Effects of dietary humic substances on pig growth performance, carcass characteristics, and ammonia emission

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ABSTRACT: Five experiments were conducted to test the effects of various dietary humic substances (HS; HS1, 2, 3, and 4, each with different fulvic and humic acid contents) on pig growth, carcass characteristics, and ammonia emission from manure. In Exp. 1, 120 pigs were allotted to 3 dietary treatments without HS (control) or with HS1 at 0.5 and 1.0% (8 pens/treatment and 5 pigs/pen) and fed diets, based on a 5-phase feeding program, from weaning (d 21.3 ± 0.3 of age) to 60 kg of BW. In Exp. 2 and 3, 384 pigs (192 for each experiment) were allotted to 3 dietary treatments without HS, with HS1, or with HS2 (0.5%) for Exp. 2 and without HS, or with HS3 or HS4 (0.5%) for Exp. 3 (8 pens/treatment and 8 pigs/pen in each experiment). Pigs were fed diets, based on a 6-phase feeding program, from weaning (25.4 ± 0.2 and 23.6 ± 0.3 d of age for Exp. 2 and 3, respectively) to 110 kg of BW. In Exp. 4, 96 pigs were weaned at 22.1 ± 0.2 d of age and allotted to 2 treatments without or with HS1 at 0.5% (6 pens/treatment and 8 pigs/pen), and in Exp. 5 96 pigs were weaned at 20.9 ± 0.3 d of age and allotted to 3 treatments without HS, or with HS3 or HS4 (0.5%; 4 pens/treatment and 8 pigs/pen). Pigs were fed the diets for at least 2 wk before they were moved to an environmental chamber to measure aerial ammonia and hydrogen sulfide for 48 h at 5-min intervals. In Exp. 1, pigs fed diets with HS1 at 0.5% had greater ($P < 0.05$) ADG during phase 3 and greater ($P < 0.05$) G:F during phases 3 and 5 than control pigs. In Exp. 2, pigs fed diets with HS1 or HS2 at 0.5% had greater ($P < 0.05$) ADG and G:F than control pigs during the entire feeding period, whereas in Exp. 3 HS3 or HS4 did not improve pig growth performance. Ammonia emission from manure was reduced by 18 or 16% when pigs were fed diets with HS1 ($P = 0.067$) or HS4 ($P = 0.054$), respectively. The results of this study indicate that the effects of dietary HS are variable but may improve growth performance of pigs and reduce ammonia emission from manure. Further research is needed to clarify these effects and the mechanisms by which HS may cause them.

Key words: ammonia, carcass characteristic, growth performance, humic substance, pig

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INTRODUCTION

Humic substances (**HS**) 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 OM in many soils (Goh and Reid, 1975) but specifically include humic acid, fulvic acid, and humin as major constituents as well as several minerals such as iron, manganese, copper, and zinc (Aiken et al., 1985). Humic and fulvic acids are high molecular weight acids with a molecular weight range between 1,000 and 300,000 Da (Stevenson, 1994). Raw materials containing HS can be mined from geographically and physically different seams. A seam with yellow to brown color (brown seam) may contain high fulvic acid, whereas a seam with dark brown to black color (black seam) may contain high humic acid and humin.

Previously, HS have been applied directly to manures of livestock to reduce ammonia emission (Ndayegamiye and Cote, 1989; Shi et al., 2001). However, supplementation as a feed additive in pig diets has not been reported. The high molecular weight acids and minerals in HS may benefit animal performance even though the actual mechanism is not yet understood. This study was conducted as a first effort to characterize HS as a potential feed supplement in pig diets. The objectives of this study were to test the effects of various HS supplements, with different compositions of fulvic and humic acids, in pig diets on growth, carcass characteristics, and room aerial ammonia concentrations.

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Table 1. Chemical composition of various humic substances (HS; DM basis)[1,2]

Item, %	HS1	HS2	HS3	HS4
Moisture	20.5 ± 0.1	17.4 ± 0.2	15.1 ± 0.1	19.5 ± 0.2
Humic acid	42.6 ± 1.5	18.4 ± 1.4	12.2 ± 1.3	54.6 ± 1.6
Fulvic acid	5.0 ± 1.2	16.8 ± 1.1	21.2 ± 1.2	1.0 ± 0.7
CP	3.4 ± 0.1	3.9 ± 0.2	3.8 ± 0.1	3.4 ± 0.1
Crude fat	0.08 ± 0.01	0.04 ± 0.01	0.08 ± 0.01	0.04 ± 0.01
Crude ash	31.8 ± 0.4	35.6 ± 0.3	42.4 ± 0.2	23.6 ± 0.3

[1]Humic substances were obtained from HumaTech, Inc. (Mesa, AZ), and the product name was Promax. Individual HS were labeled HS1: DPX48162; HS2: DPX46162; HS3: DPX4600; and HS4: DPX5800.
[2]Data are means ± SE.

MATERIALS AND METHODS

Humic Substances

Four HS (HS1, HS2, HS3, and HS4, Humatech Inc., Mesa, AZ) were produced using raw materials from different seams and processed with slightly different methods. Thus, fulvic and humic acid contents as well as chemical composition were different among the 4 HS sources tested (Table 1). Chemical composition, including the contents of moisture, CP, crude fat, and crude ash, was determined following the methods suggested by AOAC (1990). Analyses of humic acid and fulvic acid were conducted following methods suggested by Hayes

Table 2. Composition of experimental diets (as-fed basis)

Phase[1]	1	2	3	4	5	6
Ingredient, %						
Ground corn, yellow[2]	26.25	41.70	60.65	69.00	76.80	79.00
Soybean meal, dehulled	20.00	25.60	34.00	26.00	19.00	17.00
Fish meal, menhaden	3.30					
Dried whey	35.00	20.00				
Salt	0.45	0.35	0.25	0.30	0.15	0.15
VTM PM 2001[3]	4.00	3.00	2.00	2.00	1.40	1.40
Restaurant grease	3.00	3.00	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.00	1.20	1.40	1.00	0.90	0.85
Limestone	1.00	0.90	0.70	0.70	0.75	0.60
Plasma protein[4]	5.60	4.00				
Zinc oxide	0.40	0.25				
Total	100.00	100.00	100.00	100.00	100.00	100.00
Analyzed composition						
DM, %	89.6	90.2	89.2	89.3	88.9	89.4
CP, %	23.2	21.6	21.4	17.9	15.2	14.3
Calculated composition						
DM, %	91.6	90.9	89.8	89.7	89.6	89.5
ME, Mcal/kg	3.30	3.36	3.34	3.36	3.37	3.38
CP, %	22.9	21.5	21.3	18.2	15.5	14.7
Lysine, %	1.55	1.35	1.19	0.97	0.78	0.72
Cys + Met, %	0.82	0.75	0.70	0.62	0.55	0.53
Tryptophan, %	0.31	0.28	0.26	0.21	0.17	0.16
Threonine, %	1.11	0.97	0.81	0.68	0.58	0.55
Ca, %	1.18	0.92	0.74	0.62	0.59	0.52
Available P, %	0.70	0.52	0.36	0.27	0.24	0.23
Total P, %	0.85	0.72	0.64	0.54	0.50	0.49

[1]A 6-phase feeding program was used. The duration of each phase differed for each experiment, depending on the growth rate of the pigs.
[2]An equal amount of corn was replaced by the humic substances (HS). The HS1 (Exp. 1) was supplemented at 0.5 and 1.0% of the complete diets. The HS1 and HS2 (Exp. 2), and the HS3 and HS4 (Exp. 3) were supplemented at 0.5% of the complete diets. The HS1 (Exp. 4), and HS3 and HS4 (Exp. 5) were supplemented at 0.5% of the complete diets.
[3]Vitamin-mineral premix provided the following per kilogram of complete phase 1 diet: 621.8 mg of manganese as manganous oxide; 100 mg of iron as iron sulfate; 138.4 mg of zinc as zinc oxide; 12.7 mg of copper as copper oxide; 0.96 mg of iodide as ethylenediamine dihydroiodide; 0.31 mg of selenium as sodium selenate; 10,072 IU of vitamin A as vitamin A acetate; 1,100 IU of vitamin D_3; 82.5 IU of vitamin E; 5.9 IU of vitamin K as menadione sodium bisulfite; 73.2 μg of vitamin B_{12}; 18.3 mg of riboflavin; 58.5 mg of D-pantothenic acid as calcium pantothenate; 73.2 mg of niacin; and 6,446 mg of choline as choline chloride. The contents of the vitamins and minerals in phase 2, 3, 4, 5, and 6 diets were 75, 50, 50, 35, and 35% of those in the phase 1 diet, respectively.
[4]APC-920 (American Protein Corporation, Ames, IA).

Table 3. Growth performance of pigs fed diets supplemented with HS1 (Exp. 1)[1]

Item	Control	HS1 0.5%	HS1 1.0%	SEM
No. of pigs	8	8	8	
Initial BW, kg	6.45	6.45	6.53	0.15
ADG, kg/d				
Phase[2] 1	0.205	0.212	0.212	0.008
Phase 2	0.322	0.317	0.330	0.007
Phase 3	0.456[a]	0.508[b]	0.455[a]	0.012
Phase 4	0.570	0.581	0.575	0.008
Phase 5	0.785	0.846	0.762	0.030
Phase 1 to 5	0.525	0.548	0.525	0.009
ADFI, kg/d				
Phase 1	0.257	0.251	0.257	0.007
Phase 2	0.522	0.501	0.527	0.012
Phase 3	0.981	0.983	0.969	0.014
Phase 4	1.193	1.228	1.205	0.020
Phase 5	2.182[a]	1.925[b]	1.906[b]	0.065
Phase 1 to 5	1.151	1.126	1.114	0.010
G:F				
Phase 1	0.803	0.841	0.828	0.024
Phase 2	0.620	0.636	0.629	0.011
Phase 3	0.465[a]	0.516[b]	0.470[a]	0.009
Phase 4	0.478	0.474	0.478	0.014
Phase 5	0.359[a]	0.440[b]	0.400[ab]	0.014
Phase 1 to 5	0.456	0.487	0.471	0.008

[a,b]Means within a row with different superscripts differ ($P < 0.05$).
[1]HS1 (DPX48162; Promax) was obtained from HumaTech Inc. (Mesa, AZ).
[2]Phase 1 for 7 d, phase 2 for 14 d, phase 3 for 14 d, phase 4 for 48 d, and phase 5 for 15 d.

(1985). The HS4 contained the greatest amount of humic acid, whereas the HS3 contained the greatest amount of fulvic acid. Crude protein and crude fat contents were similar among the HS sources. Crude ash content was variable, with the greatest content in HS3 and the least in HS4.

Animals, Facility, and Diets

Protocols for the care and use of animals for these 5 experiments were approved by Texas Tech University Animal Care and Use Committee.

A total of 516 pigs (Camborough-22 × PIC boar, Pig Improvement Company, Franklin, KY) were used in 5 experiments. Pigs were housed in a pen (1.5 × 2.0 m for nursery pens and 2.2 × 3.8 m for grower-finisher pens; 5 to 8 pigs/pen) and fed diets containing nutrients that meet or exceed the requirements recommended by the NRC (1998) based on a 6-phase feeding program (Table 2), but the length of each period differed among experiments. For the phase 1 and 2 diets, dried whey (35 and 20%, respectively) and corn were used as the major sources of energy, and fish meal, spray-dried plasma protein, and soybean meal were used as major sources of protein (Table 2). For the phase 3, 4, and 5 diets, corn and soybean meal were used as the major sources of energy and protein, respectively. The control diet had no HS supplements, whereas treatment diets contained various HS replacing the same amount of corn in the diets. During the entire experimental periods, pigs had free access to water and the assigned diets. Body weight and feed intake of pigs were measured at the end of each phase.

Experiment 1

One hundred twenty pigs, weaned at 21.3 ± 0.3 d of age, were allotted to 3 dietary treatments: without HS (control) or with HS1 at 0.5 or 1.0%. Each treatment had 8 pens, and each pen had 5 pigs (2 barrows and 3 gilts or 3 barrows and 2 gilts/pen). Pigs were fed phase 1, 2, 3, 4, and 5 diets for 7, 14, 14, 48, and 15 d, respectively, until they reached 60 kg of BW.

Experiment 2

One hundred ninety-two pigs, weaned at 25.4 ± 0.2 d of age, were allotted to 3 dietary treatments: without HS (control), with HS1 (0.5%), or with HS2 (0.5%). Each treatment had 8 pens (4 barrow pens and 4 gilt pens) and each pen had 8 pigs. Pigs were fed phase 1, 2, 3, 4, 5, and 6 diets for 7, 14, 14, 49, 48, and 17 d, respectively, until they reached 110 kg of BW.

When pigs weighed more than 110 kg, they were transported from the Texas Tech Swine Research Center (New Deal, TX) to Seaboard Foods (Guymon, OK) for slaughter and carcass measurements. Before moving, pigs were numbered by tattoo to identify their original treatment. Hot carcass weights were obtained after slaughter just before chilling. Backfat thickness and LM depth were determined by measuring midline fat thickness (for backfat including the skin) at the last rib. Weight and percent lean of LM were also determined. Percent lean was determined on the warm carcasses before chilling. The pH and temperature were obtained from the LM between the 10th and 11th rib after 24 h of chilling. The pH of the LM was determined using a portable pH meter (Model IQ 140 pH Meter, IQ Scientific Instruments Inc., Carlsbad, CA). Hunter L (luminescence), a (redness), and b (yellowness) values were obtained using a Minolta color recorder (MiniScan XE Plus, Hunter, Reston, VA). The proportion of LM acceptable for the Japanese market was determined by selecting LM with acceptable color, texture, and firmness (all measures 3 or greater, based on a scale of 1 to 5; NPPC, 2000).

Experiment 3

One hundred ninety-two pigs, weaned at 23.6 ± 0.3 d of age, were allotted to 3 dietary treatments: without HS (control), with HS3 (0.5%), or with HS4 (0.5%). Pigs were fed the phase 1, 2, 3, 4, 5, and 6 diets for 7, 14, 14, 76, 31, and 11 d, respectively, until they reached 110 kg and were transported to Seaboard Foods (Guymon, OK) for the carcass measurements. All other procedures were identical to those in the Exp. 2.

Table 4. Growth performance of pigs fed diets supplemented with HS1 and HS2 (Exp. 2)[1]

	Control		HS1		HS2					
Item	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	SEM	Trt[2]	Sex[3]	Trt × sex[4]
No. of pigs	4	4	4	4	4	4				
Initial BW, kg	8.17	8.06	8.21	7.92	8.26	7.86	0.28	NS	NS	NS
ADG, kg/d										
Phase[5] 1	0.185	0.132	0.145	0.137	0.155	0.144	0.009	NS	NS	NS
Phase 2	0.312	0.323	0.345	0.320	0.347	0.325	0.010	NS	NS	NS
Phase 3	0.337	0.392	0.356	0.368	0.442	0.396	0.013	NS	NS	NS
Phase 4	0.686	0.590	0.670	0.645	0.647	0.619	0.012	NS	**	NS
Phase 5	0.696	0.531	0.908	0.811	0.907	0.745	0.030	*	*	NS
Phase 6	0.976	0.656	1.041	0.780	1.088	0.924	0.042	NS	*	NS
Phase 1 to 6	0.631	0.513	0.704	0.633	0.711	0.623	0.016	*	*	NS
ADFI, kg/d										
Phase 1	0.211	0.202	0.191	0.185	0.201	0.199	0.006	NS	NS	NS
Phase 2	0.572	0.535	0.552	0.565	0.571	0.544	0.011	NS	NS	NS
Phase 3	0.845	0.995	0.875	0.885	1.071	1.003	0.035	NS	NS	NS
Phase 4	1.501	1.493	1.526	1.426	1.489	1.350	0.025	NS	NS	NS
Phase 5	2.510	2.020	2.443	2.364	2.518	2.304	0.048	NS	*	NS
Phase 6	3.295	2.853	3.573	3.089	3.288	3.046	0.010	NS	**	NS
Phase 1 to 6	1.821	1.620	1.839	1.728	1.840	1.688	0.027	NS	*	NS
G:F										
Phase 1	0.878	0.614	0.765	0.733	0.758	0.702	0.035	NS	NS	NS
Phase 2	0.543	0.602	0.625	0.562	0.610	0.596	0.013	NS	NS	NS
Phase 3	0.406	0.396	0.409	0.425	0.415	0.395	0.010	NS	NS	NS
Phase 4	0.457	0.397	0.438	0.453	0.437	0.459	0.007	NS	NS	**
Phase 5	0.278	0.264	0.372	0.344	0.361	0.324	0.010	*	**	NS
Phase 6	0.296	0.225	0.298	0.253	0.329	0.304	0.011	NS	**	NS
Phase 1 to 6	0.346	0.317	0.383	0.367	0.387	0.369	0.006	*	*	NS
Mortality, %										
Phase 1 to 6	6.25	0.00	0.00	0.00	0.00	0.00	0.72	**	NS	NS

[1]HS1 (DPX48162; Promax) and HS2 (DPX46162; Promax) were obtained from HumaTech Inc. (Mesa, AZ).
[2]Effect of treatment (Trt; *$P < 0.05$; **$P < 0.01$; NS: $P > 0.05$).
[3]Effect of sex (*$P < 0.05$; **$P < 0.01$; NS: $P > 0.05$).
[4]Effect of the interaction of treatment (Trt) × sex (**$P < 0.01$; NS: $P > 0.05$).
[5]Phase 1 for 7 d, phase 2 for 14 d, phase 3 for 14 d, phase 4 for 76 d, phase 5 for 31 d, and phase 6 for 11 d.

Experiment 4

Ninety-six pigs, weaned at 22.1 ± 0.2 d of age, were divided into 6 BW groups (16 pigs/group). Within each group, pigs were allotted to 2 treatments: without HS (control) or with HS1 (0.5%). Each treatment had 6 pens, and each pen had 8 pigs. Pigs were fed the phase 1, 2, and 3 diets for 7, 14, and 31 d, respectively. On d 8 of the phase 3 diet, 8 pigs in each pen of group 1 were moved to a pen (1.2 × 2.4 m) in a ventilated environmental chamber (3.0 × 3.0 × 2.4 m) for 48 h, during which aerial ammonia and hydrogen sulfide were measured. After measurements of pigs in group 1, pigs in group 2 moved into the chamber and the treatment orders were randomly altered; this continued until all 6 groups were evaluated.

The fan inside the chamber ran continuously at a constant speed during the experimental period. A calibrated gas monitor with sensors for ammonia and hydrogen sulfide (Pac III, Draeger Safety Inc., Pittsburgh, PA) was used to measure changes of these compounds during the 48-h period, at 5-min intervals. Feed intake of pigs during the 48-h period was measured. Initial and final BW were measured before and after moving the pigs to the chamber. The first 24 h in the chamber was an acclimation period. The second 24-h period was used for data collection.

Experiment 5

Ninety-six pigs, weaned at 20.9 ± 0.3 d of age, were divided into 4 BW groups (24 pigs per group). Within each group, pigs were allotted to 3 treatments: without HS (control), with HS3 (0.5%), or with HS4 (0.5%). Each treatment had 4 pens, and each pen had 8 pigs. All other detailed methods were identical to those of Exp. 4.

Statistical Analyses

Growth performance data from Exp. 1, 2, and 3 were analyzed as a completely randomized design, with the pen as the experimental unit. A 3 × 2 factorial arrangement of treatments was used in Exp. 2 and 3, with dietary treatment and sex as the main factors. Analyses were performed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC), with treatment as the effect in

Exp. 1 and treatment, sex, and treatment × sex as the effects in Exp. 2 and 3. Treatment means were compared for statistical differences at $P < 0.05$ using the PDIFF option of GLM. Carcass performance data and Japanese Pass Rate also were evaluated for each pig. However, the carcass data were not subjected to the statistical analysis because the pigs were identified during the slaughter process only by their treatment, but not by their original pen, which was the experimental unit.

For Exp. 4 and 5, the first 24 h was used as an acclimation period and the last 24-h was used as the primary data collection period. Ammonia levels during the collection period were averaged for each replicate, and the data were analyzed using the GLM procedure of SAS. Feed intake and initial BW were used as covariates. Treatment means were compared for statistical differences at $P < 0.05$ using the PDIFF option of GLM. Regression equations were used to describe the changes of aerial ammonia during the last 24 h using the REG procedure of SAS. For the regression equations, the CONTRAST option of GLM was used to determine if the rates of change (slopes) or the initial levels (intercepts) differed between the treatments, as described (ISD, 2005).

Table 5. Carcass characteristics of pigs fed diets supplemented with HS1 and HS2 (Exp. 2)[1,2]

Item	Control	HS1	HS2
No. of carcasses	25	41	43
Carcass			
Hot carcass weight, kg	88.8	90.4	91.1
Backfat thickness,[3] mm	21.5	18.5	18.9
Lean, %	50.4	52.1	52.2
LM			
LM depth, mm	50.9	54.0	55.7
pH of the LM[4]	5.79	5.84	5.88
Minolta L[5]	43.7	41.9	41.4
Minolta a*[5]	6.16	6.01	5.67
Minolta b*[5]	7.83	5.53	5.91
Japanese pass rate,[6] %	66.7	71.4	79.0

[1]HS1 (DPX48162; Promax) and HS2 (DPX46162; Promax) were obtained from HumaTech Inc. (Mesa, AZ).
[2]Carcass data were not analyzed statistically.
[3]Last rib carcass backfat thickness.
[4]pH was obtained from the LM muscle between the 10th and 11th rib using a portable pH meter (Model IQ 140 pH Meter, IQ Scientific Instruments Inc., Carlsbad, CA).
[5]Hunter L (luminescence), a (redness), and b (yellowness) values were obtained using a Minolta color recorder (MiniScan XE Plus, Hunter, Reston, VA).
[6]Pass rate of the LM to the Japanese market was determined by color, firmness, and texture [all 3 or greater on the NPPC (2000) scale of 1 to 5].

RESULTS

Experiment 1

Pigs fed the diet with 0.5% HS1 had greater (11%, $P < 0.05$) ADG than pigs in other treatments during phase 3 (Table 3). Pigs fed the diets with 0.5 and 1.0% HS1 had lower (12 and 13%, respectively, $P < 0.05$) ADFI than pigs fed the control diet during phase 5. Gain:feed ratio of the pigs fed the diet with 0.5% HS1 was greater ($P < 0.05$) than that of the control pigs during phase 3 and phase 5. However, there were no differences among treatments in ADG, ADFI, or G:F over the entire period (phases 1 to 5).

Experiment 2

Average daily gain was the same ($P > 0.05$) between the sexes until the end of phase 3. Barrows had greater ($P < 0.05$) ADG than the gilts during phases 4, 5, 6, and the entire feeding period (Table 4). Barrows also had greater ($P < 0.05$) ADFI than the gilts during phases 5, 6, and the entire feeding period. Gain:feed ratio of barrows was greater ($P < 0.05$) than that of gilts during phases 5, 6, and the entire feeding period.

Pigs fed the diets with 0.5% of HS1 and HS2 had greater ($P < 0.05$) ADG than pigs fed the control diet during phase 5 (40 and 25%, respectively) and during the entire feeding period (17 and 14%, respectively; Table 4). Average daily feed intake of pigs was the same among the treatments during each period as well as during the entire period. Gain:feed ratios of the pigs fed the diets with HS1 and HS2 were greater ($P < 0.05$) than those of control pigs during phase 5 and during the entire feeding period. Mortality of pigs fed the HS (0%) was lower ($P < 0.01$) than that of control pigs (3.1%).

At the end of feeding period, the percentages of pigs 115 kg and heavier were 40% (25 out of 62), 64% (41 out of 64), and 67% (43 out of 64) for the control, HS1, and HS2, respectively, and those pigs were slaughtered for the carcass measurement. The results of carcass measurement were not subjected to the statistical analysis, but the mean values are shown in Table 5 for descriptive purposes.

Experiment 3

Barrows had greater ($P < 0.05$) ADG than gilts during phase 5, greater ($P < 0.05$) ADFI during phase 5, and greater ($P < 0.05$) G:F during phases 1 and 5. No differences were obtained in ADG, ADFI, G:F, and mortality between barrows and gilts during the entire feeding period.

Pigs fed diets with 0.5% of HS3 had greater ($P < 0.05$) ADG than the pigs fed the diet with 0.5% of HS4 during phase 4 and the entire feeding period (Table 6). Pigs fed diets with 0.5% HS3 had greater ($P < 0.05$) ADFI than pigs fed the control diet and HS4-supplemented diet during phases 4, 5, and the entire feeding period. Gain:feed ratio of pigs fed the control diet and the HS3 diet was greater ($P < 0.05$) than for pigs fed the HS4 diet during phase 4 and the entire feeding period. There was no difference in mortality among pigs in each dietary treatment.

Table 6. Growth performance of pigs fed diets supplemented with HS3 and HS4 (Exp. 3)[1]

	Control		HS3		HS4					
Item	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	SEM	Trt[2]	Sex[3]	Trt × sex[4]
No. of pigs	4	4	4	4	4	4				
Initial BW, kg	7.25	7.25	7.24	7.25	7.25	7.26	0.26	NS	NS	NS
ADG, kg/d										
Phase[5] 1	0.183	0.178	0.218	0.208	0.196	0.194	0.008	NS	NS	NS
Phase 2	0.283	0.306	0.327	0.352	0.321	0.316	0.011	NS	NS	NS
Phase 3	0.412	0.470	0.425	0.483	0.423	0.457	0.015	NS	NS	NS
Phase 4	0.746	0.723	0.815	0.759	0.589	0.599	0.022	*	NS	NS
Phase 5	1.070	0.838	0.964	0.919	0.958	0.863	0.021	NS	*	NS
Phase 6	0.992	1.076	0.989	0.959	0.978	0.849	0.023	NS	NS	NS
Phase 1 to 6	0.731	0.685	0.750	0.718	0.634	0.613	0.014	*	NS	NS
ADFI, kg/d										
Phase 1	0.194	0.235	0.252	0.244	0.212	0.229	0.010	NS	NS	NS
Phase 2	0.466	0.553	0.506	0.531	0.495	0.499	0.013	NS	NS	NS
Phase 3	0.738	0.768	0.683	0.790	0.723	0.725	0.015	NS	NS	NS
Phase 4	1.728	1.614	1.828	1.825	1.662	1.664	0.028	*	NS	NS
Phase 5	2.812	2.473	2.819	2.842	2.504	2.525	0.048	**	NS	NS
Phase 6	3.185	2.827	3.225	3.069	3.120	2.734	0.054	NS	*	NS
Phase 1 to 6	1.776	1.637	1.832	1.835	1.679	1.657	0.023	*	NS	NS
G:F										
Phase 1	0.943	0.768	0.880	0.848	0.936	0.844	0.021	NS	**	NS
Phase 2	0.609	0.559	0.644	0.660	0.643	0.632	0.013	NS	NS	NS
Phase 3	0.558	0.611	0.627	0.611	0.583	0.628	0.015	NS	NS	NS
Phase 4	0.432	0.449	0.445	0.416	0.355	0.361	0.010	*	NS	NS
Phase 5	0.381	0.340	0.342	0.324	0.383	0.343	0.007	NS	*	NS
Phase 6	0.311	0.384	0.308	0.314	0.314	0.312	0.009	NS	NS	NS
Phase 1 to 6	0.411	0.418	0.409	0.391	0.378	0.370	0.005	*	NS	NS
Mortality, %										
Phase 1 to 6	6.25	12.5	9.38	9.38	0.00	9.38	1.47	NS	NS	NS

[1]HS3 (DPX4600; Promax) and HS4 (DPX5800; Promax) were obtained from HumaTech, Inc. (Mesa, AZ).
[2]Effect of treatment (Trt; *$P < 0.05$; **$P < 0.01$; NS: $P > 0.05$).
[3]Effect of sex (*$P < 0.05$; **$P < 0.01$; NS: $P > 0.05$).
[4]Effect of the interaction between treatment (Trt) and sex (NS: $P > 0.05$).
[5]Phase 1 for 7 d, phase 2 for 14 d, phase 3 for 14 d, phase 4 for 49 d, phase 5 for 48 d, and phase 6 for 17 d.

After the feeding period, the percentages of pigs 110 kg and heavier were 76% (45 out of 58), 91% (53 out of 58), and 64% (39 out of 61) for the pigs in the control, the HS3, and the HS4 groups, respectively, and those pigs were slaughtered for the carcass measurement. The results of carcass measurement were not subjected to the statistical analysis, but the mean values are shown in Table 7.

Experiment 4

Initial BW, ADG, and ADFI were the same ($P > 0.60$) between the control group (14.2, 0.429, and 0.656 kg, respectively) and the HS1 group (13.8, 0.466, and 0.633 kg, respectively) with a SEM of 0.4, 0.032, and 0.022 kg, respectively, during the 48-h period in the chamber. Initial ammonia concentrations in the chamber were 0 at the beginning of each 48-h period. The first 24-h period was considered an acclimation period. The average ammonia concentration during the last 24 h from the HS1 group was 11.65 ± 0.91 ppm and tended to be lower ($P = 0.067$) than that of the control group (14.22 ± 0.83 ppm). Hydrogen sulfide was not detectable during the collection period. Changes in aerial ammonia concentration during the last 24-h collection period from both treatments were modeled as quadratic regressions (Figure 1). Both quadratic and linear slopes and intercepts of the control and HS1 were different ($P < 0.001$), indicating that the beginning and ending levels of ammonia in the HS1 group were lower than those in the control group. However, slopes for the quadratic and linear changes were greater for the HS1 group than those for the control group. Aerial ammonia concentrations followed a diurnal cycle corresponding with pig activity.

Experiment 5

Initial pig BW, ADG, and ADFI during the 48-h period in the chamber were the same ($P = 0.705$, 0.777, and 0.813, respectively) among treatments. Initial BW were 25.0, 26.4, and 25.2 kg; the ADG were 0.535, 0.521, and 0.564 kg; and the ADFI were 0.876, 0.832, and 0.882 kg for the control, HS3, and HS4 groups, respectively. Initial ammonia concentrations in the chamber were 0 at the beginning of each 48-h period. The first 24-h period was considered an acclimation period. The average ammonia concentration during the last 24 h

Table 7. Carcass characteristics of pigs fed diets supplemented with HS3 and HS4 (Exp. 3)[1,2]

Item	Control	HS3	HS4
No. of carcasses	45	53	39
Carcass			
Hot carcass wt, kg	92.0	92.9	87.7
Backfat thickness,[3] mm	17.6	18.1	17.6
Lean percent, %	53.4	53.1	52.8
LM			
LM depth	59.4	59.3	55.2
pH of the LM[4]	5.76	5.83	5.84
Minolta L[5]	40.2	39.0	40.4
Minolta a*[5]	5.48	5.11	7.02
Minolta b*[5]	7.02	6.41	9.10
Japanese pass rate,[6] %	73.3	81.8	80.8

[1]HS3 (DPX4600; Promax) and HS4 (DPX5800; Promax) were obtained from HumaTech Inc. (Mesa, AZ).
[2]Carcass data were not analyzed statistically.
[3]Last rib carcass backfat thickness.
[4]The pH was obtained from the LM muscle between the 10th and 11th rib using a portable pH meter (Model IQ 140 pH Meter, IQ Scientific Instruments Inc., Carlsbad, CA).
[5]Hunter L (luminescence), a (redness), and b (yellowness) values were obtained using a Minolta color recorder (MiniScan XE Plus, Hunter, Reston, VA).
[6]Pass rate of the LM to the Japanese market that is determined by color [3 or greater on the NPPC (2000) color scale of 1 to 5], firmness [3 or greater on the Seaboard (Seaboard Foods Inc., Guyman, OK) scale of 1 to 5], and texture [3 or greater on the Seaboard scale of 1 to 5].

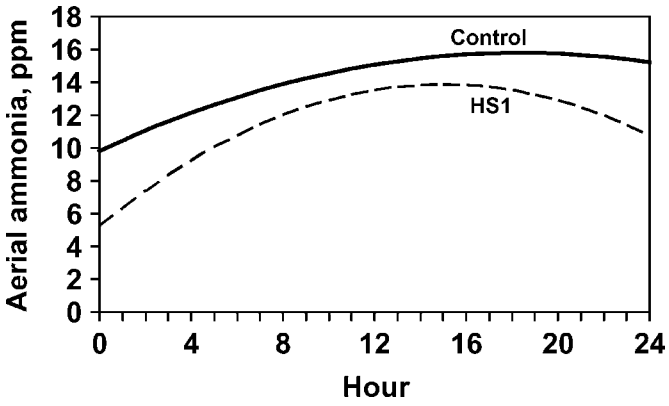

Figure 1. Concentration of aerial ammonia in the environmental chamber produced from the manure of pigs fed the control diet or the HS1-supplemented diet during the last 24 h of a 48-h collection period (indicated as 0 to 24 h on the x-axis). The HS1 was DPX48162 (Promax, HumaTech Inc., Mesa, AZ) and was supplemented at 0.5% of the complete diets. The changes in aerial ammonia concentrations were: $[\text{Ammonia}]_{\text{control}} = 9.8187 + (0.6513 \times \text{hour}) - (0.0178 \times \text{hour}^2)$ ($P < 0.001$; $R^2 = 0.92$) and $[\text{Ammonia}]_{\text{HS1}} = 5.2462 + (1.1478 \times \text{hour}) - (0.0383 \times \text{hour})$ ($P < 0.001$; $R^2 = 0.92$). Control vs. HS1 differed in the slopes ($P = 0.001$) and the intercepts ($P < 0.001$) of both the quadratic and linear responses.

from the HS4 group was 13.93 ± 0.88 ppm and tended to be smaller ($P = 0.054$) than that from the control group (16.70 ± 0.86 ppm), whereas there were no differences between the control and HS3 (16.17 ± 0.89 ppm) and between the HS3 and HS4. Hydrogen sulfide was not detectable during the collection period and was thus excluded in the data analysis. Changes of aerial ammonia during the last 24 h of collection period from all treatments were modeled as linear regressions (Figure 2). The slopes of the control and the HS3 groups were different ($P = 0.019$), but the intercepts were the same ($P = 0.233$). The rate of ammonia production from the HS3 group was smaller ($P = 0.019$) than that from the control group. The slopes from the control and the HS4 groups were the same ($P = 0.171$), but the intercept of the HS4 group was smaller than that of the control group ($P = 0.008$). The rates of ammonia production from the control and the HS4 groups were the same, but the ammonia level at the beginning of the last 24-h period in the HS4 group was smaller ($P = 0.008$) than that of the control group.

DISCUSSION

Use of HS as a supplement in pig diets is a rather novel approach. Four HS were tested in this study to characterize their effects as feed additives. This study may indicate a potential improvement in LM meat quality by humic substance supplementation. Use of HS in pig diets tended to reduce ammonia emission from pig manure. However, ADG and G:F were improved only by 2 HS, HS1 and HS2. Major differences among 4 HS used in this study were the absolute and relative contents of fulvic acid and humic acid. These differences may have contributed to different pig growth responses.

Humic substances contain minute amounts of several minerals including iron, manganese, copper, and zinc (Aiken et al., 1985). Among the minerals in HS, iron is most abundant. Iron content in the same HS used in this study was 8,700 ppm, and the relative bioavailability of the iron in HS has been reported as 71% of iron sulfate (Kim et al., 2004). Bioavailabilities of other minerals in our test materials are not known for pigs. Supplementation of HS at 0.5% of the diets contributed 31 ppm bioavailable iron that would provide additional 60 to 70% of the daily iron requirement for growing-finishing pigs. However, considering that calcium sources contain iron (0.6 to 1.0%), which can provide most of iron needs for growing-finishing pigs (NRC, 1998), economic benefits of HS as an iron supplement would be minimal.

One of the contributions of the HS from this study seems to be a potential increase in pass rate of LM for the value-added Japanese market. The average pass rate of LM to the Japanese market from the pigs fed different HS was 78.3%, and the average rate from the control group was 70.0%. A clear economic benefit would be realized for this potential improvement, although further investigation is needed to substantiate this observation.

The active components in HS that can potentially affect growth, G:F, and ammonia emission from pig

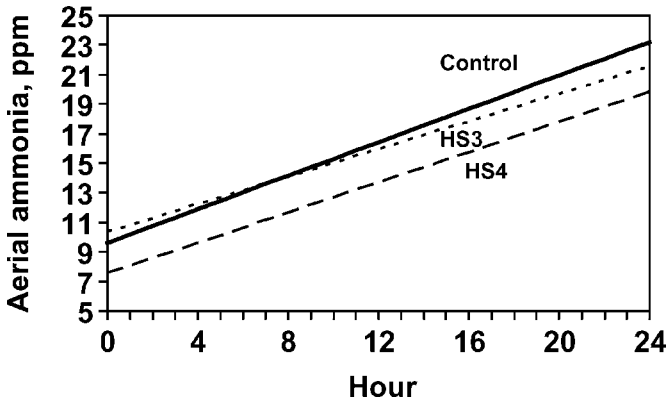

Figure 2. Concentration of aerial ammonia in the environmental chamber produced from the manure of pigs fed the control diet, the HS3-supplemented diet, or the HS4-supplemented diet during the last 24 h of a 48-h collection period (indicated as 0 to 24 h on the x-axis). The HS3 and HS4 were DPX4600 and DPX5800 (Promax, HumaTech Inc., Mesa, AZ) and were supplemented at 0.5% of the complete diets. The changes in aerial ammonia concentrations were: $[\text{Ammonia}]_{\text{control}} = (0.5662 \times \text{hour}) + 9.6196$ ($P < 0.001$; $R^2 = 0.92$), $[\text{Ammonia}]_{\text{HS3}} = (0.4677 \times \text{hour}) + 10.3222$ ($P < 0.001$; $R^2 = 0.95$), and $[\text{Ammonia}]_{\text{HS4}} = (0.5104 \times \text{hour}) + 7.5539$ ($P < 0.001$; $R^2 = 0.96$). The slopes differed ($P = 0.019$) between the control and the HS3 treatments, whereas the intercepts were not different ($P = 0.233$). The slopes did not differ ($P = 0.171$) between the control and the HS4 diets, whereas the intercepts were different ($P = 0.008$).

manure have not been clearly identified. Possible mechanisms have not yet been identified. About 35 to 55% of the HS used in this study were composed of fulvic and humic acids, and about 23 to 42% was composed of ash, whereas less than 4 and 0.1% of the composition was protein and fat, respectively (Table 1). Contents of protein and fat were consistent among the HS sources, and thus they could not be causing the different responses of the pigs observed here. Considering the amounts of fulvic acid and humic acid and their compositional differences in various HS, it can be speculated that the different fulvic and humic acid contents among various HS caused the different pig responses. The HS3 contained the greatest amount of fulvic acid but the smallest amount of humic acid than other HS, whereas the HS4 contained the smallest amount of fulvic acid but the greatest amount of humic acid than other HS. The fulvic and humic acid contents in HS1 and HS2 were intermediates between the contents of HS3 and HS4. At this moment, it is not clear if the different fulvic to humic acid ratios were the major factor that affected growth performance of pigs. The properties of fulvic acid and humic acid are relatively well characterized by plant and soil scientists (Choudhry, 1984; Aiken et al., 1985), but the effect of individual humic or fulvic acids on animal growth has not been characterized. Further investigation is needed to directly evaluate the effect of different fulvic and humic acid contents on pig responses.

Use of HS (HS1, HS3, and HS4) in pig diets reduced ammonia emission from pig manure by 3 to 18%. The typical levels of aerial ammonia in a pig farm facility range between 5 to 35 ppm (Zahn, 1997). Various government agencies suggest threshold limit values of ammonia concentration in the workplace to maintain worker health as an average of 25 ppm for a normal 8-h workday (OSHA, 1989; ACGIH, 1995). This study shows that dietary HS have a potential to reduce aerial ammonia concentrations, which may have beneficial effects on human health. The benefits of reduction of ammonia production are not limited to human well-being. Aerial ammonia levels at greater than 50 ppm reduced the growth of pigs (Drummond et al., 1978; Gustin et al., 1994), and thus the reduction of ammonia production may also be beneficial to the growth of pigs.

Humic substances were shown to inhibit urease activity. Vaughan and Ord (1991) demonstrated that the activity of a purified urease was inhibited by humic and fulvic acids obtained from an agricultural soil. Inhibition of urease was greater in acid pH. Direct application of HS to manure also reduced beef feedlot ammonia emissions (Shi et al., 2001).

This study investigated the potential benefits of supplementing HS in pig diets by comparing 4 sources of HS. Certain HS may improve growth performance, reduce ammonia emission, and potentially improve LM quality. However, the mechanisms of action related to potential improvements in growth performance and pork quality are not understood, and further investigation is needed.

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