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Effects of supplemental humic substances on growth performance, blood characteristics and meat quality in finishing pigs

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Abstract

A total of forty-eight finishing pigs were used to determine the effects of humic substances (HS) on growth performance, blood characteristics, and meat quality. The finishing pigs were assigned randomly by weight to three treatments. The dietary treatments included: 1) Control (CON; basal diet), 2) HS1 (basal diet + 5% humic substances) and 3) HS2 (basal diet + 10% humic substances). Results of the whole experimental period showed that addition of 10% HS to the diet, significantly increased average daily gain (ADG) and gain/feed (G:F) ($P < 0.05$). At the end of the experiment, the relative lymphocyte counts (% of total white blood cells) of pigs fed HS2 diet were higher ($P < 0.05$) than that of pigs fed CON diet. The Minolta color parameter a* of pigs fed HS2 was similar to that of pigs fed HS1, however, it was higher ($P < 0.05$) than that of pigs fed CON diet. The inclusion of either 5% or 10% HS significantly decreased backfat thickness ($P < 0.05$). The marbling score was increased significantly ($P < 0.05$) when diets were supplemented with HS at a level of 10%. The results of this study suggest that HS might be utilized as a feed additive in the diet. It could improve growth performance, relative lymphocyte counts and meat quality.

Keywords: Humic substances; Growth performance; Blood characteristics; Meat quality; Finishing pigs

1. Introduction

Humic substances (HS) are organic residues originating from decaying organic matter, and specifically include humic acid, fulvic acid and humin as principal constituents. The HS have been used as an antidiarrheal, analgesic, immunostimulatory, and antimicrobial agents in veterinary practices in Europe (Thiel et al., 1977; Huck et al., 1991).

Farmers utilize HS to accelerate seed germination and improve rhizome growth. These materials stimulate oxygen transport, accelerate respiration, and promote the efficient utilization of nutrients by the plant (Visser, 1987). These observations have prompted scientists to assess the specific properties of humates and their possible benefits in the improvement of health and well being in animals.

In recent years, it has been shown that humates added to the feed of poultry promote growth (Parks et al., 1996; Karaoglu et al., 2004). However, supplementation as a feed additive in pig diets has not been well reported. Therefore, this research was carried out to determine the

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effect of HS on growth performance, blood characteristics and meat quality in finishing pigs.

2. Materials and methods

2.1. Humic substances

The HS used in the current experiment were generated from seams and the main composition of HS consist of 18.8% moisture, 39.4% humic acid, 27.8% fulvic acid, 3.6% crude protein, 0.05% crude fat, 1.9% crude ash and 8.45% others. The humic and fulvic acid were determined according to the method suggested by Hayes (1985) whereas others were determined in accordance with the methods recommended by AOAC (1990).

2.2. Design, animal and diet

A total of 48 crossbred (Landrace × Yorkshire × Duroc) pigs with an average initial body weight (BW) 56.5 ± 0.28 kg (105 d of age) were used in the current experiment. At the beginning of experiment, pigs were allotted to one of three dietary treatments on the basis of initial BW. There were four pigs per pen and four pens per treatment. The dietary treatments include: 1) CON (basal diet), 2) HS1 (basal diet + 5% humic substances) and 3) HS2 (basal diet + 10% humic substances). The experimental period was 8 weeks. The pigs were allowed *ad libitum* access to feed and water throughout the experiment. All of the experimental diets (Table 1) were formulated to meet or exceed the requirements recommended of the NRC (1998).

2.3. Sampling and measurements

Body weight and feed intake were measured at the end of 4 and 8 weeks in order to determine the average daily gain (ADG), average daily feed intake (ADFI), and gain/feed (G/F). At the beginning of the experiment, two pigs were randomly selected from each pen, and blood samples were taken by jugular venipuncture. The same pigs were again bled at the week 8. The concentrations of red blood cell (RBC) counts, white blood cell (WBC) counts and relative lymphocyte counts (% of total WBC counts) in the whole blood were evaluated. All the blood parameters (RBC, WBC and lymphocyte) were evaluated utilizing an automatic blood analyzer (ADVIA 120, Bayer, USA).

At the end of the experiment, the pigs were transported to the abattoir for slaughter. The carcasses were placed in a conventional chiller at 4 °C. After 24 h chill period, carcasses were fabricated into primal cuts. Meat samples, which included lean and fat, were taken via perpendicular cut loins into 2-cm-thick chop beginning from the 10th and 11th ribs region. Backfat thickness was determined by measuring midline fat thickness. The pH of *longissimus* muscle (LM) was measured in 24 H post-mortem with an insertion glass electrode (Radiometer, Lyon, France) connected to a pH-meter (NWKbinar pH, K-21, Landsberg, Germany). The electrode was calibrated at 20 °C in buffers at pH value of 4.00 and 7.00.

Surface LM color (Minolta L*, a*, b*) was measured in triplicate on a freshly-cut surface with a Minolta Chromameter (Minolta CR 301, Tokyo, Japan). The proportion of LM acceptable for Pork Composition and Quality Assessment Procedures (NPPC, 2000) was determined via the selection of LM with acceptable color, firmness, and marbling (all measures 3 or greater, based on a scale of 1 to 5, NPPC, 2000).

2.4. Statistical analysis

In this experiment, all statistical analyses were conducted in accordance with a randomized complete block design, using the GLM procedures of SAS software package (1996). The model included the effects of block (replication) and treatment. Pen was considered as the experimental unit. Variability in the data was expressed as the standard error (SEM) of the mean and the selected level of significance was 0.05.

3. Results

3.1. Growth performance of pig

Average daily feed intake (ADFI) was unaffected ($P > 0.05$) by dietary treatments during the first 4 weeks,

Table 1
Formula and chemical composition of experimental diet (as-fed basis)

Ingredients (%)	CON	HS1	HS2
Corn	53.49	48.52	38.98
Soybean meal (CP 48%)	18.50	18.00	17.30
Wheat bran	10.00	9.50	12.00
Corn gluten meal	8.30	8.30	9.00
Soy oil	6.35	7.30	9.40
Humic substances	0.00	5.00	10.00
TCP	1.89	1.89	1.89
Limestone	0.14	0.14	0.14
L-Lysine HCl	0.26	0.26	0.26
DL-Met	0.02	0.04	0.05
Vitamin premix [a]	0.20	0.20	0.20
Mineral premix [a]	0.20	0.20	0.20
Salt	0.45	0.45	0.38
ZnO	0.10	0.10	0.10
Choline-Cl	0.10	0.10	0.10
Chemical composition [b]			
ME (MJ/kg)	14.03	14.01	13.98
Crude protein (%)	19.06	19.03	19.01
Lysine (%)	1.01	1.00	0.98
Methoinine (%)	0.38	0.38	0.38
Calcium (%)	0.79	0.79	0.78
Phosphorus (%)	0.67	0.65	0.64

[a] Supplied per kg diet: vitamin A, 11,025 IU; vitamin D_3, 11,103 IU; vitamin E, 44 IU; vitamin K (menadione bisulfate complex), 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; d-pantothenic acid (d-calcium pantothenate), 29 mg; choline,166 mg; vitamin B_{12}, 33 μg; Cu,16 mg; Fe, 165 mg; Zn, 165 mg; Mn, 12 mg; I, 0.3 mg; Co, 1.0 mg and Se, 0.3 mg.
[b] Calculated values.

Table 2
Growth performance of pigs fed diets supplemented with humic substances

Items	CON[a]	HS1[a]	HS2[a]	SEM
No of pigs	16	16	16	-
Initial body weight (kg)	56.5	56.8	56.4	-
0-4 weeks				
ADG (kg)[b]	0.631	0.613	0.640	0.030
ADFI (kg)[c]	1.702	1.724	1.634	0.054
G/F[d]	0.371	0.356	0.392	0.024
4-8 weeks				
ADG (kg)	0.723	0.805	0.878	0.029
ADFI (kg)	2.608	2.769	2.523	0.066
G/F	0.277	0.291	0.348	0.013
0-8 weeks				
ADG (kg)	0.677[e]	0.709[e]	0.759[f]	0.016
ADFI (kg)	2.115	2.247	2.079	0.060
G/F	0.321[e]	0.315[e]	0.365[f]	0.018

[a] Abbreviations: CON, basal diet; HS1, basal diet added 5% humic substances; HS2, basal diet added 10% humic substances.
[b] Average daily gain (kg).
[c] Average daily feed intake (kg).
[d] Weight gain/feed intake.
[e,f] Means in the same row with different superscripts differ ($P<0.05$).

the second 4 weeks, or the entire 8 weeks test period (Table 2). Pigs fed diets with HS2 diet had greater ($P<0.05$) ADG, G/F ratio than pigs fed HS1 and control diets throughout the feeding period.

Table 3
Blood characteristics of pigs fed diets supplemented with humic substances

Items	CON[a]	HS1[a]	HS2[a]	SEM
RBC[b] (10^6, No./mm^3)				
0 week	5.91	6.01	5.74	0.19
8 week	6.59	6.00	6.14	0.25
Difference (Wk 8-Wk 0)	0.68	-0.01	0.40	0.23
WBC[c] (10^4, No./mm^3)				
0 week	18.00	15.91	15.95	0.18
8 week	25.91	18.28	19.83	0.36
Difference (Wk 8-Wk 0)	7.90	2.37	3.88	3.61
Lymphocyte[d] (%)				
0 week	49.0	50.8	45.4	4.36
8 week	52.4	56.6	70.4	6.01
Difference (Wk 8-Wk 0)	3.4[e]	9.8[e,f]	25.0[f]	2.59

[a] Abbreviations: CON, basal diet; HS1, basal diet added 5% humic substances; HS2, basal diet added 10% humic substances.
[b] Red blood cell.
[c] White blood cell.
[d] Values are presented as percentage of total white blood cell counts.
[e,f] Means in the same row with different superscripts differ ($P<0.05$).

Table 4
Meat quality of pigs fed diets supplemented with humic substances

Items	CON[a]	HS1[a]	HS2[a]	SEM
Minolta L*[b]	40.42	38.85	40.52	2.036
Minolta a*[b]	8.04[e]	9.98[f]	10.95[f]	0.316
Minolta b*[b]	3.36	3.60	4.60	0.249
Backfat thickness (cm)	2.98[f]	2.50[e]	2.45[e]	0.101
pH	5.59	5.61	5.60	0.040
Firmness[c]	1.78	2.42	2.06	0.316
Marbling[c]	1.40[e]	2.16[e,f]	2.35[f]	0.134
Color[c]	2.25	2.33	2.67	0.240

[a] Abbreviations: CON, basal diet; HS1, basal diet added 5% humic substances; HS2, basal diet added 10% humic substances.
[b] (Minolta L*, a*, b*) were measured in triplicate on a freshly-cut surface using a Minolta Chromameter (Minolta CR 301, Tokyo, Japan).
[c] According to the NPPC (2000) that is determined by color (3 or greater on the color scale of 1 to 5), Firmness (3 or greater on the scale of 1 to 5), Marbling (3 or greater on the scale of 1 to 5).
[e, f] Means in the same row with different superscripts differ ($P<0.05$).

3.2. Blood characteristics

No significant differences were observed with regard to the RBC and WBC throughout experimental period (Table 3). The difference in relative lymphocyte counts between 0 and 8 weeks was higher in HS2 treatment than that in CON treatment ($P<0.05$).

3.3. Meat quality

The meat quality data are shown in Table 4. The Minolta color parameter a* for pigs fed HS 10% was similar to that for pigs fed HS1, however, it was higher ($P<0.05$) than that observed in pigs fed CON diet. The inclusion of HS decreased backfat thickness significantly ($P<0.05$). The marbling scores of pigs was significantly increased ($P<0.05$) when diets were supplemented with HS at a level of 10%.

4. Discussion

4.1. Growth performance of pig

Yasar et al. (2002) concluded that humic acids indeced an increase in weight gain in rats and they suggested that the improved weight gain was associated with increased feed intake and improved gain:feed ratio. Stepchenko et al. (1991) demonstrated that after the feeding diets containing 0.25% HS preparation from the age of 22 days, broilers evidenced an improvement in ADG from 5% to 7%. The HS can form a protective film on the mucus epithelium of the gastrointestinal tract against infections and toxins, thus ensuring an improved utilization of nutrients in animal feed (Islam et al., 2005).

Huck et al. (1991) reported that HS may influence, in particular, the metabolism of proteins and carbohydrates in microbes. This results in a direct devastation of bacterial cells or viral particles, which should result in improved growth performance. HS include fulvic and humic acids, which direct inhibition of bacterial and virus growth, thus reducing mycotoxin levels. They improve gut health, nutrient absorption and nutritional status in animals. In the current study, the improvement of growth was only observed during the entirety of the experimental period, whereas no significant effects were observed in each of the 4-week periods. This indicated that the effects of HS may also be associated with the administration period, and long-term administration may exert a more prominent effect. However, some inconsistent results have also been reported by other researchers, such as Rath et al. (2006), who suggested that humic acid exerted a negative effect on the growth performance of broiler chickens. The Inconsistent results observed in different studies may be attributable principally to the composition of different HS preparations, and addition levels, as well as the different animal species and ages used in different studies.

4.2. Blood characteristics

Lymphocytes play an important and integral role in the body's defenses. They function in the development of immunity. Therefore, the increased relative lymphocyte counts observed in the current study showed that HS may exert a beneficial effect on immune systems of animals. The action mechanism in humic substances is associated with their capability to form complex saccharides in the body, which function as modulators of intercellular interaction. These maintain the balance of the immune system activity, and prevent potential inadequate responses (Riede et al., 1991). Humic acid stimulates the resistance forces of the body, and result in an increase in the phagocytic activity. Terratol (2002) also previously proposed that humic acid may stimulate the production of glycoproteins, which can regulate the immune system via the maintenance of the balance of killer and T cells. However, it should also be noted that the chronic intake of humic acids has been previously associated with human diseases (Rath et al., 2006), therefore, the safety of HS application should be also evaluated in further study.

4.3. Meat quality

According to current data, the increase redness (Minolta a*) and marbling and reduced backfat thickness were observed. Similarly, Ji et al. (2006) also reported a greater redness (7.02 vs. 5.48) when pigs fed HS which included higher levels of humic acid (54.6%). However, when HS included lower levels of humic acid level (12.2%), such improvement was not observed. It appears that the humic acid may be associated with meat color, however, the precise mechanism underlying this effect currently remains unclear. Humic substances contain minute quantities of several minerals, including iron, manganese and copper (Aiken et al., 1985). Berg (2001) reported that supranutritional levels of Cu may influence pork color. This may be another reason for changes in meat color. In addition, the reduced backfat thickness and improved marbling value showed that HS may influence protein and lipid distribution. Accelerated myoglobin synthesis and fat deposit in LM can also improve the meat color (Xu and Feng, 1998). Ji et al. (2006) also reported a higher pass rate of LM to the Japanese market. This result indicated that HS supplementation could improve the appearance of meat, which is partially consistent with the result of our current study.

5. Conclusions

According to the results of current study, it is concluded that dietary supplementation with humic substances can improve growth performance, lymphocyte concentration and meat quality in finishing pigs.

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