Increasing Protein/Energy Digestion by Feeding Metal Amino Acid Chelates

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ABSTRACT

Since most gastric enzymes contain specific metals as either co-factors or in prosthetic groups, it was hypothesized that if swine were fed highly bioavailable amino acid chelates (AAC), digestion of their feed would be enhanced. Three trials were conducted. Trial 1 divided 50 grower pigs into 2 groups: the Treatment Group was fed a base diet containing 10.0% crude protein from soybean meal plus Fe, Cu, Zn, and Mn AAC. The Control Group received the same base diet with 18.0% crude protein from soybean meal but no additional minerals. Growth rates and feed conversions were measured in both groups. Trial 2 consisted of providing 2 groups of weaner pigs feed supplemented with 20 g Fe, 10 g Zn, 4 g Mn, and 0.5 g Cu per 1000 kg feed as either AAC or sulfates (IM) for 14 days. In Trial 3, 2 additional groups of similarly fed grower pigs also received the same AAC or IM formula for 14 days. Apparent digestions of gross energy and amino acids were measured Trials 2 and 3. There were no significant differences in weight gains or feed conversions in pigs receiving less protein plus AAC compared to herdmates ingesting more protein and no AAC (Trial 1). There was a 7% to 8% increase in apparent energy digestion by pigs consuming AAC compared to pigs receiving IM ($P < 0.05$) (Trials 2 and 3). Apparent digestion of the essential amino acids, histidine, leucine, lysine, methionine, phenylalanine, and valine, also increased in AAC groups ($P < 0.05$) (Trials 2 and 3). This study suggested that protein and energy could be reduced in feed containing AAC without compromising swine performance. This would lower feed costs and could also potentially reduce environmental pollution resulting from animal waste.

INTRODUCTION

The usual focus of mineral supplementation in swine nutrition relates to their bioavailabilities and subsequent involvements in promoting growth, production, reproduction, and health. Little attention has been given to the roles of minerals in enhancing digestion.

Dietary protein and lipids are partially hydrolyzed by enzymes that have been secreted into the gastrointestinal tract from mucosal tissue and the pancreas.[1-4] Many of these hydrolytic enzymes require Zn, Mn, Mg, Cu, and/or Fe in their prosthetic groups or as co-factors.[4,5]

A few studies have reported that inclusion of certain trace elements, such as Cu or Zn, will enhance digestion of dietary protein.[6-8] If, however, the protein enters the ileum, very little subsequent digestion or absorption occurs. Instead the amino acids from the ingested protein tend to be oxidized resulting in ammonia production.[9-11] Other studies have reported significantly greater oxidation of dietary amino acids in the gastrointestinal tracts of Zn-deprived animals suggesting insufficient Zn-related hydrolytic activity occurred in the upper portion of the gastrointestinal tract.[12-14]

Several studies have demonstrated that when nutritionally essential trace minerals are chelated with amino acids (AAC) (AAFCO # 57.142),[15] the gastrointestinal absorption of those metals is enhanced.[16-23] This greater absorption has been reported to result in increased mineral-related metabolic activity.[18,20,21,23] To date, however, little work has been directed towards the effects of mineral bioavailability on digestion. One group reported that supplementing a blend of Fe, Zn, Mn, and Cu AAC increased the activities of maltase, lactase, saccharase, trehalase, and cellobiose ($P < 0.05$) in rat intestines, but these investigators did not relate the individual enzymatic activities to specific metals.[24]

While this current study was not designed to measure enzymatic activity in the gastrointestinal tract, the above mentioned studies contributed to developing a hypothesis that if pigs' diets were supplemented with more efficiently absorbed AAC instead of inorganic minerals (IM), it may result in greater digestion of the pigs' feed presumable through increased metalloenzyme hydrolytic activity. To test the hypothesis, this study was designed and divided into 3 trials. The first trial examined the effect of AAC on feed conversion with different protein concentrations in the feed ration. Following that initial trial, 2 additional trials examined the apparent digestion of individual dietary components supplemented with AAC or IM in pigs at different ages.

MATERIALS AND METHODS

Trial 1

Fifty Large White castrated male grower pigs of similar age and weight were removed from a larger herd, numbered, and, based on odd/even numbers, randomly divided into 2 groups of 25 animals each. One group was labeled Treatment and the other Control. The 2 groups were placed in similar pens adjacent to each other. All had ad libitum access to water.

The Treatment Group received a base feed containing 10.0% crude protein from soybean meal plus a non-nutritive filler and an AAC[15] mineral supplement containing 20 g Fe, 10 g Zn, 4 g Mn, and 0.5g Cu per 1000 kg feed (Albion, Clearfield, Utah). The feed was labeled Treatment Feed. The presence of the supplemental minerals in the Treatment Feed was confirmed by plasma emission spectrophotometry prior to administration.[25,26] The AAC supplement was fed in addition to the vitamin/mineral concentrate normally included in the base feed. The Control Group received the same base feed labeled Control Feed. It contained 18.0% crude protein from soybean meal and no supplemental minerals. Other than the quantity of soybean meal fed to create the different protein amounts and the inclusion of the AAC mineral supplement and a non-nutritive filler to replace the removed protein in the Treatment Feed, the feed provided to the 2 groups were identical and in all other respects met National Research Council guidelines.[8]

The study commenced when the pigs weighed 74.6 kg ± 1.3 kg and concluded 30 days later. The pigs were weighed at the commencement of the study and again at its conclusion. Fresh feed was supplied once daily. The feeds were provided ad libitum to both groups but each pig received approximately the same amount of total feed, by weight, daily. Mean daily weight gains and feed conversions for both groups were calculated at the conclusion of the study. The calculated data were analyzed for differences using a T-test. A P value of <0.05 was considered significant.

Trial 2

Thirty six male Large White pigs were weaned at Day 30. On Day 31, they were randomly allocated, by liter, into 2 groups of 18 pigs each and placed in 2 pens adjacent to each other. One pen was labeled Treatment Group and the other Control Group. Each group was fed Diet 1 described in Tables 1 and 2 ad libitum for 14 days. All pigs had ad libitum access to water.

In addition to the base feed, the Treatment Group, received an AAC[15] supplement (Albion, Clearfield, Utah) that provided 20g Fe, 10 g Zn, 4 g Mn, and 0.5g Cu per 1000 kg finished feed. The Control Group received the same base feed plus equivalent amounts of the same supplemental minerals but in the form of sulfates (IM).[15] Diet 1 was divided into 2 equal parts. One half was blended with AAC while the other half was blended with IM, and, prior to initiating the trial, Fe, Zn, Mn and Cu presences from either source (AAC or IM) were confirmed by plasma emission spectrophotometry.[25,26] The 2 feeds were labeled Treatment Feed or Control Feed so that the Treatment Group received the AAC supplement and the Control Group received the IM supplement.

Chromic oxide (Cr_2O_3) was used as a non-nutritive marker to allow calculations for apparent nutrient digestibility. It was blended in the feeds concurrently with the mineral supplements. The presence of Cr_2O_3 in the 2 feeds was confirmed by inductively coupled plasma atomic emission spectroscopy (ICP AES) prior to study initiation.[26] Amino acids in the feeds were determined using an auto-analyzer.[27] Gross energy values from the feeds were determined via a bomb calorimeter.[28] Both the amino acids and gross energy values in the feeds were ascertained prior to commencement of the study.

Fresh fortified feed was supplied daily for 14 days at the same time each day. Since apparent nutrient digestibility evaluations were based on comparative concentrations of Cr_2O_3 in the feed and feces, no records were maintained on daily feed intake.

On Day 14 of the study, fecal samples were obtained rectally from each pig and analyzed for gross energy, amino acid content and Cr_2O_3. The Cr_2O_3 assays were performed by ICP AES,[26] the amino acids by an auto-analyzer,[27] and the gross energy by a bomb calorimeter.[28] Each assay was performed on each of the fecal samples.

The values for apparent digestibility of gross energy and individual amino acids were calculated using the relative comparison of Cr_2O_3 contained in the feed and the individual fecal samples collected at the termination of the trial.

Statistical analysis of the data from the 2 groups employed a paired T-test using Systat Version 10 (Systat Software, Inc, Palo Alto, California) to determine significance. A *P* value of <0.05 was considered significant.

Trial 3

Thirty six male Large White pigs ranging between 45 and 50 days of age were removed from a larger herd of grower pigs, numbered at the time of removal, and based on odd/even numbers, randomly allotted into 2 groups of 18 pigs each. The Treatment Group and the Control Group, were housed in 2 identical pens adjacent to each other and provided Diet 2, described in Tables 1 and 2. Both groups of animals received supplemental Fe (20 g/1000 kg), Zn (10 g/1000 kg), Mn (4 g/1000 kg), and Cu (0.5 g/1000 kg) as either AAC[15] (Albion, Clearfield, Utah) or as IM (sulfates).[15] The presences of minerals, from either source, were confirmed in the feeds by ICP AES prior to feeding.[25,26] Chromic oxide was also added to both feeds as a non-nutritive marker and its presence verified by ICP AES.[26] The amino acid content of the feeds and each ration's apparent gross energy were also determine prior to feeding.[27,28]

Each group of pigs received its assigned feed daily ad libitum for 14 days. The Treatment Group received the feed containing the AAC supplement and the Control Group received the feed containing IM supplement. All feed was provided daily between 1700 and 1800 hours. Water was provided ad libitum.

On Day 14, fecal samples were obtained rectally from each pig and each assayed for Cr_2O_3,[26] amino acids,[27] and apparent gross energy.[28] The results of the individual assays

Table 1. Diets 1 and 2 Fed in Trials 2 and 3

Ingredient	Ingredient Composition of Trial Rations, %	
	Diet 1	Diet 2
Ground corn meal	12.75	21.75
Dehulled barley, rolled	20.00	19.00
Dehulled oat groats	18.00	17.00
Soybean meal, 44% ext	15.00	13.00
Fish meal	5.00	4.00
Dried skim milk	9.00	7.00
Corn germ meal	4.00	4.00
Feed rice	9.00	5.00
Wheat bran		3.00
Dry torula yeast	1.00	1.00
Sugar	2.00	2.00
Calcium carbonate	1.00	1.50
Dicalcium phosphate	1.50	
Salt	0.50	0.50
Vitamin/mineral compound*	1.00	1.00
Cr_2O_3	0.25	0.25
Total	**100.00**	**100.00**
*Vitamin/mineral compound(1 kg to supply)		
Vit A, IU	4,000,000	
Vit D, IU	200,000	
Vit B1, mg	250	
Vit B2, mg	1,000	
Vit B6, mg	200	
Vit B12, mg	7	
Vit PP, mg	5	
Vit K, mg	250	
d-Pantothenic acid, mg	3	
Choline chloride, mg	100,000	
DL-methionine, mg	20,000	
Lysine, mg	10,000	
BHT, mg	1,000	
Calcium	100	
Iron, mg	30,000	
Iodine, mg	200	
Manganese, mg	3,000	
Copper, mg	4,000	
Zinc, mg	13,000	

from each group were statistically analyzed using a paired T-test and significant differences determined by Systat Version 10 (Systat Software, Inc., Palo Alto, California). A P value of <0.05 was considered significant.

RESULTS

At the conclusion of Trial 1, the Treatment Group had a mean weight of 101.8 kg/pig compared to a mean weight of 102.6 kg/pig for the Control Group. There was no significant difference in their weights ($P > 0.05$). The mean feed conversion of the Treatment Group was 1:1.79. The Control Group, which did not receive AAC, had a mean feed conversion of 1:1.72. There was no significant difference in feed conversion between the 2 groups ($P > 0.05$).

Relative mean differences for apparent digestibilities of gross energy and individual amino acids between groups in Trials 2 and 3 are shown in Table 3. The apparent digestions of the energy and amino acids of the Control Groups were set at 100% in Table 3. The apparent digestions of energy and amino acids in the Treatment Groups were then compared to the apparent digestions of the Control Group as percentage increases over the Control Groups' apparent digestions.

Results indicated that the amount of energy extracted from the feeds increased approximately 7% to 8% in the 2 trials when diets were supplemented with AAC compared to IM ($P < 0.05$).

Table 2. Analysis of Feed Provided in Trials 2 and 3.

Nutrient	Analyzed Nutrient Content of Trial Rations, %	
	Diet 1	Diet 2
Dry matter	88.25	88.12
Crude protein	21.18	19.32
Ether extract	3.80	3.63
Crude fiber	2.54	2.69
Nitrogen-free extract	54.60	56.43
Ash	6.13	6.05

Apparent digestibilities of the essential individual dietary amino acids were significantly greater ($P < 0.05$) in the pigs fed AAC compared to IM except in the cases of threonine, isoleucine, and arginine (Table 3). The mean increases in apparent digestibilities of all amino acids analyzed were 4.5% in Trial 2 and 3.1% in Trial 3. If one were to consider only the mean increases of those essential amino acids where digestibilities were significantly improved by the addition of AAC to the feed, the improvements were 6.6% in Trial 2 and 4.75% in Trial 3. Regardless of which mean comparison is chosen, the differences in apparent amino acid digestions in pigs consuming AAC were significantly greater ($P < 0.05$) compared to pigs receiving IM.

DISCUSSION

This study hypothesized that the increased absorption of the AAC form of minerals would augment apparent digestion of amino acids and gross energy from pig feed. While these 3 trials appear to substantiate the hypothesis, the exact mechanism of this observed increase in nutrient utilization remains to be precisely elucidated.

One possible explanation that should be examined is that the greater absorption of the AAC form of minerals into the mucosal tissues may have resulted in an increased supply of essential trace elements that were subsequently used as co-factors or as prosthetic groups in the hydrolytic enzymes that were secreted into the gastrointestinal tract following their production in the mucosal tissues. An increased quantity of digestive enzymes in the gastrointestinal tract would presumably result in greater digestion of feedstuffs.

If, as reported by Kirchessner et al,[29] pigs under normal feeding conditions only utilize 30% to 35% of the protein in their feed, and if AAC supplementation resulted in increased enzymatic activity in the gastrointestinal tract, this would probably be manifest as increased protein digestion. The data in these trials tend to support this supposition although the improved protein digestion has not been linked to a specific metal in this study. Even though less protein was provided in the diet of the Treatment Group in Trial 1, it appeared the pigs receiving supplemental AAC were able to digest more of the protein they consumed. That greater digestion was manifest as a feed conversion ratio that was equivalent to pigs fed more dietary protein without AAC. It appeared that the Control Group, while ingesting more protein, had lower digestion efficacy compared to the Treatment Group.

When Trials 2 and 3 were examined, they appeared to support the conclusions generated in Trial 1. Amino acid digestion was significantly increased in pigs receiving AAC compared to IM in Trials 2 and 3. In Trials 2 and 3, equivalent dietary amounts of both sources of minerals were equally available to the pigs to activate enzymes already created and secreted into the gastrointestinal tracts. The fact that the Treatment Groups had significantly greater amino acid digestions compared to the Control Groups seems to point to the greater absorption of the AAC into the mucosal tissues for production/activation of gastric enzymes. If the hydrolytic enzymes were already produced and secreted into the gastrointestinal tracts and simply activated by the introduction of the minerals into the gastrointestinal tracts through feed ingestion, then apparent digestions of the amino acids would have been the same for both groups. Because there was a significant difference in apparent digestions of amino acids and energy between groups, the differences had to come from the greater absorption of the AAC and the subsequent involvements of those minerals in production and/or activation of digestive enzymes prior to their introduction into the gastrointestinal tracts.

An alternative explanation for the observation in these trials has been suggested by the work of Jen et al.[30] They reported that apparent digestion of protein is related to the percent of protein in the diet. When the intake of dietary protein is increased, apparent digestion of that dietary protein also increases, but only up to a certain point. Once a plateau is reached, additional intake of dietary protein no longer increases its apparent digestion. The data of Jen et al[30] could be interpreted to apply to Trial 1. In this first trial, feed conversions in both groups were the same even though the Control Group ingested approximately twice as much protein. When Trials 2 and 3 are factored into the study, the conclusions of Jen et al[30] no longer seem to apply. In Trials 2 and 3, both groups were fed the same amounts of protein in those isonutrient trials, but the Treatment Groups digested more of the ingested protein.

By definition, an AAC is "the product resulting from the reaction of a metal ion from a soluble metal salt with amino acids."[15] Based on that definition, one might further argue that if a protein digestion plateau described by Jen et al[30] had not been attained in these trials, the addition of more amino acids from the chelates would have stimulated increased apparent digestion. This seems very unlikely. The AAC contributed only 0.66 g of amino acids per 1000 kg feed, or 0.007%. This is an insignificant contribution.

While the data in Table 3 demonstrated greater apparent digestion of energy from the feeds with AAC provided to the pigs, the source of that energy was not identified. Energy can be derived from protein, carbohydrates and/or lipids. All 3 energy sources were found in the pigs' rations and all 3 became available after the ingested feeds were digested with the aid of hydrolytic metalloenzymes. A future study should be undertaken to ascertain which energy source is most affected by feeding AAC. The data derived from that study would be most helpful in reformulating a feed ration.

Most strains of feeder pigs have previously been evaluated and their specific nutrient requirements for maximum weight gains determined. Trials 2 and 3 were not designed to measure weight gains or feed efficiency. They were designed to look at the increased digestibility of specific amino acids in the feed as a way to explaining the observations

Table 3. Relative Percent Increase in Apparent Nutrient Utilization from Pigs Fed Metal Amino Acid Mineral Sources Compared to Pigs Fed Metal Sulfate in Trials 2 and 3

	Trial 2		Trial 3	
	Control Group	Treatment Group	Control Group	Treatment Group
Gross energy	100.0	108.4 *	100.0	107.1*
Essential amino acids				
Arginine	100.0	100.3	100.0	99.6
Histidine	100.0	104.5*	100.0	103.3*
Isoleucine	100.0	100.2	100.0	100.0
Leucine	100.0	108.9*	100.0	104.7*
Lysine	100.0	108.8*	100.0	106.2*
Methionine	100.0	107.7*	100.0	106.1*
Phenylalanine	100.0	105.3*	100.0	104.2*
Threonine	100.0	100.6	100.0	100.2
Valine	100.0	104.5*	100.0	104.0*

*Significant percent increase of treatment group over control group within the trial ($P < 0.05$).

noted in Trial 1. As a result of what was learned in Trials 2 and 3 coupled with Trial 1, one could anticipate that if the pigs were fed for optimum performance, improvement from changes in dietary nutrients would not be expected to result in increased weight gains. The improvements would occur from increased efficiency of nutrient utilization, that is, less feed/unit of gain. Some attractive possibilities for feed ration reformulation should be explored.

One reformulation possibility worthy of further examination is since AAC bioavailability is greater, mineral intake from the AAC source could potentially be reduced while still maintaining a level of performance equivalent to "normal" rates obtained when using IM supplements. Lowering mineral intake with AAC may not enhance protein digestion as efficiently, but the lower concentration of minerals in the feces may have a positive environmental impact.[31] Balancing feed efficiency against environmental concerns needs to be explored.

Measurable benefits may also result from a decrease in dietary requirements for amino acids and energy due to increased digestibility of those amino acids and energy present in feed containing greater amounts of AAC. With lower protein intake from feed, the resulting nitrogenous waste excreted into the environment should be less because more protein from the feed would be utilized by the pig supplemented with AAC rather then being excreted in the manure. It is estimated that the average pig fed IM only utilizes 30% to 35% of the nitrogen in its feed. The rest is excreted into the environment.[29] If protein levels in the feed could be reduced, due to improved digestibility, without compromising swine performance, the impact of the reduction could have a 2-fold effect. First, there would be a measurable decrease in total ration costs due to feeding less protein/energy. Second, the reduced feed intake would automatically reduce the amount of manure requiring disposal.

Manure containing high nitrogen is creating ground water pollution, which is rapidly becoming a major problem in swine production.[29,32] Based on the increased efficiency observed in these trials, it would be expected that both amino acid and energy content of swine feed could potentially be reduced by at least 8% with no decrease in animal production if AAC were included in the swine diets. This potential protein (Trials 1, 2, and 3) and energy reduction (Trials 2 and 3) coupled with comparable weight gains previously obtained without AAC but with more feed (Trial 1) would be manifest as an increase in feed efficiency in pigs. Increase feed efficiency coupled with the lower feed requirement for energy and amino acids would result in an increased profitability of pigs. More work is required to refine these suggestions.

It was noted in this study that digestion efficiency in younger pigs receiving AAC in their diets was greater than in older pigs. The exact reason remains to be elucidated. Perhaps as the animals mature, their digestive systems became more efficient.

CONCLUSION

This study with weaner and grower pigs demonstrated that the inclusion of 20 g Fe, 10 g Zn, 4 g Mn, and 0.5 g Cu, as amino acid chelates, in 1000 kg of swine feed resulted in a 7% to 8% increase in apparent gross energy digestion ($P < 0.05$). Concurrently, the feeding of these metal amino acid chelates also significantly increased the apparent digestion of the essential amino acids, histidine, leucine, lysine, methionine, phenylalanine, and valine ($P < 0.05$).

With the increases in apparent digestions of energy and essential amino acids, it may be possible to reduce protein and energy intakes in commercial swine feeds without compromising swine performance. Initial trials demonstrated equivalent feed conversion ratios in pigs fed less protein compared to pigs receiving more protein. If a lower protein ration plus amino acid chelates were adopted, the feeding of these rations could potentially have a positive impact on the environment by reducing the excretion of nitrogen compounds in swine feces. It may also have an economic impact for the swine producer by reducing feed costs.

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