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Research Paper

Growth Responses and Antimicrobial Resistance in Broiler Chickens Added with Humic Substances from Vermicompost

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Abstract
The objective of the study was to evaluate the productive responses and presence of antimicrobial resistance (AMR) in E. coli and Streptococcus isolates from the excreta of chickens subjected to weekly changes of feed supplemented with AGP, HS and their combination.Six hundred one-day-old chicks were penned in groups of 25 and randomly assigned to four treatments: 1) Standard corn/soybean meal diet supplemented with BMD and coccidiostats (St+AGP) throughout the study, 2) Standard diet and sorghum/soybean meal/canola meal diet alternated weekly, with BMD and coccidiostats (Alt+AGP), 3) Same as 2) plus 0.30% HS (Alt+AGP+HS), and 4) Same as 2) but without AGP, and 0.30% HS (Alt+HS). The growth performance and carcass measurements were registered up to 42 days. At the end of the study, excreta samples were taken to test the AMR against different antimicrobials in E. coli and Streptococcus isolates. At 42 days of age, the body weight was higher (P < 0.009) and from 1-42 the weight gain was also higher (0.009) in St+AGP and Alt+AGP+SH broilers compared to the other treatments; but the feed conversion was lower (P < 0.023) in Alt+HSregarding the rest of the treatments. The breast (P < 0.004) and the carcass (P < 0.047) yield were higher in broilers fed Alt+AGP+HS compared to the other treatments.On average, a high proportion of E. coli isolates were resistant to most of the antibiotics tested: ampicillin (90%), carbenicillin (77%), cephalothin (63%), cefotaxime (19%), ciprofloxacin (60%), chloramphenicol (75%), gentamicin (33%), norfloxacin (54%), and S/T (54%). The lowest AMR rates were observed for nitrofurantoin and amikacin with only one isolate of E. coli in Alt+AGP+HS showing AMR, but none in the other treatments.On average, a high proportion of Streptococcus isolates were 100% resistant to most of the antibiotics tested, with exception of tetraciclyn (82.50%), S/T (76.88%) and vancomicyn (50%).In conclusion, broilers fed Alt+HS had lower body weight at 42 days and lower weight gain and feed conversion from 1-42 days compared to St+AGP and Alt+AGP+HS; in addition, the Alt+AGP+HS chickens showed higher breast and carcass yield. However, none of the treatments were able to reduce the presence of AMR in the E. coli and Streptococcus isolates from excreta.
Keywords: *Broiler chickens, Humic substances, Production, Antimicrobial resistance*

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I. Introduction

Humic substances (HS), which are derived from leonardite and lignite, have been studied for decades due to their well-documented beneficial effects in preventing and controlling various medical conditions in both animals and humans (Domínguez-Negrete et al., 2021; Angeles et al., 2022a; Maguey-González et al., 2022). HS primarily consist of humic acids (HA), fulvic acids (FA), and humins, all of which are formed through a complex biochemical transformation of organic matter (Domínguez-Negrete et al., 2021; Maguey-González et al., 2023).In a recent review, it was reported that the addition of HS extracted from vermicompost has shown improved live-weight, growth rates and feed intakesby improving immune functions and gut health in broiler chickens.The main benefits of HS in the gastrointestinal tract stem from the formation of barriers in the epithelial mucosa due to their colloidal properties and stimulation of mucin production, as well as the promotion of probiotic microbiota development, with positive changes in bacterial fermentation patterns, resulting in improved intestinal health and integrity (Angeles et al., 2022a; Maguey-González et al., 2022; López-García et al., 2025). It was recently reported that, by simulating the chicken digestive tract in vitro, the addition of humic acid (HA) extracted from vermicompost caused a trend of increasing bacterial counts as the HA concentration increased in the simulated crop, while in the simulated intestine, HA increased counts of Salmonella Enteritidis, Escherichia coli, Clostridium perfringens, and Bacillus subtilis. These results suggested that HA could be used as a prebiotic (Maguey-González et al., 2023), although the mechanism of action is unknown. Another area of study regarding HS is their potential to reduce the spread of antimicrobial resistance (AMR) factors in broiler chickens, thereby minimizing potential health risks in animals and humans (Pipová et al., 2025). In poultry AMR is a significant global health issue since antibiotics are still commonly used for growth promotion in feeds, disease prevention, and treatment, which creates conditions that encourage the development of resistant bacteria(Zahari et al., 2023; Chen et al., 2025; Sana et al., 2025).In a previous study, it was shown that HS of commercial origin has the capacity to reduce the presence of AMR-carrying bacteria (Pipová et al., 2025), however, the effectiveness of HS extracted from vermicompost to reduce the presence of AMR in birds has not been evaluated until now. The objective of the study was to evaluate the productive responses and presence of AMR in E. coli and Streptococcus isolates from the excreta of chickens subjected to weekly changes of feed supplemented with AGP, HSand their combination.

II. Materials And Methods

Extraction and characterization of humic substances

The HS was extracted from vermicompost produced with sheep manure through alkaline extraction followed by acid precipitationas previously described (Domínguez-Negrete et al. 2021; Maguey-González et al., 2022). In previous studies, the chemical features of HS were reported. Briefly, the physicochemical analysis reported by Domínguez-Negrete et al. (2021) indicated a pH of 7.8, an ash content of 23.2%, a HA to FAratio of 1.59 (47.1% HA and 29.6% FA) and an estimated aromaticity of 53.8%. The cation exchange capacity (CEC) was 79.4 cmol(+)/kg. UV–Vis spectra exhibited a λ_max at 260 nm and an E4/E6 ratio of 4.3, while FTIR analysis revealed characteristic absorption bands at 3400, 1715, 1600, and 1230 cm^{-1} (Maguey-González et al., 2023). The chemical properties and the flatstructures with aromaticityof HA andFA molecules were reported elsewhere (Angeles et al., 2022b).

Birds, management and treatments

Six hundred one-day-old male Ross 308 chicks were used. The environment of the facilities was manually controlled, with canvas curtains and gas brooders. During rearing, the temperature was initially set at 32 °C and gradually decreased at a rate of 2 °C each week until reaching 26 °C after 21 days. The lighting program was 23 h of light for 1 h of darkness for the first seven days, and from day eight on, 20 h of light for 4 h of darkness were used. At arrival, broilers were housed in 40-floor pens of 1 × 1.5 m equipped with a bell drinker and a hanging hopper feeder; a layer of five cm of sawdust was placed on the floor of each pen. Twenty five broilers were housed in each pen and were randomly assigned to four treatments: 1) Standard corn/soybean meal diet supplemented with bacitracin methylene disalicylate (BMD) and coccidiostats (St+AGP) throughout the study, 2) Standard diet and sorghum/soybean meal/canola meal diet alternated weekly, with BMD and coccidiostats (Alt+AGP), 3) Same as 2) plus 0.30% HS (Alt+AGP+HS), and 4) Same as 2) but without AGP, and 0.30% HS (Alt+HS).BMDwas added at a dose of 55 g/ton of feed; nicarbazin was used as coccidiostat from 1 to 21 days at a dose of 125 g/ton of feed, and salinomycin from 22 to 42 d at a dose of 60 g/ton of feed.Water and feed were freely available during the whole production trial. The composition and nutrient content of the experimental diets are shown in Table 1.

Table 1. Feed ingredient composition and nutrient content of the experimental diets.

Item	Standard	Sorghum/canola	Standard	Sorghum/canola	Standard	Sorghum/canola
Groundcorn	53.57	0.00	53.14	0.00	64.16	0.00
Groundsorghum	0.00	54.87	0.00	51.27	0.00	71.52
Soybeanmeal	33.39	28.20	37.92	32.92	25.93	10.96
Soy protein	3.71	3.13	0.00	0.00	0.00	0.00
Canola meal	0.00	4.00	0.00	6.00	0.00	8.00
Vegetable oil	4.69	5.06	5.64	6.53	5.64	5.24
Dicalciumphosphate	1.75	1.73	0.84	0.78	1.50	1.47
Calcium carbonate	1.60	1.53	1.38	1.29	1.41	1.30
Sodiumbicarbonate	0.36	0.43	0.35	0.37	0.46	0.55
Salt	0.19	0.11	0.22	0.18	0.13	0.04
DL-Methionine	0.23	0.27	0.12	0.20	0.17	0.16
L-Lysine·HCl	0.11	0.23	0.09	0.13	0.24	0.39
L-Threonine	0.00	0.04	0.02	0.02	0.08	0.09
Cholinechloride	0.10	0.10	0.08	0.08	0.05	0.05
Vitamin premix[1]	0.10	0.10	0.05	0.05	0.05	0.05
Mineral premix[2]	0.10	0.10	0.05	0.05	0.05	0.05
Coccidiostate	0.05	0.05	0.05	0.05	0.05	0.05
BMD	0.05	0.05	0.05	0.05	0.05	0.05
Pigments	0.00	0.00	0.00	0.03	0.03	0.03

Nutrientcomposition						
ME, kcal/kg	3000	3000	3100	3100	3100	3100
CP, %	23.77	24.33	19.41	20.57	19.00	20.50
Digestible Lys, %	1.19	1.19	1.00	1.00	0.95	0.95
DigestibleMet, %	0.46	0.46	0.38	0.38	0.43	0.43
DigestibleThr, %	0.79	0.79	0.65	0.65	0.61	0.61
Ca, %	1.00	1.00	0.90	0.90	0.85	0.85
AvailableP, %	0.50	0.50	0.45	0.45	0.43	0.43

[1]Each kg provided: 6500 IU Vit A; 2000 IU Vit D3; 15 IU Vit E; 1.5 mg Vit K; 1.5 mg thiamine; 5 mg riboflavin;35 mg niacin; 3.5 mg pyridoxine; 10 mg pantothenicacid; 1500 mg choline; 0.6 mg folicacid; 0.15 mg biotin;0.15 mg Vit B12.
[2]Each kg provided:100.0 mg Mn; 100 mg Zn; 50 mg Fe; 10 mg Cu; 1.0 mg I.

Data collection, excreta samples and laboratory tests

Productivity was recorded from 1–14, 1–28 and 1–42 days of age. At the end of the study, excreta samples were taken from 12 broilers per treatment kept in individual crates. After birds were weighed on day 42, broilers were moved to the crates to collect the excreta using metal trays beneath the floor; the excreta collected from two broilers of the same treatment were mixed for each sample.To evaluate the presence of antimicrobial resistance (AMR) in gram negative bacteria,E. coli isolates were selected using the Kirby-Bauer technique, including ampicillin, carbenicillin, cephalothin, cefotaxime, ciprofloxacin, chloramphenicol, nitrofurantoin, amikacin, gentamicin, netilmicin, norfloxacin, sulfamethoxazole/trimethoprim (ST)(Thermo Fisher Scientific, Waltham, MA, United States). E. coli ATCC 35218 was used as control. Cultures were adjusted to 0.5 McFarland scale tubes and streaked onto Mueller-Hinton agar using a swab. Antibiotic discs were then added, and the plates were incubated for 24 h. For gram positive bacteria, Streptococcus isolates were selected and were tested against penicillin, ampicillin, dicloxacillin, ceftriaxone, ciprofloxacin, clindamycin, enrofloxacin, tetracycline, chloramphenicol, gentamicin, S/T and vancomycin. The isolates were cultured in blood agar for the isolation of Streptococcus, hemolysis was observed and it was identified with the catalase test, oxidation/reduction and CAMP esculin test.Staphylococcus aureus ATCC 25923was used as control. At the end of the incubation period, zones of inhibition were measured, classifying the bacteria as susceptible or resistant to the antimicrobials used, according to the Clinical and Laboratory Standards Institute.Isolates with resistance to more than 3 antimicrobials were classified as multidrug resistance (MDR).

The results were subjected to ANOVA using GLM procedures of SAS and six replicate pens per treatment. The growth performance and carcass measurements were analyzed using a completely randomized design. Before analysis, percentage values were transformed to arcsine. RAM results are expressed as percentages.

III. Results And Discussion

The growth performance of broilers from1 to 42 days of age is shown in Table 2. At 14 days of age, the body weight was higher ($P < 0.002$) and from 1-14 days the weight gain was higher ($P < 0.003$) but the feed conversion was lower ($P < 0.045$) in broilers fed Alt+AGP compared to the other treatments.At 28 days of age the body weight was higher ($P < 0.001$) and from 1-28 days the weight gain was higher ($P < 0.001$) but the feed conversion was lower ($P < 0.005$) in Alt+HS birds compared to the other treatments; in addition, the feed conversion was lower in Alt+AGP and Alt+AGP+HS compared to St+AGP.At 42 days of age, the body weight was higher ($P < 0.009$) and from 1-42 the weight gain was also higher (0.009) in St+AGP and Alt+AGP+SH broilers compared to the other treatments; but the feed conversion was lower ($P <0.023$) in Alt+HSregarding the rest of the treatments.The breast ($P < 0.004$) and the carcass ($P < 0.047$) yield were higher in broilers fed Alt+AGP+HS compared to the other treatments. The addition of HS enhanced the productivity of broilers from 1-28 days of age and showed similar productivity compared to the St+AGP from 1-42 days of age; in addition, broilers fed HS had higher yield of the breast and carcass at the end of the study. Improvements in productivity and carcass yield have been previously reported in chickens supplemented with HS of different origin including those from vermicompost (Angeles et al., 2022a; Maguey-Gonzalez et al., 2022). In a study in which broilers were subjected to stable and sudden dietary changes it was found that under stable digestive conditions andafter experiencing sudden dietary changes, the feed conversion ratio and the number of GC in the jejunum of HS-fed broilersbehaves closely to that of AGP-fed broilers (López-Garcia et al., 2023).In a recent report in which broilers also experienced sudden dietary changes the productive variables, tibia responses, thickness and area of the jejunal villus, number of neutral, sulfated and non-sulfated GC, and mucin-2 and ocludin gene expression in jejunum were improved in HS-fed birds compared to those with added AGP, regardless of the feeding system; these results confirmed themucoprotectiveaction of HS in the jejunal epithelium (López-Garcia et al., 2025). The forementioned findings support the improved productivity of HS-fed broilers in the present study.

Table 2. Growth performance of broilers from 1 to 42 days of age.

	St+AGP[a]	Alt+AGP	Alt+AGP+HS	Alt+HS	SEM[b]	P <
Bodyweight, g						
Day 1, g	46.1	47.3	46.6	47.0	0.599	0.494
Day 14, g	366.6[c]	371.0[c]	369.6[c]	390.8[d]	4.203	0.002
Day 28, g	1250.8[c]	1258.4[c]	1271.0[c]	1348.6[d]	13.610	0.001
Day 42, g	3098.7[c]	2777.6[d]	2983.4[c]	2800.2[d]	64.260	0.009
Productive performance from 1-14 days						
Feedintake, g/day	30.85	31.81	31.56	32.22	0.418	0.164
Weightgain, g/day	22.90[c]	23.12[c]	23.07[c]	24.56[d]	0.298	0.003
Feedconversion	1.35	1.38	1.37	1.31	0.018	0.079
Productive performance from 1-28 days						
Feedintake, g/day	78.49	71.25	73.78	66.09	3.182	0.078
Weightgain, g/day	43.03[c]	43.25[c]	43.73[c]	46.49[d]	0.481	0.001
Feedconversion	1.82[c]	1.65[d]	1.69[d]	1.42[e]	0.068	0.005
Productive performance from 1-42 days						
Feedintake, g/day	131.63	117.99	129.08	110.20	2.976	0.059
Weightgain, g/day	72.68[c]	65.01[d]	69.92[cd]	63.17[d]	1.534	0.009
Feedconversion	1.81[e]	1.81[e]	1.85[e]	1.74[f]	0.011	0.023
Breast, g	772.32	700.23	879.23	694.35	34.64	0.057
Breast, %	22.73[c]	22.84[c]	24.93[d]	21.79[c]	0.53	0.004
Carcass, g	1068.17	950.17	1041.64	946.23	42.15	0.265
Carcass, %	53.76[e]	53.30[e]	56.03[f]	51.31[e]	0.97	0.047

[a]St+AGP,standard corn/soybean meal diet supplemented with bacitracin methylene disalicylate (BMD) and coccidiostats throughout the study;Alt+AGP, standard diet and sorghum/soybean meal/canola meal diet alternated weekly, with BMD and coccidiostats;Alt+AGP+HS, same as 2) plus 0.30% HS;Alt+HS, same as 2) but without AGP, and 0.30% HS.
[b] Standard error of the mean.

In Table 2 the results of antimicrobial resistance of E. coli are presented. E. coli was found in all excreta samples (100%) regardless of the treatment.Alt+AGP birds showed reduced AMR for ampicillin, carbenicillin, cephalothin, cefotaxime and gentamicin but increased AMR for chloramphenicol and netilmicin compared to St+AGP broilers.In Alt+AGP+HS birds reduced AMR for ampicillin, cephalothin and gentamicin but increased AMR for cefotaxime, gentamicin, nitrofurantoin, amikacin and netilmicin was observed regardingSt+AGP broilers.In Alt+AGP+HS birds diminished AMR for cephalothin, cefotaxime, gentamicin and netilmicin, but higher AMR for ciprofloxacine norfloxacin, S/T and chloranphenicol were seen related to St+AGP broilers.It can also be observed that only one isolate of E. coli in Alt+AGP+HS showed AMR to nitrofurantoin and amikacin, but none in the other treatments. On average, a high proportion of E. coli isolates were resistant to most of the antibiotics tested: ampicillin (90%), carbenicillin (77%), cephalothin (63%), cefotaxime (19%), ciprofloxacin (60%), chloramphenicol (75%), gentamicin (33%), norfloxacin (54%), and S/T (54%). These results indicate that HS was unable to reduce the presence of AMR factors in E. coli from the excreta samples.Opposite to our findings, reduction or removal of AMR against 19 antimicrobials tested in E. coli isolates from the ceca of broilers added with HS was recently reported (Pipová et al., 2025). The presence of AMR depends on many complex interactions within the different animal production systems (Chen et al., 2025; Sana et al., 2025), and it has been shown that the resistance rates are higher in countries in which the use of antimicrobials is not or is slightly regulated (Moawad et al., 2018; Montoro-Dasi et al., 2020). The resistance rate of E. coli isolates observed in the present experiment are much greater than those observed by Pipová et al. (2025) and are inside the range found in previous reports (Moawad et al., 2018; Montoro-Dasi et al., 2020). There were four MDR isolates in St+AGP with resistanceto 5-9 antimicrobials, four in Alt+AGP with resistance to 4-8 antimicrobials, five in Alt+AGP+SHwith resistance to 5-10 antimicrobials and five in Alt+SH with resistance to 4-8 antimicrobials. These results agree with a previous report (Montoro-Dasi et al., 2020).

Table 2. Antimicrobial resistance of E. coli isolates.

	St+AGP[a]	Alt+AGP	Alt+AGP+HS	Alt+HS	Average
E. coli, number	6	6	6	6	
E. coli, %	100.00	100.00	100.00	100.00	100.00
Ampicillin	100.00	75.00	83.33	100.00	89.58
Carbenicillin	83.33	58.33	83.33	83.33	77.08
Cephalotin	75.00	66.67	66.67	41.67	62.50
Cefotaxime	25.00	0.00	50.00	0.00	18.75
Ciprofloxacin	58.33	58.33	58.33	66.67	60.42
Norfloxacin	50.00	50.00	50.00	66.67	54.17
S/T[b]	50.00	50.00	50.00	66.67	54.17
Gentamicin	33.33	16.67	66.67	16.67	33.33
Chloranphenicol	66.67	83.33	66.67	83.33	75.00
Nitrofurantoin	0.00	0.00	16.67	0.00	4.17
Amikacin	0.00	0.00	16.67	0.00	4.17
Netimicin	16.67	33.33	50.00	0.00	25.00

[a]St+AGP, standard corn/soybean meal diet supplemented with bacitracin methylene disalicylate (BMD) and coccidiostats throughout the study; Alt+AGP, standard diet and sorghum/soybean meal/canola meal diet alternated weekly, with BMD and coccidiostats; Alt+AGP+HS, same as 2) plus 0.30% HS; Alt+HS, same as 2) but without AGP, and 0.30% HS.
[b] sulfamethoxazole/trimethoprim.

In Table 3 the results of antimicrobial resistance of Streptococcus are presented. Alt+AGP birds showed a similar number of Streptococcus isolates (5), a slight reduction of AMR for S/T but similar resistance for the rest of the antimicrobials tested compared to St+AGP broilers.Alt+AGP+SH birds showed four Streptococcus isolates, a slight reduction of AMR for S/T but similar resistance for the rest of the antimicrobials tested compared to St+AGP broilers.Alt+SH birds showed four Streptococcus isolates, reduced AMR for tetraciclyne and S/T and similar resistance for the rest of the antimicrobials tested compared to St+AGP broilers.On average, a high proportion of Streptococcus isolates were 100% resistant to most of the antibiotics tested, with exception of tetraciclyn (82.50%), S/T (76.88%) and vancomicyn (50%). All the Streptococcus isolates, regardless of treatments, were MRD with resistance to 9-11 antimicrobials. These results indicate that HS was unable to reduce the presence of AMR factors in Streptococcus from the excreta samples. The high AMR rates found in Streptococcus agree with other reports. It has been shown that S. pneumoniaeshows AMR against β-lactams (such as penicillin and cephalosporines),fluoroquinolones, macrolides, S/T and tetracycline (Zahari et al., 2023; Rajput et al., 2024). Likewise, in excreta samples taken from healthy broilers, a high rate of AMR ofS.gallolyticus isolates against tetracycline, doxycycline and lincomycin was observed (Nomoto et al., 2013). Similarly, in healthy chickens in which S. suis was isolated, unusually high levels of resistance against tetracycline (100%), clindamycin (100%) and erythromycin (95%) and intermediate resistance against penicillin (35%) and ceftriaxone (15%)were found (Nhung et al., 2020).

Table 3.Antimicrobial resistance of Streptococcusisolates.

	St+AGP[a]	Alt+AGP	Alt+AGP+HS	Alt+HS	Average
Streptococcusnumber	5	5	4	4	
Streptococcus, %	83.33	83.33	66.67	66.67	75.00
Penicillin	100.00	100.00	100.00	100.00	100.00
Ampicillin	100.00	100.00	100.00	100.00	100.00
Dicloxacillin	100.00	100.00	100.00	100.00	100.00
Ceftriaxone	100.00	100.00	100.00	100.00	100.00
Ciprofloxacin	100.00	100.00	100.00	100.00	100.00
Clindamicyn	100.00	100.00	100.00	100.00	100.00
Enrofloxacin	100.00	100.00	100.00	100.00	100.00
Tetraciclyne	90.00	90.00	87.50	62.50	82.50
Chloramphenicol	100.00	100.00	100.00	100.00	100.00
Gentamicin	100.00	100.00	100.00	100.00	100.00
S/T[b]	90.00	80.00	75.00	62.50	76.88
Vancomicyn	50.00	50.00	50.00	50.00	50.00

[a]St+AGP, standard corn/soybean meal diet supplemented with bacitracin methylene disalicylate (BMD) and coccidiostats throughout the study; Alt+AGP, standard diet and sorghum/soybean meal/canola meal diet alternated weekly, with BMD and coccidiostats; Alt+AGP+HS, same as 2) plus 0.30% HS; Alt+HS, same as 2) but without AGP, and 0.30% HS.
[b] sulfamethoxazole/trimethoprim.

IV. Conclusion And Recommendation

Broilers fed Alt+HS had lower body weight at 42 days and lower weight gain and feed conversion from 1-42 days compared to St+AGP and Alt+AGP+HS; in addition, the Alt+AGP+HS chickens showed higher breast and carcass yield. However, none of the treatments were able to reduce the presence of AMR in the E. coli and Streptococcus isolates from excreta. HS extracted from vermicompost should continue to be evaluated, and the dose, time of use and other conditions that help eliminate the presence of AMR in poultry farming should be clarified.

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