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Effect of Flavomycin on Performance, Gut Morphology and Intestinal Microflora in Broilers

J.J. Ni, T.T. Ju and X.S. Piao
State Key Laboratory of Animal Nutrition, China Agricultural University,
100193 Beijing, China

Abstract: This study was conducted to determine the effects of flavomycin on performance, gut morphology and the intestinal microflora of broilers. One hundred and twenty six, 1 day old, Arbor Acres broilers were divided into three treatments which were applied to six cages of birds with seven birds per cage. The treatments consisted of an unsupplemented basal diet or similar diets supplemented with 5 mg kg^{-1} of flavomycin or virginiamycin. Broiler performance was measured from days 0-21, 22-42 and for the overall experiment. On days 21 and 42, one bird per pen was euthanized to evaluate intestinal morphology and intestinal microflora. During the period from days 0-21, the weight gain ($p = 0.04$) and feed conversion ($p = 0.03$) of birds fed flavomycin or virginiamycin were improved compared with birds fed the unsupplemented diet. However, broiler performance was unaffected by treatment during the period from days 22-42 and during the overall experiment ($p>0.05$). Both flavomycin and virginiamycin significantly ($p<0.05$) increased villus height and reduced crypt depth in the duodenum, jejunum and ileum on day 21st while intestinal morphology was largely unaffected on day 42th. Cecal *E. coli* and Salmonella colony forming units in flavomycin or virginiamycin treated broilers were significantly lower ($p<0.01$) than those in the control group on both day 21st and 42th. The results indicate that flavomycin improved the performance, gut morphology and intestinal microflora of broilers in the starter phase. Based on these results, supplementation with antibiotics in the starter phase would result in the greatest economic benefit.

Key words: Flavomycin, virginiamycin, performance, gut morphology, intestinal microflora, broiler

INTRODUCTION

Flavomycin, also known as moenomycin and bambermycin has been used exclusively as an antimicrobial growth promoter in animal production systems (Kling *et al*., 1976; Moeller *et al*., 1976; Flachowsky and Richter, 1991; Celik *et al*., 2001; Ashayerizadeh *et al*., 2009). Flavomycin inhibits bacterial growth by competitive inhibition of the enzyme that catalyses the transglycosylation reaction during peptidoglycan biosynthesis (Van Heijenoort, 2001). It suppresses certain microorganisms and thus contributes to an improved equilibrium of the microflora in order to increase the efficiency of broiler digestion (Smith and Tucker, 1975; Bolder *et al*., 1999).

Flavomycin improves the intestinal morphology by increasing the villus height and decreasing the crypt depth to increase the efficiency of nutrient absorption (Markovic *et al*., 2009). Virginiamycin has also been used for many years as a growth promoter (Barrow, 1998). Virginiamycin destroys the synthesis of bacterial protein and is active against gram-positive bacteria. The nutrient-sparing effect of virginiamycin is due to a reduction in nutrient breakdown by the intestinal microflora of the chicks (Henry *et al*., 1986). Reports regarding the supplementation of broiler diets with flavomycin have been focused on various aspects of broiler productions including performance (Leeson, 1984; Denli *et al*., 2003b), intestinal morphology (Markovic *et al*., 2009), intestinal microflora (Bolder *et al*., 1999), short-term effects (Fairchild *et al*., 2001) and long-term effects (Markovic *et al*., 2009). However, there are few reports which have examined multi-faceted observations of the effects of flavomycin on the combination of performance, intestinal morphology and intestinal microflora in both starter and grower phases in broilers. Therefore, the objective of the current study was to evaluate the effect of flavomycin on performance, gut morphology and intestinal microflora in both starter and grower phases in broilers.

Corresponding Author: Xiang Shu Piao, State Key Laboratory of Animal Nutrition, China Agricultural University, No. 2 Yuanmingyuan West Road, 100193 Beijing, P.R. China

MATERIALS AND METHODS

The Animal Care and Use Protocol was approved by the China Agricultural University Animal Care and Use Committee (Beijing, China).

Experimental design: One hundred and twenty six, 1 day old, Arbor Acres broilers (Beijing Arbor Acres Poultry Breeding Limited, Beijing, China), weighing an average of 43.8 g were obtained from a commercial hatchery. The birds were divided into three groups with each group assigned to six cages with seven broilers per cage. Three diets were fed consisting of an unsupplemented control diet or similar diets supplemented with 5 mg kg^{-1} of flavomycin (Huvepharma N.V., Antwerp, Belgium) or virginiamycin (Huvepharma N.V., Antwerp, Belgium). The diets were formulated to meet the National Research Council (1994) requirements (Table 1).

Animals and housing facilities: The broiler chicks were housed in wire-floored cages (90×60×40 cm). Diets and water were available during the entire experimental period. Room temperature was maintained at 33°C from days 0-3 and then gradually reduced 3°C per week, to eventually maintain a constant temperature of 24°C from days 21-42. During the whole experimental period, a 24 h constant light and air ventilation schedule was maintained. Vaccinations were performed according to the program of the lab during the 1st 4 weeks.

Table 1: Composition of basal diets and nutrient levels

Basal diets	Starter phase days 1-21	Grower phase days 22-42
Ingredients (percent as-fed)		
Corn	56.35	60.15
Soybean meal	33.12	29.30
Fish meal	4.50	3.57
Soybean oil	1.95	3.07
Dicalcium phosphate	1.00	1.05
Vitamin-mineral premix[1]	1.00	1.00
Limestone	1.48	1.32
Salt	0.25	0.25
Chromic oxide	0.25	0.25
DL-methionine (98%)	0.10	0.05
Nutrient levels[2]		
Metabolizable energy (kcal kg^{-1})	3000.00	3100.00
Crude protein (%)	21.63	20.12
Calcium (%)	1.02	0.93
Total phosphorus (%)	0.69	0.64
Lysine (%)	1.22	1.01
Methionine + cysteine (%)	0.86	0.72
Threonine (%)	0.81	0.76
Tryptophan (%)	0.26	0.22

[1]The premix provided the following (per kilogram of compound feed): zinc, 60 mg; iron, 95 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.35 mg; selenium, 0.3 mg; Vitamin A, 10,000 IU; Vitamin D_3, 2,750 IU; Vitamin E, 30 IU; Vitamin K_3, 2 mg; Vitamin B12, 12 g; riboflavin, 6 mg; nicotinic acid, 40 mg; pantothenic acid, 12 mg; pyridoxine, 3 mg; biotin, 0.2 mg; choline chloride, 800 mg; [2]All nutrient levels except metabolizable energy were analyzed and values were the means of duplicate determinations

Performance and nutrient digestibility: Broilers and feeders were weighed on days 0, 21 and 42. On day 21st and 42th, body weight and feed intake were measured after a 12 h fast to determine weight gain, feed intake and feed conversion. The total excreta from each cage were collected from days 19-21 and from days 40-42 and then the excreta were weighed and dried at 60°C for 72 h. The dried excreta samples were ground to pass through a 40 mesh screen and mixed thoroughly before analysis. The dry matter, crude protein, calcium and total phosphorus contents were determined according to AOAC (1990) and gross energy content was measured using an adiabatic bomb calorimeter (Parr 6300 Calorimeter, Moline, IL). The amino acid content of the diets was determined according to AOAC (2000) using an Amino Acid Analyzer (Hitachi L-8800, Tokyo, Japan).

Small intestinal morphology: On day 21st and 42th, one bird per pen was randomly selected and euthanized for sampling. The middle sections of the duodenum, jejunum and ileum were aseptically isolated, flushed with 0.9% salt solution, fixed with 10% formaldehyde-phosphate buffer and kept at 4°C for microscopic assessment of mucosa morphology. Villus height and crypt depth were measured according to Li *et al.* (1990) with some modifications. In brief, fixed intestinal samples were prepared using conventional paraffin embedding techniques. Samples were sectioned at 6 μm thickness and stained with hematoxylin and eosin. Villus height and crypt depth were measured under 10×40 magnification using an Olympus CK 40 microscope (Olympus Optical Company, Shenzhen, China). At least fifteen well-oriented, intact villus were selected to measure the length in triplicate for each bird.

Bacteriological analysis: Cecal survival of Lactobacillus, *E. coli* and Salmonella were determined according to the method of Mikkelsen *et al.* (2003) with certain modifications. In brief, the frozen cecal samples were transferred to 4°C for a10 h incubation. Thereafter, 1 g of digesta was taken and serially diluted 10 fold with sterile physiological saline resulting in dilutions ranging from 10^{-1}-10^{-8} for enumeration. *E. coli* were cultivated on MacConkey agar (Beijing Haidian Microbiological Culture Factory, Beijing, China), Lactobacillus were cultured in DeMan Rogosa-Sharpe culture (Beijing Haidian Microbiological Culture Factory, Beijing, China) and Salmonella was cultivated on Salmonella Shigella agar (Beijing Haidian Microbiological Culture Factory, Beijing, China). Each dilution was determined in triplicate and the result was the average of three replicates. Lactobacillus was planted in Hungate Roll Tubes. *E. coli* and Salmonella were fostered in plates. All tubes and plates

were incubated at 37°C for 36 h. The microbial enumerations in digesta were expressed as $\log_{10}$ Colony Forming units per gram. Bacteria were enumerated by a visual count of colonies using the best replicate set from dilutions that resulted in 30-300 colonies per plate or tube.

Statistical analysis: All data were subjected to statistical analysis in a randomized complete block design using the General Linear Model procedures of SAS (SAS Institute, Cary, NC) with the cage serving as the experimental unit. Differences among treatments were separated by Student Newman Keul's multiple range test $p<0.05$ were considered significant.

RESULTS

Performance and nutrient digestibility: The results of antibiotic inclusion on broiler performance are shown in Table 2. During the period from days 0-21, the weight gain ($p = 0.04$) and feed conversion ($p = 0.03$) of birds fed flavomycin or virginiamycin were improved compared with birds fed the unsupplemented diet. However, broiler performance was unaffected by treatment during the period from days 22-42 and during the overall experiment ($p>0.05$). The effects of antibiotic inclusion on the apparent digestibility of dry matter, energy, crude protein, calcium and phosphorus are shown in the Table 3. In the starter phase, the digestibility of energy in the flavomycin treatment was greater ($p = 0.02$) than the control. In the grower phase, there were no differences in the digestibility of dry matter, energy, crude protein, calcium and phosphorous as a result of dietary treatement.

Table 2: Effect of flavomycin on performance for broiler chickens

Effects	Control	Flavomycin	Virginiamycin	SEM	p-value
0-21 days					
Weight gain (g day^{-1})	29.27^{b}	30.80^{a}	30.80^{a}	0.46	0.04
Feed intake (g day^{-1})	42.40	41.40	42.60	0.48	0.22
Feed conversion	1.46^{a}	1.34^{b}	1.38^{ab}	0.03	0.03
22-42 days					
Weight gain (g day^{-1})	64.20	65.60	66.70	3.14	0.85
Feed intake (g day^{-1})	120.60	121.30	123.30	3.49	0.85
Feed conversion	1.89	1.86	1.87	0.05	0.93
0-42 days					
Weight gain (g day^{-1})	46.70	48.20	48.80	1.79	0.86
Feed intake (g day^{-1})	81.50	81.30	82.90	2.50	0.92
Feed conversion	1.75	1.69	1.71	0.02	0.21

Table 3: Effect of flavomycin on nutrient digestibility for broiler chickens

Nutrient digestibility	Control	Flavomycin	Virginiamycin	SEM	p-value
Day 21					
Dry matter	0.77	0.79	0.78	0.008	0.15
Energy	0.82^{a}	0.85^{b}	0.83^{ab}	0.006	0.02
Crude protein	0.67	0.69	0.67	0.009	0.13
Calcium	0.64	0.65	0.65	0.013	0.60
Phosphorous	0.67	0.70	0.68	0.016	0.56
Day 42					
Dry matter	0.82	0.84	0.82	0.008	0.40
Energy	0.86	0.88	0.87	0.007	0.08
Crude protein	0.78	0.81	0.79	0.009	0.07
Calcium	0.54	0.56	0.55	0.022	0.92
Phosphorous	0.63	0.63	0.63	0.015	0.97

^{a,b}Means in the same row with different superscripts differ ($p<0.05$)

Small intestinal morphology: The data shown in the Table 4 shows the effects of antibiotic inclusion on small intestinal morphology on day 21st. Both flavomycin and virginiamycin significantly ($p<0.05$) increased villus height and reduced crypth depth in the duodenum, jejunum and ileum on day 21st. Table 5 shows the effects of antibiotic inclusion on small intestinal morphology on day 42th. There is no significant difference in intestinal morphology in the duodenum, jejunum and ileum among the treatments with the exception that supplementation with flavomycin and virginiamycin decreased crypt depth in the ileum compared with the control ($p = 0.02$).

Microbial populations of cecal digesta: The effect of antibiotic inclusion on the intestinal microflora of broiler chickens is shown in Table 6. Cecal *E. coli* and Salmonella colony forming units in both flavomycin and virginiamycin treated broilers were significantly lower ($p<0.01$) than those in the control group on day 21st and 42th. Lactobacillus numbers in birds fed flavomycin and virginiamycin were not significantly reduced in comparison with the control.

Table 4: Effect of flavomycin on small intestinal morphology on day 21st

Morphology	Control	Flavomycin	Virginiamycin	SEM	p-value
Duodenum (µm)					
Villus height	1214.4^{a}	1338.70^{b}	1325.4^{b}	23.23	<0.01
Villus width	129.4^{a}	136.00^{b}	136.7^{b}	1.77	0.02
Crypt depth	129.1^{a}	116.50^{b}	119.2^{b}	2.74	0.02
Jejunum (µm)					
Villus height	838.0^{a}	900.67^{b}	891.1^{b}	14.79	0.02
Villus width	119.8	126.80	126.2	3.07	0.24
Crypt depth	120.5^{a}	106.40^{b}	102.0^{b}	4.60	0.03
Ileum (µm)					
Villus height	604.9^{a}	637.90^{b}	630.7^{ab}	8.91	0.05
Villus width	134.7	140.10	139.6	1.65	0.06
Crypt depth	109.5^{a}	100.40^{b}	101.3^{b}	2.09	0.02

Table 5: Effect of flavomycin on small intestinal morphology on day 42th

Morphology	Control	Flavomycin	Virginiamycin	SEM	p-value
Duodenum (µm)					
Villus height	1685.1	1701.3	1696.7	9.46	0.48
Villus width	176.2	178.2	178.0	1.68	0.65
Crypt depth	236.9	235.6	236.8	2.03	0.88
Jejunum (µm)					
Villus height	1275.1	1286.9	1289.8	4.58	0.09
Villus width	152.9	155.5	153.8	1.76	0.57
Crypt depth	124.6	121.5	121.8	1.45	0.27
Ileum (µm)					
Villus height	1191.8	1201.1	1200.2	5.50	0.44
Villus width	132.5	137.1	135.8	1.74	0.19
Crypt depth	152.0^{a}	146.4^{b}	146.8^{b}	1.43	0.02

^{a,b}Means in the same row with different superscripts differ ($p<0.05$)

Table 6: Effect of flavomycin on broiler intestinal microflora (log10 CFU g^{-1} feces)

Microflora	Control	Flavomycin	Virginiamycin	SEM	p-value
Day 21					
Lactobacillus	8.66	8.44	8.40	0.10	0.18
E. coli	8.93[a]	7.89[b]	7.91[b]	0.10	<0.01
Salmonella	7.97[a]	7.19[b]	7.21[b]	0.05	<0.01
Day 42					
Lactobacillus	8.45	8.12	8.05	0.13	0.10
E. coli	8.17[a]	7.13[b]	7.16[b]	0.12	<0.01
Salmonella	6.46[a]	5.09[b]	5.11[b]	0.10	<0.01

[a, b]Means in the same row with different superscripts differ ($p<0.05$)

DISCUSSION

Some studies have shown a positive response to antibiotics in broilers and turkeys during the entire growth phase (Caston and Leeson, 1992; Celik *et al*., 2001; Denli *et al*., 2003a, b; Demir *et al*., 2008). However, in the current experiment, supplementation with either flavomycin or virginiamycin only increased weight gain and improved feed conversion in the starter phase. These results are in close agreement with previous results for broilers (Leeson, 1984) and hens (Fairchild *et al*., 2001). In the starter phase, the improvement in the digestibility of energy in the flavomycin treatment compared with the control is similar to the report by Huang *et al*. (2005). Waldroup *et al*. (1970) indicated an improvement in weight gain of 4 weeks broilers supplemented with 2 mg kg^{-1} flavomycin.

The results of the grower phase of the current experiment were in agreement with Waldroup *et al*. (1970) who also showed no difference in broiler weight at an older age. Prasad *et al*. (1972) also showed little advantage in body weight or feed utilization with 8 weeks flavomycin supplementation. These results suggested that the growth-promoting effect of flavomycin declined with age of the bird. In the starter phase of the current experiment, the finding that supplementation with flavomycin or virginiamycin increased villus height are in agreement with some earlier reports (Baurhoo *et al*., 2007; Markovic *et al*., 2009). A taller villus provides greater surface area for absorption (Solomon and Tullett, 1988). Miles *et al*. (2006) found a shorter villus compared with the control in the duodenum and ileum of broilers given virginiamycin. However, there were more villus per unit area which would also increase absorptive surface area. In the starter phase, the broilers given flavomycin or virginiamycin had shorter crypt depths in the duodenum, jejunum and ileum. This result is similar to that of Gunal *et al*. (2006). Enterocytes undergo a continual cycle of proliferation in the intestinal crypt, maturation and migration up the villus with desquamation at the tip of the villus. Accelerated replacement of enterocytes requires energy and proteins which can reduce growth and the development of other tissues and organ systems (Markovic *et al*., 2009).

Thus, a decreased crypt depth could be related to the increase in growth observed in the current experiment. In the current experiment, broilers given flavomycin or virginiamycin induced lower shedding rates of *E. coli* and Salmonella in comparison with control. Not only were the individual levels of *E. coli* and Salmonellas shedding reduced by the treatment but also the total number of broilers which carried the bacteria to market age was lower. These results are in close agreement with the reports on chicken (Bolder *et al*., 1999) and piglets (Dealy and Moeller, 1976). Lactobacillus in flavomycin or virginiamycin treatments was not significantly reduced compared with the control. This result agrees with the observation by Fairchild *et al*. (2001). Brenes *et al*. (1989) also demonstrated that flavomycin did not affect Lactobacillus in the intestinal microflora of broilers.

CONCLUSION

In this study, the results indicate that flavomycin improved the performance, gut morphology and intestinal microflora of broilers. Based on these results, supplementation with antibiotics in the starter phase would result in the greatest economic benefit.

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