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Effects of Antibiotics, Zinc Oxide or a Rare Earth Mineral-Yeast Product on Performance, Nutrient Digestibility and Serum Parameters in Weanling Pigs*

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ABSTRACT : Two experiments were conducted to compare the effects of feeding a newly-developed rare earth mineral-yeast product, zinc oxide (ZnO) or antibiotics on the performance, nutrient digestibility and serum parameters of weanling pigs. In experiment 1, 150 crossbred barrows (24 d old and 6.28 kg BW) were fed one of five dietary treatments consisting of an unsupplemented basal diet or the basal diet supplemented with antibiotics (33 ppm tiamulin and 100 ppm chlortetracycline), ZnO (1,500 or 2,500 ppm) or 0.1% peptide-bound rare earth mineral-yeast. In experiment 2, 576 crossbred barrows (28 d old and 7.20 kg BW) were fed the same diets as those used in experiment 1 modified only by the addition of 1.0% Celite 545 to all diets as a digestibility marker. However, the negative control was not included. In experiment 1, weight gain was significantly lower ($p<0.05$) for pigs fed the negative control than for pigs fed diets supplemented with antibiotics, ZnO, or rare earth mineral-yeast. Pig performance did not differ between pigs fed the four supplemented diets. In experiment 2, there were no differences in performance between pigs fed diets supplemented with antibiotic, ZnO or rare earth mineral-yeast. The digestibility of dry matter, crude protein, calcium, phosphorus and energy were significantly ($p<0.01$) higher on the rare earth mineral-yeast diet than on diets supplemented with ZnO. In addition, pigs fed the diet supplemented with rare earth mineral-yeast had significantly ($p<0.05$) higher digestibility of histidine, lysine, threonine and valine than pigs fed the ZnO supplemented diets. Digestibility coefficients for pigs fed antibiotics tended to be intermediate to those of pigs fed rare earth mineral-yeast or ZnO. In conclusion, the performance of pigs fed rare earth mineral-yeast was basically equal to that of pigs fed antibiotics or ZnO indicating that rare earth mineral-yeast can be successfully used as a growth promoter in diets fed to nursery pigs. The effects of rare earth mineral-yeast appeared to be mediated through improvements in nutrient digestibility. (**Key Words :** Piglets, Zinc, Rare Earth Mineral-yeast, Performance, Digestibility, Serum Parameters)

INTRODUCTION

Antibiotics have been used in livestock production for over 50 years (Kim et al., 2005). When used at subtherapeutic levels in swine feeds, antibiotics improve growth rate and feed efficiency, reduce mortality and morbidity and improve reproductive performance (Cromwell, 2002). Antibiotics are also widely used at intermediate levels to prevent disease and at therapeutic levels to treat disease in various species of livestock (Cromwell, 2002). However, due to concerns about residues in animal foods and the potential for the development of microbial resistance to antibiotics, the possibility exists for the implementation of a complete ban in the use of antibiotics in animal feed (Adjiri-Awere and Van Lunen, 2005). As a consequence, the development of alternatives to antibiotics is receiving considerable attention (Turner et al., 2001).

Pharmacological levels of zinc in the form of zinc oxide (ZnO) are commonly added to nursery pig diets because they improve pig performance (Poulsen, 1995; Smith et al., 1997; Hill et al., 2000) and reduce the incidence of diarrhea after weaning (Kavanagh, 1992). However, a concern with feeding pharmacological levels of zinc to pigs is that the application of manure containing high levels of zinc to soil can negatively impact the environment (Berenguer et al.,

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2008). Therefore, it would be desirable if the beneficial effects of feeding ZnO could be obtained at lower levels of supplementation (Han and Thacker, 2009) or if alternative growth promoters could be developed.

Rare earth elements in the diet can significantly improve animal performance (Redling, 2006; Schoene, 2009). The term rare earth element encompasses the elements scandium, yttrium, lanthanum and the 14 chemical elements following lanthanum called lanthanides (Foerster et al., 2008). Since organic mineral sources have been reported to have higher bioavailability than inorganic mineral sources (Hahn and Baker, 1993; Case and Carlson, 2002), a rare earth mineral-yeast product has recently been developed in Korea as a feed additive for use with swine. However, its potential to act as an alternative to antibiotics in nursery pig diets has not been extensively studied. Therefore, the aim of the present study was to compare the effects of feeding rare earth mineral-yeast with ZnO and antibiotic addition on the performance, nutrient digestibility and serum parameters of weanling pigs.

MATERIALS AND METHODS

Production of rare earth mineral-yeast product

A mixture of rare earth minerals was obtained from the Rare Earth Mineral Mine operated by the Baotu Metal Company (Hohhot, Inner Mongolia). The mixture contained 35.3% lanthanum oxide, 25.2% cerium oxide, 10.2% praseodymium oxide and 29.3% of several other contaminating minerals which were present in trace amounts. In order to produce the rare earth mineral-yeast product used in the present experiment, the rare earth material was first mixed with water in a ratio of one part rare earth minerals to nine parts water. This solution was then mixed with 99% pure acetic acid in a ratio of one part rare earth solution to two parts acetic acid and then heated at 90°C until the solution became clear.

Saccharomyces cerevisiae yeast (Danbaoli Yeast Company, Guangdong Province, China) was cultured in Yeast and Mold medium (Difco Laboratories, Franklin Lakes, NJ) for 48 h at 24°C with constant addition of oxygen. The medium was centrifuged at 15,000 rpm for 15 min and the yeast was subsequently collected. The yeast was mixed with purified water in a ratio of one part yeast and 10 parts water and left to stand for 24 h. The concentration of yeast in this mixture was 1×10^{10} CFU. The yeast was then cultured with 1% protease enzyme (Collupulin MR, DSM Nutritional Products, Heeerlen, The Netherlands) at an initial pH of 4.0 for 48 h to make the yeast-peptide solution. Following this, the solution was neutralized by the addition of 3 ml of 40% NaOH to 100 ml of yeast-peptide solution. Visual observation under a microscope confirmed that lysis of the yeast cell wall had occurred.

The rare earth solution described above was mixed with the yeast peptide solution in a ratio of one part rare earth solution to 5 parts yeast-peptide solution. Binding of the rare earth mineral to the yeast-peptide was confirmed by electrophoresis. The solution was then dried and the dried product was ground through a 1 mm screen (Cyclotec Grinder Model 1093, Foss Group, Hillerod, Denmark). The rare earth-yeast peptide mixture was then mixed with rice bran as a carrier. The final product contained 50% yeast, 43.4% rice bran, 6% rare earth minerals and 0.6% collupulin (manufacturer's specifications). The procedure has been patented by the Korean Intellectual Property Office (Daejeon, Korea) under patent number 10-2009-0064064 and the product is marketed under the trade name Lanthanum-Yeast by the Celltech Company located in Umsung, Korea.

Experiment 1

In experiment 1, 150 crossbred barrows (Landrace× Yorkshire×Duroc; 24±3 d of age and 6.28±1.12 kg BW) were blocked based on initial weight and litter of origin and allotted to one of five dietary treatments in a randomized block design. The basal diet was formulated based on corn (extruded and expanded), soybean meal (regular and fermented), milk based products (lactose, whey powder and milk powder complex) and spray dried porcine plasma. The basal diet contained 100 ppm zinc as $ZnSO_4$ (35.5% zinc).

The five dietary treatments consisted of the basal diet fed without supplementation (negative control) or the basal diet supplemented with antibiotics (33 ppm tiamulin and 100 ppm chlortetracycline; positive control), ZnO (1,500 or 2,500 ppm) or 0.1% peptide bound rare earth mineral-yeast. At this inclusion level, the product provided 60 mg of rare earth minerals per kg of diet comprising 21 mg/kg lanthanum, 15 mg/kg cerium and 6 mg/kg praseodymium. Prior to mixing in the diet, antibiotics, ZnO or rare earth mineral-yeast were premixed with fiber powder and then added to the feed mixer.

The experiment was conducted in three phases with the phase 1 diet (d 1 to 7) formulated to provide 1.75% lysine, 1.18% threonine and 1.08% methionine plus cystine, while the phase 2 diet (d 7 to 21) was formulated to provide 1.56% lysine, 1.09% threonine and 0.88% methionine plus cystine, and the phase 3 diet (d 21 to 28) was formulated to provide 1.44% lysine, 0.98% threonine and 0.81% methionine plus cystine. All diets were fed in meal form and their nutrient levels met or exceeded NRC (1998) requirements for the nursery pig.

During the 28 day study, the pigs were housed in an environmentally regulated house in 1.4×1.8 m pens located over a wire slatted floor. Air temperature was controlled at 32°C during the first 7 days and the temperature was

decreased by 1°C every three days until it reached 25°C at the end of the experiment. There were with six pens per treatment and each pen housed five barrows. Each pen had two feeders and a nipple waterer to provide free access to feed and water. Body weights and pen feed consumption were measured weekly to evaluate weight gain, feed intake and feed conversion.

Experiment 2

This experiment was conducted to evaluate the effects of antibiotics, ZnO or rare earth mineral-yeast supplementation on performance, nutrient digestibility, and serum parameters. A total of 576 crossbred barrows (Landrace×Yorkshire×Duroc; 28±3 d of age and 7.20±0.78 kg BW) were blocked based on initial body weight and litter of origin, and allotted to one of four dietary treatments in a randomized block design. The pigs were fed a mixture of the phase 1 and 2 diets (phase 1: phase 2 = 50:50) used in experiment 1.The negative control was not used in this experiment.All diets contained 1.0% Celite 545 (Fluka Chemika/Biochemika, Buchs, Switzerland) as a digestibility marker.

During the 14 day experiment, the pigs were housed in an environmentally regulated container house in 2.0 × 2.4 m pens located over a wire slatted floor. There were nine pens per treatment and each pen housed 16 barrows. Each pen had a three-hole feeder and a nipple waterer to provide free access to feed and water. Air temperature in the house was controlled at 32°C during first 7 days and the temperature was decreased by 1°C every day until it reached 25°C at the end of the experiment.

Fecal samples were collected from each pen on days 11, 12, 13, and 14. The fecal samples from the four collections were pooled by placing the feces into an aluminum pan and stirring with a rubber spatula. All fecal samples were stored in sealed plastic bags at -60°C. Prior to analysis, the fecal samples were freeze dried for 72 h, allowed to equilibrate at room temperature for 24 h and then ground through a 1.0 mm screen with a Cyclotec Grinder (Model 1093, Foss Group, Hillerod, Denmark). Digestibility coefficients for

Table 1. Ingredient composition (% as fed) of the basal diet used to determine the effects of antibiotics, zinc oxide (ZnO) or rare earth mineral-yeast on performance, nutrient digestibility and blood parameters in weanling pigs

	Phase 1	Phase 2	Phase 3
Yellow corn, extruded	11.00	0.00	0.00
Yellow corn, expanded	3.50	36.28	47.78
Bakery by-product	5.00	7.00	9.00
Lactose	10.00	0.00	0.00
Soybean meal	8.00	16.50	30.00
Soybean meal, fermented	10.00	3.75	0.00
Whey powder	20.76	10.00	5.00
Skim milk powder	10.00	7.50	0.00
Spray dried plasma	5.00	2.50	0.00
Fishmeal	2.50	0.00	0.00
Lard	0.00	2.00	2.50
Soybean oil	4.50	3.00	0.00
Limestone	0.00	0.30	0.13
Tricalcium phosphate	0.00	0.00	0.60
Monocalcium phosphate	1.32	0.64	0.00
Salt	0.00	0.20	0.30
Calprona[1]	1.05	0.73	1.25
Fine sugar	3.00	3.00	0.00
DL-methionine	0.26	0.16	0.18
L-lysine-HCl	0.06	0.25	0.31
Threonine	0.04	0.00	0.12
Choline chloride, 50%	0.20	0.20	0.20
Vitamin-mineral premix[2]	0.30	0.30	0.30
Fiber powder+treatment[3]	3.51	3.19	2.33

[1] 76% Sodium propionate, Verdugt, Tiel, The Netherlands.

[2] The vitamin and mineral premix provided the following per kg of diet: Fe, 100 mg; Cu, 10 mg; Mn, 20 mg; Zn, 100 mg; I, 0.35 mg; Se, 0.20 mg; vitamin A, 20,000 IU; vitamin D_3, 2,000 IU; vitamin E, 100 mg; vitamin K, 3 mg; thiamin, 4 mg; riboflavin, 7.0 mg; pyridoxine, 5 mg; vitamin B_{12}, 0.05 mg; pantothenic acid, 16 mg; niacin, 35 mg; biotin, 0.18 mg; folic acid 1.3 mg; choline, 350 mg.

[3] Fiber powder was mixed with antibiotics, ZnO or rare earth mineral-yeast.

nutrients were calculated using the equations for the indicator method described by Schneider and Flatt (1975).

On d 14, one pig in each pen (N = 9), weighing closest to the average body weight for that pen, was chosen for blood sampling. Blood samples (about 7 ml) were collected by anterior vena cava puncture using plain vacutainer tubes (Serum Clot Activator, Greiner Bio-one, Kremsmunster, Austria) after a 6 h fast. The samples were then centrifuged at 4°C for 15 min at 1,500 rpm. Serum was collected and stored at -25°C until needed for analysis.

Chemical analysis

Samples of the diets and feces were analyzed in triplicate according to the methods of the Association of Official Analytical Chemists (AOAC, 1990). Analyses were conducted for moisture (AOAC method 930.15), crude protein (AOAC method 984.13), ash (AOAC method 942.05), crude fiber (AOAC 978.10) and ether extract (AOAC method 920.39). Calcium was determined by a Shimazu AA625 Atomic Absorption Spectrophotometer (Shimazu, Kyoto, Japan), and phosphorus was analyzed using a UV-vis. Spectrophotometer (Hitachi, Tokyo, Japan). Zinc was determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS Model Elan DRCe, Perkin Elmer, Wiesbaden, Germany). An amino acid analysis of the feed and feces was performed using a L8500-Hitachi Amino Acid Analyzer (Hitachi, Tokyo, Japan) after hydrolysis for 24 h in 6 M HCl. Performic acid hydrolysis was performed for analysis of sulfur-containing amino acids. Gross energy was measured using an Adiabatic Oxygen Bomb Calorimeter (Model 1241, Parr Instrument Co., Molin, IL). Celite (HCL-Insoluble Ash) analysis was conducted according to the description provided by Prabucki et al. (1975). The analyzed chemical composition of the experimental diets is shown in Table 2.

The concentrations of total protein, albumin, glutamic-oxaloacetic transferase, glutamic-pyruvic transferase, potassium, blood urea nitrogen, total cholesterol, high density lipoprotein, low density lipoprotein, and triglyceride in serum were determined using commercial kits (Bayer, Terrytown, NY) following the procedures recommended for an Automatic Biochemical Analyzer (Model ADVIA 1650, Bayer, Terrytwon, NY). The concentration of immunoglobulin G was analyzed using a commercially available Nephelometry kit (Bade Behring, Schwalbach, Germany) conducted using a Model BN II Nephelometer (Bade Behring, Schwalbach, Germany). Magnesium, iron, and total iron binding capacity were analyzed using commercial kits supplied by Roche (Basel, Switzerland). Zinc and copper were analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS Model Elan DRCe, Perkin Elmer, Wiesbaden, Germany). Serum cortisol, somatomedin-C, and tumor necrosis factor were analyzed using radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA), chemiluminescent immunoassay (Diagnostic Products Corporation, Los Angeles, CA), and enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN), respectively.

Table 2. Chemical analysis (%) of diets fed to determine the effects of antibiotics, zinc oxide (ZnO) and rare earth mineral-yeast on performance, nutrient digestibility and blood parameters in weanling pigs[1, 2]

	Phase 1	Phase 2	Phase 3
Crude protein	25.84	23.92	21.98
Crude fiber	1.50	2.82	3.33
Ether extract	6.52	6.68	6.71
Ash	6.86	6.94	6.78
Calcium	0.91	0.87	0.87
Phosphorus	0.70	0.69	0.66
Essential amino acids			
Arginine	1.35	1.18	1.08
Histidine	0.67	0.56	0.56
Isoleucine	1.07	0.98	0.89
Leucine	2.10	1.99	1.88
Lysine	1.75	1.56	1.44
Methionine+cystine	1.08	0.88	0.81
Phenylalanine	1.16	1.11	1.07
Threonine	1.18	1.09	0.98
Valine	1.21	1.15	0.99

[1] The basal diet was fed either unsupplemented or supplemented with antibiotic, ZnO, or rare earth mineral-yeast.

[2] The zinc content of the control diet ranged from 98 to 108 ppm zinc, the 1500 ppm zinc diets ranged from 1,491 to 1,541 ppm zinc while the diets containing 2,500 ppm zinc ranged from 2,459 to 2,522 ppm zinc.

Table 3. Effects of antibiotics, zinc oxide (ZnO), or rare earth mineral-yeast on the performance of weanling pigs (Exp. 1)[1,2]

	Control	Antibiotics	1,500 ppm ZnO	2,500 ppm ZnO	Rare earth mineral yeast	SEM	p values
Weight gain (g/d)	302[b]	353[a]	352[a]	369[a]	359[a]	14.0	0.02
Feed intake (g/d)	467	518	530	558	501	22.6	0.10
Feed conversion	1.55	1.47	1.50	1.52	1.41	0.05	0.22

[1] Six replicate pens of five pigs per pen for the performance data.
[2] Means in the same row with same or no superscript do not differ (p>0.05).

Statistical analysis

The performance and digestibility data were analyzed as a randomized block design using the General Linear Model Procedure (General AOV/AOCV) of the Statistix for Windows program (Analytical Software, Tallahassee, FL, 1996). Pigs were blocked on the basis of initial body weight and the pen was considered the experimental unit for analyses of performance and digestibility data. The individual pig was considered the experimental unit for analyses of data regarding blood parameters. The model included the effects of replication (i.e., block), treatment, and replication×treatment (error). The significance of differences between means was determined by the Least Significant Difference (LSD) method for performance, digestibility, and blood parameter data. Differences were considered significant at the level of $p<0.05$ and highly significant at the level of $p<0.01$.

RESULTS

In experiment 1, weight gain was lower ($p<0.05$) for pigs fed the negative control than for pigs fed diets supplemented with antibiotics, ZnO (both 1,500 and 2,500 ppm) or rare earth mineral-yeast (Table 3). Weight gain did not differ ($p>0.05$) between pigs fed the four supplemented diets. In addition, feed intake and feed conversion were unaffected ($p>0.05$) by dietary treatment. In experiment 2, there were no differences ($p>0.05$) in weight gain, feed intake or feed conversion between pigs fed diets supplemented with antibiotics, ZnO or rare earth mineral-yeast (Table 4).

The apparent fecal digestibility coefficients for various nutrients contained in the experimental diets are shown in Table 5. The digestibility of dry matter, crude protein, calcium, phosphorus and energy were significantly ($p<0.01$) higher for pigs fed the rare earth mineral-yeast diet than the diets supplemented with ZnO. Digestibility coefficients for calcium, phosphorus and energy were also higher ($p<0.01$) for pigs fed the rare earth mineral-yeast diet than the diet supplemented with antibiotics. Pigs fed the antibiotic supplemented diet had significantly ($p<0.01$) higher digestibility coefficients for dry matter, calcium and phosphorus than pigs fed diets supplemented with 1,500 or 2,500 ppm ZnO.

In terms of the essential amino acids, pigs fed the diet supplemented with rare earth mineral-yeast had significantly ($p<0.05$) higher digestibility of histidine, lysine, threonine and valine than pigs fed either of the ZnO supplemented diets. In addition, pigs fed the diet supplemented with rare earth mineral-yeast had significantly ($p<0.05$) higher digestibility coefficients for isoleucine, leucine, methionine and phenylalanine than pigs fed the diet supplemented with 1,500 ppm ZnO. Digestibility coefficients for pigs fed antibiotics tended to be intermediate to those of the pigs fed rare earth mineral-yeast or ZnO. Digestibility coefficients for histidine, lysine, methionine, phenylalanine, threonine and valine were significantly ($p<0.05$) higher for pigs fed the diet supplemented with lanthanum oxide than the diet supplemented with antibiotics.

There were no differences between the treatments for serum total protein, albumin, blood urea nitrogen, triglyceride, total cholesterol, high and low density lipoprotein cholesterol, calcium, copper, iron, potassium, magnesium, immunoglobulin G, total iron binding capacity, and tumor necrosis factor-α concentration (Table 6). Pigs fed ZnO had higher ($p<0.01$) serum zinc concentrations than pigs fed antibiotic or rare earth mineral-yeast supplemented diets. Glutamic-oxaloacetic transferase and glutamic-pyruvic transferase were also higher ($p<0.01$) for pigs fed ZnO than for pigs fed the antibiotic or rare earth mineral-yeast supplemented diets.

Table 4. Effects of antibiotics, zinc oxide (ZnO) or rare earth mineral-yeast on the performance of weanling pigs (Exp. 2)[1]

	Antibiotics	1,500 ppm ZnO	2,500 ppm ZnO	Rare earth mineral-yeast	SEM	p values
Weight gain (g/d)	304	297	306	306	21.2	0.99
Feed intake (g/d)	527	536	534	521	27.9	0.98
Feed conversion	1.79	1.85	1.77	1.71	0.09	0.72

[1] Nine replicate pens of sixteen pigs per pen.

Table 5. Effects of antibiotics, zinc oxide (ZnO) or rare earth mineral-yeast on nutrient digestibility (Exp. 2)[1,2]

	Antibiotics	1,500 ppm ZnO	2,500 ppm ZnO	Rare earth mineral-yeast	SEM	p-values
Dry matter	95.19[a]	93.83[b]	93.98[b]	95.46[a]	0.30	<0.01
Crude protein	74.51[ab]	71.55[b]	72.33[b]	78.34[a]	1.38	<0.01
Calcium	56.59[b]	46.98[c]	48.50[c]	65.10[a]	1.69	<0.01
Phosphorus	54.87[b]	43.07[c]	38.52[c]	66.11[a]	2.09	<0.01
Energy	83.51[b]	81.42[b]	81.33[b]	86.89[a]	0.80	<0.01
Essential amino acids						
Arginine	87.01	83.07	86.97	87.31	2.38	0.56
Histidine	80.27[b]	77.30[b]	77.29[b]	85.50[a]	1.11	<0.01
Isoleucine	72.17[ab]	64.98[b]	72.51[ab]	77.53[a]	2.96	0.05
Leucine	79.63[a]	74.37[b]	79.70[a]	81.69[a]	1.70	0.04
Lysine	81.45[b]	79.42[b]	80.32[b]	85.15[a]	0.95	<0.01
Methionine	83.49[b]	83.67[b]	86.76[a]	87.32[a]	0.79	<0.01
Phenylalanine	74.21[b]	73.75[b]	75.41[ab]	78.96[a]	1.32	0.05
Threonine	76.19[b]	75.13[b]	75.28[b]	81.19[a]	1.58	0.04
Valine	65.88[b]	58.42[b]	61.32[b]	76.54[a]	3.32	<0.01

[1] Six replicate pens of sixteen pigs per pen for the digestibility data.
[2] Means in the same row with the same or no superscript do not differ (p>0.05).

DISCUSSION

Experiment 1 demonstrated more rapid growth for pigs fed either of the ZnO supplemented diets than for pigs fed the negative control while both experiments 1 and 2 showed that the performance of pigs fed ZnO was essentially equal to that of the antibiotic supplemented pigs. This data supports previous studies which demonstrated that pharmacological doses of ZnO stimulate performance in weanling pigs (Hahn and Baker, 1993; Carlson et al., 1999;

Table 6. Effects of antibiotics, zinc oxide (ZnO) or rare earth mineral-yeast on serum parameters of weanling pigs (Exp. 2)[1,2]

	Antibiotics	1,500 ppm Zinc	2,500 ppm Zinc	Rare earth mineral-yeast	SEM	p-values
Total protein, g/dl	4.57	4.67	4.63	4.40	0.15	0.60
Albumin, g/dl	2.96	3.02	3.12	2.92	0.12	0.66
Blood urea nitrogen, mg/dl	11.61	11.21	11.73	10.29	0.75	0.53
Triglyceride, mg/dl	54.1	62.2	55.8	68.9	6.80	0.42
Total cholesterol, mg/dl	81.56	88.56	97.56	94.33	4.43	0.08
HDL cholesterol, mg/dl	37.89	39.78	42.22	43.00	2.34	0.41
LDL cholesterol, mg/dl	34.78	39.56	45.11	41.56	2.85	0.10
Calcium, mg/dl	11.09	11.22	11.10	11.33	0.18	0.75
Copper, μg/dl	175	172	175	182	21.00	0.99
Iron, μg/dl	125	109	115	138	18.00	0.69
Potassium, mmol/dl	6.23	6.73	7.31	6.60	0.39	0.28
Magnesium, mg/dl	2.48	2.39	2.51	2.56	0.08	0.48
Zinc, μg/dl	100[c]	225[b]	304[a]	98[c]	21.00	<0.01
Cortisol, μg/dl	3.53	2.19	1.81	2.69	0.49	0.12
Somatomedin-C, ng/ml	112	87	107	124	10.00	0.10
Immunoglobulin G, mg/dl	247	282	222	215	36.00	0.55
Total iron binding capacity, μg/dl	522	498	521	486	26.00	0.72
Tumor necrosis factor-α, pg/ml	132	141	129	120	14.00	0.78
Glutamic-oxaloacetic transferase, U/L	50.0[b]	69.0[a]	81.8[a]	44.8[b]	4.60	<0.01
Glutamic-pyruvic transferase, U/L	36.6[b]	58.7[a]	59.1[a]	34.0[b]	2.90	<0.01

[1] Nine replicate pens of sixteen pigs per pen for the serum data.
[2] Means in the same row with the same or no superscript do not differ (p>0.05).

Hill et al., 2000). However, there are some reports in which no growth-promoting benefits were observed (Fryer et al., 1992; Tokach et al., 1992; Schell and Kornegay, 1996). Our data indicate that feeding 1,500 ppm ZnO resulted in similar performance to feeding 2,500 ppm ZnO which supports the findings of Hill et al. (2001) who observed that responses to ZnO reached a plateau at about 1,500 ppm.

It has previously been suggested that ZnO promotes the growth of weanling pigs by controlling pathogenic bacterial scours (Holm and Poulsen, 1996; Katouli et al., 1999, Huang et al., 1999). However, other work has indicated that ZnO promotes growth in early and conventionally weanling pigs regardless of diarrhea incidence or effect on intestinal microbial numbers (Hahn and Baker, 1993; Li et al., 2001). The ZnO supplemented pigs used in our experiment exhibited improved performance but did not exhibit diarrhea, suggesting that mechanisms other than the antimicrobial effects of ZnO supplementation are responsible for the improvements in pig performance observed in the present experiment.

In comparison with the diets containing antibiotics or rare earth mineral-yeast, the present experiment demonstrated reductions in the digestibility of dry matter, calcium, and phosphorus as a result of feeding either 1,500 or 2,500 ppm ZnO. This finding was somewhat surprising given the fact that Li et al. (2001) and Li et al. (2006) have shown that zinc supplementation improves gut morphology by increasing villous height and reducing crypt depth in the small intestine thus potentially increasing the absorptive capacity of the small intestine. In addition, Hedemann et al. (2006) reported that high dietary zinc increased the activity of several enzymes in the pancreatic tissue (amylase, carboxypeptidase, chymotrypsin, trypsin and lipase) and it might reasonably be expected that such an increase would result in improvements in nutrient digestibility. However, such was not the case with the present experiment.

Serum zinc concentrations were elevated for pigs fed diets supplemented with 1,500 or 2,500 ppm ZnO. Previous studies with zinc supplemented pigs have also noted elevated levels of serum zinc (Hahn and Baker, 1993; Hill et al., 2001; Case and Carlson, 2002). To our knowledge, this study is the first to report elevated levels of glutamic-oxaloacetic transferase and glutamic-pyruvic transferase in ZnO supplemented pigs. Elevated levels of these enzymes are typically associated with liver damage (Giboney, 2005). Jensen-Waern et al. (1998) reported that zinc concentrations in liver tissue were 4.5 times higher in weanling pigs fed diets supplemented with 2,500 ppm ZnO than in controls. In addition, microscopic examination of zinc supplemented pigs showed increased lipid accumulation in hepatocytes. Therefore, future work should be conducted to determine whether or not pharmacological levels of dietary zinc alter liver function.

The pig performance data from experiment 1 showed improved weight gain for piglets fed rare earth mineral-yeast supplemented diets compared with the negative control. In both experiments 1 and 2, the performance of pigs fed rare earth mineral-yeast was similar to that of the pigs fed the antibiotic or ZnO supplemented diets indicating that rare earth mineral-yeast can substitute for antibiotics and pharmacological levels of zinc in diets fed to nursery pigs.

Previous studies have indicated that rare earth elements stimulate performance in weanling pigs under both Chinese (Shen et al., 1991; Yuan, 1994; He and Xia, 1998; He et al., 2001) and western conditions (Rambeck et al., 1999; Borger, 2003; Eisele, 2003). Our results support the findings of He and Xia (1998) who observed that weight gain in weanling pigs was increased by 5% to 23% under the influence of rare earth elements. Similar improvements in weight gains were reported by Li et al. (1992) and He and Xia (1998). Under western conditions, Rambeck et al. (1999) reported that weight gain was improved by as much as 5% and feed conversion by as much as 7% by the addition of rare earth elements.

Our data indicate that the improvements in pig performance due to rare earth mineral-yeast supplementation were primary the result of improved nutrient digestibility. We observed an increase in the digestibility of calcium, phosphorus, gross energy and several essential amino acids including histidine, lysine, threonine and valine as a result of feeding rare earth mineral-yeast. This supports earlier work which reported that rare earth elements can improve digestibility and utilization of nutrients in the diets of pigs and broilers (Li et al., 1992; Zhu et al., 1994; Lu and Yang, 1996).

As rare earth elements are poorly absorbed from the gastrointestinal tract (Krafka, 1999; He et al., 2001), it might be that rare earth mineral-yeast influences the microbial environment in the gut and this could be the mechanism through which rare earth minerals improve digestibility and utilization of nutrients in the diet (Li et al., 1992). Several authors have reported that the bacterial growth may be stimulated by low concentrations of rare earth elements both *in vitro* (Muroma, 1958) and *in vivo* (Monafo et al., 1976). Future work on the effects of rare earth minerals on the types and numbers of intestinal microorganisms may provide further insight into the mechanisms through which rare earth minerals function.

Although the focus of this study was on rare earth minerals, we can't rule out the possibility that the effects of the rare earth mineral-yeast product on pig performance were due to the presence of the yeast and not due to the presence of rare earth minerals at all. Previous research has demonstrated that yeast culture can benefit weanling pigs by enhancing growth, nitrogen balance and nutrient

digestion (Mathew et al., 1998; van der Peet-Schering et al., 2007; Shen et al., 2009).

The overall results of this study indicate that rare earth mineral-yeast may be an effective substitute for antibiotics and ZnO as a means of growth promotion in diets fed to weanling pigs. An issue with feeding pharmacological levels of zinc to pigs is that application of their manure to soil can negatively impact the environment (Berenguer et al., 2008). Pigs fed 3,000 ppm zinc excreted almost four times more zinc in their feces as pigs fed 500 ppm zinc (Case and Carlson, 2002). Zinc accumulation in the soil has been implicated in reducing plant growth (Chaney, 1993) while leaching of zinc from soils treated with swine manure may lead to pollution of lakes, streams and costal waters (Li and Shuman, 1997; Hsu and Lo, 2001; Martinez and Motto, 2001). The fact that 60 ppm rare earth minerals produced similar levels of growth promotion as 1,500 or 2,500 ppm zinc suggests that the use of organic rare earth minerals as a growth promoter could minimize the environmental impact of intensive swine operations and reduce the amount of minerals in swine manure that could potentially pollute the environment.

In conclusion, the performance of pigs fed rare earth mineral-yeast was basically equal to that of the pigs fed antibiotic or ZnO supplemented diets indicating that rare earth mineral-yeast can be successfully used as a growth promoter in diets fed to nursery pigs. The effects of rare earth mineral-yeast appeared to be mediated through improvements in nutrient digestibility.

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