Pathological, Bacteriological and Biochemical Studies on the Effect of Formic Acid in Broiler Chickens

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Abstract

A total of 100 apparently healthy Hubbard chicks, one day of age were used in this study to investigate the effect of formic acid on body performance. Biochemical and histopathological changes beside its effect on intestinal bacterial growth in broiler were also studied. Cloacal swabs were collected from all chicks for bacteriological examination at day one of age. Eighteen chicks were positive and the distribution of the bacterial agents was 13 for single infection (E. coli, Corynebacterium species and Salmonella species) and 5 in case of mixed infection (Streptococcus, Corynebacterium species, Staphylococcus aureus and E. coli). Serological identification of the isolated E. coli revealed O78 (4) and O157 (2), while, the obtained Salmonella serotypes were S. Typhimurium (3) and S. Enteritidis (1). Sixty bacterial free chicks were divided into 3 groups (20 birds, each), 1st group served as control. The 2nd and 3rd groups received 1 ml and 2 ml formic acid/ liter drinking water, respectively for 30 days. Formic acid in both doses induced a significant increase in body weight gain, total proteins, albumin and globulins coupled with significant decrease in total lipids, cholesterol and triglyceride. Meanwhile, A/G Ratio calcium, phosphorous, magnesium, zinc, sodium and potassium insignificantly increased beside insignificant decrease in liver enzymes (AST, ALT and ALP) and uric acid, creatinine and intestinal bacterial content as well as improved the feed consumption and feed conversion rate. Histopathologically, spleen and bursa showed hyperplasia of lymphocytes in white pulp. Hepatic tissue particularly the 3rd group had mild fatty changes and hydropic degeneration. Also renal tubules of undergo mild hydropic degeneration. In conclusion the use of formic acid as feed additive in chicken broiler ration may act as growth promoter and exhibits positive impact on biochemical parameters, intestinal and immune organs histology beside reduction of colonization of bacteria in intestinal wall.

Keywords: Broiler, Formic Acid, Bacteriology, Pathology, Biochemistry

Introduction

Poultry industry is one of the most important sources of protein all over the world [1]. Feed additives induce high growth and efficient feed conversion [2]. Antibiotic growth promoters and antibiotic resistance are clearly connected and increased concern of researchers to use other alternatives like organic acids as feed additives in poultry production [3]. Organic acids have a long history of being utilized as food additives to prevent food deterioration and extend the shelf life of perishable food ingredients [4]. They are used in poultry diets to elicit a positive response in body growth [5] and as alternative for antibiotic growth promoters [6].

This study aimed to investigate the influence of formic acid on body performance, biochemical parameters beside its pathological effect. Also the changes in populations of bacteria inhabiting the gastrointestinal tract of broiler chickens were investigated.
Material and Methods

**Birds**

A total of 100 apparently healthy one day-old Hubbard broiler chicks nearly equal in the live body weight (45.22-48.10 gm) were used in this study. Cloacal swabs were collected from each chick for bacteriological examination.

**Experimental design**

Post bacteriological examination, 60 chicks free from any bacterial infection were chosen and divided into 3 groups (20 chicks each). The first group served as control group, while, 2nd group received 1 ml formic acid/liter drinking water and 3rd group received 2 ml formic acid/liter drinking water for 30 days (from 1st day of age up to 30th day of age).

**Body weight**

Chicks were individually weighed at the beginning of the experiment and then at 1st day post supplementation for determination of the body weight gain and feed conversion ratio.

**Bacteriological examination**

At 1st day post treatment, five chicks from each group were slaughtered and the intestine was exposed ligated at both sides and its contents were taken aseptically. One gram of caecal content was suspended in a tube containing sterile 0.9% normal saline solution (1:1). Then the solutions were mixed on vortex. Serial dilutions of samples were made up to 6th dilution. 0.1 ml of each dilution was poured and spread uniformly on nutrient agar, for total bacterial count and MacConkey’s agar for caecal coliform count. All plates were incubated at 37°C for 48 hrs. Colonies were counted by pour plate method [7].

**Serological identification**

Antisera of for the serotyping of E. coli isolates were used for the identification of somatic antigen “O” using slide agglutination test [8].

Seroalogical identification of the isolated strains of Salmonella was performed using slide agglutination for identification of somatic antigen while flagellar antigen was identified by tube agglutination test [8].

**Blood samples**

At 1st, 7th and 14th day post supplementation, five chicks from each group were slaughtered and blood samples were taken to obtain clear serum for the estimation of the total protein [9] albumin [10] the globulin was calculated as difference between total protein and albumin, total lipid [11], triglyceride [12] cholesterol [13], transaminases (AST and ALT) [14], ALP [15] Uric acid [16] creatinine [17], calcium [18], inorganic phosphorus [19], sodium and potassium [20] and zinc [21].

**Pathological examination**

Specimens were taken from the internal organs of the sacrificed chicks and fixed in 10% neutral buffered formalin. Five micron thick paraffin sections were prepared and stained with hematoxylin and eosin and examined microscopically [22].

**Statistical analysis**

The data were analyzed using PASW Statistics (SPSS version 18.0 for Windows [23]. The statistical analysis was performed by analysis of variance (ANOVA) with the fixed effect of Formic acid supplementation and the other investigated parameters as dependent variables. Bacteriological data were transformed to Log10 estimates before further analysis. Duncan’s multiple range tests were used for comparing the means.

**Results**

Bacteriological and biochemical results are summarized in Tables (1-4). The formic acid addition to the 2nd group, intestine showing finger like villi with normal structure that increase in height (Figure1A), but birds of 3rd group had thickening in the columnar epithelium and abundant goblet cells (Figure 1B). Bursa of fabricus in 2nd group showed narrowing of interstitial connective tissue, beside mild hyperplasia in lymphocytes (Figure1C). In 3rd group, the lymphoid tissue of bursa undergone moderate to severe hyperplasia (Figure1D), besides more
narrowing in the interstitial connective tissue. Spleen in 2nd group showed severe hyperplasia in both red and white pulps (Figure1E), liver in 3rd group had mild vacuolar degeneration and sporadic areas of fatty changes (Figure1F), with heterophilic infiltration and hydropic degeneration Figure1G). In 3rd group, showing degenerative changes, round cells proliferations, severe fatty change, hemorrhages and coagulative necrosis (Figure1H). Kidneys in 3rd group with more vacuolar and hydropic degeneration of the renal tubules (Figure1I).

Table 1: The proportion of bacterial agents isolated from cloacal swabs of apparently healthy one day-old Hubbard broiler chicks (n=100)

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Serological identification of isolated E. coli and Salmonellae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E. coli (6)</td>
</tr>
<tr>
<td></td>
<td>Sero group</td>
</tr>
<tr>
<td>Single isolates</td>
<td>6</td>
</tr>
<tr>
<td>E. coli species</td>
<td>4</td>
</tr>
<tr>
<td>Salmonella species</td>
<td>3</td>
</tr>
<tr>
<td>Mixed isolates</td>
<td>2</td>
</tr>
<tr>
<td>Staphylococcus aureus + E. coli</td>
<td>Total</td>
</tr>
</tbody>
</table>

Table 2: The effect of formic acid in Microbial balance (log10 CFU/g) in gastrointestinal tract and body performance of Hubbard broiler chickens (n=5)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Microbial balance (log10 cfu/g) in gastrointestinal tract</th>
<th>Initial body weight</th>
<th>Final body weight</th>
<th>Weight gain</th>
<th>FC</th>
<th>FCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total count</td>
<td>Coliform</td>
<td>Lactobacillus</td>
<td>Coliform</td>
<td>Lactobacillus</td>
<td>Coliform</td>
</tr>
<tr>
<td>Healthy broiler</td>
<td>8.10±0.92</td>
<td>5.57±0.88</td>
<td>3.19±0.21</td>
<td>47.84±1.19</td>
<td>1210.06±5.07*</td>
<td>1162.22±8.84</td>
</tr>
<tr>
<td>formic acid 1ml</td>
<td>7.15±0.77</td>
<td>4.59±0.63</td>
<td>2.67±0.18</td>
<td>48.10±1.41</td>
<td>1234.12±3.87**</td>
<td>1186.02±4.07*</td>
</tr>
<tr>
<td>formic acid 2ml</td>
<td>7.04±0.89</td>
<td>4.26±0.58</td>
<td>2.52±0.15</td>
<td>45.32±1.30</td>
<td>1241.95±4.94**</td>
<td>1196.63±6.32**</td>
</tr>
</tbody>
</table>

FC=feed consumption  
FCR= Feed Conversion rate  
* Significant at P < 0.05  
** Significant at P < 0.001
### Table 3: The effect of formic acid on biochemical parameters of chicken broilers (n=5)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver function</th>
<th>lipid profile</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T.Protein</td>
<td>Albumin</td>
</tr>
<tr>
<td>Healthy broiler</td>
<td>5.39±0.31</td>
<td>2.90±0.26</td>
</tr>
<tr>
<td>Formic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1ml, 1st day</td>
<td>7.06±0.57*</td>
<td>3.95±0.31*</td>
</tr>
<tr>
<td>7th day</td>
<td>6.78±0.19*</td>
<td>3.70±0.21*</td>
</tr>
<tr>
<td>14th day</td>
<td>5.41±0.22</td>
<td>3.01±0.44</td>
</tr>
<tr>
<td>2ml, 1ml, 1st day</td>
<td>7.11±0.61*</td>
<td>3.89±0.30*</td>
</tr>
<tr>
<td>7th day</td>
<td>6.79±0.41*</td>
<td>3.58±0.15*</td>
</tr>
<tr>
<td>14th day</td>
<td>5.36±0.28</td>
<td>3.05±0.36</td>
</tr>
</tbody>
</table>

### Table 4: The effect of formic acid in some minerals of chicken broilers (n=5)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Uric acid (mg/dl)</th>
<th>creatinine (mg/dl)</th>
<th>Ca (mg/dl)</th>
<th>Ph (mg/dl)</th>
<th>Mg (g/dl)</th>
<th>Na (mmol/L)</th>
<th>K (mmol/L)</th>
<th>Zinc (Ug/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy broiler</td>
<td>5.64±0.44</td>
<td>1.83±0.38</td>
<td>8.78±0.32</td>
<td>5.48±0.62</td>
<td>3.75±0.21</td>
<td>141.60±1.37</td>
<td>4.15±0.49</td>
<td>147.07±7.13</td>
</tr>
<tr>
<td>Formic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1ml, 1st day</td>
<td>5.25±0.32</td>
<td>1.64±0.21</td>
<td>9.16±0.47</td>
<td>6.19±0.31</td>
<td>3.99±0.44</td>
<td>144.16±1.94</td>
<td>4.97±0.70</td>
<td>154.20±9.50</td>
</tr>
<tr>
<td>7th day</td>
<td>5.43±0.22</td>
<td>1.70±0.17</td>
<td>8.96±0.34</td>
<td>5.27±0.49</td>
<td>3.89±0.28</td>
<td>142.73±1.84</td>
<td>4.82±0.44</td>
<td>151.42±8.32</td>
</tr>
<tr>
<td>14th day</td>
<td>5.69±0.24</td>
<td>1.85±0.37</td>
<td>8.80±0.29</td>
<td>5.50±0.22</td>
<td>3.70±0.50</td>
<td>141.63±1.49</td>
<td>4.18±0.28</td>
<td>148.31±9.55</td>
</tr>
<tr>
<td>2ml, 1ml, 1st day</td>
<td>5.16±0.18</td>
<td>1.70±0.21</td>
<td>9.25±0.31</td>
<td>6.21±0.36</td>
<td>4.06±0.37</td>
<td>144.47±1.69</td>
<td>4.74±0.63</td>
<td>152.50±8.96</td>
</tr>
<tr>
<td>7th day</td>
<td>5.44±0.21</td>
<td>1.79±0.19</td>
<td>9.11±0.28</td>
<td>6.14±0.42</td>
<td>3.71±0.39</td>
<td>142.09±1.63</td>
<td>4.60±0.63</td>
<td>147.31±5.18</td>
</tr>
<tr>
<td>14th day</td>
<td>5.55±0.30</td>
<td>1.81±0.15</td>
<td>8.95±0.45</td>
<td>5.43±0.55</td>
<td>3.65±0.47</td>
<td>141.41±1.78</td>
<td>4.20±0.72</td>
<td>146.95±6.92</td>
</tr>
</tbody>
</table>
Figure 1: The pathological findings in the tissues of broiler chickens received formic acid in their ration: A) Small intestine of chickens, 2nd group show finger like villi with elongation (H&EX 200); B) thickening in the lining epithelium and abundant goblet of small intestine of chickens, 3rd group (H&EX 400); C) Bursa of fabricius of chickens 2nd group show mild hyperplasia (H&EX 200); D) Bursa of fabricius of chickens 3rd group, had hyperplasia (H&EX 200); E) Spleen of chickens, 3rd group, with severe hyperplasia (H&E X 400); F) liver of chickens, 2nd group, show heterophilic infiltration, vacuolar degeneration and mild fatty changes (H&EX 200); G) liver of chickens, 3rd group, hydropic degeneration, fatty change and heterophilic infiltration in liver of chickens (H&E X 400); H) liver of chickens, 3rd group, fatty changes (H&E X 400) and I) Kidneys of chickens, 2nd group, with vacuolar degeneration & congestion (H&EX 400).

Discussion

Broiler chickens supplemented with formic acid in the drinking water with both doses showed a significant increase in body weight gain and improved feed conversion rate. Our results were in conformity with those previously reported in broilers received formic acid [24-25]. Acidified increased body weight gain [26]. Growth promoting effect of formic acid may be due to its positive effect on digestion by inducing a slower passage of feed in the intestinal tract, a more efficient absorption of the necessary nutrients [27]. Also the improved body weight gain was explained previously by the decrease in the number of pathogenic bacteria in small intestine [28] and the beneficial effect of acidifiers on gut flora [29].

Our findings revealed that, broilers supplemented with formic acid in both doses showed significant increase in total proteins, albumin and globulin beside insignificant decrease in A/G Ratio. Similar increase in serum protein was recorded Adil et al. [30] in chickens fed organic acid. Increase globulin in broilers supplying with organic acids [31]. Increase in total protein in broiler chickens in our study may be due to organic acids increased gastric proteolysis and improved the digestibility and absorption of protein and amino acids as reported earlier by Samanta et al. [32].

Analysis of lipids profile of the broiler received formic acid showed significant decrease total lipids, cholesterol and triglyceride in broilers. Alike the findings of Kamal and Ragaa [33] who supplemented broiler with organic acid. Serum total lipids and triglyceride significantly decreased by
dietary acidifiers [34-35]. Organic acid induced the decrease in total lipid in hens [36]. Organic acids decreased total lipid cholesterol and triglyceride in quail [37].

The obtained results showed that formic acid resulted in insignificant increase in calcium, phosphorous, magnesium, zinc, sodium and potassium. The obtained results are in agreement with the results in a study conducted in broilers received formic acid [6]. Acidic anion has been shown to complex with calcium, inorganic phosphorous, zinc, magnesium, sodium and potassium which results in an improved digestibility of these minerals [38]. Also, it was reported that organic acid improved digestibility of calcium, phosphorous, magnesium, zinc, sodium and potassium in broiler chickens [39]. Insignificant increase in this minerals may be due to organic acids induce lowering of gastrointestinal tract pH, which lead to increased absorption of these minerals from the gut into the blood stream [40].

Formic acid induced the insignificant decrease in liver enzymes (AST, ALT and ALP), uric acid and creatinine. These results are in full agreement with Adil et al. [41] in broiler received organic acid and with Abdel-Azeem et al. [42] in growing rabbits received citric acid. Organic acids up to 3% had no effects on liver and kidney function in broiler [43]. Reduced liver enzymes could be resulted from improvement of the physiological condition of liver and the increase in hepatic metabolic reserve [44].

The present investigation revealed that broiler chickens supplied with formic acid in both doses show insignificant reduction in total bacterial count, Coliform (E. coli and other coliform) and Lactobacillus in intestinal tract. Same observation was previously recorded where other organic acid (Galli acid) induced the reduction in the total bacterial count in intestine [45]. Also, organic acids reduced colonization of pathogens on the intestinal wall [46]. Moreover, Gauthier [47] stated that organic acids cause a reduction of the bacteria in the colon. Organic acids can penetrate the bacterial cell wall and disrupt the normal physiology of certain types of bacteria [48]. In addition, organic acids supplementation has pH reducing properties in various gastrointestinal segments of broiler chicken lead to reduction of pathogenic bacteria [34]. The way of action of organic acids seems to be related to a reduction of pH in the upper intestinal tract, interfering with the growth of undesirable bacteria and modifying the intestinal flora [49].

The histomorphological changes in villi could be considered as an indicator for a responding in the functioning activity of the absorptive organs (villi) and healthy elongated villi in chickens lead to high absorption efficiency as in [50]. Tappenden and McBurney [51] stated that increased villi heights with the most organic acids was attributed to the reduction of many intestinal pathogens or non pathogens growth and decreasing the inflammatory reactions at the intestinal mucosa. In the immune organs (bursa and spleen) hyperplasia of lymphocytes in different cases was observed. Similar observations were previously recorded [52].

**Conclusion**

It could be concluded that, formic acid supplementation of great value on modern poultry production as it act as growth promoter and exhibits some benefits effect on the biochemical parameters intestine and the immune organs histology beside inhibition of colonization of pathogenic bacteria in intestinal wall of chickens.

**Conflict of interest**

The authors declare no conflict of interest.

**References**


الملخص العربي

دراسات باثولوجية بكتريولوجية وبيوكيميائية على تأثير حمض الفورميك في كتاكيت التسمين

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1. قسم الباثولوجيا: مهندس بحوث صحة الحيوان-الزراعي
2. قسم الكيمياء: مهندس بحوث صحة الحيوان-الزراعي
3. قسم البيتكريولوجيا: مهندس بحوث صحة الحيوان-الزراعي
4. قسم البيتكريولوجيا: مهندس بحوث صحة الحيوان-الزراعي

كان الهدف من هذه الدراسة هو معرفة تأثير حمض الفورميك على وزن الجسم على بعض الوظائف البيوكيميائية والتغيرات الباثولوجية بجانب تأثير حمض الفورميك على نمو البكتيريا. لذلك تم جمع 100 مسحة من فتحة المجمعي من كتاكيت بقر عمر بحميرة واحدة تم تتم مع بصحة جيدة وذلك لفحصها ببيتكريولوجيا. ونهاية البكتريولوجي للمسحات المأخوذة من الكتاكيت السلبية وحد عدد عينات تم عزل البكتيريا منها وزع اللاحالي عدوى من فردة 18.21 % (13) وعديد مختلفة

مسحة وحود السالمونيلا تيفينيوريوم في عدد 20 مسحة والسلمونيلا انترديرس في عدد 1 مسحة

تم استخدام عدد 20 كتاكي خالية من أي نوع من البكتيريا في تلك التجربة وتقييمها إلى 3 مجموعات متساوية تحتوى كلا منها على 20 كتاكي المجموعة الأولى استخدمت كمجمعة ضابطة المجموعة الثانية كتاكيت تم عزلهم حمض الفورميك بجرعه 1 مل من مياه الشرب والمجمعي الثالثة كتاكيتم تم عزلهم حمض الفورميك بجرعه 2 مل/لتر من مياه الشرب. يتم إعطاء حمض الفورميك لمدة ثلاثين يوماً من اليوم الأول من العمر حتى اليوم الثلاثين من العمر. يتم دراسة كفاءة حمض الفورميك على وزن الجسم ومعدل التحويل الغذائي. يتم جمع عينات عند اليوم الأول، السابع، والثامن عشر من نهاية استعداد حمض الفورميك. لقياس بعض الوظائف البيوكيميائية. يتم ذي 5 كتاكي من كل مجموع بعد نهاية استخدام حمض الفورميك. وتم ربط الاعمام من الجانبين واخذها لفحص مكوناتها ببيتكريولوجيا للدراسة تأثير حمض الفورميك على البكتيريا الموجودة بالاعمام. وتم تجميع إجزاء من الاعمام. الكبد والكلي والطحال والبرستع اليوم الأول، السابع، والثامن عشر من نهاية استخدام حمض الفورميك. لدراسة تأثير حمض الفورميك على تلك الاعمام ببيتكريولوجيا.

وقد أظهرت النتائج إستغلال حمض الفورميك الجرعيين ادى إلى حدوث زيادة معنوية في وزن الجسم المكاسب.

الروتين الكلي، البروتين الكلي، الزالو، والجليوبولين بالبكتيريا، ونسبة معنوية في النزول الكلي، الكليستيرول، الدهون الثلاثي، زيادة غير معنوية في مستوى الكالسيوم في الحمضيات، ماشيكسوم، والببتايسوم والزلاك، ونسبة غير معنوية في نشاط القنوات الابيك. ALT، ALP

بكتريولوجيا حمض الفورميك ادى إلى زيادة في طول وسمن الجسم. كما ادى إلى نشاط قوي في معظم الأجسام المذاعية

يجب أن نذكر أن هذه الدراسة أن حمض الفورميك له دور في صناعة الدواجن الحديثة حيث انه يعمل كمضخ للنمو حيث ادى إلى تعدين ملحوظ في وزن الجسم المكاسب ومعامل التحويل الغذائي. وادى إلى تسنين وزن الفكين والكلية وвлقلي الدهون والكليستيرول والدهون الثلاثي في معدل الدم. وتكثيف عدد البكتيريا الموجودة بالاعمام وتحسين في جدار الاعمام. واتى ذلك تأكيدها بالبيتكريولوجيا. لذلك نوصي باستخدام حمض الفورميك نظراً لقاطه الآثاره الضارة على الجسم وتثبيتها المهم على وزن الجسم ومعامل التحويل الغذائي.