Relative Availability of Iron in Mined Humic Substances for Weanling Pigs*

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ABSTRACT : Humic substances include several biological active and inactive compounds that are commonly used for improving soil fertility. Use of humic substances in swine diets is a novel concept. Humic substances contain 8,700 mg/kg of iron but its bioavailability is unknown. This study was conducted to test the bioavailability of iron in humic substances for nursery pigs. One hundred twenty five pigs (Newsham, Colorado Springs, CO) were not given supplemental iron while nursing for 21 d. Pigs were weaned on d 21 and allotted to one of five treatments (four control treatments with different levels of supplemented iron; 0, 30, 70 and 88 mg/kg from $FeSO_4$ and one treatment with 70 mg/kg iron from humic substances). Pigs were fed diets for 5 wk *ad libitum* and water was accessible freely. Body weight and feed intake were measured weekly. Blood samples were taken from pigs on d 28 to measure the number of red blood cells and hemoglobin concentration. Pigs fed a diet with the humic substances grew faster ($p<0.05$) during the first week postweaning, but performance was not different during the entire 5 wk period. Feed intake and gain/feed were the same among treatments. The slope ratio technique was used to estimate relative iron bioavailability. The concentration of blood hemoglobin did not respond to dietary iron levels using this model. However, the number of red blood cells (106/μl) was modeled by 4.438+0.017× 'iron (mg/kg) from $FeSO_4$'+0.012×'iron (mg/kg) from the humic substances'. Based on the comparison between the slopes (0.012 from humic substances and 0.017 from $FeSO_4$), iron in humic substances was 71% as available as the iron in $FeSO_4$. The slopes for dietary feed intake of $FeSO_4$ and the iron in humic substances did not differ ($p>0.05$). Humic substances can replace $FeSO_4$ as an alternative iron source for pigs at 71% relative bioavailability. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 9 : 1266-1270)***

Key Words : Bioavailability, Humic Substances, Iron, Nursery, Pigs

INTRODUCTION

Humic substances are defined as 'a series of relatively high-molecular-weight, yellow to black colored substances formed by secondary synthesis reactions' (Stevenson, 1994). Humic substances can include most of the organic matter in most soils (Goh and Reid, 1975) but specifically include humic acids, fulvic acids, and humin as major constituents as well as several minerals such as iron, manganese, copper, and zinc (Aiken et al., 1985). Among the minerals in humic substances, iron is most abundant.

Use of humic substances in pig diets is a rather novel approach. Previously humic substances have been applied to reduce ammonia emission from manures of livestock either by dietary supplementation or application to manure (Ndayegamiye and Cote, 1989; Shi et al., 2001). However, dietary supplementation with humic substances in pig diets has not been reported. Organic acids and minerals in humic substances may benefit animal performance even though the actual mechanism is not yet understood. This study was conducted as the first effort to characterize humic substances as a feed supplement for use in pig diets. The objective of this study was to measure the bioavailability of iron in humic substances relative to iron sulfate.

MATERIALS AND METHODS

Humic substances

Humic substances were obtained from Humatech Inc. (Mesa, Arizona) and the registered commercial product name was Promax®. Humic substances are naturally-occurring, mined products containing trace minerals (including iron, manganese, zinc and copper), organic acids (including fulvic and humic acids) and other organic compounds (including humin). Promax® contained 8,700 mg/kg iron as assayed by atomic absorption spectrophotometry.

Design, animal and diet

One hundred twenty five pigs were used to determine the bioavailability of iron in humic substances. None of the pigs were given supplemental iron while nursing for 21 d. Pigs were weaned at 21 d of age and allotted to one of five dietary treatments including four standards (S1, S2, S3 and S4) and a humic substances treatment (HS). Each treatment had five replicates and five pigs were in each pen replicate.

The basal diet contained corn and fat as major energy

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Table 1. Composition of the basal diet

Ingredient	%
Ground corn, yellow	59.86
Soybean meal, dehulled	2.00
Dried skim milk [a]	25.00
Plasma protein[b]	7.00
Vegetable oil	1.00
Monosodium phosphate	0.70
Calcium propionate	2.50
Iodized salt	0.20
Choline	0.50
Vitamin premix[c]	0.22
Trace mineral premix[d]	0.22
Fe supplement[e]	0.80
Total	100.00
Calculated composition:	
ME, Mcal/kg	3.26
CP, %	26.00
Lysine, %	1.50
Fe (total), mg/kg	27.00

[a] International Ingredient Corp. St. Louis. Missouri.

[b] APC-920 (American Protein Corp, Ames. IA).

[c] The vitamin premix provided the following per kilogram of complete diet: 4,433 IU vitamin A as vitamin A acetate, 484 IU vitamin D_3, 36.3 IU vitamin E, 1.6 IU vitamin K as menadione sodium bisulfite, 32.2 μg vitamin B_{12}, 8.1 mg riboflavin, 25.8 mg D-pantothenic acid as calcium pantothenate, 32.2 mg niacin and 972 mg choline as choline chloride.

[d] The trace-mineral salt provided the following per kilogram of complete diet: 110 mg manganese as manganous oxide, 55.5 mg zinc as zinc sulfate, 11.2 mg copper as copper sulfate, 608 mg magnesium as magnesium oxide, 144 mg calcium as calcium propionate and 0.24 mg selenium as sodium selenite.

[e] Basal diets contained 0.8% of iron supplement and the compositions of iron supplements for each treatment are shown in Table 2.

sources and dried skim milk, plasma protein, and soybean meal as major protein sources (Table 1). The basal diet contained 27 mg/kg total iron or 15 mg/kg bioavailable iron based on NRC (1998) values. Graded levels of iron sulfate were supplemented to the basal diet resulting in four diets at rates of 0, 30, 70 and 88 mg/kg of additional iron, respectively. Humic substances were supplemented at 0.8% to provide 70 mg/kg of additional total iron to the basal diet. The HS diet was designed to provide approximately 90% of the iron requirement of nursery pigs (NRC, 1998).

Pigs had free access to their experimental diets and water during the 35 d experimental period. Pig weights and feed intakes were measured weekly. Three pigs from each pen were selected randomly at 28 d and blood samples were obtained over sodium heparin from the vena cava to measure the number of red blood cells and hemoglobin concentrations. This study was approved by Texas Tech University Animal Care and Use Committee (# 01124).

Chemical analysis

Iron content of the diets was measured by atomic absorption spectrophotometry as described by Lee and Clydesdale (1979) and Acda et al. (2002). The number of red blood cells, hemoglobin content, the number of white blood cells, and packed cell volume were measured as described below.

The Unopette® microcollection system (Becton Dickinson & Company, Franklin lakes, NJ) containing 1.98 ml of 3% acetic acid was used for total Leukocyte counts. A 20 μl of whole blood was withdrawn using a capillary pipette (dilution ratio 1:100), and was inserted into the Unopette® and diluted. Unopettes were left for 10 min and then 20 μl drawn into the capillary tube and inserted into two wells on a Bright-line hemacytometer (Hauser Scientific Horsham, PA). Wells were divided into a grid of nine, 1 mm^2. The total number of cells in nine squares was counted using a light microscope (10×). The two counts were averaged if they were within 5% of each other to determine the total leukocyte, $10^3/\mu l$ (Howard and Matsumoto, 1977).

The Unopette® microcollection system (Becton Dickinson & Company) containing 1.99 ml of diluents, a mixture of sodium azide and sodium chloride in HPLC grade water, was used for erythrocyte determination. A 10 μl of whole blood was drawn using a capillary pipette (dilution ratio 1:200) and was inserted into the Unopette® and diluted. Samples were allowed to stand for 10 min and then 10 μl of the sample was drawn into the capillary tube and inserted into two wells on a Bright-line hemacytometer (Hauser Scientific Horsham, PA). Using a light microscope (430×), the erythrocytes were counted using the middle 1mm grid square which was dived into 5×5 squares. The middle and four counter sub squares were counted. An average of the two wells (within 5%) were taken and multiplied by 10,000/mm^3 (Howard and Matsumoto, 1977).

A 20 μl of whole blood was drawn into a 40 mm Statspin microhematocrit tube (Statspin Technologies, Norwood MA) to determine pack cell volume (PCV). The capillary tube was sealed and all tubes were placed into a 12 position Hematocrit rotor CritSpin® Digital Reader (S120, Norwood, MA) to determine PCV.

Hemoglobin contents were determined using the Drabkin's method (Balasubramaniam and Malathi, 1992). Drabkin's reagent (Sigma-Aldrich, St. Louis, MO) was mixed with 1,000 ml of HPLC grade water and 0.5ml of 30% BRJi-35 Solution (Sigma-Aldrich). A 50.0 ml of this solution was mixed with one vial of Hemoglobin Standard Preparation (Sigma-Aldrich) making 18 g hemoglobin per dL of whole blood. Using a Microtest™ u-bottom tissue culture plate (Becton Dickinson & Company, Franklin Lakes, NJ), a standard curve was obtained and 20 μl of each blood sample was plated with the Drabkin's reagent. The plate was analyzed using the BIO-RAD Model 2550 EIA reader (Hercules, CA).

Table 2. Composition of iron supplement

	Standard				HS[a]
	S1	S2	S3	S4	
Corn starch	100.0	98.1	95.6	94.5	0.00
$FeSO_4$	0.00	1.88	4.35	5.50	0.00
HS[a]	0.00	0.00	0.00	0.00	100.00
Calculated composition					
Fe, mg/kg	0.00	30.0	70.0	88.0	70.0
Analyzed composition					
Fe, mg/kg	0.00	12.0	54.0	69.0	88.0

[a] Humic substances (Promax®, Humatech, Mesa, AZ).

Statistical analysis

Data were analyzed as a completely randomized design. The pen was the experimental unit. The statistical analysis was performed with the General Linear Models procedure (PROC GLM) in SAS/STAT® software (SAS Inst. Inc., Cary, NC). Least-squares means, probability of differences, and standard errors were used to evaluate the differences among the treatment groups. Data from one pen of HS group was excluded because of known contamination in the feeder. Thus, observations were five for all the treatments except for the HS. The General Linear Models procedure (PROC GLM) was also programmed to perform the statistical analysis used for relative bioavailability studies as described by Littell et al. (1997) and Kim and Easter (2001). Regression equations were obtained between the number of red blood cell and additional iron intake from different iron sources. Slopes from regressions were compared to obtain the relative bioavailability of HS compared with iron sulfate.

RESULTS AND DISCUSSION

Based on chemical analyses, the iron contents of the diets were 0, 12, 54, 69, and 88 mg/kg for S1, S2, S3, S4 and HS, respectively (Table 2).

Initial weights of pigs were the same among the treatments (Table 3). Final weights of pigs were the statistically similar among the treatment. Pigs fed HS had numerically 9.0% higher weight gain compared with pigs fed the S1 diet. Average daily gain (ADG) was different among the treatments during the wk 1. The HS group had higher ($p<0.05$) ADG than pigs fed S1 and S4 whereas ADG was similar for pigs fed S2 and S3 diets. Average daily gain of the pigs during the wk 2 to 5 did not differ ($p>0.05$) among the treatments. Overall, ADG of the pigs were the same ($p>0.05$) among treatments even though pigs in the HS group had numerically 16% greater ADG than pigs in the S1 group.

Average daily feed intake of the HS group was higher ($p<0.05$) than those of the S1 and S4 groups during the wk1

Table 3. Growth performance of nursery pigs (3 to 8 wk of age)[a]

	Added dietary iron, mg/kg[b]					SEM
	$FeSO_4$				HS	
	0	12	54	69	88	
Body wt, kg						
Initial	6.23	6.42	6.31	6.22	6.30	0.198
Final	12.80	13.59	13.72	13.52	13.95	0.409
Average daily gain, g/d						
Wk 1	-3[c]	29[cd]	23[cd]	-25[c]	57[d]	9.5
Wk 2	108	145	121	152	127	9.2
Wk 3	192	192	228	249	222	11.6
Wk 4	260	288	276	270	293	14.3
Wk 5	383	371	411	395	394	12.3
All	188	205	212	208	219	7.3
Average daily feed intake, g/d						
Wk 1	86[c]	128[cd]	112[cd]	77[c]	175[d]	13.2
Wk 2	182[c]	280[d]	217[cd]	221[cd]	199[cd]	15.5
Wk 3	355	365	394	427	419	14.1
Wk 4	470[c]	564[d]	482[cd]	537[cd]	523[cd]	19.3
Wk 5	661	712	716	705	737	21.7
All	351	410	384	393	411	12.5
Gain/feed						
Wk 1	-0.12	0.22	-0.15	-0.55	0.36	0.148
Wk 2	0.59	0.55	0.55	0.71	0.64	0.036
Wk 3	0.54	0.55	0.57	0.58	0.53	0.023
Wk 4	0.53	0.51	0.58	0.50	0.56	0.019
Wk 5	0.58	0.52	0.57	0.57	0.54	0.014
All	0.53	0.50	0.55	0.53	0.53	0.008

[a] Sample size equals 5 pens per treatment except for HS (humic substances), Promax® treatment which had 4 pens.

[b] Iron levels are analyzed values. Calculated values were 0, 30, 70, 88 and 70 mg/kg, respectively.

[cd] Means with a different superscript differ ($p<0.05$).

Table 4. Measures of hematology of nursery pigs at 4 wk postweaning[a]

	Added dietary iron, mg/kg[b]					
	$FeSO_4$				HS	SEM
	0	12	54	69	88	
Hemoglobin, g/dL	6.22	6.49	6.98	6.46	6.49	0.44
RBC, 10^6 cell/μL[c]	4.42[d]	4.76[de]	4.89[de]	5.37[e]	5.26[e]	0.22
PCV, %	31.2	32.1	31.4	30.4	29.7	1.16
WBC, 10^3 cell/μL	13.1	10.4	14.9	12.6	11.4	1.51

[a] Sample size equals 5 pens per treatment except for Promax® treatment which had 4 pens.
[b] Iron levels are analyzed values. Calculated values were 0, 30, 70, 88 and 70 mg/kg, respectively.
[c] Linear effect of dietary iron ($p<0.01$).
[cd] Means with a different superscript differ ($p<0.05$).

(Table 3). The S2 group had higher ($p<0.05$) ADFI than S1 during the wk 2 and wk 4. There were no differences ($p>0.05$) in ADFI among the treatments during the wk 3 and wk 5. Overall, ADFI was the same ($p>0.05$) among pigs in each the treatment, even though the HS group had numerically 17% greater ADFI than that of the S1 group. Gain:feed ratio was the same ($p>0.05$) among the treatments during the each week as well as during the entire experimental period.

Hemoglobin concentration was the same ($p>0.05$) among the treatments. Blood hemoglobin concentrations did not respond to the dietary iron supplementation levels. The numbers of red blood cells (RBC) per μl of blood from the S4 and HS groups were higher ($p<0.05$) than that of the S1 group (Table 4). The number of RBC increased ($p<0.01$) linearly as dietary iron supplementation increased. Pack cell volume and the number of white blood cells were the same ($p<0.05$) among the treatments.

Pigs were fed the diets with varying levels of dietary iron supplemented for 5 weeks but hemoglobin concentrations did not respond to dietary iron content. Instead, the number of RBC responded in a sensitive manner to dietary iron content. Determination of the status of functional iron in an animal can be carried out by measuring the number of red blood cells, hemoglobin levels, and the size and shape of red blood cells (Cavill, 2002; Parvanta et al., 2003). In this study, the best indicator was the number of RBC but the blood hemoglobin content did not respond to dietary iron levels. The number of RBC and hemoglobin contents was positively correlated (Cavill, 2002; Parvanta et al., 2003), but it is not known why this correlation was weak in this study.

Bioavailability of iron in humic substances relative to iron sulfate was calculated by measuring the change of the number of RBC as iron intake changed (Figure 1). The numbers of RBC from the pigs received varying amounts of

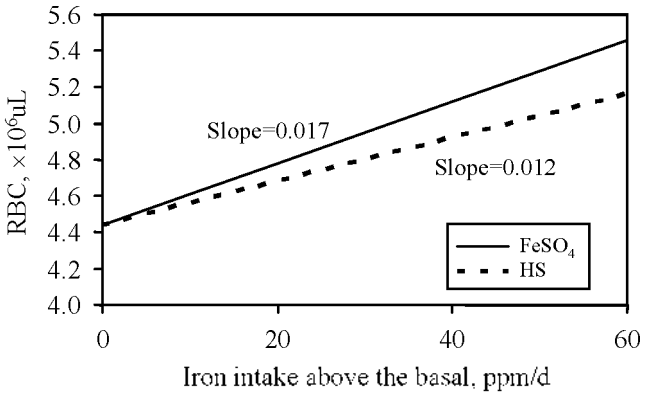

Figure 1. Standard curve for weanling pigs fed iron sulfate based on individual pen feed intake. The slope of the regression equation for pens fed humic substances (HS) indicate that the iron in HS was 71% as available as the iron in iron sulfate ($FeSO_4$), based on the relative slopes of the following equation (RBC=4.438+0.017× Fe from $FeSO_4$ and RBC=4.438+0.012×Fe from HS) and relative bioavailability of iron in HS (%) was obtained by (slope of $FeSO_4$/slope of HS)×100.

supplemental iron were plotted and the regressions were obtained. The changes in RBC number from the pigs received iron sulfate as a source of additional iron were modeled as 4.4386+0.017×'iron from $FeSO_4$' (R^2=0.87, $p<0.05$). The changes of RBC number from the pigs received HS as a source of additional iron was modeled as 4.438+0.012×'iron from HS' (R^2=0.87, $p<0.05$). The slopes from these regressions were 0.017 and 0.012 for iron sulfate and HS, respectively. The slope of HS did not differ statistically from the slope of $FeSO_4$ ($p>0.05$). From the slopes, the relative bioavailability (0.012/0.017) of iron in HS was 71% as bioavailable as the iron in iron sulfate. Relative bioavailabilities of other iron sources were compared to iron sulfate including iron methionine (68.3%; Lewis et al., 1996), iron in spray dried blood cells (24.0%; Anderson and Easter, 1999), and iron in defluorinated phosphate (58.5%; Kornegay, 1972). Harmon et al. (1969) showed that ferrous carbonate is was an effective dietary iron supplement. In this study, humic substances with 8,700 mg/kg iron and 71% relative bioavailability can be used as a source of iron for pig diets.

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