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The addition of humic substances extracted from vermicompost enhances the growth performance and the antioxidant status of weaning pigs

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Abstract

The aim of the study was to assess the effect of humic substances (HS) extracted from vermicompost on the growth performance, fecal score, bone ash content and antioxidant status in muscles and blood serum of weaned pigs from 1-42 days postweaning. Two-hundred 22-day weaned pigs were assigned to four treatments: 1= Positive control diet with colistin (PC), 2= Negative control diet without antibiotic or HS (NC), 3 and 4= Diets with 2 500 and 5 000 ppm of HS, respectively. Growth performance, fecal consistency, dry matter and ashes of metatarsus and the 1,1-diphenyl-2-picryl-hydrazyl radical scavenging activity, iron reducing ability and thiobarbituric acid reactive substances in muscles and blood were registered. Results were subjected to ANOVA and regression analysis. Linear increasing responses ($P < 0.05$) were found in the body weight at 42 days postweaning, the ADG from 1-7, 8-21, 22-42 and 1-42 and the gain:feed ratio from 1-7, 22-42 and 1-42 days postweaning, whilst the fecal score decreased linearly ($P < 0.01$) from 1-7, 8-21 and 1-42 days postweaning due to the increasing dietary HS concentration. The antioxidant potential of 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity ($P < 0.05$) in loin muscle and blood serum at three and seven days, and in fillet muscle at seven days postweaning increased linearly due to the increasing dietary HS concentration. It is concluded that the addition of HS improved the growth performance from 1-42 days postweaning and the antioxidant status in muscles and blood serum of weaned pigs at three and seven days postweaning.

Keywords: Weaned pigs; Colistin; Humic substances; Performance; Antioxidant status.

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Study contribution

Humic substances (HS) have been studied as growth promoters and health enhancers in pigs with encouraging results in recent years. The primary sources of HS are lignite and leonardite mines; however, HS can also be recovered from organic waste that has been humified, such as compost and vermicompost created with animal manure. The current study was conducted to assess the incorporation of various levels of HS recovered from vermicompost in weaned pig meals from 1 to 42 days postweaning. The main benefits noticed were higher body weight and weight gain, lower feed efficiency and fecal score, and higher antioxidant status in muscles and blood serum from pigs fed HS after weaning. The findings suggest that HS extracted from vermicompost enhanced growth and antioxidant status in a manner similar to HS obtained from leonardite and lignite when added to weaned pig feeds.

Introduction

Weaning is one of the most stressful events in a pig's life, affecting productivity and predisposing to digestive disorders in the short and medium term. Piglets face dietary, social, environmental, or infectious stressors, leading to profound negative changes in the digestive physiology, immune response, behavior, and growth performance.[1, 2, 3] In response to this, growth promoter antibiotics (GPA) are added in feeds, which help to control the growth of pathogenic bacteria and to reduce the risk of diseases. However, it has been stated that prolonged use of GPA can cause antimicrobial resistance to antibiotics in pathogenic bacteria, putting animal and human health at risk.[4, 5]

Alternatives to reduce the use of GPA in animals while maintaining efficient production and obtaining high-quality, low-cost foods are being sought.[2, 6] Humic substances (HS) are potential alternatives as growth promoters as they have been shown to improve production efficiency and health in animals.[7, 8, 9] The most concentrated commercial sources of HS are leonardite and lignite, which are obtained from mines, and are extensively used to improve crop yields in agriculture; to a lesser extent, they are used as growth promoters in animals.[10, 11, 12] The primary components of HS are humic acids (HA), fulvic acids (FA), and humins; these are organic constituents of soil, rivers, lakes, and oceans.[13, 14] The side chains of HS contain functional groups that confer coloidal, spectral, electrochemistry, and ion exchange properties.[10, 14]

In weaning pigs supplemented with HS from leonardite, improved growth performance and mineral status, reduced serum biomarkers, indicative of oxidative stress, and decreased incidence of diarrhea, have been reported.[1, 2, 15] For more than three decades, HS has been used to treat gastrointestinal disorders and has been recommended to be used orally in horses, dogs, pigs, and birds for the treatment of diarrhea, dyspepsia, and acute poisoning.[7] HS also has been linked to improved mineral utilization in plants and animals.[9, 10] Increased ash and Ca content in tibia bone in HS-fed broilers,[16–18] as well as, increased percentage, thickness, and hardness of eggshells in HS-supplemented laying hens and pheasants have been reported.[19–21] Improved femur mineralization and increased milk Ca and Fe content have also been reported in HS-added rabbits[22] and cows.[23]

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In HS-fed weaned pigs, increased serum alkaline phosphatase, Ca, P, and Mg have been found.[1, 15] However, in pigs, the effects of HS from any source on bone mineralization have not been investigated. HS from vermicompost caused higher ashes retention, and tibia mineralization in broiler chickens.[9, 12] Until now, the possibility that HS from vermicompost improve mineral assimilation, and hence, bone mineralization in piglets at weaning has not been evaluated.

Regarding the antioxidant properties of HS, in weaned pigs added with sodium humate, increased total antioxidant capacity and reduced malondialdehyde, a marker of oxidative stress, were observed.[2] In piglets born from HS-added sows, reduced TBA products concentration, lipid hydroperoxides, and protein carbonyl groups and increased superoxide dismutase and catalase activity were found.[24] In weaning pigs fed a commercial source of HS and challenged with an *Escherichia (E.) coli* LPS, higher glutathione concentrations and lower glutathione peroxidase activities were also reported.[25] In pigs, the possibility that HS from vermicompost may improve the antioxidant response in piglets experiencing the weaning stress has not been evaluated either.

In recent reports, HS extracted from vermicompost has been evaluated in broiler chickens, added in the drinking water or feed, with promising results in the productive parameters and health.[12, 26] HS extracted from vermicompost are still in an early humification process and are considered immature, compared to aged HS sources like lignite and leonardite;[26] however, the results in broiler chickens supplemented with HS from vermicompost indicate similar effects on productive performance and health as those seen with HS from other sources, mainly lignite and leonardite. In pigs, the supplementation with HS extracted from vermicompost has not been evaluated as an option to reduce the use of GPA. Therefore, the current study aimed to assess the effect of humic substances (HS) extracted from vermicompost on the growth performance, fecal score, bone ash content and antioxidant status in muscles and blood serum of weaned pigs from 1–42 days postweaning.

Materials and methods

Ethical statement

This study was reviewed and approved by the Institutional Subcommittee for the Care and Use of Experimental Animals of the Universidad Nacional Autónoma de México, protocol number: SICUAE.DC-2021/1-1.

Extraction of humic substances

The HS were extracted and isolated from a vermicompost prepared with sheep manure. The concentrations of HA (47.1 %), FA (29.6 %) and ashes (23.2 %), as well as the functional groups, elemental analysis, crystal types, chemical properties and the flat structures of HS with aromaticity from the vermicompost were previously published.[26, 27] In brief, sheep manure was pre-decomposed for two weeks before being inoculated with red Californian earthworm and the vermicompost was harvested three months later. Prior to extraction, the vermicompost was dried at room temperature and passed through a metal mesh to homogenize the particle size.

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The extraction and isolation of HS was carried out in three phases as described by Stevenson.[28] In the first phase, 100 kg of dried vermicompost were deposited in a container and mixed with 400 L of sodium hydroxide (NaOH, 0.5 M), the mixture was homogenized, and allowed to stand for 24 hours. To separate the solid and liquid fractions, the mixture was decanted. The liquid fraction was collected in a plastic container. In the second phase, 300 L of 0.1 M NaOH was added to the remaining solid fraction and the same procedures used in the first phase were repeated.

The remaining solid fraction was homogenized with 100 L of water in the third phase, preceded by the same procedures as before. The three extracted liquid fractions were mixed and homogenized before being placed in plastic-covered drying troughs inside a natural-ventilated greenhouse. In each trough, 50 L of the extract were placed and allowed to dry at room temperature for seven days. After that, the solids were collected and dried for 48 hours at 55°C in a forced air oven (Shel Lab, Cornelius, OR, USA) before grinding it in a Thomas Willey mill with a 0.1 mM diameter screen.

Animals, treatments and management

Two hundred piglets ([Large white×Landrace]×Large white) weaned at 22 days of age with an average body weight of 5.58 ±0.69 kg, were assigned to four treatments based on litter of origin, sex, and weaning weight in a randomized complete block design. The treatments were: 1= positive control (PC) basal diet containing colistin at a dose of 40 ppm, 2= negative control (NC) diet without GPA, 3= diet without GPA and added with 2 500 ppm of HS, and 4) diet without GPA and added with 5 000 ppm of HS. Each treatment had ten replicate pens, each with five piglets at the beginning for a total of 50 piglets per treatment. At weaning, piglets were housed in a closed concrete room with controlled temperature (28 to 30°C during the first two weeks) via a heating system for the next three postweaning weeks. The weaning room had raised pens in slatted floor at 38 cm height, and gridded 115 cm wide × 150 cm long floor, for an effective surface area of 1.7 m^2. A nipple drinker and a hopper type feeder (with six holes) were provided in each pen.

Piglets had free access to water. Each experimental unit received three daily feed rations, with an average of 200 grams per ration initially, which was increased or decreased based on daily consumption per pen. The first week a Phase 1, and the following two weeks, a Phase 2 diet were offered (Table 1). At 22 days postweaning, piglets were moved to concrete floor pens, and were kept there until 42 days postweaning. The building was partially open, with curtains to isolate from the external environment; each pen was provided with an automatic feeder and drinker. A phase 3 diet was offered (Table 1).

At weaning, piglets were weighed individually, and then every seven days, until the end of the trial to calculate the average daily gain (ADG). Feed offered and rejected was recorded weekly and average daily feed intake (ADFI) was estimated. The gain:feed ratio (G:F) was calculated by dividing the ADG by the ADFI. The fecal consistency score (FS) of each pen was evaluated in the mornings from day 1 to 42, based on a visual assessment,[29] using a scale from 0 to 3, where: 0 = feces with normal consistency, 1 = pasty feces; 2 = semi-liquid feces, and 3 = very liquid feces. Before scoring, the floor under the raised pens was washed with water to

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Table 1. Experimental diets

Item, kg	Phase 1	Phase 2	Phase 3
Yellow corn	393.90	467.00	618.10
Oat	50.00	40.00	0.00
Soybean meal, 44 %	120.00	200.00	306.70
Isolated soy protein	76.30	48.50	0.00
Fish meal	50.00	0.00	0.00
Dried whey	246.90	164.60	0.00
Corn oil	33.50	42.60	37.40
Limestome	4.30	6.10	8.20
Dicalciun phosphate	8.70	13.30	13.50
L-lysine·HCl, 78 %	4.10	5.00	4.60
L-threonine, 98 %	1.20	1.30	1.20
DL-methionine, 98 %	1.80	1.90	1.30
L-tryptophane, 98 %	0.30	0.20	0.10
L-valine, 98 %	0.20	0.60	0.20
Salt	5.00	5.00	5.00
Vitamins* and mineral** premix	2.00	2.00	2.00
Choline chloride	1.00	0.80	0.80
Colistine	1.00	1.00	1.00
Calculated nutrient composition			
Metabolizable energy, kcal/kg	3 400	3 400	3 350
Crude protein %	22.5	20.2	19.5
Digestible lysine %	1.50	1.35	1.23
Digestible threonine	0.88	0.79	0.73
Digestible sulfur aminoacids %	0.82	0.74	0.68
Digestible tryptophane %	0.25	0.22	0.20
Digestible valine %	0.95	0.86	0.78
Calcium %	0.85	0.80	0.60
Digestible phosphorus %	0.54	0.45	0.35

* Each kilogramm of feed provided: vitamin A, 4 250 IU; vitamin D3, 800 IU; vitamin E, 32 IU; vitamin K3, 1.5 mg; biotin, 120 µg; cyanocobalamin, 16 µg; choline, 250 mg; folic acid, 800 µg; niacin, 15 mg; pantothenic acid, 13 mg; pyridoxine, 2.5 mg; riboflavin, 5 mg; thiamine, 1.25 mg.
** Each kilogramm of feed provided: Zn, 120 mg; Fe, 100 mg; I, 0.80 mg; Co, 0.60 mg; Mn, 4 mg; Se, 0.25 mg; Cu, 14 mg.

remove overnight feces. The FS was immediately verified in the feces within the floor area of each pen; the evaluation lasted one hour to ensure enough feces in each pens to facilitate the FS. The scoring was carried out by the same person throughout the experiment. The daily score per pen was averaged each week and for the total experimental period.

Slaughtering and sampling

10 piglets at weaning were selected, and at three and seven days postweaning, one piglet per pen, were selected, slaughtered and sampled. Previous to the slaughtering, a blood sample per animal was collected in polypropylene vacutainer tubes, centrifuged at 3 500 rpm for 10 min at 4 °C to obtain the serum; the samples were stored in 1.5 mL microvials and frozen at -20 °C until determinations. Subsequently, the animals were desensitized with a penetration captive bolt gun, and exsanguinated by cutting the anterior vena cava, as indicated in the Official Mexican

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Norm, NOM-033-ZOO-1995 (Humane slaughtering of domestic and wild animals). The psoas major and longissimus dorsi muscles, between the 9–11th ribs, were sampled and placed in a cold chamber until reaching 4 °C; the pH was measured using a HI 99.163 potentiometer for meat, connected to a glass puncture electrode (HANNA Instruments Mexico, Mexico City, Mexico). The antioxidant capacity and lipid oxidation were evaluated subsequently. The left anterior metacarpus was obtained for dry matter and ash determinations.

Laboratory determinations

To determine the antioxidant capacity of the psoas major and longissimus dorsi muscles, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and iron reducing ability (FRAP) were determined, following standardized procedures.[30, 31] In brief, 5 g of meat were homogenized in 25 mL of phosphate buffer (IKA T25 homogenizer) for 1 min; subsequently, the homogenate was centrifuged for 30 min at 10 000 rpm at 4 °C, and then passed through a Whatman No. 4 filter paper. The filtrate was stored at -20 °C using eppendorf tubes until further analysis. The DPPH radical inhibition was determined by mixing 25 µL of the extract with 975 µL of the DPPH solution in test tubes, and were incubated in the dark for 1 h at room temperature. The absorbance /was read at 515 nm, using anhydrous methanol as blank spectrum; the absorbance of each sample was recorded (AS), as well as the absorbance of the reaction blank (ADPPH) after 1 h of reaction time. Results were expressed as mg Trolox equivalents/g meat. Subsequently, the percentage inhibition of DPPH (% inhibition) was calculated with the next formula:

$$\text{Inhibition of DPPH,\%} = \{(\text{ADPPH} - \text{AS}) \div \text{ADPPH}\} \times 100$$

This result was introduced to the equation of the standard curve to obtain the result in trolox equivalents/g meat. For FRAP determinations, a 25 µL aliquot of the extract was mixed with 975 µL of a FRAP solution (2.5 mL 2,4,6-tripyridyl-s-triazine acid [TPTZ] 40 mM, 2.5 mL $FeCl_3$ 20 mM and 25 mL 0.3 mM acetate buffer, pH 3.6) in test tubes. The absorbance was read at 593 nm using a UV/VIS spectrophotometer (GENESYS 10S UV-Vis Thermo scientific), readings were taken 6 min after the start of the reaction; results were expressed as mg trolox equivalents/g meat. For the determinations in blood serum samples, the same procedures were used, and the results were expressed as µM trolox/mL of serum.

The thiobarbituric acid reactive substances (TBARS) test was used to measure the lipid oxidation as previously described.[32] In brief, 5 g of meat were homogenized in an ice bath with 20 mL of trichloroacetic acid (TCA) solution for 1 min. The homogenate was centrifuged for 20 min at 10 000 rpm at 4 °C. The supernatant was filtered into light-protected tubes and stored at -20 °C for further analysis. TBARS were determined in 1 mL of the extract mixed with 1 mL of TBA in test tubes. The tubes were heated for 30 min at 95 °C, cooled, centrifuged, and then read using an absorbance of 530 nm. The results were expressed as mg malondialdehyde (MDA)/g of meat. The dry matter in the metacarpus was determined after 24 h in a horizontal flow hot air oven (Terlab SA de CV, Zapopan, Jalisco, Mexico) at 105 °C, and the ashes were measured in a furnace (Furnatrol I Type 1, 8 200; Thermolyne, Guadalajara, Jalisco, Mexico) after 6 h at 600 °C.

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Statistical analysis

For the analysis of the growth performance and FS results, the data was subjected to analysis of variance using a complete randomized block design; the block was defined by the group of parity. There were four treatments with ten pen replications each with five piglets at the beginning, four piglets at seven days of age and three piglets thereafter; the experimental units was the pen. For the growth performance and FS, an analysis was carried out for each period, that is, 1-7, 8-21, 22-42 and 1-42 days of age. For the analysis of metacarpus measurements and the antioxidant capacity in muscles and serum, the same statistical model was used and the same block criteria; the experimental unit was the piglet. The effects of treatments for the antioxidant status were analyzed within each day of sampling, at three and seven days of age, independently.

For all the analysis, the independent variable was the treatment and the dependent variables were the growth performance, FS, metacarpal measurements and the antioxidant status in muscles and serum. Regression analysis was used to calculate the estimated relationship (lineal and quadratic) between the addition of increasing levels of HS (0: negative control, 2 500 and 5 000 ppm) and the independent variables. The least significant difference procedure was used to assess the difference among treatments. The tables show the least squares means and the standard error of the mean. Significance was defined as $P \leq 0.0500$, whereas a tendency for significance was defined as $0.0500 < P \leq 0.1000$.

Results

Growth performance

Table 2 shows the results of the productive performance of weaned pigs from 1-42 days postweaning. From 1–7 days postweaning, the PC and NC piglets had lower ADG ($P < 0.0018$) and G:F ($P < 0.007$) compared to those added with 2 500 and 5 000 ppm HS. From 8–21 days postweaning, the PC piglets had similar ADG compared to the rest of the groups; but the ADG was lower $P < 0.0973$) in NC compared to piglets receiving 2 500 and 5 000 ppm HS. At 42 days postweaning, the body weight ($P < 0.0418$) and the ADG ($P < 0.0486$) was lower and the G:F tended to be lower ($P < 0.0875$) from 22–42 days postweaning in the PC and NC pigs compared to those fed 5000 ppm HS; pigs receiving 2 500 ppm HS showed intermediate responses. From 1–42 days postweaning, the NC pigs showed the lowest ($P < 0.0151$) and those receiving 5 000 ppm HS had the highest ADG; the PC and pigs fed 2 500 ppm HS exhibited intermediate ADG. The PC and NC pigs showed the lowest ($P < 0.0346$) and those receiving 5 000 ppm HS had the highest G:F; while pigs fed 2 500 ppm HS exhibited intermediate G:F. Linear increasing responses were found in the body weight at 42 days postweaning ($P < 0.0136$), the ADG from 1–7 ($P < 0.0002$), 8-21 ($P < 0.05$), 22–42 ($P < 0.0167$) and 1–42 ($P < 0.0033$) days postweaning, and the G:F from 1-7 ($P < 0.0001$), 22–42 ($P < 0.0331$) and 1–42 ($P < 0.0049$) days postweaning due to the increasing dietary HS concentration.

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Fecal score and metacarpal measurements

The FS from 1–42 days postweaning and the metacarpal weight and ash content at seven days postweaning are shown in Table 3. The FS from 1–7 ($P < 0.0335$), 8–21 ($P < 0.0099$) and 1–42 ($P < 0.0002$) days postweaning, was highest in NC, intermediate in PC and pigs fed 2 500 ppm HS, and was lowest with 5 000 ppm HS. The FS decreased linearly from 1–7 ($P < 0.0067$), 8–21 ($P < 0.0017$) and 1–42 ($P < 0.0001$) days postweaning as the addition of HS in the feed increased. There were no differences among treatments in metacarpal dry matter and ash content at seven days postweaning.

Antioxidant responses

The results of TBARS, FRAP and DPPH in the psoas major and longissimus dorsi muscles and FRAP and DPPH in serum are shown in Table 4. In the psoas major muscle, TBARS, FRAP and DPPH at three days postweaning and TBARS and FRAP at seven days postweaning were similar among treatments. At seven days postweaning, the DPPH in mg Eq. trolox/g meat ($P < 0.0088$) and % inhibition ($P < 0.0082$) were highest in pigs with 2 500 ppm HS and intermediate in pigs with 5 000 ppm HS, and were lowest in NC and PC groups. In the longissimus dorsi muscle, TBARS and FRAP were similar among treatments at three and seven postweaning days. At three days postweaning, the DPPH in mg Eq. trolox/g meat ($P < 0.0165$) and % inhibition ($P < 0.0111$) were higher in pigs with 2 500 and 5 000 ppm HS compared to those in the NC and PC groups. At seven days postweaning, the DPPH in mg Eq. trolox/g meat ($P < 0.0605$) and % inhibition ($P < 0.1000$) tended to be highest in pigs with 2 500 and 5 000 ppm HS, were intermediate in NC and were lowest in PC groups.

In serum, FRAP was no different among treatments at three and seven days postweaning. At three days postweaning, the DPPH in mg Eq. trolox/g meat ($P < 0.0001$) and % inhibition ($P < 0.0001$) were higher in pigs receiving 2 500 and 5 000 ppm HS compared to those in the NC and PC groups. At seven days postweaning, the DPPH in mg Eq. trolox/g meat ($P < 0.0180$) and % inhibition ($P < 0.0180$) were highest in pigs receiving 2 500 and 5 000 ppm HS, were intermediate in NC and were lowest in the PC groups. Linear increasing responses were found in DPPH in mg Eq. trolox/g meat ($P < 0.05$) and % inhibition ($P < 0.05$) in the psoas major muscle at seven days postweaning and in the longissimus dorsi muscle at three and seven days postweaning due to the increasing dietary HS concentration.

Discussion

The improvements in the final body weight, ADG and G:F observed in weaned pigs added with HS in the present study agree with previous publications.[1, 2, 15, 33] Higher ADG up to 21 days postweaning compared to a control group was reported in piglets added with 1 % sodium humate.[33] Weaned pigs fed diets added with 20 g/kg leonardite or lignite, showed higher body weight at 21 days postweaning and ADG on the second and third week postweaning compared to a control group.[15]

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Table 2. Productive performance of weaned pigs supplemented with humic substances from 1 to 42 days postweaning

Item	PC[1]	NC	Humic substances, ppm			
			2 500	5 000	SEM[a]	P-value
From 1–7 postweaning days						
Initial body weight, kg	6.04	6.02	6.00	5.96	0.230	0.9958
Body weight at day 7, kg	6.19	6.07	6.32	6.34	0.236	0.8412
Feed intake, g/day	149.7	149.7	161.2	160.8	5.729	0.2684
Weight gain, g/day	22.1^{b}	7.9^{b}	45.8^{c}	54.6^{c}	8.643	0.0018
Gain:feed ratio	0.13^{b}	0.05^{b}	0.28^{c}	0.34^{c}	0.050	0.0007
From 8–21 postweaning days						
Body weight at day 21, kg	8.51	8.13	8.54	9.00	0.301	0.2599
Feed intake, g/day	254.8	257.9	273.2	288.1	12.920	0.2157
Weight gain, g/day	165.	146.8	158.4	189.5	12.191	0.0973
Gain:feed ratio	0.73	0.56	0.57	0.65	0.040	0.2918
From 22–42 postweaning days						
Body weight at day 42, kg	16.24^{b}	15.36^{b}	16.99^{bc}	18.46^{c}	0.785	0.0418
Feed intake, g/day	754.9	689.3	751.4	775.0	35.846	0.3885
Weight gain, g/day	368.4^{b}	344.3^{b}	402.4^{bc}	450.7^{c}	28.039	0.0486
Gain:feed ratio	0.49	0.50	0.54	0.58	0.028	0.0875
From 1–42 postweaning days						
Feed intake, g/day	487.3	455.6	493.6	510.3	20.557	0.2805
Weight gain, g/day	242.9^{bc}	222.4^{b}	261.7^{bc}	297.6^{d}	15.021	0.0151
Gain:feed ratio	0.50^{b}	0.49^{b}	0.53^{bc}	0.58^{c}	0.023	0.0346

Note: [a] Standard error of the mean: [b, d] different superscripts within a row indicate significant differences ($P < 0.0500$).
[1] Positive control (PC): basal diet containing colistin (40 ppm) as growth promoter antibiotic; negative control (NC): basal diet without growth promoter antibiotic.

Table 3. Fecal score from 1 to 42 days postweaning and the metacarpal weight and ash content of weaned pigs at seven days postweaning

Item	PC[1]	NC	Humic substances, ppm			
			2 500	5 000	SEM[a]	P-value
Feces consistency score						
1–7 days	0.59^{bc}	0.77^{b}	0.40^{c}	0.36^{c}	0.098	0.0335
8–21 days	0.70^{b}	0.89^{b}	0.55b^{bc}	0.35^{c}	0.110	0.0099
22–42 days	0.45	0.64	0.40	0.41	0.106	0.3429
1–42 days	0.58^{b}	0.77^{c}	0.47^{bd}	0.37^{d}	0.059	0.0002
Metacarpal measurements						
Dry matter, %	33.2	31.56	32.08	32.15	0.524	0.2128
Dry matter, g	4.81	4.65	4.63	4.78	0.338	0.4829
Ash, %	32.01	31.66	33.64	33.06	1.176	0.0646
Ash, g	1.53	1.47	1.56	1.58	0.116	0.9710

Note: [a] Standard error of the mean; [b, d] different superscripts within a row indicate a significant difference ($P < 0.0500$).
[1] Positive control (PC): basal diet containing colistin (40 ppm) as growth promoter antibiotic; negative control (NC): basal diet without growth promoter antibiotic.

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Table 4. Antioxidant capacity and lipid oxidation in the psoas major and longissimus dorsi muscles and in blood serum at three and seven days postweaning

Item	PC[1]	NC	Humic substances, ppm			
			2 500	5 000	SEM[a]	P-value
Psoas major muscle						
3 days postweaning						
FRAP, mg Eq. trolox/g meat[2]	0.06	0.06	0.06	0.07	0.006	0.3931
DPPH, mg Eq. trolox/g meat	0.41	0.44	0.47	0.53	0.041	0.2121
DPPH, % inhibition	17.67	18.81	20.34	22.76	1.798	0.2316
TBARS, mg MDA/g meat	0.19	0.15	0.24	0.23	0.044	0.4326
7 days postweaning						
FRAP, mg Eq. trolox/g meat	0.06	0.05	0.06	0.06	0.005	0.3708
DPPH, mg Eq. trolox/g meat	0.59^{bc}	0.51^{b}	0.71^{d}	0.61^{cd}	0.038	0.0088
DPPH, % inhibition	25.16^{bc}	21.54^{b}	30.2^{d}	26.05^{cd}	1.66	0.0082
TBARS, mg MDA/g meat	0.24	0.24	0.19	0.26	0.036	0.5520
Longissimus dorsi muscle						
3 days postweaning						
FRAP, mg Eq. trolox/g meat	0.04	0.03	0.04	0.05	0.05	0.2421
DPPH, mg Eq. trolox/g meat	0.34^{b}	0.38^{b}	0.50^{c}	0.51^{c}	0.043	0.0165
DPPH, % inhibition	15.31^{b}	17.36^{b}	22.59^{c}	23.54^{c}	1.929	0.0111
TBARS, mg MDA/g meat	0.17	0.14	0.16	0.18	0.031	0.8389
7 days postweaning						
FRAP, mg Eq. trolox/g meat	0.04	0.03	0.03	0.04	0.005	0.2114
DPPH, mg Eq. trolox/g meat	0.41	0.54	0.59	0.59	0.048	0.0605
DPPH, % inhibition	18.86	24.75	26.85	26.49	2.25	0.0619
TBARS, mg MDA/g meat	0.16	0.13	0.2	0.24	0.04	0.2643
Blood serum						
3 days postweaning						
FRAP, mg Eq. trolox/g meat	7.2	6.72	7.54	7.13	0.294	0.2821
DPPH, mg Eq. trolox/g meat	13.07^{b}	11.90^{b}	14.98^{b}	22.90^{c}	1.251	0.0001
DPPH, % inhibition	7.81^{b}	7.01^{b}	9.12^{b}	14.54^{c}	0.857	0.0001
7 days postweaning						
FRAP, mg Eq. trolox/g meat	6.38	6.63	6.12	6.2	0.271	0.5607
DPPH, mg Eq. trolox/g meat	14.91^{b}	13.02^{b}	15.36^{b}	22.01^{c}	2.03	0.0180
DPPH, % inhibition	9.07^{b}	7.78^{b}	9.38^{b}	13.93^{c}	1.39	0.0180

Note: [a] Standard error of the mean; [b, d] different superscripts within a row indicate a significant difference ($P < 0.0500$).

[1] Positive control (PC): basal diet containing colistin (40 ppm) as growth promoter antibiotic; negative control (NC): basal diet without growth promoter antibiotic.

[2] FRAP = antioxidant power of ferric radicals; DPPH = Scavenging activity of 1,1-diphenyl-2-picrylhydrazyl radicals; TBARS = thiobarbituric acid-reactive substances.

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Weaned pigs fed 2 000 mg/kg dietary sodium humate, had higher body weight at 16 and 30 postweaning days, and ADG, ADFI and G:F from 1–30 postweaning days, compared to the control group.[2] Furthermore, higher body weight at day 40 postweaning and higher ADG and ADFI from 14–40 days postweaning were found in weaned pigs added with 0.25 % leonardite.[1]

Even though the actual mechanism has not been fully understood, several theories have been suggested to explain the enhanced growth performance in pigs and other farm animals added with different sources of HS.[9] For example, a positive effect of HS could be explained by increased metabolic activity of cell membranes, participation in ion transport, and acceleration of oxidative processes that stimulate vital functions due to increased nutrient uptake.[15, 33, 34] More frequently, the addition of HS in animals have been associated with the formation of a film on the mucus epithelium of the gastrointestinal tract[9, 35, 36] improving its morphology, increasing the activity of several enzymes, with the subsequent improvement in nutrient digestion and utilization, especially protein and trace elements,[37–39] as well as, the stabilization of the intestinal microbiota and enhancement of the immune response.[2, 15, 40]

For more than three decades, HS has been used to treat diarrhea, dyspepsia, and acute intoxications in animals such as horses, swine, and poultry. HS has been attributed antiphlogistic, adsorptive, antitoxic, and antimicrobial properties.[7, 35] However, scientific data of the HS use in the prophylaxis of diarrhea in piglets is scarce. In previous research, leonardite and lignite cannot completely prevent diarrhea in piglets after weaning, but they significantly reduced its severity and related mortality,[15] and it was also confirmed, that sodium humate can be an effective option for the partial replacement of a high therapeutic dose of zinc oxide in treatment of serious diarrheal ETEC infection.[41] Furthermore, lower diarrhea rate was reported from 1–30 days postweaning in piglets fed diets added with sodium humate.[2] These findings are in agreement with the results of the present study, in that lower FS were observed in HS-fed pigs.

HS are thought to be the most complex natural ligands, with a high potential to form chelates with various ions, which has been linked to improved mineral utilization in plants and animals.[9, 10, 27] Increased ash and Ca content in tibia bone in HS-fed broilers,[16–18] as well as, increased percentage, thickness, and hardness of eggshells in HS-supplemented laying hens and pheasants have been reported.[19–21] Improved femur mineralization and increased milk Ca and Fe content have also been reported in HS-added rabbits[22] and cows.[23] In HS-fed weaned pigs, increased serum alkaline phosphatase, Ca, P, and Mg have been found.[1, 15] In HS-fed weaned pigs, controversial results have been found in the serum or body levels of some minerals. In one hand, increased serum alkaline phosphatase, Ca, P, and Mg,[1, 15] but on the other hand, a certain loss of Mn and Se from the body, have been reported in HS-fed weaned pigs.[33] In HS-fed broiler chickens, increased ash, Ca and P content in tibia bone have been reported.[27] In the present study, metatarsal weight and ash content were similar among treatments at seven days postweaning. This could have partially been due to the fact that broiler chickens had been added with HS for longer periods of time, with a range between 21 and 36 days.[27]

The determination of antioxidant capacity by the DPPH technique allows the activity of specific compounds or extracts to be evaluated using the free radical

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DPPH in anhydrous methanol solution.[30, 31] This free radical is susceptible to react with antioxidant compounds through a process characterized by the yielding of an hydrogen atom provided by the antioxidant agent.[42] High values (as mg Eq. trolox/g meat or % inhibition) for this variable indicate the capacity of the evaluated extract to capture free radicals or inhibit their formation.[43] The weaning process in pigs has been shown to increase reactive oxygen species and TBARS by 50 % after 28-day postweaning compared to preweaned levels,[44] and to decrease the antioxidant capacity of the jejunum and colon, after 21-day postweaning.[45] These changes have been associated to the nutritional, immunological, social and environmental stress caused by weaning;[1, 3, 15] affecting the physiological and biochemical state of the organism, leading to oxidative stress.[2]

The psoas major muscle shows oxidative metabolic activity with fatty acid chains taken and oxidized, producing ATP through aerobic pathways, generating more free radicals; demanding, a greater amount of antioxidants making it more prone to oxidation.[46] Our results indicate that HS consumption for three days postweaning was not enough to provoke significant changes in DPPH in the psoas major muscle, as was significantly observed at seven days postweaning. On the other hand, increases in DPPH at three and seven days postweaning were found in the longissimus dorsi muscle; this was most likely due to the fact that this muscle has high glycolytic metabolic activity that uses glucose and fat as energy fuels; this muscle generates ATP through anaerobic glycolysis, which produces less ATP per cycle, making it less susceptible to oxidation, sparing more antioxidant agents with inhibition capacity of free radicals.[46] Furthermore, the high levels of DPPH detected in serum at three and seven days after weaning support the high levels of this index in the muscles. These results demonstrate that in weaning pigs, dietary HS increases the meat free radical inhibition capacity, and indeed, could be used to prevent the oxidative stress in piglets triggered by the weaning process.

The higher DPPH in serum found at three and seven days postweaning agree with previous research, in which reduced active TBA product concentrations and increased serum superoxide dismutase and catalase activity of HS-fed weaning pigs was reported.[24] In HS-fed weaned pigs, LPS-challenged, increased serum glutathione and reduced glutathione peroxidase was reported.[25] Reduced serum biomarkers of oxidative stress (8-iso PGE_2 and 8-iso $PGF_{2\alpha}$) in HS-fed weaned pigs have been also found.[15, 47] Additionally, increased total superoxide dismutase activity and total antioxidant capacity and reduced content of malondialdehyde have also been detected in weaned pigs added with sodium humate,[2] as well as, increased total antioxidant capacity.[1] All these research likewise show improvements in the antioxidant capacity of HS-weaned pigs, however, the explanation of the main mechanisms of action of HS on the antioxidant status of piglets remain unclear.

In *in vitro* and *in vivo* studies on the HS antioxidant potential have revealed that quinones are reducible fragments and phenols are electron-donating fragments with antioxidant characteristics in contrast to electron-accepting quinones.[48, 49] Besides, it has been proposed that the *in vivo* antioxidant effects of HS are influenced by the composition of their structure; for example, a high concentration of quinones can result in the production of large amounts of oxygen reactive substances that foster oxidative stress and lipid peroxidation, thereby overpowering the muscle's antioxidant response. However, it is oxygen-containing functional groups,

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primarily carboxylic and phenolic groups, that are able to account for the compound's antioxidant properties.[1, 50]

The mechanism of action of HS behind the improved antioxidant status in animals is still under elucidation. The most outstanding theories indicate that HS may act systemically or locally in the digestive system.[9] Some studies have revealed that HS can be assimilated and transferred to different tissues of the body. HS particles were found in all sections of the small intestine and the lymph nodes associated with the intestine, the urinary bladder and trachea in young pigs.[51] HA distribution was also observed in several tissues, including the skin, blood serum, liver, muscle, and digestive tract of rats in previous studies using ^{125}I-HA.[52]

These findings suggest that HS could be absorbed in the intestine and transported in the blood serum to different organs of the body, where they could directly capture and reduce the overproduction of reactive oxygen species and free radicals, preventing the oxidative stress in piglets triggered by the weaning process. Another likely explanation for HS's antioxidant properties stems from its ability to form protective layers in the digestive mucosa, preventing the penetration of pathogenic bacteria and toxins, from bacteria and feeds, that could harm the digestive mucosa, as well as, a probable stabilization of the microbial communities, lessening intestinal challenges and, indirectly, the generation of free radicals within the body.[9]

Conclusions

The addition of HS from vermicompost to piglets feed at weaning linearly improved the body weight at 42 days postweaning and the ADG and G:F ratio from 1–42 days postweaning. Conversely, the FS had linear decreasing responses as dietary HS addition increased. Linear increments of DPPH radical and inhibition capacity was observed in longissimus dorsi muscle and blood serum at three and seven days postweaning, and in the psoas major muscle at seven days postweaning in response to the addition of increasing dietary HS. The results suggest that HS from vermicompost could be used as growth promoter and enhancer of the antioxidant responses in weanling pigs, as does HS from any other source.

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Data availability

All relevant data are within the manuscript and its supporting information files.

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Conflicts of interest

The authors have no conflict of interest to declare in regard to this publication.

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