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Effects of humic acids supplementation on pig growth performance, Nitrogen digestibility, odor and ammonia emission.

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.

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EFFECTS OF HUMIC ACIDS SUPPLEMENTATION ON PIG GROWTH PERFORMANCE, NITROGEN DIGESTIBILITY, ODOR AND AMMONIA EMISSION

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RESUMEN

Se llevaron a cabo tres experimentos, en los que cuales se suplementó ácidos húmicos en la dieta de cerdos y se evaluaron los efectos en los parámetros de crecimiento, digestibilidad de N, emisiones de amonio y olor de las heces. En el primer experimento, se dividió en bloques a un grupo de 16 cerdos (41 ± 3 d) según su peso inicial. Los tratamientos dietéticos fueron: 1) Control (CNTRL: dieta basal), 2) HA-2 (dieta basal + 2 g de Huminfeed (HA, Huminfeed WSG, Humintech GmbH, Grevenbroich, GER, 75% de ácidos húmicos). Basados en estos tratamientos, se midieron los parámetros productivos: ganancia diaria promedio (ADG), ingesta diaria promedio de alimento (ADFI) y la ganancia/alimento (G:F). Ningún parámetro mostró diferencias significativas entre los tratamientos ($P>0.05$). Para el segundo experimento, se usaron 150 cerdos post destete (York Landrace × Hypor, BW inicial: 6 kg ± 0,430 kg), estratificados de acuerdo a su peso inicial. Las dietas experimentales fueron: 1) Control (CNTRL: dieta basal), 2) HA-2 (dieta basal + 2 g de Huminfeed) y 3) HA-4 (dieta basal + 4 g de Huminfeed). Los parámetros productivos no mostraron diferencias significativas entre tratamientos, con excepción de la ganancia diaria promedio durante la quinta semana de experimentación, que presentó una tendencia a disminuir mientras mayor cantidad de ácidos húmicos suplementada ($P<0.05$). Se realizó un balance de la emisión de N y NH3 usando el método Kjeldahl. Para esto, se recolectaron heces y orina en el día 35 del experimento durante 5 días consecutivos. En este experimento, se encontraron diferencias significativas en la ingesta de N ($P \leq 0.04$), mostrando una reducción lineal y cuadrática. Además, la excreción de N en las heces presentó una disminución lineal ($P = 0.02$). Las emisiones de NH3 fueron menores mientras mayor era la cantidad de ácidos húmicos suplementada; sin embargo, no hubo diferencias significativas ($P \geq 0.10$). Del mismo modo, las variables (agradable, irritabilidad e intensidad) mejoraron cuando se suplementó más ácidos húmicos, mostrando una disminución cuadrática y lineal ($P<0.05$). El tercer experimento consistió en administrar los ácidos húmicos directamente a los purines de cerdos (266 g de heces y 534 g de orina) para medir la emisión de NH3 a las 48 h y a las 120 h, utilizando el mismo método que en el segundo experimento. La tasa de producción de NH3 fue mayor durante las primeras 48h; sin embargo, no se obtuvieron diferencias significativas entre tratamientos ($P>0.05$).

Palabras clave: Ácidos húmicos, nitrógeno, amoniaco.

ABSTRACT

Three experiments were carried out, in which humic acids were supplemented in the diet of pigs and its effects on growth performance, N digestibility, ammonium emissions and odor of feces were evaluated. In the first experiment, 16 pigs (41 ± 3 d) were divided in blocks according to its initial BW. The dietary treatments included: 1) Control (CNTRL: basal diet), 2) HA-2 (basal diet + 2 g of Huminfeed (HA; Huminfeed WSG, Humintech GmbH, Grevenbroich, GER, 75% humic acids). Based on these treatments, productive parameters were measured by weighing average daily gain (ADG), average daily feed intake (ADFI) and obtaining the gain/feed (G:F). None of the parameters showed significant differences between treatments (P>0.05). In second experiment, 150 post-weaning pigs (York Landrace × Hypor, Initial BW: 6kg ± 0.430 kg) were used and stratified according their initial BW. The treatments were the folowing: 1) Control (CNTRL: basal diet), 2) HA-2 (basal diet + 2 g of Huminfeed) and 3) HA-4 (basal diet + 4 g of Huminfeed). The growth performance parameters did not show significant differences across treatments, with the exception of ADG during the fifth week of the experiment which showed a decreasing linear trend (P<0.05). A balance of N and NH3 emission was made using the Kjeldahl method, for this, stool and urine were collected on the 35th day of the experiment for 5 consecutive days. In this experiment, significant differences were found in N intake (P ≤ 0.04) showing a trend for a linear and quadratic reduction and the N excretion in feces presented a linear decrease (P = 0.02). The NH3 emissions were lower when higher amounts of humic acids were supplemented; however, there were no significant differences (P ≥ 0.10). Likewise, the variables (pleasant, irritability and intensity) improved when more HA was supplemented showing a linear and quadratic decrease (P<0.05). The third experiment consisted of administering the humic acids directly to the slurry (266 g of feaces and 534 g of urine) from Crossbred pigs to evaluate the emission of NH3 at 48h and at 120h, using the same method as in Exp 2. The NH3 production rate was larger during the first collection period; however, no experimental differences were obtained across the treatments (P>0.05).

Keywords: Humic acids, nitrogen, ammonia.

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INTRODUCTION

Pork is the third most consumed protein source of animal origin in Ecuador. For this reason, it is necessary to promote this production system through integration of factors to improve its productivity, enhancing food security (Abrahale et al., 2019).

Among the principal factors, altering swine production system we find: health problems during production, little implementation of innovative measures and the breach of sanitary standards suggested. These factors can cause problems on feed conversion and therefore animal productivity. In addition, appropriate nutrient supplementation is another factor considered to enhance nutrient deposition and decrease nutrient waste. For instance, enhancing N digestibility can be associated with reduction of ammonia excretion (In Montagnini, 2017). Ecuador's government, through its institution Agrocalidad controls swine through the Program of Good Livestock Practices, which is based on the OIE Terrestrial Animal Health Code. A key component within this program is prevention of accumulation of effluent gases (OIE, 2018). Ammonia is the main gas emitted from pig slurry. This is because the fragmentation of the protein in the digestive tract of the pig produces as a byproduct NH3. This gas has polluting potential and produces uncomfortable odor. By reducing odor producing compounds excreted in the urine and faeces would lead to improve air quality, achieving optimum standards of production. Therefore, given the aforementioned background, it is proposed to incorporate alternatives for feeding pigs with non-traditional additives, such as humic acids; to establish the quantities in the diet that cause benefits in the sustainable production of pigs (Taylor-Pickard and Spring, 2008).

Humic acids have been administered in the diet of animals and used to reduce the incidence of diarrhea in piglets, in the improvement of weight gain and feed conversion, and in the decrease of release of ammonia and nitrates in animals (Hristov et al, 2015). Additionally, humic acids have been used directly in the slurry of pigs to reduce NH3 emissions. Therefore, the objective of the present study was to determine the effect of humic acids on measuring production parameters, N digestibility, ammonia emissions and odors in pigs (Plaza et al, 2002).

MATERIALS AND METHODS

The study was conducted following the Guidelines for Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010).

EXPERIMENT 1

Animal and Housing

A total of 16 crossbred piglets (41 ± 3 d) were obtained from a commercial farm at weaning. Pigs were individually housed in a common commercial nursery, which contained 16 pens. Each pen (0.58 × 0.52m), had wooden floor, metal grills, automatic water trough and wood feeder. The building contained radiant tube heaters, vent boards and a curtain which controlled air inlet. The room temperature was maintained at 30 ° C during de first week and temperature was decreased 1 ° C each week. Relative humidity was 65% and light was provided during 24 hours. At arrival, pigs were weighted, stratified according to the initial weight (8.69 ± 1.16 kg) and assigned randomly to one of two treatments. All of the pigs were placed into the pens and immediately started to receive the experimental treatments.

Experimental treatments

Two experimental diets consisted of 1) Commercial diet (Pronaca, Ecuador) without humic acids (Ctrl, n = 8); and 2) Commercial diet with 2g of Huminfeed (HA-2; Huminfeed WSG, Humintech GmbH, Grevenbroich, GER, 75% Humic Acids, n = 8). The piglets were individually fed and received commercial pelleted diet. The basal diet was the commercial feed which was formulated to meet nutrient specifications for initial phase, according to NRC (2012). Therefore, it contained (DM basis) 3300 kcal/kg of ME, 19% of CP, a 1,1% of SID Lysine, 0.85% of Ca and 0.4% available P. In the case of the HA-2 diet, the amount of HA was added by top dressing mixed with 18 g of ground corn to the commercial diet; while in Cntrl diet was administered 18 g of ground corn.

Performance parameters

Initial body weight (BW) was measured at the beginning and at the end of each dietary phase, and, every seven days (day 0, 7, 14, 21 and 28). Average daily gain (ADG) was calculated for each phase. In addition, ADFI and G:F, were calculated for each feeding period with the values obtained from daily measurements. Presence of diarrhea was recorded daily throughout the study, using a scale from 0 to 4, where: solid feaces = 0, hard feaces = 0, soft feaces = 1, stool feaces = 2, liquid feaces = 3 and feaces with blood = 4.

Statistical Analysis

The study involved a completely randomized block design where blocks were formed with the initial weight and each piglet pen location. Each block contained 2 pens. The experimental unit was the pen. Parametric data were analyzed using the SAS procedure (SAS Inst. Inc., Cary, NC) as a completely randomized block design. Diarrhea prevalence was analyzed using the Chi square. Statistical differences were declared when value of $P \leq 0.05$ and trends were considered at: $0.05 > P < 0.10$.

EXPERIMENT 2

Animal and Housing

A group of 150 piglets (York Landrace × Hypor; Initial BW: 6kg ± 0,430 kg) procured from a commercial farm at weaning were used in this study during 49 days. Animals fed a basal diet containing three different quantities of humic acids (HA). During the study, pigs were housed since day 21 until day 70 of age in a barn (12 × 4 m) whose construction has raised floors, brick walls, roof composed of fibre tiles, automatic troughs and 30 bell feeders. The temperature of the sheds was measured, the first week was at 30°C and decreased 1 ° C each week. Also, relative humidity was 65% and remained 24 hours of light. For this study, 30 pens housed all of the animals at a density of 0.30 m^2 / piglet.

Experimental treatments

Experimental treatments consisted of commercial diet (Pronaca, Ecuador), which were fed ad libitum in a pellet form in 2 phases, from weaning to 49 d (pre-starter) with an ADFI of 0.410 kg/animal, and from d 49 to 70 d (starter) with an ADFI of 0.975 kg/animal. Humic acids were supplemented by top dressing the quantity of Huminfeed assigned mixed with 18 g of ground corn to the commercial diet, used in the first experiment, in the particular doses described in the experimental treatments (0, 2 and 4 g of Huminfeed). The control (Cntrl) was without HA supplementation. The second treatment consisted in commercial diet plus supplementation of 2 g/pig/day of Huminfeed (HA-2); and the third experimental treatment consisted in the supplementation of 4 g/pig/day of Huminfeed (HA-4) to commercial diet.

Performance parameters

Growth, feed intake and feed conversion rate were measured weekly. Individual pig body weight was measure at the beginning and at the end of each dietary phase, and, every seven days (day 0, 7, 14, 2, 28, 35, 42 and 49) and ADG was calculated for each phase. As well as ADFI and G:F, both were calculated for each feeding period with the values obtained from daily measurements of feed consumption.

Nitrogen balance

For the analysis of Nitrogen intake in the experimental treatments, basal diets samples were sent to a commercial laboratory (Agrocalidad Lab). For obtaining the N excretion and retention in feaces and urine, samples were collected from 3 pens/treatment selected randomly on day 14 of the experiment (55 to 59 days of pig´s age) for 5 consecutive days to measure nitrogen digestibility. The pens were chosen randomly and the samples were individually labeled according to the selected pen. On the sixth day, a composite sample was made. For the collection of the samples, funnel-shaped baskets lined with plastic were used. This type of collector was used for the purpose of separating faeces from urine. After 24 hours, the collected was placed in plastic containers. The 10% of the slurry collected was taken, weighed and dehydrated for 48 h in an oven at 60 ° C. Later, they were ground in a rotary mill and the dry sample was weighed and the sample was sent to a laboratory (Agrocalidad Lab) bromatological analysis to obtain the percentage of N excreted in faeces. Urine samples collected simultaneously, containers were placed with 50 ml of 20% HCl. This allowed the sample to be preserved for further analysis. A sample of 10% of the collected volume was taken a composite sample was sent to a commercial laboratory (Agrocalidad Lab) to obtain the percentage of N excreted in urine and feaces was determined by Kjeldahl method (Bradstreet, 1965).

Ammonia excretion measurement

Fecal samples and urine samples (1:2) were collected and homogenized (266 g of feaces and 534 g of urine). During the transportation to the Soil Laboratory of the IASA Agricultural Engineering Faculty, the samples were refrigerated (5° C). Three samples per treatment were processed, using the same methods described in the sampling for Nitrogen measurement. For the determination of NH3 emissions from samples of slurry was carried out following the methodology reported by Hristov et al. (2009), using the Kjeldahl method. Briefly, each sample was placed in a sealed glass bottle (250 ml) kept in water bath at 25° C. Each bottle was attached to a vacuum system and two bottles containing 100ml of 0.9 M sulfuric acid (H2SO4). The vacuum system pulled air (rate of 1.2 ± 0.2L/min) from the bottle containing the samples and NH3 – N was trapped. Ammonia emitted was captured and measured (mg / L) in the H2SO4 at 24 h and at 48 h. The sulfuric acid (H2SO4) of acid traps was replaced every 24 hours.

Odor evaluation

For measuring odor characteristics, on day 24 of the experiment, the purines of the pigs of 3 pens / treatment randomly selected were collected. The feces were collected after the pigs had received the different diets corresponding to the treatments. The samples were marked according to the corresponding treatments and stored in glass bottles at room temperature for 24 hours. At 24 hours, trained personal evaluated odor characteristics. Each sample had a nominal evaluation sheet with a nominal odor scale with three variables (pleasant, irritability and intensity) as described by Shiffman and Williams (1996). Each variable had a scale from 0 to 8. For each odor evaluation, each evaluator was allowed to sniff each sample and give a score for each variable.

Statistical Analysis

Results of performance parameters, nitrogen measurements, ammonia excretion measurements were analyzed using the SAS procedure (SAS Inst. Inc., Cary, NC) as a completely randomized block design. The study involved a block design where blocks were formed with the initial weight and each piglet pen location. Each block contained 2 pens. The experimental unit was 1 pen. Therefore, the parametric data were analyzed using the SAS procedure (SAS Inst. Inc., Cary, NC) as a completely randomized block design.

The conditional ANOVA with SAS statistical package (Version 9.0, SAS Inst., Inc., Cary, NC) was used to process the information from odor evaluation given by the participants involved. The statistical model used included fixed effects. The random selection of the participants was considered as a random effect within the model and considered as an error associated with the F-test. Turkey´s studentized range test (P 0.05) was used to determine the differences within the participant and diet means.

EXPERIMENT 3

Animal and Housing

Crossbred pigs of a commercial farm were used in the study. The pigs were housed in individual pens which consisted in metal tiestalls. Animals were fed on plastic feeders and metal automatic troughs. Feaces and urine produced by the animals end up separately in sedimentation ponds.

Treatments

The study consisted of two treatments: No addition of HA to purines (Cntrl); and addition of humic acids to purines (HA) at a rate of 5mg of HA per gram of purines. In addition, all of the animals received a basal diet which was formulated meeting the nutrient specifications of NRC (2012); without dietary supplementation of humic acids.

Sampling for Ammonia excretion measurement

All the feaces and urine end up separately in their corresponding pond because each pig had a urine collector harness. A considerable amount of feaces and urine were collected from ponds. Six homogenous samples of urine:feaces (534g: 266 g) were prepared. Three samples were randomly assigned to Cntrl and remaining three received HA treatment. For the determination of NH3 emissions from samples of slurry was carried out following the methodology proposed by Ndegwa (2009), using the same Kjeldahl method, as described for experiment 2.

Statistical Analysis

The results of the completely random design of the treatments were analyzed. using GLM procedure of SAS (SAS Inst., Inc., Cary, NC). F-test was obtained.

RESULTS AND DISCUSSION

EXPERIMENT 1

Performance parameters were not significantly different (Table 1, $P \geq 0.01$) across treatments during the experimental period. However, total body weight gain was slightly greater in piglets supplemented with HA than piglets who received Cntrl treatment. Meanwhile, a trend of lower dry matter intake was observed during the first 7 days by piglets supplemented with humic acids, compared to piglets under the control regime ($P = 0.10$; Table 1). Additionally, piglets supplemented with HA, showed a tendency to improve daily gain and gain efficiency within the first 21 d (7% improvement, $P \leq 0.08$), within the 4 groups corresponding to the lower stratum of the blocks under study (data not shown). However, caution must be taken interpreting these results due to the limited number of observations. The incidence of diarrhea was not altered with the HA supplementation during the course of the experiment at any interval measured ($P>0.24$). The incidence of diarrhea within the 4 groups of the lower stratum of initial weight had a numerical decrease of 25% ($P<0.1$) in piglets supplemented with humic acids (Figure 1).

In contrast to the study reported by Ji et al (2006), at the 5th week of the experiment, weaned piglets fed 0.5% HA showed a significant increase on ADG but not with pigs fed 1% HA. Later, during the 8th week period, the mentioned study obtained significant changes between treatments in growth parameters: ADFI decreased, ADG increased and G:F increased. These results suggest that growth parameters depend on the concentration of HA and the period of HA supplementation. Nevertheless, the effects are equivocal, as suggested by Weber et a. (2014) where their findings where similar to our results.

Table 1. Effect of humic acids supplementation on pig performance (Exp.1)

Item	Treatments[1]		SEM[2]	*P*-value[3]
	Ctrl	HA-2		
Initial BW, kg	8.73	8.66	0.42	0.69
Final BW, kg	19.04	19.03	0.61	0.97
ADG, kg/animal/d				
d 0 to 7	0.21	0.20	0.02	0.82
d 0 to 14	0.25	0.25	0.01	0.92
d 0 to 21	0.35	0.34	0.01	0.68
d 0 to 28	0.37	0.37	0.01	0.89
ADFI, kg/animal/d				
d 0 to 7	0.36	0.35	0.016	0.10
d 0 to 14	0.39	0.39	0.016	0.52
d 0 to 21	0.48	0.48	0.016	0.55
d 0 to 28	0.59	0.59	0.016	0.59
G:F				
d 0 to 7	0.58	0.59	0.07	0.88
d 0 to 14	0.63	0.64	0.03	0.73
d 0 to 21	0.72	0.70	0.02	0.81
d 0 to 28	0.63	0.63	0.02	0.76

[1]Ctrl = Commercial diet and 0g of humic acids; HA-2 = Commercial diet and 2g of Huminfeed (75% of humic acids).
[2]Standard error of the mean, n=8.
[3]Observed Significance level for the difference between treatments.

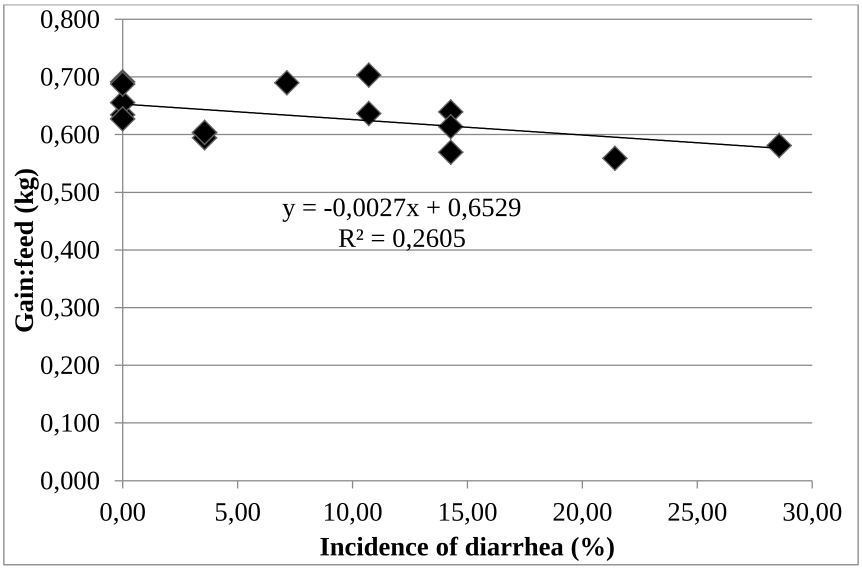

Figure 1. Incidence of diarrhea (%) in the efficiency of gain (kg). Humic acid supplementation did not influence significantly the incidence on diarrhea during the experimental period. Piglets of 4 groups of lower stratum of initial BW had a decrease of the 25% (P>0.05).

EXPERIMENT 2

Performance parameters

Pig growth performance did not show significant differences across treatments except in the fifth week of experiment. Animals fed Cntrl diet had greater ADG compared to HA-2 and HA-4 treatments, showing a trend for a linear decrease ($P = 0.02$, Table 2). This results were unexpected, considering that Ji et al. (2006) observed in their study that the inclusion of HA at 0.5% in the diet of weaning pigs increases weight gain. On the other hand, experiment 1 showed that HA supplementation has no influence on the pig growth performance and HA have shown to demonstrate different pig growth responses as in other production animals. The study of Yalcin et al. (2006) in laying hens of 22 to 40 weeks, showed that HA do not generate significant changes in production parameters when humic substance at 0.15% (35% of humic acids and 6% of fluvic acids) were added to the diet. These results coincide with the absence of differences between treatments present in this experiment, which shows that the effects of HA are variable. Wang et al (2008) reported benefit in their total weight gain when using humic substances at 5 an 10% during 8 weeks; however, during the first four-week period there were no significant effects of HA in ADFI. Feed intake was not altered by experimental treatments (Table 2, $P > 0.05$). This indicates that the effects of HA administration may be associated with the time of administration. Long-term HA administration may have a more prominent effect.

In the study done by Weber et al. (2014), 448 crossbred weanling pigs were supplemented with humic substance (2 ug/kg; 50% of humic acids) during 35 d. In this study, it was concluded that HA has no effects on growth performance, which means that performance parameters as ADG, ADFI and G:F had no impact. However, in this experiment there were no evidence of a significant difference in ADG and feed conversion ratio across treatments.

Table 2. Effects of humic acids supplementation (0, 2, 4) g on pig growth performance (d 1 to 70) (Exp. 2).

	Treatments[1]				*P*-value		
Item	Ctrl	HA-2	HA-4	SEM[2]	Treatment	Linear[3]	Quadratic[3]
Initial BW, kg	5.90	5.95	5.97	0.063	0.70	0.42	0.81
d7	6.91	6.97	7.00	0.086	0.76	0.47	0.92
d14	9.09	9.07	8.93	0.138	0.68	0.42	0.75
d21	12.50	12.50	12.17	0.256	0.59	0.38	0.61
d28	15.53	15.63	15.46	0.355	0.95	0.90	0.77
d35	19.72	19.25	19.14	0.451	0.64	0.38	0.75
d42	22.85	22.63	22.69	0.426	0.93	0.80	0.79
d49	27.43	27.49	27.62	0.571	0.97	0.82	0.96
ADFI, kg/animal							
d1 to 7	0.18	0.18	0.17	0.004	0.20	0.11	0.43
d8 to14	0.42	0.41	0.39	0.010	0.18	0.07	0.83
d15 to 21	0.64	0.64	0.63	0.011	0.80	0.52	0.92
d22 to d28	0.90	0.90	0.94	0.021	0.40	0.20	0.65
d29 to 35	1.09	1.08	1.11	0.023	0.61	0.45	0.52
d36 to 42	1.22	1.24	1.23	0.029	0.97	0.81	0.98
d43 to 49	1.39	1.37	1.40	0.037	0.84	0.84	0.58
ADG, kg/animal							
d1 to 7	0.15	0.15	0.15	0.008	0.99	0.94	0.89
d8 to 14	0.31	0.30	0.28	0.046	0.22	0.09	0.72
d15 to 21	0.49	0.49	0.47	0.026	0.74	0.53	0.67
d22 to d28	0.44	0.46	0.49	0.031	0.51	0.27	0.77
d29 to 35	0.60	0.52	0.53	0.019	0.02	0.02	0.08
d36 to 42	0.46	0.48	0.51	0.030	0.49	0.24	0.96
d43 to 49	0.66	0.70	0.70	0.034	0.56	0.32	0.70
ADFI, kg/animal							
d1 to d28	0.53	0.53	0.53	0.008	0.97	0.87	0.85
d29 to d49	1.24	1.23	1.25	0.027	0.88	0.84	0.65
d1 to 49	1.78	1.76	1.78	0.033	0.90	0.92	0.67
ADG, kg/animal							
d1 to d28	0.35	0.35	0.34	0.012	0.98	0.91	0.90
d29 to d49	0.57	0.57	0.58	0.015	0.81	0.69	0.62
d1to 49	0.92	0.91	0.92	0.024	0.95	0.86	0.79
G:F				0.000			
d1 to d28	1.59	1.58	1.57	0.058	0.96	0.79	0.95
d29 to d49	2.19	2.20	2.19	0.058	0.99	0.95	0.92
d1 to 49	1.95	1.96	1.94	0.046	0.96	0.84	0.83

[1]Ctrl = Commercial diet and 0g of humic acids; HA-2 = Commercial diet and 2g of Huminfeed (75% of humic acids); HA-4 = Commercial diet and 4g of Huminfeed.
[2] Standard error of the mean, n = 10.
[3]Observed Significance level for the difference between treatments. Orthogonal contrast of adding humic acids to Ctrl diet.

Nitrogen measurement

The effects of the treatments were significant, presenting a trend for a linear and quadratic decrease on nitrogen intake (P<0.05). Also, a linear decrease in nitrogen excreted in faeces (P < 0.05) and a linear increase in apparent digestibility, as the amount of HA, increased in the diet. In addition, an increasing trend was detected for apparent digestibility (P<0.05). The percentages of N excreted in feces are similar to the percentages presented in the study conducted by Portejoie et al. (2004). Nevertheless, there were not significant differences across treatments for Nitrogen excreted in urine, N retention and total N excretion (P>0.05) (Table 3). These results are opposite to previous reports. Hobbs, et al. (1996) reported humic acids decreased N excreted in pig slurry and in consequence odorants were reduced.

Table 3. Effects of humic acids supplementation on N utilization and ammonia emissions (Exp. 2).

	Treatments[1]				*P*-value		
Item	Ctrl	HA-2	HA-4	SEM	Treatment	Linear[3]	Quadratic[3]
N intake, g/d	36.08	35.61	33.94	0.061	0.00	0.00	0.04
Fecal N excreted, g	6.39	6.09	5.21	0.071	0.02	0.01	0.23
AD (%)[4]	82.34	82.91	84.76	0.208	0.04	0.02	0.33
Urinary N, g	8.65	7.71	8.68	0.368	0.66	0.98	0.40
Total N excretion (g)	15.05	13.79	13.90	0.369	0.47	0.31	0.54
N retention (g)	21.07	21.82	20.06	0.360	0.37	0.36	0.29
N retention (%)	58.23	61.15	58.79	1.114	0.69	0.86	0.46

[1]Ctrl = Commercial diet and 0g of humic acids; HA-2 = Commercial diet and 2g of Huminfeed (75% of humic acids); HA-4 = Commercial diet and 4g of Huminfeed.
[2] Standard error of the mean, n = 5.
[3]Orthogonal contrast of adding humic acids to Ctrl diet. Observed Significance level for the difference between treatments.
[4] AD = Apparent digestibility

Ammonia excretion

During the first 48 h, more ammonia (g) was collected in Cntrl than in HA treatments, although differences were not statistically significant (P>0.05; Table 4).

This result confirms what was demonstrated in the study carried out by Ji et al (2006) that shows that the inclusion of 0.5% in the diet of pigs produces a reduction of ammonia emissions around 15 to 20%. On the other hand, the more liquid the slurry, the less NH3 we will find because it is a water soluble gas. Furthermore, the experiment obtained the expected results based on the study in which ammonia emissions were reduced by 3 to 18% in the feces by adding 4 g of acids in the pigs´ diet (Ji et al, 2006).

The recommended limit of NH3 excreted for maintaining odor control and maintaining air quality is 2.19 g animal/day (Sutton et al., 1998). The results of the experiment were 1.03, 0.75 and 0.64 NH3 g/animal/day of the Cntrl, HA-2 and HA-3 treatments respectively (Table 4). Emission of NH3 at 48 hours in this experiment were 32% of what is described by the limit when animas are fed HA.

Table 4. Daily emissions of nitrogen in the form of ammonia (g of ammonia per treatment) of the 2 days of measurement (Exp. 2).

	Treatments[1]				*P*-value		
Item	Ctrl	HA-2	HA-4	SEM[2]	Treatment	Linear[3]	Quadratic[3]
NH3(24h) g/1 d	0.42	0.23	0.31	0.134	0.48	0.49	0.35
NH3(24h) g/2 d	0.39	0.42	0.23	0.026	0.23	0.28	0.26
NH3 (48 h) g	0.65	0.48	0.42	0.025	0.19	0.11	0.50
Sum 1d + 2 d	0.82	0.65	0.53	0.066	0.52	0.31	0.39
Total NH3 excretion, g/1 d	0.67	0.36	0.47	0.065	0.45	0.42	0.35
Total NH3 excretion, g/2 d	0.62	0.67	0.35	0.044	0.24	0.18	0.28
Total NH3 excretion, g/48h	1.03	0.75	0.64	0.088	0.17	0.10	0.54
Sum 1d + 2 d	1.30	1.02	0.81	0.105	0.47	0.28	0.91

[1]Ctrl = Commercial diet and 0g of humic acids; HA-2 = Commercial diet and 2g of Huminfeed (75% of humic acids); HA-4 = Commercial diet and 4g of Huminfeed.
[2] Standard error of the mean, n = 5.
[3]Observed Significance level for the difference between treatments. Orthogonal contrast of adding humic acids to Ctrl diet.

Odor evaluation

The HA-2 and HA-4 treatments showed a further improvement in the evaluated variables (intensity, irritation and pleasantness) ($P<0.05$). In percentage terms, the odor of purines was 55% less intense, 53.75% less irritating and 60% more pleasant. Odor characteristics improved with humic acids supplementation (Table 5). This could be explained by the theory that humic acids improve apparent digestibility ($P<0.05$, Table 3) of the diet as a result of maintaining optimal pH within the intestine. This should result in lower levels of nitrogen excretion and less odor (Sutton et al., 1998). This coincides with results from an experiment conducted by Texas A and M University, which found that the humic acids added to the diet decreases the volatile ammonia in feces and also reduces the odor by 64% (Islam et al, 2005). However, no significant decrease in ammonia excretion was shown in this experiment ($P>0.05$, Table 3) but a significant difference across treatments was demonstrated in odor evaluation.

Table 5. Average values of odor evaluation of each of the treatments with humic acids supplementation (0, 2, 4g) (Exp. 2).

Item[4]	Treatments[1]			SEM[2]	*P*-value		
	Ctrl	HA-2	HA-4		Treatment	Linear[3]	Quadratic[3]
Intensity	4,5	4.94	3.59	0.43	<0.001	<0.001	<0.001
Irritation	4,74	5.13	3.67	0.37	<0.001	<0.001	<0.001
Pleasantness	4,70	5.00	3.21	0.37	<0.001	<0.001	<0.001

[1]Ctrl = Commercial diet and 0g of humic acids; HA-2 = Commercial diet and 2g of Huminfeed (75% of humic acids); HA-4 = Commercial diet and 4g of Huminfeed.
[2] Standard error of the mean, n = 5.
[3]Observed Significance level for the difference between treatments. Orthogonal contrast of adding humic acids to Ctrl diet.
[4]Odor intensity: 0 = No intensity, 8 = Most intense; Irritation: 0 = No irritability, 8 = Most irritable; Pleasantness: 0 = Very pleasant; 8 = Extremely unpleasant.

EXPERIMENT 3

The average ammonia emission from slurry was reduced with HA during the first 48 h collection period than in Cntrl without showing a significant difference ($P>0.05$). On the other hand, during the 120 h period, the Cntrl showed to have a smaller ammonia production than HA treatment. Therefore, the NH3 production rate was greater during the first 48 h. Nevertheless, there were not significant differences (P>0.05) (Table 6).

Humic acids have demonstrated to inhibit urease. This follows the inhibition of volatilization of ammonia to the environment. Urease inhibition is one of the manure treatment techniques used for reducing ammonia emissions. Ji et al. (2006) used HA in manure at 0.5% obtaining a reduction of ammonia emissions by 16 to 18%. Therefore, the results of this experiment were not the expected but ammonia emitting potential on manure independent of environmental factors was measured.

Table 6. Emissions of nitrogen in the form of ammonia (g of ammonia per treatment) of 48h and 120h of measurement (Exp. 3).

	Treatments[1]			
Item, g	Ctrl	HA	SEM[2]	*P*-value
NH3 (48h)	1.14	1.01	0.24	0.79
NH3 (120h)	0.39	0.42	0.37	0.28

[1]Ctrl = 0g of humic acids added; HA = 5g of Huminfeed added per gram of purines (75% of humic acids).

[2] Standard error of the mean, n = 10 pens per treatment.

[3]Observed Significance level for the difference between treatments.

CONCLUSIONS

Use of supplementation of HA as a novel approach was tested in the current study to identify its effects as a feed additive and when applied in pig manure. HA have shown to potentially affect growth performance, N digestibility, ammonia emissions and manure odor, using one source of HA. These studies showed that HA in diet significantly increased the N and ammonia emissions were reduced but not significantly; which led to improve the odors produced by pig manure. However, potential improvements in growth performance with HA were not evidenced. Further research is required, particularly to denote HA mechanisms of actions. Nevertheless, it is considered that the benefits of ammonia reduction may benefit growth in pigs as well as human wellbeing and the environment. HA are an available additive to achieve some of the benefits the swine production; however, its results are variable depending on the production management and environment conditions.

Regarding results from the experiments carried out, it is suggested to continue with the evaluation of the dietary supplementation of humic acids in longer periods and from different sources of HA to determinate the real potential of HA in growth performance. Also, HA supplementation could be separately applied in weaning, growing and finishing pig to evaluate and determine comparative differences of the effect depending on the growth stage. A further consideration involves biochemical processes to understand of protein decay and N digestion that determines NH3 production and NH3 production rate. In addition, investigation of physical, kinetic and thermodynamic influences on slurry odor characteristics to determine further methods to attenuate odor with HA application.

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