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 FeSO₄ 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 FeSO₄’+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 FeSO₄), iron in humic substances was 71% as available as the iron in FeSO₄. The slopes for dietary feed intake of FeSO₄ and the iron in humic substances did not differ (p>0.05). Humic substances can replace FeSO₄ 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 sources.
Table 1. Composition of the basal diet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground corn, yellow</td>
<td>59.86</td>
</tr>
<tr>
<td>Soybean meal, dehulled</td>
<td>2.00</td>
</tr>
<tr>
<td>Dried skim milk a</td>
<td>25.00</td>
</tr>
<tr>
<td>Plasma proteinb</td>
<td>7.00</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>1.00</td>
</tr>
<tr>
<td>Monosodium phosphate</td>
<td>0.70</td>
</tr>
<tr>
<td>Calcium propionate</td>
<td>2.50</td>
</tr>
<tr>
<td>Iodized salt</td>
<td>0.20</td>
</tr>
<tr>
<td>Choline</td>
<td>0.50</td>
</tr>
<tr>
<td>Vitamin premixa</td>
<td>0.22</td>
</tr>
<tr>
<td>Trace mineral premixd</td>
<td>0.22</td>
</tr>
<tr>
<td>Fe supplementf</td>
<td>0.80</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Calculated composition:

- ME, Mcal/kg: 3.26
- CP, %: 26.00
- Lysine, %: 1.50
- Fe (total), mg/kg: 27.00

The trace-mineral salt provided the following per kilogram of complete diet: 4,433 IU vitamin A as vitamin A acetate, 484 IU vitamin D₃, 36.3 IU vitamin E, 1.6 IU vitamin K as menadione sodium bisulfite, 32.2 µg vitamin B₁₂, 8.1 mg riboflavin, 25.8 mg D-pantothenic acid as calcium pantothenate, 32.2 mg niacin and 972 mg choline as choline chloride.

The trace-mineral salt was 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).

Fe PROAVAILABILITY IN HUMIC SUBSTANCES

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 1.99 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 mm². 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, 10⁷/µl (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 1 mm² grid square which was divided 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/mm² (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 0.5 ml of 30% BRJ-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 MicrotestTM 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).
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 wk 1.
Table 4. Measures of hematology of nursery pigs at 4 wk postweaning

<table>
<thead>
<tr>
<th>Added dietary iron, mg/kg</th>
<th>FeSO₄</th>
<th>HS</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/dL</td>
<td>6.22</td>
<td>6.49</td>
<td>0.44</td>
</tr>
<tr>
<td>RBC, 10⁶ cell/µL</td>
<td>4.42d</td>
<td>4.76e</td>
<td>4.89e</td>
</tr>
<tr>
<td>PCV, %</td>
<td>31.2</td>
<td>32.1</td>
<td>1.16</td>
</tr>
<tr>
<td>WBC, 10⁶ cell/µL</td>
<td>13.1</td>
<td>10.4</td>
<td>1.51</td>
</tr>
</tbody>
</table>

*Sample size equals 5 pens per treatment except for Promax® treatment which had 4 pens.

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

1 Linear effect of dietary iron (p<0.01).

2 Means with a different superscript differ (p<0.05).

Chae. 2002. Influence of single dose of Fe dextran 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 (FeSO₄), based on the relative slopes of the following equation (RBC=4.438+0.017×Fe from FeSO₄ and RBC=4.438+0.012×Fe from HS) and relative bioavailability of iron in HS (%) was obtained by (slope of FeSO₄/slope of HS)×100.

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 (FeSO₄), based on the relative slopes of the following equation (RBC=4.438+0.017×Fe from FeSO₄ and RBC=4.438+0.012×Fe from HS) and relative bioavailability of iron in HS (%) was obtained by (slope of FeSO₄/slope of HS)×100.

REFERENCES
