The effect of flavomycin and probiotic combination on the growth performance and duodenal histology of broiler chickens

Elhassan M.M.O1, Hamad S2, Atif Hamza3, Lemiaa Eissa1, Asia I. Adam4, Elseory A.M.A4,5 and Hamid I.I3

1Department of Anatomy, College of Veterinary Medicine, University of Bahri, Khartoum, Sudan
2Department of Food Safety of Animal Origin, College of Veterinary Medicine, University of Bahri, Khartoum, Sudan
3Poultry Research Section, Animal Production Research Center, Khartoum, Sudan
4Department of Anatomy, Faculty of Veterinary Medicine, University of Khartoum, Sudan
5Department of Anatomy, College of Veterinary Medicine, King Faisal University, Saudi Arabia

*Corresponding Author: Mortada Elhassan, University of Bahri, College of Veterinary Medicine, P.O. Box 1660, Khartoum North, Khartoum, Sudan, Tel: +249912291449; Email: mortadamahgoub@bahri.edu.sd

Received Date: Jun 27, 2022 / Accepted Date: Jul 15, 2022 / Published Date: Jul 18, 2022

Abstract

This study investigated the effect of probiotic (Bacillus licheniformis) and flavomycin combination, using different doses, on growth performance and intestinal histology of broilers. Two hundred and ten one-day-old unsexed broiler chickens were allocated into five groups (based on the dose of each additive per ton of feed): A received no additives; B (probiotic 1.6x1012 CFU + flavomycin 4 g); C (probiotic 1.6x1012 CFU + flavomycin 20 g); D (probiotic 3.2x1012 CFU/ton + flavomycin 4 g/ton); E (probiotic 3.2x1012 CFU + flavomycin 20 g). On day 21 of age, group E showed the lowest (p<0.05) body weight gain (BWG). Low feed intake (FI) was observed in group B, C, and D. Low values (p<0.05) of feed conversion ratio (FCR) were recorded in group B, C, and D. Group E showed the highest value (p<0.05) of FCR. On day 42 of age, FCR was noticeably lower (p<0.05) in group B, C and D compared to group A and E. All groups received feed additives revealed a significant rise (p<0.05) in villus height (VH). The crypt depth (CD) was higher in group B and C. Group D demonstrated higher (p<0.05) villus to crypt ratio (V:C). In conclusion, the combination of high dose of the probiotic, Bacillus licheniformis, and low dose of flavomycin had beneficial effects on the performance as well as the intestinal histology of broiler chickens. Not only did the coadministration of high doses of the two additives had no benefits on the performance at six weeks of age, but also might be detrimental during starter period.

Keywords: Bacillus licheniformis; Broilers; Flavomycin; Intestine; Performance


Copyright: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. Copyright © 2022; Elhassan M.M.O
Introduction

The use of antibiotics as growth promotors has been restricted or even strictly prohibited in many countries worldwide. This is mainly due to the great concerns regarding the antibiotic residues [1-3]. It is well known that the antibiotic residues in poultry meat can pose a potential hazard to human health such as imbalance of intestinal microbiota, and bacterial resistance to antibiotics [4]. As a result, this situation has led to adopt alternative strategies [5]. One of these strategies is the use of natural feed additives such as probiotics [6]. Probiotics are defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" [7]. Several microorganisms are utilized as probiotics, including Bacillus licheniformis bacteria [8]. Flavomycin, also known as flavophospholipol, is an antibiotic which considered as another possible alternative to several antibiotics used as growth promoters in poultry diet. It is noteworthy that flavomycin can be administered without concerns about tissue residues, due to the fact that its large molecules cannot be absorbed by the gastrointestinal tract of birds [9]. Consequently, the residues of flavomycin were not detected even when added to diet in high doses. For instance, an oral dose of 20 ppm of flavomycin did not cause tissue residues in chicken [10]. Moreover, flavomycin is characterized by its low impact on beneficial bacteria existed in broiler gut [10]. It has been also reported that flavomycin reduced the shedding of potentially pathogenic bacteria in chicken feces [11]. The use of probiotics and flavomycin in broilers has been reported to improve intestinal health along with intestinal morphology, both of which resulted in the enhancement of nutrient absorption and subsequently growth performance [12-15]. On the other hand, results obtained by using either probiotics or flavomycin were sometimes controversial [16]. However, the benefits emerged from utilizing probiotics and flavomycin as feed additives may well justify the continuity of research on their positive effects [8,17]. In this respect, little is known about the use of the two additives when given in combination despite the availability of a commercial product containing both flavomycin and Bacillus licheniformis. Study conducted by wang et al. [8] on such product revealed a synergistic effects of the probiotic Bacillus licheniformis (1.35x10^{12} CFU/ton) and flavomycin (5 g/ton) on broiler chickens as compared to the administration of flavomycin alone. However, it is envisioned that more benefits could be obtained by determining the optimum dose of each additive when fed in combination. Therefore, the present study aimed at examining the effect of different combinations of probiotic (Bacillus licheniformis) and flavomycin on the performance and the duodenal histology of broiler chickens.

Materials and Methods

Experimental design: The experiment was carried out in closed house at Animal Production Research Center, Poultry Research Section, Sudan. Two hundred and ten one-day-old unsexed broiler chicks (Arbor acres) were utilized in this study. The birds were weighted individually to obtain the average initial body weight. Afterwards, the chicks were allocated randomly into five treatment groups, using randomized complete block design. Each group comprised 42 birds which were divided into six replicates of 7 birds each. The replicate was represented by a pen with a dimensions of 1 × 1 m² and wood shavings on the floor. The five dietary treatment groups were: group A received no additives; group B received Bacillus licheniformis 1.6x10^{12} CFU/ton of feed + flavomycin 4 g/ton of feed; group C received Bacillus licheniformis 1.6x10^{12} CFU/ton of feed + flavomycin 20 g/ton of feed; group D received Bacillus licheniformis 3.2x10^{12} CFU/ton of feed + flavomycin 4 g/ton of feed; group E received 3.2x10^{12} CFU/ton of feed + flavomycin 20 g/ton of feed (Table 1). The high and low doses of the probiotic were determined according to the guidelines given by the manufacturer. The low dose of flavomycin in this study was approximately similar to the dose...
The effect of flavomycin and probiotic combination on the growth performance and duodenal histology of broiler chickens

DOI: https://doi.org/10.36811/jvsr.2022.110019

utilized by Wang et al. [8]. The high dose of flavomycin was calculated based on its safe use, in particular the lack of tissue residues [10].

<table>
<thead>
<tr>
<th>Group</th>
<th>Probiotic¹ (CFU/ton)</th>
<th>Flavomycin² (g/ton)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>1.6X10¹²</td>
<td>4</td>
</tr>
<tr>
<td>C</td>
<td>1.6X10¹²</td>
<td>20</td>
</tr>
<tr>
<td>D</td>
<td>3.2X10¹²</td>
<td>4</td>
</tr>
<tr>
<td>E</td>
<td>3.2X10¹²</td>
<td>20</td>
</tr>
</tbody>
</table>

CFU/ton= Colony forming unit per ton feed
¹B-ACT®, Huvepharma N.V., Antwerp, Belgium. Each gram contains 3.2 X10⁹ CFU of Bacillus licheniformis (DSM 28710)
²FLAVOMYCIN® 80, Huvepharma N.V., Antwerp, Belgium. Each 100 g of the product contain 8 g of flavomycin.

The basal diet given to the chicks was calculated to meet the nutrients requirement of broilers in the starter (1 to 23 days of age) and finisher (24 to 42 days of age) periods (Table 2) as recommended by the National Research Council [18]. Mash feed was then formulated using a horizontal mixture. Birds were allowed to access water and feed ad libitum. Continuous lighting program was applied throughout the experiment. The house temperature had been set at 33 °C from day 1 to day 3 before it was reduced gradually to 24 °C until the end of the experiment.

**Growth Performance:** Growth performance was evaluated according to the calculations of body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) at the end of weeks 3 and 6 of age (days 21 and 42).

**Samples Collection:** On day 42, 6 broiler chickens from each group were selected (one bird from each replicate). Birds were then slaughtered manually by making a fast-single ventral cut in the neck using a sharp knife with straight surface. The small intestine was detached from the other visceral organs and samples from the mid of the duodenum were taken for histological investigations. Samples were fixed in 10% neutral buffered formalin for 5 days, and processed for routine histological technique. Thirty tissue sections (6 for each treatment) were stained with hematoxylin and eosin. Sections were then examined under light microscope connected to a digital camera (Optica microscopes, Italy). Histological measurements were then determined using a software program provided with the camera. Ten well-oriented villi with prominent lamina propria were selected from each section. The villus height (VH) was measured from the base to the tip of each villus. In addition, ten well-defined intestinal crypts were also selected for measurement in each section. Therefore, a total of 600 readings (300 for villi and 300 for intestinal crypts) were carried out; each group was representing by 120 readings (60 for villi and 60 for intestinal crypts).

**Statistical Analysis:** Statistical analysis was conducted using one-way ANOVA in statistical software (SPSS version 21.0, IBM Corporation, New York, USA). Significant differences were determined at p<0.05 and Duncan’s multiple range test was applied to separate the treatment means.
The effect of flavomycin and probiotic combination on the growth performance and duodenal histology of broiler chickens

DOI: https://doi.org/10.36811/jvsr.2022.110019

JVSР: July-2022: Page No: 01-07

Table 2: Composition and chemical analysis of the basal diet used in the experiment.

<table>
<thead>
<tr>
<th>Feed ingredients</th>
<th>Type of Feed mixture</th>
<th>Starter (1-23)</th>
<th>Finisher (24-42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorghum</td>
<td></td>
<td>66.06</td>
<td>66.04</td>
</tr>
<tr>
<td>Ground nut cake</td>
<td></td>
<td>26.58</td>
<td>21.02</td>
</tr>
<tr>
<td>Wheat bran</td>
<td></td>
<td>0.00</td>
<td>4.50</td>
</tr>
<tr>
<td>Broiler concentrate*</td>
<td></td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Lime stone</td>
<td></td>
<td>1.10</td>
<td>0.50</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td></td>
<td>0.00</td>
<td>0.20</td>
</tr>
<tr>
<td>Salt</td>
<td></td>
<td>0.15</td>
<td>0.34</td>
</tr>
<tr>
<td>Methionine</td>
<td></td>
<td>0.50</td>
<td>0.05</td>
</tr>
<tr>
<td>Lysine</td>
<td></td>
<td>0.16</td>
<td>0.00</td>
</tr>
<tr>
<td>Beef tallow</td>
<td></td>
<td>0.00</td>
<td>2.15</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td></td>
<td>0.25</td>
<td>0.10</td>
</tr>
<tr>
<td>Antimycotoxin</td>
<td></td>
<td>0.20</td>
<td>0.10</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>ME kcal/kg</td>
<td></td>
<td>3089</td>
<td>3196</td>
</tr>
<tr>
<td>Crude protein %</td>
<td></td>
<td>22.0</td>
<td>20.3</td>
</tr>
<tr>
<td>Crude fiber %</td>
<td></td>
<td>4.4</td>
<td>4.5</td>
</tr>
<tr>
<td>Ether extract %</td>
<td></td>
<td>3.9</td>
<td>5.7</td>
</tr>
<tr>
<td>Calcium %</td>
<td></td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Available phosphorus %</td>
<td></td>
<td>0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>Lysine %</td>
<td></td>
<td>1.20</td>
<td>1.02</td>
</tr>
<tr>
<td>Methionine %</td>
<td></td>
<td>0.90</td>
<td>0.72</td>
</tr>
</tbody>
</table>

* Each kg of broiler concentrate contains: metabolizable energy 1900 Kcal, crude protein 35%, crude fat 2.6 %, crude fibre 4.5%; Ca 6.5%, total phosphorus 5.5%; lysine 10%; methionine 3%; vitamin A 200,000 IU; vitamin D₃ 40,000 IU; vitamin E 500 mg; vitamin K₃ 40 mg; vitamin B₁ 300 mg; vitamin B₂ 100 mg; vitamin B₃ 200 mg; vitamin B₆ 40 mg; vitamin B₁₂ 200 mg; niacin 600 mg, folic acid 20 mg; biotin 2 mg; choline chloride 10,000 mg; Fe 800 mg; Zn 1,000 mg; Mg 29 mg; Cu 160 mg; I 8 mg; Mn 1,200 mg; Se 3 mg. ME= Metabolizable energy.

Results

Growth performance: Table 3 shows the growth performance of the broiler chickens utilized in this study. On day 21 of age, group D showed the highest (p<0.05) BWG (591.7 g) whereas the lowest (p<0.05) BWG was observed in group E (499.9 g). Remarkable reduction (p<0.05) in FI was observed in group B (714.2 g), C (656.0 g), and D (695.4 g) when compared to group A (837.9 g) and E (822.5 g). Significantly lower values (p<0.05) of FCR were recorded in group B (1.25), C (1.17), and D (1.17) compared to group A (1.47). The highest value (p<0.05) of FCR was noted in group E (1.64). On day 42 of age, the highest (p<0.05) BWG was observed in group B (2244.8 g). Significantly lower (p<0.05) FI was noted in group B (3298.1 g), C (3255.5 g), and D (3246.4 g) when compared to FI in group A (3434.6 g). Broilers in groups B, C and D displayed noticeably lower (p<0.05) FCR (1.47, 1.49, 1.48 respectively) as compared to FCR in group A (1.68) and E (1.62).
The effect of flavomycin and probiotic combination on the growth performance and duodenal histology of broiler chickens

DOI: https://doi.org/10.36811/jvsr.2022.110019

Table 3: Body weight gain (g), feed intake (g) and feed conversion ratio of broiler chickies on days 21 and 42 of age.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 21</th>
<th>Day 42</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BWG</td>
<td>FI</td>
</tr>
<tr>
<td>A</td>
<td>557.0a</td>
<td>837.9a</td>
</tr>
<tr>
<td>B</td>
<td>566.1a</td>
<td>714.2b</td>
</tr>
<tr>
<td>C</td>
<td>559.7a</td>
<td>656.0c</td>
</tr>
<tr>
<td>D</td>
<td>591.7b</td>
<td>695.4d</td>
</tr>
<tr>
<td>E</td>
<td>499.9c</td>
<td>822.5a</td>
</tr>
<tr>
<td>SEM</td>
<td>8.4</td>
<td>8.3</td>
</tr>
</tbody>
</table>

Different superscripts within the same column indicate significant difference (P<0.05).
BGW=Body weight gain, FI=Feed intake, FCR=Feed conversion ratio. SEM=Standard error of mean.

Intestinal histology: Table 4 shows the histological parameters of the duodenum of the broiler chickens in this study. All treatment groups received feed additives revealed a significant rise (p<0.05) in VH when compared to control group. In addition, the VH in group C (1735.7 µm) and D (1655.7 µm) was significantly higher as compared to group B (1506.2 µm) and E (1439.1 µm). The CD was remarkably (p<0.05) higher in group B (208.4 µm) and C (214.0 µm) when compared to CD in group A (189.9 µm). The CD in group D (199.5 µm) and E (197.5 µm) did not show a significant difference (p>0.05) as compared to control group (189.9 µm). Birds in group D had substantially higher (p<0.05) V:C (8.59) compared to birds in group A (7.28), B (7.33), and E (7.71).

Table 4: Villus height and crypt depth (µm), and villus to crypt ratio in different treatment groups of broilers on day 42 of age.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>VH</th>
<th>CD</th>
<th>V:C</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1336.8a</td>
<td>189.9a</td>
<td>7.28a</td>
</tr>
<tr>
<td>B</td>
<td>1506.2b</td>
<td>208.4b</td>
<td>7.33b</td>
</tr>
<tr>
<td>C</td>
<td>1735.7c</td>
<td>214.0b</td>
<td>8.06bc</td>
</tr>
<tr>
<td>D</td>
<td>1655.7c</td>
<td>199.5ab</td>
<td>8.59c</td>
</tr>
<tr>
<td>E</td>
<td>1439.1b</td>
<td>197.5ab</td>
<td>7.71ab</td>
</tr>
<tr>
<td>SEM</td>
<td>3.00</td>
<td>0.50</td>
<td>2.20</td>
</tr>
</tbody>
</table>

Different superscripts within the same column indicate significant difference (P<0.05).
VH = Villus Height, CD = Crypt Depth, V:C = Villus to crypt ratio. SEM= Standard error of mean.

Discussion

Similarly, most of the combinations of *Bacillus licheniformis* and falvomycin utilized in this study displayed marked improvement in the growth performance of broilers during the first three weeks of age. In contrast, it has been reported that the combination of flavomycin and *Bacillus licheniformis* had no discernable improvement in the performance of broilers during starter period [8]. In this study, the combination of higher doses of the two additives during the same period might have been even detrimental to the broiler chicks as they exhibited poor FCR compared to broilers given neither of the additives. However, the aforementioned negative impact of the combination of these additives during the relatively fast-growing stage of chickens warrant further investigations. In this study, the combinations containing different doses of *Bacillus licheniformis* and falvomycin had substantial beneficial results on growth performance at six weeks of age as compared to...
birds received no additives. This is in accord with the finding reported by Wang et al. [18] who stated that the dietary addition of *Bacillus licheniformis* and flavomycin in combination was more effective compared to flavomycin when given alone. Additionally, the combined effect of probiotics and flavomycin on body weight gain has been also reported in broiler chickens at day 38 of age [19]. On the other hand, despite the positive effects of the two additives when given in combination, it is essential to indicate that the coadministration of high doses of *Bacillus licheniformis* and flavomycin in this study had no beneficial effects on growth performance of broiler chickens at six weeks of age. It is well documented that the histological features of the small intestine of broiler chicks are improved by dietary addition of either probiotics, *Bacillus spp.* [8,17] or flavomycin [8,20]. In this study, all treatment groups received feed additives have shown remarkable increase in VH as compared to the group received no additives. Besides, the V:C was essentially more pronounced in chickens received higher dose of the probiotic and lower dose of flavomycin. Taken into account the results of the growth performance in this study, it is most likely that the combination of higher dose of probiotic and lower dose of flavomycin had more beneficial effects not only on performance but also on intestinal histology of broiler chickens. In conclusion, the present study revealed that the combination of higher dose of the probiotic, *Bacillus licheniformis*, and lower dose of flavomycin had beneficial effects on the performance as well as the intestinal histology of broiler chickens. In contrast, the coadministration of high doses of the probiotic and flavomycin had no benefits on the growth performance at six weeks of age. The high doses of the two additives might have been even detrimental during starter period.

References


The effect of flavomycin and probiotic combination on the growth performance and duodenal histology of broiler chickens

DOI: https://doi.org/10.36811/jvsr.2022.110019

JVSР: July-2022: Page No: 01-07

Doi: https://doi.org/10.1186/s13567-018-0530-1

Doi: https://doi.org/10.1128/cmr.16.2.175-188.2003

Doi: https://doi.org/10.1093/ps/78.12.1681


Doi: https://doi.org/10.1111/jpn.13454


Doi: https://doi.org/10.3382/ps.2011-01844

Doi: https://doi.org/10.3382/ps.2011-01844


Doi: https://doi.org/10.3389/fmicb.2020.618144