JPSA
Japan Poultry Science Association

http://www.jstage.jst.go.jp/browse/jpsa
doi:10.2141/jpsa.0150077

Effects of Flavomycin, *Bacillus licheniformis* and Enramycin on Performance, Nutrient Digestibility, Gut Morphology and the Intestinal Microflora of Broilers

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The effects of Flavomycin, *Bacillus licheniformis* and Enramycin on broiler performance, nutrient digestibility, gut morphology and the intestinal microflora were studied in a 42-d experiment. A total of 288, one-day-old, male, Arbor Acres broilers were randomly assigned to 1 of 4 dietary treatments with 12 pens per treatment and 6 birds per pen. The treatments were comprised of a control diet without supplementation, a diet supplemented with 5 ppm Flavomycin, a diet supplemented with the combination of 5 ppm Flavomycin and 1.35×10^9 CFU/kg *Bacillus licheniformis*, as well as a diet supplemented with 5 ppm Enramycin. The average daily gain (ADG) and feed conversion ratio (FCR) of birds fed the diet with Flavomycin combined with *Bacillus licheniformis* and the Enramycin diet were improved ($P<0.05$) compared with the control diet. The digestibility of dry matter, energy, and calcium for birds fed the combination of Flavomycin and *Bacillus licheniformis* and the Enramycin diet were also enhanced compared with the control diet. All additives improved the villus height and crypt depth in the duodenum, jejunum and ileum on d 21. In addition, reduced numbers of cecal *E. coli* ($P<0.01$) were found in birds fed all three supplemented diets on d 42. In conclusion, supplementation with Flavomycin and *Bacillus licheniformis* in combination or Enramycin would appear to be superior to supplementation with Flavomycin alone. All three supplemented diets were superior to the control.

Key words: broilers, gut morphology, intestinal microbes, nutrient digestibility, performance

J. Poult. Sci., 53: 128–135, 2016

Introduction

Antibiotics are commonly used as feed additives to improve the health and performance of livestock as well as for therapeutic and prophylactic purposes (Jones and Ricket, 2003). The introduction of these agents coincided with the introduction of intensive animal rearing (Butaye *et al*., 2003). Antibiotics stabilize the bacterial population present in the digestive tract resulting in improved performance and reduced morbidity and mortality due to clinical and subclinical diseases (Miles *et al*., 2006).

Flavomycin, also known as moenomycin or bambermycin, is a fermentation product of *Streptomyces ghaenaensis* (Kling *et al*., 1976; Moeller *et al*., 1976; Ni *et al*., 2012). It is a phosphorus-containing glycolipid and is non-absorbable (Wasiliewski *et al*., 1965). It inhibits peptidoglycan synthesis by inhibiting peptidoglycan polymerases through impairment of the trans-glycolase activities of penicillin-binding proteins (Van *et al*., 1987). Flavomycin enhances the normal avian gut flora instead of disrupting it (Humbert *et al*., 1991; Manning *et al*., 1994). Its supplementation in broiler diets has been shown to improve weight gain and feed conversion without altering the predominant species of microorganisms present in the gastrointestinal tract (Evangelisti *et al*., 1975). Flavomycin also increases the villus height and decreases the crypt depth to enhance the efficiency of nutrient absorption (Markovic *et al*., 2009; Ni *et al*., 2012).

Bacillus licheniformis, a gram-positive bacterium, is classified as a 'Generally Recognized as Safe'' (GRAS) microorganism by the United States Food and Drug Administration (Wang *et al*., 2004). *Bacillus* have been suggested to have probiotic effects (Sanders *et al*., 2003). The microbial populations in the gastrointestinal tract of poultry play a key role in the normal digestive processes and in maintaining animal health. In piglets, ingestion of *Bacillus licheniformis*

Received: May 11, 2015, Accepted: October 19, 2015
Released Online Advance Publication: December 25, 2015
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spores has been shown to increase growth, feed conversion, and meat quality (Kyriakis *et al*., 1999; Alexopoulos *et al*., 2004).

Enramycin is a linear-ring, peptide antibiotic produced by Streptomyces fungicidicus and was first isolated in 1967 (Higashiide *et al*., 1968). It consists of two major components namely Enramycin-A (C107 H138 N26 O31 Cl2) and Enramycin-B (C106 H140 N26 O31 Cl2). It inhibits gram-positive bacteria such as Clostridium and Streptoccocus by destroying bacterial cell walls (EI-Husseiny *et al*., 2008). It is highly stable, both in dry form and in aqueous solution, over a pH range of 3.5 to 7.5 (Inoue *et al*., 2010).

Enramycin is regularly used in the animal feed industry as an antibiotic. Both Kamran *et al*. (2013) and EI-Husseiny *et al*. (2008) have shown that diets supplemented with Enramycin (120 g/ton feed) significantly increased weight gain, feed intake and feed conversion of broilers. Emramycin inclusion in feed also decreased the numbers of E. coli in the intestinal tract (Amaechi and Iheanetu, 2014).

Flavo combi® (Huvepharma N. V. Antwerp, Belgium) is a commercial product which contains both Flavomycin and *Bacillus licheniformis*. Athough Flavomycin and *Bacillus licheniformis* have been fed separately to broilers, there are no reports in the literature of these additives being fed in combination. It is not known if they provide any synergistic effects. The objective of the present study was to determine whether the effects of this combination are superior to Flavomycin fed alone. In addition, an attempt was made to compare the effects of these products on the performance, nutrient digestibility, gut morphometric indices and the intestinal microflora of broilers fed diets containing Enramycin.

Materials and Methods

Bird Husbandry

One-day-old, male, Arbor Acres broiler chicks were obtained from the Beijing Arbor Acres Poultry Breeding Company (Beijing, China). The broiler chicks were housed in wire-floored cages ($90\times60\times40$ cm^3) and raised under environmentally controlled conditions in which the temperature was gradually decreased from 33 to 24℃ by reducing the temperature 3℃ a week for the first 3 weeks. The temperature was then maintained at 24℃ until the end of the experiment. The mean relative humidity was set at 45 to 55% and was kept within this range. Supplemental lighting was provided on a continuous basis. All birds were provided feed and water *ad libitum*. All procedures were approved by the Animal Care and Use Committee of China Agricultural University (Beijing, China).

Experimental Products

The Flavo Combi® used in this experiment is a complex product with a content of 40 g Flavomycin and 1.08×10^{13} CFU *Bacillus licheniformis* per kg. The concentrations of Flavomycin and Enramycin in the other products were both 80 g/kg. All three products were provided by Huvepharma N. V. (Antwerp, Belgium).

Diets and Experimental Design

A total of 288, one-day-old, male, Arbor Acres broilers were randomly assigned to 1 of 4 dietary treatments with 12 pens per treatment and 6 birds per pen. The treatments comprised of a control diet without supplementation, a diet supplemented with 5 ppm Flavomycin, a diet supplemented with the combination of 5 ppm Flavomycin and 1.35×10^9 CFU/kg *Bacillus licheniformis* as well as a diet supplemented with 5 ppm Enramycin. All diets were formulated to meet the nutritional levels for broiler chickens recommended by the National Research Council (1994). The birds were fed starter diets from d 0 to 21 and grower diets from d 22 to 42. The composition of the experimental diets is listed in Table 1.

Sample Collection and Laboratory Analysis

All birds were weighed individually after their arrival from the hatchery. The birds were also weighed on d 21 and 42 after a 12 h fast. Feed bags were weighed at the same time and these values were used to calculate average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR).

On d 19 and 40, all feed and excreta were removed from the feeders and collection trays. A weighed amount of feed was then added to the feeders in sufficient quantity that the feeders would not run out of feed during the 3 d collection period. The amount of feed consumed by birds in each pen during these three days was measured, and all excreta from each pen was collected and then feathers and spilled feed were removed and the excreta weighed. A 500 gram sample of excreta was removed and dried at 60℃ for 72 h. Feed and dried excreta samples were subsequently ground to pass through a 40-mesh screen and thoroughly mixed before analyses.

The dry matter, crude protein, calcium, and phosphorus content of the diets were determined according to the methods of the Association of Official Analytical Chemists (AOAC, 1990) whereas the gross energy content was measured with an Isoperibol Bomb Calorimeter (Model 1281, Parr, Moline, IL, USA). Feed samples were hydrolyzed with 6 *N* HCl for 24 h at 110℃ (AOAC Method 999.13) and subsequently analyzed for their amino acid (AA) content using an AA Analyzer (Hitachi L-8900, Tokyo, Japan). Methionine and cystine were determined as methionine sulfone and cysteic acid after cold performic acid oxidation overnight and hydrolyzed with 7.5 *N* HCl for 24 h at 110℃ (AOAC Method 994.12). Tryptophan was determined after LiOH hydrolysis for 22 h at 110℃ (AOAC Method 998.15) using High Performance Liquid Chromatography (Agilent 1200 Series, Santa Clara, CA, USA).

One bird per cage was randomly selected and slaughtered on d 21 and 42. Immediately after euthanasia, a 2-cm sample of the duodenum (taken at the midpoint of the duodenum), the jejunum (midway between the entrance to the bile duct and Meckel's diverticulum) and the ileum (at the distal end of the lower ileum) were aseptically isolated, flushed with 0.9% salt solution, fixed with 10% formaldehyde-phosphate buffer and kept at 4℃ for microscopic assessment of mucosa

Table 1. **Composition of basal diets and nutrient levels**

	Starter phase (d 0−21)	Grower phase (d 22−42)
Ingredient (% as-fed)		
Corn	56.60	60.40
Soybean meal	33.12	29.30
Soybean oil	1.95	3.07
Fish meal	4.50	3.56
Dicalcium phosphate	1.00	1.05
Limestone	1.48	1.32
Salt	0.25	0.25
DL-methionine (98%)	0.10	0.05
Premix[1]	1.00	1.00
Nutritional content[2] (% as fed)		
Crude protein	23.32	20.40
Calcium	1.02	0.92
Total phosphorus	0.69	0.64
Lysine	1.36	1.14
Threonine	0.90	0.80
Tryptophan	0.26	0.22
Methionine + cysteine	0.82	0.74
ME, (MJ/kg)	13.02	12.96

[1] Premix supplied per kg diet: vitamin A, 11,000 IU; vitamin D, 3,025 IU; vitamin E, 22 mg; vitamin K_3, 2.2 mg; thiamine, 1.65 mg; riboflavin, 6.6 mg; pyridoxine, 3.3 mg; cobalamin, 17.6 μg; nicotinic acid, 22 mg; pantothenic acid, 13.2 mg; folic acid, 0.33 mg; biotin, 88 μg; choline chloride, 500 mg; iron, 48 mg; zinc, 96.6 mg; manganese, 101.76 mg; copper, 10 mg; selenium, 0.05 mg; iodine, 0.96 mg; cobalt, 0.3 mg.
[2] Analyzed values except for ME.

morphology. A portion of the cecum containing digesta was cut away and both ends were tied with a cotton thread. The tied off ceca were packed and then frozen by immersion in liquid nitrogen and stored at −80℃ until analysis.

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 a thickness of 5 μm and then placed on a glass slide and stained with hematoxylin and eosin. Villus height and crypt depth were measured under 10 times magnification using an Olympus CK 40 microscope (Olympus Optical Company, Shenzhen, China). The villus height and crypt depth was measured in triplicate for each bird.

Cecal numbers of *Lactobacillus* and *E. coli* were determined according to the method of Mikkelsen *et al*. (2003) with some modifications. In brief, the frozen cecal samples were transferred to an incubator at 4℃ for 10 h of incubation. Thereafter, 1 g of digesta was serially diluted 10 fold with sterile physiological saline resulting in dilutions ranging from 10^{-1} to 10^{-7}. *E. coli* were cultivated on MacConkey agar (Beijing Haidian Microbiological Culture Factory, Beijing, China), while *Lactobacillus* were cultured on DeMan Rogosa-Sharpe media (Beijing Haidian Microbiological Culture Factory, Beijing, China). Each dilution was determined in triplicate and the average of 3 replicates was recorded. *Lactobacillus* and *E. coli* were cultured in disposable culture dishes. All dishes were incubated at 37℃ for 24 h. The microbial enumerations in the digesta are expressed as $\log_{10}$ CFU per gram. Bacteria were enumerated by a visual count of colonies using the best replicated set from dilutions that resulted in 30 to 300 colonies per dish.

Statistical Analysis

Analysis of variance was conducted for a randomized block design using the General Lineal Model (GLM) procedures of the Statistical Analysis System Institute (Cary, NC). Statistical significance was declared at $P<0.05$. If significant effects were found, individual means were compared using Tukey's Multiple Range Test. Values in the tables are means and pooled S.E.M.

Results

Broiler Performance

From d 0 to 21, there were no differences in the performance of birds fed the diets supplemented with any of the growth promoters compared with the control diet. From d 22 to 42, the FCR for birds fed the diets supplemented with Flavomycin and *Bacillus licheniformis* or Enramycin were improved in comparison with the control and Flavomycin diets. From d 0 to 42, the ADG of birds fed all three supplemented diets was improved compared with the control while the FCR of birds fed the combination of Flavomycin and *Bacillus licheniformis* or the Enramycin diet was improved compared with that of birds fed Flavomycin alone or the control.

Nutrient Digestibility

The effect of the dietary additives on the apparent digestibility of dry matter, energy, crude protein, calcium and

Table 2. **Effects of dietary Flavomycin, *Bacillus licheniformis* and Enramycin supplementation on broiler performance**

	Control	FLV	FLC	ENR	SEM	*P*-value
0–21 days						
ADG, g	34.4	35.5	35.4	35.6	0.50	0.23
ADFI, g	41.5	42.0	41.9	42.2	0.73	0.92
FCR	1.21	1.18	1.18	1.18	0.01	0.47
22–42 days						
ADG, g	79.6	82.3	82.0	81.9	1.46	0.52
ADFI, g	145.8	146.0	138.8	140.0	2.49	0.09
FCR	1.83^{a}	1.78^{a}	1.70^{b}	1.71^{b}	0.03	0.03
0–42 days						
ADG, g	56.6^{b}	59.0^{a}	59.3^{a}	59.2^{a}	0.79	0.04
ADFI, g	93.4	94.2	90.8	90.7	1.27	0.22
FCR	1.65^{a}	1.60^{a}	1.53^{b}	1.54^{b}	0.02	$<$0.01

Control: basal diet; **FLV**: basal diet+5 ppm Flavomycin; **FLC**: basal diet+5 ppm Flavomycin+1.35×10^{9} CFU/kg *Bacillus licheniformis*; **ENR**: basal diet+5 ppm Enramycin.
$^{a, b}$ Means in the same row with different superscripts differ ($P<0.05$).

Table 3. **Effect of dietary Flavomycin, *Bacillus licheniformis* and Enramycin supplementation on nutrient digestibility of broilers**

	Control	FLV	FLC	ENR	SEM	*P*-value
Day 19 to 21						
Dry matter	0.68^{c}	0.70^{bc}	0.71^{b}	0.73^{a}	0.01	$<$0.01
Energy	0.74^{c}	0.75^{bc}	0.76^{ab}	0.77^{a}	0.01	$<$0.01
Crude protein	0.73	0.74	0.74	0.75	0.01	0.19
Calcium	0.38^{c}	0.40^{bc}	0.43^{ab}	0.45^{a}	0.01	$<$0.01
Phosphorous	0.50^{b}	0.52^{b}	0.53^{b}	0.56^{a}	0.01	$<$0.01
Day 40 to 42						
Dry matter	0.70^{c}	0.73^{b}	0.77^{a}	0.74^{b}	0.01	$<$0.01
Energy	0.76^{b}	0.79^{a}	0.81^{a}	0.79^{a}	0.01	$<$0.01
Crude protein	0.50^{c}	0.56^{b}	0.66^{a}	0.57^{b}	0.02	$<$0.01
Calcium	0.36^{b}	0.37^{b}	0.49^{a}	0.44^{a}	0.02	$<$0.01
Phosphorous	0.74^{b}	0.77^{b}	0.83^{a}	0.81^{a}	0.01	$<$0.01

Control: basal diet; **FLV**: basal diet+5 ppm Flavomycin; **FLC**: basal diet+5 ppm Flavomycin+1.35×10^{9} CFU/kg *Bacillus licheniformis*; **ENR**: basal diet+5 ppm Enramycin.
$^{a–c}$ Means in the same row with different superscripts differ ($P<0.05$).

phosphorous are shown in Table 3. From d 19 to 21, the digestibility of dry matter, energy and calcium in broilers fed the combination of Flavomycin and *Bacillus licheniformis* diet or the Enramycin diet were greater ($P<0.05$) than for birds fed the control diet. The digestibility of phosphorous for birds fed the Enramycin treatment was significantly higher ($P<0.05$) than for birds fed the other three diets. Values for birds fed Flavomycin alone were intermediate to those of birds fed the combination of Flavomycin and *Bacillus licheniformis* or Enramycin and the control diet.

From d 40 to 42, the digestibility of dry matter, energy and crude protein in birds fed the Flavomycin, Flavomycin and *Bacillus licheniformis* or Enramycin diets were significantly improved ($P<0.05$) compared with the control diet. The digestibility of calcium and phosphorous for birds fed the Flavomycin and *Bacillus licheniformis* or Enramycin diets were significantly ($P<0.05$) higher than for birds fed the control diet. The digestibility of all nutrients for birds the Flavomycin and *Bacillus licheniformis* treatment were significantly higher ($P<0.05$) than for birds fed Flavomycin alone with the exception of energy.

Small Intestinal Morphology

The data displayed in Table 4 shows the effects of the dietary additives on the small intestinal morphology of broiler chickens on d 21. All diets supplemented with growth promoters significantly ($P<0.05$) increased the villus height, and the villus:crypt ratio while decreasing the crypt depth in the duodenum, jejunum and ileum in comparison with birds fed the control diet.

Table 5 shows the effects of the dietary additives on the small intestinal morphology of the broilers on d 42. There was no significant difference in the gut morphology in the duodenum ($P>0.05$) for any treatment. However, the villus height in the jejunum and ileum in birds fed the combination

Table 4. **Effect of dietary Flavomycin, *Bacillus licheniformis* and Enramycin supplementation on the small intestinal morphology of broilers (d 21)**

	Control	FLV	FLC	ENR	SEM	*P*-value
Duodenum (μm)						
Villus height	1626[b]	1752[a]	1764[a]	1765[a]	12.16	<0.01
Crypt depth	225[a]	210[b]	208[b]	207[b]	3.13	<0.01
Villus: Crypt ratio	7[b]	8[a]	8[a]	8[a]	0.09	<0.01
Jejunum (μm)						
Villus height	987[b]	1042[a]	1045[a]	1045[a]	9.46	<0.01
Crypt depth	188[a]	173[b]	165[b]	163[b]	4.34	<0.01
Villus: Crypt ratio	5[b]	6[a]	6[a]	6[a]	0.15	<0.01
Ileum (μm)						
Villus height	786[b]	820[a]	829[a]	834[a]	8.50	<0.01
Crypt depth	166[a]	151[b]	145[b]	144[b]	3.59	<0.01
Villus: Crypt ratio	4[b]	5[a]	5[a]	6[a]	0.14	<0.01

Control: basal diet; **FLV**: basal diet+5 ppm Flavomycin; **FLC**: basal diet+5 ppm Flavomycin+1.35×10^9 CFU/kg *Bacillus licheniformis*; **ENR**: basal diet+5 ppm Enramycin.
[a, b] Means in the same row with different superscripts differ ($P<0.05$).

Table 5. **Effect of dietary Flavomycin, *Bacillus licheniformis* and Enramycin supplementation on the small intestinal morphology of broilers (d 42)**

	Control	FLV	FLC	ENR	SEM	*P*-value
Duodenum (μm)						
Villus height	1925	1948	1955	1960	26.28	0.32
Crypt depth	318	307	310	306	7.89	0.72
Villus: Crypt ratio	6	7	6	6	0.18	0.32
Jejunum (μm)						
Villus height	1367[b]	1388[ab]	1403[a]	1403[a]	7.83	0.02
Crypt depth	196	187	175	173	8.19	0.22
Villus: Crypt ratio	7	8	8	8	0.32	0.08
Ileum (μm)						
Villus height	1186[b]	1226[a]	1238[a]	1243[a]	8.18	<0.01
Crypt depth	188	178	171	170	8.17	0.41
Villus: Crypt ratio	6	7	7	7	0.28	0.09

Control: basal diet; **FLV**: basal diet+5 ppm Flavomycin; **FLC**: basal diet+5 ppm Flavomycin+1.35×10^9 CFU/kg *Bacillus licheniformis*; **ENR**: basal diet+5 ppm Enramycin.
[a, b] Means in the same row with different superscripts differ ($P<0.05$).

of Flavomycin and *Bacillus licheniformis* diet or the Enramycin diet was both improved ($P<0.05$) compared with birds fed the control diet.

Microbial Populations in the Cecal Digesta

The effect of the dietary additives on the intestinal microflora of broiler chickens is shown in Table 6. The *Lactobacillus* colony forming units in the ceca of birds fed the Enramycin diet were significantly ($P<0.05$) lower than for birds fed the control diet, the Flavomycin diet or the Flavomycin combined with *Bacillus licheniformis* diet on d 21 ($P=0.02$) and d 42 ($P<0.01$), while no differences were found between birds fed the Flavomycin diet and the Flavomycin and *Bacillus licheniformis* diet compared with the control diet. *E. coli* numbers in the cecum of birds fed the Flavomycin, Flavomycin and *Bacillus licheniformis* or Enramycin diets tended to be lower ($P=0.09$) in comparison with the control diet during the starter period while they were significantly decreased ($P<0.01$) during the grower period.

Discussion

In the present study, no differences were found in ADG during either the starter or grower phases but differences were observed over the entire experimental period. During the grower phase and over the entire experimental period, only the FCR in birds fed the combination of Flavomycin and *Bacillus licheniformis* or the Enramycin diet was improved indicating that Flavomycin combined with *Bacillus licheniformis* and Enramycin has a greater effect on broiler performance than Flavomycin fed alone. The lack of a response in ADG and ADFI is consistent with previous findings for broiler chickens or turkeys (Mohamed *et al*., 2008; Haque *et al*., 2010; Wasilewska *et al*., 2010).

Table 6. **Effect of dietary Flavomycin, *Bacillus licheniformis* and Enramycin supplementation on the cecal microflora of broilers (log_{10} CFU g^{-1} digesta)**

	Control	FLV	FLC	ENR	SEM	*P*-value
Day 21						
Lactobacillus	8.57[a]	8.3[a]	8.14[a]	7.25[b]	0.26	0.02
E. coli	7.14	6.60	5.94	5.82	0.38	0.09
Day 42						
Lactobacillus	8.16[a]	7.83[a]	7.55[a]	6.48[b]	0.27	<0.01
E. coli	5.57[a]	5.05[b]	4.98[b]	4.18[c]	0.17	<0.01

Control: basal diet; **FLV**: basal diet+5 ppm Flavomycin; **FLC**: basal diet+5 ppm Flavomycin+1.35×10^9 CFU/kg *Bacillus licheniformis*; **ENR**: basal diet+5 ppm Enramycin.
[a–c] Means in the same row with different superscripts differ ($P<0.05$).

There is an abundance of data in the scientific literature on the benefits of antibiotics in improving the growth and health of poultry. For example, Hooge *et al*. (2003) showed that the addition of bacitracin methylene disalicylate followed by virginiamycin to broiler diets increased body weight compared with a negative control. Diets supplemented with Flavomycin and Enramycin have also been shown to improve the feed conversion of broiler chickens and turkeys (Esteive *et al*., 1997; Parks *et al*., 2001; Kamran *et al*., 2013).

The digestibility of most nutrients in birds fed the combination of Flavomycin and *Bacillus licheniformis* or Enramycin diets was enhanced compared with the control. Nutrient digestibility was also improved in birds fed Flavomycin alone but this improvement in digestibility was not reflected in improved performance in the current experiment. Similar results were reported by Haque *et al.* (2010).

For young chicks, a longer villus increases the absorptive surface of the intestines, while shorter crypt depths indicate lower tissue turnover as well as a lower demand for tissue development (Khodambashi *et al*., 2012). In the starter period, additive inclusion increased the villus height and reduced the crypt depth and thus increased nutrient digestibility compared with the control, which was also demonstrated by Baurhoo *et al*. (2007) and Parsaie *et al*. (2007). During the grower phase, broilers fed diets supplemented with Flavomycin or Enramycin showed no improvement in gut morphology in comparison with the control diet with the exception of an increase in villus height in the jejunum and ileum. This result is in accordance with the study of Gunal *et al*. (2006) and may be explained by the fact that an increase in age may reduce the beneficial effects of these growth promoters.

Enterocytes undergo a continued cycle of proliferation in the intestinal crypt, cell maturation and migration up the villus with desquamation at the tip of the villus. The depth of the crypts is correlated with cell replacement rate (Savage *et al*., 1997). Accelerated replacement of enterocytes requires energy and proteins and the diversion of these nutrients can reduce the growth rate of broilers as well as the development of other tissues and organ systems (Markovicva *et al*., 2009).

The gastrointestinal tract is the front line of defense against the constant invasion of microbes (Markovicva *et al*., 2009). The makeup of the gastrointestinal fauna of the bird is an important factor in improving poultry performance and flock health (Markovicva *et al*., 2009). In the current experiment, birds fed diets containing the feed additives showed significant reductions in the abundance of *E. coli* during the grower phase but not during the starter phase. This result may be explained by changes in the environment (i.e. a build up of manure over time) and possible cumulative effects of feed additive inclusion. When the birds were young, pathogens were rarely seen so the effect of antibiotic inclusion may be limited during this period. This conclusion is in close agreement with previous research (Kling *et al*., 1976; Jang *et al*., 2007; Hassan *et al*., 2010; Amaechi *et al*., 2014).

Of potential concern is the fact that *Lactobacillus* numbers were decreased in birds fed the Enramycin diet. This finding was also made by Butaye *et al*., (2003) and Brenes *et al*., (1989). *Lactobacillus* are generally considered to be beneficial bacteria which compete with pathogens in order to reduce their number in the gastrointestinal tract. However, the performance of birds fed the Enramycin diet was still improved relative to the control suggesting that the reduction in *Lactobacillus* was not sufficient to negatively affect broiler performance.

On the basis of the overall results of this experiment, supplementation with Flavomycin and *Bacillus licheniformis* and Enramycin would appear to be superior to supplementation with Flavomycin alone while all three additives were superior to the control. Therefore, given a choice, poultry producers should use these additives in preference to no additives or Flavomycin alone.

Acknowledgments

Financial support was received from Huvepharma N. V. (Antwerp, Belgium) and the National Natural Science Foundation of China (No. 31372316).

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