters and on possible immunoprotective properties of IQ. The impact of IQ supplementation on the digestibility of starch also needs to be further researched.

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