

Productive responses and nitrogen balance in broilers fed with humic substances in the drinking water

Respuesta productiva y balance de nitrógeno en pollos adicionados con sustancias húmicas en el agua de bebida

Sergio Gómez-Rosales^{1*} , María Angeles¹ , Jesús Maguey-González² 

¹Centro Nacional de Investigación Disciplinaria en Fisiología y Mejoramiento Animal, Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias. Km. 1 Carretera a Colón, Ajuchitlán, Colón, Querétaro, México. CP 76280. ²Facultad de Estudios Superiores Cuautitlán, Universidad Nacional Autónoma de México. Km. 1 Carretera a Colón, Ajuchitlán, Colón, Querétaro, México. CP 76280. *Responsible author and for correspondence: Gómez-Rosales Sergio, Av. del Marqués 3100-40. Col. Centro Sur. Querétaro, Querétaro, México. CP 76090. gomez.sergio@inifap.gob.mx angeles.lourdes@inifap.gob.mx magueyjesus@gmail.com

ABSTRACT

Humic substances obtained from vermicompost are an option to improve productivity and reduce ammonia emissions in broiler houses. The objective of the study was to evaluate the addition of 20% raw (RWL) or pasteurized (PWL) vermicompost leachate in the drinking water on production and carcass variables, broiler and litter chemical composition and gain, and nitrogen retention and losses in broilers from 21 to 45 days of age. A conventional diet supplemented with antibiotic growth promoters was offered throughout the experiment. Results indicate that breast performance was improved ($P < 0.05$) in broilers fed 20 % PWL in the drinking water compared to broilers that drank the RWL or water alone. The addition of 20 % RWL or PWL did not improve nutrient retention in chicks or litter or nitrogen balance or losses in 21- to 45-day-old chicks. PWL can be added to broiler drinking water to improve breast performance.

Keywords: broilers, wormcomposting leachate, humic substances, nitrogen, ammonia.

RESUMEN

Las sustancias húmicas obtenidas de lombricompostas son una opción para mejorar la productividad y reducir las emisiones de amoníaco en las casetas de pollos de engorda. El objetivo del estudio fue evaluar la adición de lixiviado de lombricomposta crudo (LLC) o pasteurizado (LLP) al 20 % en el agua de bebida sobre las variables productivas y de canal, la composición y ganancia de componentes químicos de pollos y camas y la retención y pérdidas de nitrógeno en pollos de 21 a 45 días de edad. Se ofreció una dieta convencional adicionada con antibióticos promotores del crecimiento durante todo el experimento. Los resultados indican que se mejoró ($P < 0.05$) el rendimiento de la pechuga en pollos adicionados con 20 % de LLP en el agua de bebida comparados con los pollos que bebieron el LLC o solo agua. La adición de 20 % de LLC o LLP no mejoró la retención de nutrientes en los pollos o las camas ni el balance o pérdidas de nitrógeno en pollos de 21 a 45 días de edad. El LLP se puede adicionar en el agua de bebida de pollos para mejorar el rendimiento de la pechuga.

Palabras Clave: pollos, lixiviado de lombricomposta, sustancias húmicas, nitrógeno, amoníaco.

INTRODUCTION

Humic substances (HS) have been evaluated for some years as growth-promoting and health-enhancing additives in broilers and layers ([Sanmiguel et al., 2014](#); [Arif et al., 2019](#)). Research results in broilers supplemented with HS indicate improvements in body weight, feed conversion, carcass weight and intestinal villus morphology ([Ozturk et al., 2012](#); [Taklimi et al., 2012](#); [Disethle et al., 2017](#)). Higher digestibility and retention of energy, nitrogen and ash have also been observed in chickens supplemented with HS in the drinking water ([Gomez-Rosales and Angeles, 2015](#)).

The main components of HS are humic acids (HA), fulvic acids (FA) and humins; they originate from the decomposition of organic matter, are very common in nature and are naturally present in drinking water, soil and lignite. HSs are molecules with a three-dimensional structure containing an aromatic core with heterocyclic oxygen and nitrogen; in the side chains there are functional groups that confer colloidal, spectral, and electrochemical and ion-exchange qualities ([Lehmann and Kleber, 2015](#); [Piccolo et al., 2019](#)). SH can be found in concentrations of between 8-12% in composts and vermicomposts prepared with different sources of organic matter and domestic animal manure ([Gómez et al., 2013](#)). HS are also found, although in smaller amounts, in the liquid (leachate) that drains from vermicompost beds after irrigation.

Two of the proposed mechanisms of action suggest that HSs act: 1) by increasing membrane permeability due to their detergent effect, as they behave as natural surfactants and can adsorb on different surfaces including biological membranes increasing nutrient absorption ([Gad El-Hak et al., 2012](#); [Disethle et al., 2017](#)) and 2) as detoxifying agents in the intestine due to their reducing power in the absorption of nitrates, fluorides and heavy metals ([Taklimi et al., 2012](#); [Orsi, 2014](#)). HS can inhibit soil and rumen ammonia production, which has been associated with increased efficiency of microbial protein synthesis ([Zhang et al., 2013](#); [Terry et al., 2018](#)). Also, HS reduce ammonia emissions from manure of pigs supplemented with different sources of SH by a probable reduction of bacterial urease activity ([Ji et al., 2006](#)) and ammonia concentration in fresh excreta of chickens supplemented with SH ([Maguey-Gonzalez et al., 2018](#)).

Broilers are fed high-protein diets, which can cause ammonia excesses inside the intestine ([Qaisrani et al., 2015](#); [Lemme et al., 2019](#)), causing mucosal damage such as reduced villus height and crypt depth ([Feng-Xiang et al., 2012](#); [Zhang et al., 2015](#)). Increased ammonia emissions from litter excreta are also present inside the houses, due to high N excretion, causing reductions in weight gain and increased feed conversion ([Zhang et al., 2015](#)); in addition to damage to mucus flow, ciliary action and mucous membranes of the respiratory tract ([Wang et al., 2020](#); [Zhou et al., 2020](#)); reduced specific antibody titers and other immune functions, with increased susceptibility to disease and

increased mortality ([Wei et al., 2015](#); [Zhou et al., 2020](#)). In a previous study, it was observed that broilers supplemented with a vermicompost leachate (WL) had higher N retention as the WL dose was increased, with respect to the control group ([Gomez-Rosales and Angeles, 2015](#)); reductions of up to 30 % in ammonia content were also observed in excreta from chickens supplemented with HS extracted from vermicompost ([Maguey-Gonzalez et al., 2018](#)).

Obtaining vermicompost from animal excreta represents a sustainable option for nutrient recycling and mitigation of toxic gas emissions such as ammonia, and they are also a renewable source of HS that when added to broiler feeds have the capacity to improve growth, nitrogen retention and reduce ammonia emissions from excreta ([Maguey-Gonzalez et al., 2018](#); [Domínguez-Negrete et al., 2019](#)). In previous work in chickens supplemented with HS extracted from vermicompost the feeds offered to the chickens were devoid of growth-promoting antibiotics ([Gomez-Rosales and Angeles, 2015](#); [Maguey-Gonzalez et al., 2018](#); [Domínguez-Negrete et al., 2019](#)), but the positive effects of HS on growth and nitrogen use in chickens supplemented with HS and growth-promoting antibiotics in the same diet are unknown. This information is important since in Mexico the addition of growth-promoting antibiotics in feed is a common practice. With the above background, a study was designed to evaluate the addition of raw or pasteurized vermicompost leachate on production and carcass variables, composition and gain of chemical components of broilers and litter, and nitrogen retention and losses in broilers from 21 to 45 days of age.

MATERIAL AND METHODS

Location, animals and experimental design

The study was conducted at the National Center for Disciplinary Research in Physiology and Animal Improvement of the National Institute of Forestry, Agriculture and Livestock Research (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias,) INIFAP). The protocol was reviewed and approved by the Animal Use Ethics Committee and complied with the Mexican Official Standard ([NOM-062-ZOO, 1999](#)). A total of 421 Ross 308 chicks aged 21 to 45 days, housed on the floor (17-18 chicks/cage), with eight replicates per treatment, were fed a diet formulated with corn and soybean paste to meet the nutrient recommendations of the strain, offered free access. Bacitracin methylene disalicylate (BMD) at 11% was included as a growth-promoting antibiotic at a dose of 500 ppm equivalent to 55 ppm of active ingredient; and salinomycin at 12% at a dose of 500 ppm equivalent to 60 ppm of active ingredient for the prevention of coccidiosis. Treatments were as follows: 1) broilers received drinking water directly from a storage tank connected to bell drinkers, 2) 80 % tank water was mixed with 20 % raw WL (RWL) and 3) 80 % tank water was mixed with 20 % pasteurized WL (PWL). The WL was obtained from

vermicompost prepared from sheep manure. In treatment 2, before mixing the WL with the drinking water, it was filtered using cotton cloth (diaper cloth). In treatment 3, the WL was filtered and heated at 65 °C for 1 h before mixing with water. In treatments 2 and 3, the water-WL mixture was put into 20-L plastic jugs and connected to bell troughs. The contents of HA, FA and total humic acids (THA) were 0.30, 0.33 and 0.66 %, respectively.

Recording of production variables

The body weight of the chicks was recorded at the beginning and end of the study and the daily weight gain (DWG, g/d) was estimated as the difference between the final weight (42 d) and the initial weight, divided by the experiment day number. Feed offered and rejected was also monitored to estimate the daily feed intake (DFI, g/d). Feed conversion (FC) was calculated by dividing the DFI by DWG. Daily water consumption (DWCg, ml/d) was quantified and water consumption/feed consumption was calculated. Mortality was recorded daily. At the end of the experiment and after a 12 h fast, five broilers were slaughtered from each pen and carcass and breast weights were recorded. Carcass and breast weights were expressed in grams (g) and the yield was calculated as a percentage (%), dividing by body weight.

Sampling and laboratory analysis

At the beginning of the experiment, before putting the chickens in pens, the floor was cleaned by placing 11 kg of new sawdust and a sample was taken; at the end, the complete litter was weighed and a sample was taken from each pen. At the beginning of the experiment, three groups of chickens from the same flock of chickens used in the experiment were slaughtered; at the end, three chickens were slaughtered per pen with an average weight similar to the average weight per pen. Feed samples were also taken every week.

Litter samples were freeze-dried and ground using a 2 mm sieve. Slaughtered chickens were plucked and feather and body weights were recorded and analyzed separately. The whole body was ground in a meat grinder and a representative sample was taken. The body and feathers were freeze-dried separately and ground. Feed samples were ground using a 2 mm sieve. In litter, broiler carcass, feather and feed samples, dry matter (DM), ash (A) and nitrogen (N) were determined. All laboratory determinations were carried out following recommendations [AOAC \(2019\)](#).

Calculations of the chemical composition of chickens and litter

The following formulas were used to estimate total DM, A and N of litter and broilers at the beginning and end of the experiment in each pen:

DM, A and N in litters, kg = % DM, A and N × weight of litters;

*DM, A and N in chickens, kg * = (% de DM, A and N of chickens × weight of chickens) + (% de DM, A and N of feathers × weight of feathers, kg);*

*The sum of the N of the body and feathers was made in amounts equivalent to the weight of the body and feathers at the beginning and at the end of the experiment.

Total feed consumption, kg = DFI × number of chickens per pen × 24 days;

Total consumption of DM, kg = % DM of feed × total feed consumption;

N total intake, kg = DM total intake × % N in feed;

Calculations of gain of chemical components of chickens and litter

The following formulas were used to calculate the gain of chemical components in broilers per pen:

DM gain in chickens, kg = DM total of chickens at the end – DM total of chickens at the beginning of the experiment;

C gain in chickens, kg = total C of chickens – Total C of the chicks at the beginning of the experiment;

N gain in chickens, kg = Total N of chicks at the end – Total N of chicks at the beginning of the experiment-;

The following formulas were used to calculate the gain of chemical components in the litter per pen:

DM gain in the litter, kg = total DM in the litter at the end – total DM in the litter at the beginning of the experiment;

*C gain in litter, kg = Total C in litter at the end –
Total C in litter at the beginning of the experiment;;*

*N gain in litter, kg = Total N in litter at the end –
Total N in litter at the beginning of the experiment;*

Calculations of nitrogen retention and losses

The following formulas were used to obtain the N retention in kg and percentage in broilers and litter per pen:

*N retention in chickens, kg = total N in chicks at 45 days –
– total N in chicks at 21 days;;*

N retention in chickens, % = $\frac{\text{Total N in chickens at 45 days} - \text{Total N in chickens at 21 days}}{\text{Total N consumption}} \times 100$

N retention in litter, kg = total N in litter at 45 days – total N in litter at 21 days;

N retention in beds, % = $\frac{\text{Total N in beds at 45 days} - \text{Total N in beds at 21 days}}{\text{Total N Consumption}} \times 100$

*N retention in broilers and litter, kg = total N in broilers and litter at 45 days –
total N in broilers and litter at 21 days*

N retention in chickens and litter, % = $\frac{\text{Total N in chickens and litter at 45 days} - \text{Total N in chickens and litter at 21 days}}{\text{Total N consumption}} \times 100$

The amount of N lost in total, per chicken and per kg of chicken produced was calculated as follows:

Total N lost, kg = Total N consumption, kg – N retention in broilers and litter, kg;

N lost per chicken produced, g/d = $\left(\frac{\text{Total N lost, kg}}{\text{Number of chickens}} \right) \div 24;$

N lost per chicken produced, g/d = $\left(\frac{\text{Total N lost, kg}}{\text{Total produced kilograms}} \right) \div 24;$

Statistical analysis

The results were subjected to analysis of variance under a completely randomized design, using the General Linear Models procedures of the SAS statistical package. Prior to the analysis of variance, assumption verification was carried out. The GDP and CDA variables were transformed using the multiplicative inverse and all variables expressed as percentages were transformed to arcsine to comply with the assumption of normality. Statistical differences between means were analyzed using the least significant difference methodology at $P < 0.05$.

RESULTS AND DISCUSSION

Production and carcass variables

Table 1 shows the productive variables, carcass and breast weight and yield. Initial weight, final weight, feed consumption, weight gain, feed conversion, water consumption, and water consumption/feed consumption and mortality were similar among the three treatments evaluated. These findings do not agree with those of a study in chickens added with RWL in the drinking water ([Gomez-Rosales and Angeles, 2015](#)), obtaining benefits in final weight, weight gain and feed conversion; but using diets devoid of antibiotic growth promoter and anticoccidial products. One of present work purposes was to clarify whether the beneficial effects of WL on broiler growth could be maintained despite the presence of BMD and salinomycin; however, productivity results show that the benefits of WL previously observed using antibiotic-free diets were lost. WL pasteurization was carried out with the intention of eliminating the naturally present microorganisms and nullifying any possible growth-promoting effect that the beneficial flora might exert; as has been suggested in tests of plant growth added with WL, as a source of HS ([Canellas et al., 2015](#); [Olivares et al., 2015](#)). Another important difference was that in the previous work a WL from a vermicompost, made with pig and sheep manure containing 0.47, 0.14 and 0.61 % of HA, FA and AHT, respectively, was used ([Gomez-Rosales and Angeles, 2015](#)); and in the present work a WL obtained from a vermicompost, made with sheep manure containing 0.30, 0.33 and 0.66 % of HA, FA and THA, respectively, was used.

Breast yield was higher ($P < 0.05$) in broilers that drank RWL with respect to those that drank only water and RWL (Table 1). Carcass and breast weight and yield were similar between treatments. In a previous study, higher carcass yield was obtained from chickens supplemented with HS extracted from worm composting ([Domínguez-Negrete et al., 2019](#)). Higher carcass weight and carcass yield have also been reported in chickens supplemented with increasing amounts of HS in feed and drinking water compared to the control group not supplemented with HS ([Ozturk et al., 2010](#); [Ozturk et al., 2012](#)). In a previous report it was found that HS subjected to heating for more than 40 min retain their detergent properties, but present a lower electron transfer capacity of labile chemical

groups that are lost during heating (Visser, 1985); probably by reducing the reactions associated with electron exchange between HS and different acceptors, and highlighting only the surfactant effect, increasing the permeability of the membranes; causing the higher breast yield with PWL, compared to RWL.

Table 1. Productive variables, carcass and breast weight and yield

	Water	Worm composting leachate		Standard error of the mean
		Raw	Pasteurized	
Body weight				
Day 21, kg	0.66	0.64	0.66	0.011
Day 45, kg	2.23	2.23	2.26	0.031
DFI, g/day	142.48	141.92	142.87	1.922
DWG, g/day	84.48	87.21	88.09	3.482
DFI/DWG	1.69	1.65	1.64	0.056
DWCg, ml/day	310.82	300.44	299.79	8.747
DWCg/DFI	2.19	2.11	2.08	0.065
Mortality, %	2.50	3.57	2.86	1.336
Breast, g	488.61	488.12	497.49	11.360
Breast, %	23.55 ^a	23.43 ^a	24.67 ^b	0.397
Carcass, g	1193.31	1204.44	1205.26	23.301
Carcass, %	57.58	57.89	59.82	0.751

Productive variables n= 8. Breast and carcass n= 40.^{a-b} Different letters in the same row show statistically different values (P < 0.05).

Composition and gain of chemical components of broiler chickens

The chemical composition of the slaughtered chickens at the beginning and end of the experiment and the gain of chemical components are presented in Table 2. The initial chemical composition was not analyzed statistically because only a representative sample of the chickens was taken. There were no statistical differences in the chemical composition or chemical component gain of the chickens at the end. The results are not consistent with improvements in protein use efficiency and N and A retention in chickens supplemented with HS (Gomez-Rosales and Angeles, 2015; Disetlhe *et al.*, 2017), through the excreta collection method.

Table 2. Chemical composition of chickens at the beginning and end of the experiment and gain of chemical components

	Water	Worm composting leachate		Standard error of the mean
		Raw	Pasteurized	
Chemical composition at 21 days				
Dry matter, %	26.79	26.79	26.79	ND
Ash, %	7.78	7.78	7.78	ND
Nitrogen, %	12.06	12.06	12.06	ND
Chemical composition at 45 days				
Dry matter, %	33.15	33.72	34.46	0.809
Ash, %	8.23	7.77	7.8	0.307
Nitrogen, %	12.11	11.95	12.13	0.065
Gain of chemical components per pen at				
Dry matter, kg	9.74	9.78	10.55	0.352
Ash, kg	2.31	2.09	2.19	0.127
Nitrogen, kg	1.18	1.16	1.22	0.048

Chemical composition n= 24. Gain of chemical components n= 8, ND = not determined. ^a No significant statistical differences were observed between treatments (P > 0.5).

It has been reported that HS are able to regulate N availability for plants in the soil, due to their adsorptive properties, binding directly with ammonia or stimulating the activity of disintegrating bacteria that facilitate N uptake through the roots ([Canellas et al., 2015](#); [Olivares et al., 2015](#)). It was expected that this same effect could be carried out in the chicken intestine, reducing ammonia levels released in the digestive tract, improving health, production and nutrient retention ([Qaisrani et al., 2015](#); [Lemme et al., 2019](#)). Probably the presence of BMD and salinomycin counteracted the effects of HS observed in previous work.

Table 3 shows the chemical composition of the litter at the beginning and end of the experiment and the gain of chemical components. The chemical components at the beginning were not statistically analyzed because only a representative sample of the litter was taken. There were no statistical differences in the chemical composition or chemical component gain of the litter at the end. Previous studies have reported that HS can inhibit urease activity present in soil bacteria ([Zhang et al., 2013](#)) and ammonia emissions from excreta of pigs and chickens supplemented with HS ([Ji et al., 2006](#); [Maguey-Gonzalez et al., 2018](#)).

In the present study, it was expected that HS would bind to ammonia inside the intestine and in the litter, reducing N losses by volatilization, and consequently, higher N retention in the litter at the end. Probably the presence of the antibiotics counteracted the effects of HS in the litter.

Table 3. Chemical composition of the litters at the beginning and end of the experiment and gain of chemical components

	Worm composting leachate			Standard error of the mean
	Water	Raw	Pasteurized	
Chemical composition at 21 days				
Dry matter, %	97.66	97.66	97.66	ND
Ash, %	4.77	4.77	4.77	ND
Nitrogen, %	1.53	1.53	1.53	ND
Chemical composition at 45 days				
Dry matter, %	70.87	71.61	69.29	2.936
Ash, %	17.89	18.19	18.15	0.53
Nitrogen, %	3.6	3.51	3.54	0.074
Gain of chemical components per pen at				
Dry matter, kg	10.46	9.4	9.6	1.199
Ash, kg	3.26	3.16	3.17	0.213
Nitrogen, kg	0.61	0.55	0.56	0.054

Chemical composition and gain of chemical components n= 8. ND = not determined. ^a No significant statistical differences were observed between treatments (P > 0.5).

Retention and losses of nitrogen from broilers and litter

Table 4 shows the results of nitrogen balance and loss during the experiment. None of the variables analyzed showed differences between treatments. These findings do not coincide with the higher breast yield, which could suggest that there was greater efficiency in the use of N for muscle protein synthesis, nor with the higher N retention reported in a previous work in chickens supplemented with RWL ([Gomez-Rosales and Angeles, 2015](#)). The results are not consistent with improvements in protein use efficiency and N and A retention in chickens supplemented with HS ([Gomez-Rosales and Angeles, 2015](#); [Disetlthe et al., 2017](#)).

The lack of differences in N balance coincides with the lack of differences in broiler productivity. N retention efficiency in broilers, litter and broilers + litter was 41, 28 and 69 %, respectively. N retention in broilers using the culling method was lower than reported nitrogen retention (of 61-80 %) in broilers used in nitrogen balance studies subjected to restricted feeding ([Gómez et al., 2012](#); [Gomez-Rosales and Angeles, 2015](#)); but is more in line with nitrogen retention (of 29-43 %) in free-fed broilers ([Gomez and Angeles, 2011](#)).

Table 4. Retention and losses of total nitrogen

		Worm composting		Standard error of the mean
	Water	Raw	Pasteurized	
Nitrogen balance				
N content of the diet, %	3.75	3.75	3.75	ND
Daily N intake, g	4.94	4.93	4.96	0.055
Total N intake, kg	2.07	2	2.08	0.095
N retention in broilers, kg	0.85	0.83	0.91	0.04
N retention in chickens/N consumption, %	40.73	40.96	42.22	1.123
N retention in litter, kg	0.61	0.55	0.56	0.054
N retention in litter/N consumption	29.23	27.09	27.26	1.646
N retention in broilers + litter, kg	1.49	1.42	1.46	0.087
N retention in broilers and litter/N consumption, %	69.96	67.84	68.93	2.52
Nitrogen losses per pen and per chicken				
Total N lost, kg	0.58	0.61	0.6	0.118
Total N lost, kg, %	28.08	30.56	29.06	1.079
N lost/chicken live/day, g	1.37	1.48	1.41	0.133
% N lost/chicken live/day, kg	0.62	0.68	0.63	0.058

Nitrogen balance and nitrogen losses n= 8. ND = not determined. ^a No significant statistical differences were observed between treatments (P > 0.5).

Theoretically, the rest of the N not recovered in the broilers was excreted to the litter, i.e., 59% of the N excreted by the broilers was expected to be recovered; however, only 28% of N was recovered in the litter, so it is assumed that the missing N was lost as ammonia to the environment, i.e., 31% of the N consumed and excreted was released from the litter by volatilization inside the house during the 24-day period that the broilers remained in the experiment. In litter, N losses in the form of ammonia are due to microbial mineralization of urea and uric acid accounting for up to 80 % of the total nitrogen excreted ([Zhang et al., 2015](#)).

N losses per chicken and per kg of chicken indicate that an average of 1.42 and 0.65 g of N per day were volatilized, respectively; considering that 0.216 g of H is required for the formation of 1 g of ammonia from 1 g of N; estimating that per chicken and per kg of chicken produced, 1.72 and 0.78 g of ammonia were generated. The rate of ammonia formation from litter depends mainly on ambient temperature and humidity, and the rate of accumulation inside the house depends on house size, number of chickens and degree of ventilation ([Feng-Xiang et al., 2012](#); [Wei et al., 2015](#)). However, it should be taken into account that when ammonia is released from the litter, it is first inspired by the chick before it is distributed in the house environment. If the house is adequately ventilated, the harmful effects of ammonia on the chicken may be slight; but if the ventilation is not adequate, the chicken will remain exposed to the toxicity of the gas for a longer period of time, because

it will be constantly breathing it in. Therefore, it is necessary to continue searching for feed and environmental management alternatives to mitigate the emissions of polluting gases such as ammonia inside the houses, in order to reduce their detrimental impacts on the productivity and health of the birds.

CONCLUSIONS

The results indicate that breast performance can be improved in broilers fed diets supplemented with antibiotic growth promoters and 20% PWL in the drinking water. The addition of 20 % RWL or PWL did not improve nutrient retention in broilers or litter, nor N balance or losses in broilers 21 to 45 days of age.

CITED LITERATURE

ARIF M, Alagawany M, Abd El-Hack ME, Saeed M, Arain MA, Elnesr SS. 2019. Humic acid as a feed additive in poultry diets: A review. *Iranian Journal of Veterinarian Research*. 20(3):167–172. <https://pubmed.ncbi.nlm.nih.gov/31656520/>

ASSOCIATION of Official Analytical Chemists. 2019. *Official methods of analysis of AOAC International*. 21st Edition. Arlington, VA, USA. ISBN 0-935584-77-3. <https://www.aoac.org/official-methods-of-analysis-21st-edition-2019/>

CANELLAS LP, Olivares FL, Aguiar NO, Jones DL, Nebbioso A, Mazzei P, Piccolo A. 2015. Humic and fulvic acids as biostimulants in horticulture. *Scientia Horticulturae*. 196(11): 15–27. <https://doi.org/10.1016/j.scienta.2015.09.013>

DISETLHE ARP, Marume U, Mlambo V, Dinev I. 2017. Humic acid and enzymes in canola-based broiler diets: Effects on bone development, intestinal histomorphology and immune development. *South African Journal of Animal Science*. 47(6): 914-922. <http://dx.doi.org/10.4314/sajas.v47i6.19>

DOMÍNGUEZ-NEGRET A, Gómez-Rosales S, Angeles ML, López-Hernández LH, Reis-de Souza TC, López-García Y, Zavala FA, Tellez IG. 2019. Effect of the addition of humic substances as growth promoter in broiler chickens under two feeding regimens. *Animals*. 9(12): 1-15, e1101. <http://doi:10.3390/ani9121101>

FENG-XIAN W, Bin X, Xiao-Fei H, Shao-Yu L, Fu-Zhu L, Quan-You S, Yu-Ping J, Lin-Yi W. 2012. The Effect of Ammonia and Humidity in Poultry Houses on Intestinal Morphology and Function of Broilers. *Journal of Animal and Veterinary Advances*. 11(19): 3641-3646. <https://doi.org/10.1080/00071668.2020.1752912>

GAD EL-HAK SH, Ahmed AM, Moustafa YMM. 2012. Effect of Foliar Application with Two Antioxidants and Humic Acid on Growth, Yield and Yield Components of Peas (*Pisum sativum* L.). *Journal of Horticultural Science & Ornamental Plants*. 4(3): 318–328. <https://doi.org/10.5829/idosi.jhsop.2012.4.3.262>

GOMEZ S, Angeles ML. 2011. Effects of an enzymatically hydrolyzed yeast and yeast culture combined with flavomycin and monensin on finishing broiler chickens. *International Journal of Poultry Science*. 10(6): 433-439. <https://doi.org/10.5713/ajas.2011.11316>

GÓMEZ S, Angeles ML, Mojica MC, Jalukar S. 2012. Combination of an enzymatically hydrolyzed yeast and yeast culture with a direct-fed microbial in the feeds of broiler chickens. *Asian-Australasian Journal of Animal Science*. 25(5): 665 – 673. <http://dx.doi.org/10.5713/ajas.2011.11316>

GÓMEZ-ROSALES S, Angeles ML, Núñez-Hernández G, Figueroa-Viramontes U. 2013. Metodologías para la elaboración de compostas y lombricompostas de excretas de ganado de leche. Centro Nacional de Investigación Disciplinaria en Fisiología y Mejoramiento Animal, INIFAP-SAGARPA. Colón, Querétaro. *INIFAP*. 20: 1-52. ISBN: 978-607-37-0219-5. <https://pdfs.semanticscholar.org/581d/3131800723d95415a9a9b691ed672523ac41.pdf>

GOMEZ-ROSALES S, Angeles ML. 2015. Addition of a worm leachate as source of humic substances in the drinking water of broiler chickens. *Asia-Australasian Journal of Animal Science*. 28(2): 215-222. <http://dx.doi.org/10.5713/ajas.14.0321>

JI F, McGlone JJ, Kim SW. 2006. Effects of dietary humic substances on pig growth performance, carcass characteristics, and ammonia emission. *Journal of Animal Science*. 84(9): 2482-2490. <https://doi.org/10.2527/jas.2005-206>

LEHMANN J, Kleber M. 2015. The contentious nature of soil organic matter. *Nature*. 528(11): 60–68. <https://doi.org/10.1038/nature16069>

LEMME A, Hiller P, Klahsen M, Taube V, Stegemann J, Simon I. 2019. Reduction of dietary protein in broiler diets not only reduces n-emissions but is also accompanied by several further benefits. *Journal of Applied Poultry Research*. 28(4): 867–880. <https://doi.org/10.3382/japr/pfz045>

MAGUEY-GONZALEZ JA, Michel MA, Baxter MFA, Tellez G, Moore PA, Solis-Cruz B, Hernandez-Patlan D, Merino-Guzman R, Hernandez-Velasco X, Latorre JD, Hargis BM, Gomez-Rosales S, Tellez IG. 2018. Effect of humic acids on intestinal viscosity, leaky gut and ammonia excretion in a 24 hr feed restriction model to induce intestinal permeability in broiler chickens. *Animal Science Journal*. 89(7): 1002–1010.

<https://doi.org/10.1111/asj.13011>

OLIVARES FL, Aguiar NO, Rosa RCC, Canellas LP. 2015. Substrate biofortification in combination with foliar sprays of plant growth promoting bacteria and humic substances boosts production of organic tomatoes. *Scientia Horticulturae*. 183(1): 100–108.

<http://dx.doi.org/10.1016/j.scienta.2014.11.012>

ORSI, M. 2014. Molecular dynamics simulation of humic substances. *Chemical and Biological Technologies in Agriculture*. 1(1): 1-14. <https://doi.org/10.1186/s40538-014-0010-4>

OZTURK E, Ocak N, Turan A, Erener G, Altop A, Cankaya S. 2012. Performance, carcass, gastrointestinal tract and meat quality traits, and selected blood parameters of broilers fed diets supplemented with humic substances. *Journal of the Science of Food and Agriculture*. 92(1): 59–65. <https://doi.org/10.1002/jsfa.4541>

OZTURK E, Ocak N, Coskun I, Turhan S, Erener G. 2010. Effects of humic substances supplementation provided through drinking water on performance, carcass traits and meat quality of broilers. *Journal of Animal Physiology and Animal Nutrition*. 94(1): 78–85. <https://doi.org/10.1111/j.1439-0396.2008.00886.x>

PICCOLO A, Spaccini R, Savy D, Drosos M, Cozzolino V. 2019. The Soil Humeome: Chemical Structure, Functions and Technological Perspectives. In *Sustainable Agrochemistry*. S. Vaz Jr. (ed.) Springer Nature Switzerland AG. 187-222. https://doi.org/10.1007/978-3-030-17891-8_7

QAISRANI SN, Van Krimpen MM, Kwakkel RP, Verstegen MWA, Hendriks WH. 2015. Dietary factors affecting hindgut protein fermentation in broilers: A review. *World's Poultry Science Journal*. 71(1): 139–160. <https://doi.org/10.1017/S0043933915000124>

SANMIGUEL PRA, Aguirre PWJ, Rondón BIS. 2014. Perspectivas sobre el uso de sustancias húmicas en la producción aviar. *Revista CES Medicina Veterinaria y Zootecnia*. 9(1): 104–113. <http://www.scielo.org.co/pdf/cmvez/v9n1/v9n1a10.pdf>

SECRETARÍA de Gobernación. [NOM-062-ZOO] Norma Oficial Mexicana. 1999. Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio. Ciudad de México: *Diario Oficial de la Federación*. https://www.anmm.org.mx/bgmm/1864_2007/2002-138-3-295-298.pdf

TAKLIMI SM, Ghahri H, Isakan MA. 2012. Influence of different levels of humic acid and esterified glucomannan on growth performance and intestinal morphology of broiler chickens. *Agricultural Science*. 3(5): 663-668. <https://doi.org/10.4236/as.2012.35080>

TERRY SA, Ramos AFO, Holman DB, McAllister TA, Breves G, Chaves AV. 2018. Humic substances alter ammonia production and the microbial populations within a RUSITEC fed a mixed hay - Concentrate diet. *Frontiers in Microbiology*. 9(7): 1-19. <https://doi.org/10.3389/fmicb.2018.01410>

VISSER SA. 1985. Physiological action of humic substances on microbial cells. *Soil Biology and Biochemistry*. 17(4): 457-462. [https://doi.org/10.1016/0038-0717\(85\)90009-4](https://doi.org/10.1016/0038-0717(85)90009-4)

WANG W, Shi Q, Wang S, Zhang H, Xu S. 2020. Ammonia regulates chicken tracheal cell necroptosis via the LncRNA-107053293/MiR-148a-3p/FAF1 axis. *Journal of Hazardous Materials*. 386(10): 1-12, e121626. <https://doi.org/10.1016/j.jhazmat.2019.121626>

WEI FX, Hu XF, Xu B, Zhang MH, Li SY, Sun QY, Lin P. 2015. Ammonia concentration and relative humidity in poultry houses affect the immune response of broilers. *Genetics and Molecular Research*. 14 (2): 3160-3169. <https://doi.org/10.4238/2015.April.10.27>

ZHANG WZ, Chen XQ, Zhou JM, Liu DH, Wang HY, Du CW. 2013. Influence of Humic Acid on Interaction of Ammonium and Potassium Ions on Clay Minerals. *Pedosphere*. 23(4): 493–502. [https://doi.org/10.1016/S1002-0160\(13\)60042-9](https://doi.org/10.1016/S1002-0160(13)60042-9)

ZHANG J, Li C, Tang X, Lu Q, Sa R, Zhang H. 2015. Proteome changes in the small intestinal mucosa of broilers (*Gallus gallus*) induced by high concentrations of atmospheric ammonia. *Proteome Science*. 13(1): 1-14. <https://doi.org/10.1186/s12953-015-0067-4>

ZHOU Y, Liu QX, Li XM, Ma DD, Xing S, Feng JH, Zhang MH. 2020. Effects of ammonia exposure on growth performance and cytokines in the serum, trachea, and ileum of broilers. *Poultry Science*. 99(5): 2485–2493. <https://doi.org/10.1016/j.psj.2019.12.063>