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# Effect of Formic Acid Supplementation on Some Serum Biochemical Parameters of Broiler Chickens

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**Abstract:** The aim of this study was to evaluate the effect of adding different concentrations of the formic acid to the drinking water on some serum biochemical parameters of broiler chickens aged between 21 and 42 days. In a completely randomized design, Eighty-four, one-day-old broiler chicks were divided into 4 groups with three replicates of each group and seven birds per each replicate. The organic formic acid was added to the drinking water in the following concentration (0.1%, 0.2%, and 0.3%) represented the treatment group (T2, T3 and T4) respectively, while in the control group (T1); the drinking water was free of formic acids. Blood samples were collected at age of 21 and 42 days. At age of 21 days, data indicated that the first treatment group (T2) was showed a significant increase ($P\leq0.05$) in serum total protein and albumin and significant decrease ($P\leq0.05$) in each of serum cholesterol, triglycerides and serum glucose. The second treatment group (T3) was also showed a significant increase ($P\leq0.05$) in serum total protein and albumin, in addition to serum cholesterol but it was showed a significant decrease ($P\leq0.05$) in both of serum triglycerides and serum glucose. Whereas the third treatment group (T4) was showed a significant increase ($P\leq0.05$) in each of serum total protein, albumin, cholesterol, and serum triglycerides, but it was showed non-significant differences ($P>0.05$) in serum glucose concentration. The result of the blood samples that were collected at 42 days of age indicated a presence of a significant decrease ($P\leq0.05$) in serum total protein level and non-significant differences ($P>0.05$) in both of serum albumin and serum cholesterol level, but it was showed a significant increase ($P\leq0.05$) in both serum triglycerides and glucose levels in the first treatment group (T2). The second treatment group (T3) showed a significant increase ($P\leq0.05$) in each of serum total protein, triglycerides and serum cholesterol levels, but it was showed non-significant differences ($P>0.05$) in serum albumin level and non-significant decrease ($P>0.05$) in serum glucose levels. The result from the third treatment group (T4) indicated non-significant differences ($P>0.05$) in each of serum total protein, albumin, and serum cholesterol levels, and a non-significant decrease in serum glucose levels. These results showed the importance of adding organic formic acid to the drinking water on the improvement of some serum biochemical parameters of broiler chickens.

**Key words:** Formic acid, Serum biochemical parameters, Broiler chickens.

## Introduction:

Broiler chicken meat is considered one of the main sources of animal protein where the poultry production rate was about 25% of the total global production of meat [(1)]. Broiler chicken meat is characterized by their high nutritional value as it is rich in the essential elements in addition to its easy digestion [(2)]. Poultry production has consistently in recent years to find the ways to improve the growth rates in order to obtain the

highest conversion efficiency of food as well as reduction of the negative effects of the diseases that lead to heavy economic losses. One of the most important ways to control the negative effect of the diseases was the introduction of the antibiotics in the poultry production [3]. However, use of these antibiotics on a large scale has led to the development of the bacterial resistance to antibiotics. A study done by (Nayak and Kenney 2002) [4] on four turkey flocks showed that about 25% of *Salmonella Sp.* were resistant to one or more antibiotics, including tetracycline, streptomycin, trimethoprim, gentamycin, and tobramycin. In addition to that, use of antibiotics leads to a cumulative harmful effect on broiler chicken meat as it changes the bacterial environment and leads to weakening in the performance of the poultry production [5]. Therefore, the researchers attempt to find an alternative to antibiotics to control the negative effect of the diseases and among these alternatives to antibiotics was the use of the organic acids and the addition of probiotic to poultry feeds [6]. Organic acids are weak acids that are commonly found in fruit juices and fermented foods and that are added to foods as preservative agents [7]. Organic acids have been used for decades in feed preservation, for protecting feed from microbial and fungal destruction or to increase the preservation effect of fermented feed. Organic acids have increasingly and successfully been supplemented broiler feeding. 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 [8]. Several studies demonstrated that supplementation of organic acids to broiler diets increased growth performance, reduced diseases and management problems [9-14]. Moreover, several authors have investigated the effects of broiler feed supplementation with propionic and formic acids and their salts, either alone [15-17] or in combination [12, 18, 19], on growth performance and carcass traits, but the results are incongruous. Furthermore, studies simultaneously comparing the effects of those acids at various inclusion levels in the feed are scarce. In addition to very few studies that have reported data on blood plasma metabolites or immune response in broilers chickens after addition of those substances [20].

The aim of the present study was to evaluate the effect of adding different concentrations of the organic formic acid to the drinking water on some serum biochemical parameters of broiler chickens.

## Materials and Methods:

This study was conducted in the chicken field of the poultry section in the Animal and Fish Research Center \ Agricultural Research Directorate \ Ministry of Science and Technology \ Baghdad \ Iraq, which included the field and laboratory study. Eighty-four, mixed sexes broiler chickens (Rose Type), one-day-old, were used for the experiment. These broiler chickens were distributed randomly inside a closed room into four groups as the following:

- Control group (T1): Drinking water was free of formic acid.

- First treatment group (T2): Drinking water was treated with 0.1% formic acid.
- Second treatment group (T3): Drinking water was treated with 0.2% formic acid.
- Third treatment group (T4): Drinking water was treated with 0.3% formic acid.

Each group was further sub-divided into three replicates of 7 birds per replicate. A photo period of 24 hours \ day was maintained since the start of the experiment and until the end of the sixth week by using the electric lamps. The ingredients and the chemical composition of the diets presented in Table 1. They were analyzed by using N.R.C (1994) procedure. Feed and water were provided *ad libitum* during the experiment. The experiment was carried out according to the guidelines of the Ethics Committee of the Animal and Fish Research Center \ Agricultural Research Directorate \ Ministry of Science and Technology for the humane care and use of animals in research.

**Table (1) The ingredients and the chemical composition of the diets.**

| Ingredient | Composition Percentage |
|---|---|
| **Yellow corn** | 43 |
| **wheat** | 12 |
| **Barley** | 9 |
| **Soybean meal** | 20 |
| **Animal protein concentrate** | 12 |
| **Oil** | 3 |
| **Limestone** | 0.7 |
| **Salt** | 0.3 |
| **Total** | 100% |
| **Calculated levels** | |
| **Crude protein (%)** | 21.14 |
| **Metabolizable Energy (Kcal/kg$^{-1}$)** | 3032 |
| **Protein-energy ratios** | 143 |
| **Lysine (%)** | 0.82 |
| **Methionine + Cysteine (%)** | 0.73 |
| **Crude fiber (%)** | 2.16 |

Blood samples were collected at 21 and 42 days of age from the wing vein and centrifuged at 3000 rpm for 5 minutes by using Hettich centrifuge to separate the serum samples and the obtained sera were aspirated into sterile vials and kept in deep freezer (-20°C) for the later analysis of the serum biochemical parameters that include the serum total protein [21], albumin [21], cholesterol [21], triglycerides and glucose [21]

and these analysis was estimated by using the Vegasys chemical analyzer device (AMS Co., Italy) in the Animal and Fish Research Center \ Agricultural Research Directorate \ Ministry of Science \ Baghdad \ Iraq.

Statistical analysis was performed with statistical program (Statistical Analysis System) 2001. The one-way analysis of variance (ANOVA) was used and the means were compared by Duncan's multiple range tests at the level of 5% probability to compare the results between the control group and the treatment groups. Data were expressed as mean ± standard error of mean. The values were considered statistically significant when p-value ≤ 0.05.

## Results and Discussion:

The results in Table (2) indicates the serum biochemical analysis of the broiler chickens at 21 days of age. These data showed a significant increase (P≤0.05) in the concentration of serum total protein and albumin in the treatment groups (T2, T3 and T4) in comparison with the control group (T1).

Serum cholesterol concentration was significantly increased (P≤0.05) in the treatment groups (T3 and T4) while it was significantly decreased (P≤0.05) in the treatment group (T2) in comparison with the control group (T1). Serum triglycerides concentration showed a significant decrease (P≤0.05) in the treatment groups (T2 and T3), while it was significantly increased (P≤0.05) in the treatment group (T4) in comparison with the control group (T1). Serum glucose concentration was also significantly decreased (P≤0.05) in the treatment groups (T2 and T3), but it was non-significantly increased (P>0.05) in the treatment group (T4) in comparison with the control group (T1).

**Table (2) Serum biochemical analysis of the broiler chickens at 21 days of age.**

| Treatments | Total Protein g/dl | Albumin g/dl | Cholesterol mg/dl | Triglycerides mg/dl | Glucose mg/dl |
|---|---|---|---|---|---|
| **T1 (Control)** | 2.97 ± 0.24 c | 1.506 ± 0.17 bc | 150 ± 10.11 b | 120 ± 5.53 b | 361.66 ± 3.69 a |
| **T2 (0.1%)** | 3.84 ± 0.31 a | 1.566 ± 0.98 b | 134 ± 2.34 c | 111.66 ± 2.73 c | 235.33 ± 3.52 b |
| **T3 (0.2%)** | 3.86 ± 0.31 a | 1.813 ± 0.01 a | 154.33 ± 6.64 b | 114.66 ± 9.20 c | 293.33 ± 7.05 b |

| Treatments | Total Protein g/dl | Albumin g/dl | Cholesterol mg/dl | Triglycerides mg/dl | Glucose mg/dl |
|---|---|---|---|---|---|
| **T4 (0.3%)** | 3.64 ± 0.12 b | 1.793 ± 0.06 a | 169.33 ± 7.68 a | 140.33 ± 8.55 a | 370.0 ± 10.26 a |

The data in Table (2) showed the serum biochemical analysis of the broiler chickens at 42 days of age. These results showed a significant decrease (P≤0.05) in serum total protein concentration of the treatment group (T2) and significant increase (P≤0.05) in the treatment group (T3) but there were non-significant differences (P>0.05) in the treatment group (T4) in comparison with the treatment group (T1). Serum albumin concentration showed non-significant differences (P>0.05) between the treatment groups (T2, T3, and T4) and the control group (T4). There were also non-significant differences (P>0.05) in serum cholesterol concentration between the treatment group (T2 and T4) and the control group (T1), while it was significantly increased (P≤0.05) in the treatment group (T3) in comparison with the control group (T1). Serum triglycerides concentration was significantly decreased (P≤0.05) in the treatment group (T4), while it was significantly increased (P≤0.05) in the treatment groups (T2 and T3) in comparison with the control group (T1). Serum glucose concentration showed a significant increase (P≤0.05) in the treatment group (T2) and non-significant decrease (P>0.05) in the treatment groups (T3 and T4) in comparison with the control group (T1).

**Table (3) Serum biochemical analysis of the broiler chickens at 42 days of age.**

| Treatments | Total Protein g/dl | Albumin g/dl | Cholesterol mg/dl | Triglycerides mg/dl | Glucose mg/dl |
|---|---|---|---|---|---|
| **T1 (Control)** | 3.846 ± 0.15 b | 1.77 ± 0.14 | 149.33 ± 8.74 b | 121.33 ± 7.03 b | 3055.33 ± 2.60 b |
| **T2 (0.1%)** | 3.686 ± 0.19 c | 1.803 ± 0.12 | 157.66 ± 5.17 b | 129.33 ± 7.83 a | 356.33 ± 4.35 a |
| **T3 (0.2%)** | 3.883 ± 0.13 a | 1.793 ± 0.72 | 170.66 ± 8.78 a | 123.33 ± 9.24 a | 289.33 ± 4.17 c |
| **T4 (0.3%)** | 3.840 ± 0.10 b | 1.836 ± 0.04 | 157.66 ± 4.97 b | 114.33 ± 4.80 c | 259.33 ± 5.81 c |

In this experiment, the addition of formic acid to the diet resulted in reducing the mortality rate and this result agrees with previous results of (Sallh and Al Hussary, 2009) [22], were they also showed that a decrease in the mortality rate as a result of using a probiotics containing organic acids in the diet of the broiler chickens. (Brz_oska et al., 2013) [23] was also reported that the organic acid in the diet was enhanced the growth and reduced the mortality rate of the broiler chickens.

In this study, the addition of formic acid to the diet showed significant decrease in serum total cholesterol levels. It is well known that acidifiers enhance gut health by stimulating the growth of beneficial bacteria while inhibiting the pathogenic bacteria. Beneficial bacteria like Lactobacillus sp. have high bile salt hydrolytic activity which is responsible for deconjugation of bile salts [24]. Deconjugated bile acids are less soluble, hence less absorbed in the intestine and are more likely to excrete cholesterol and its fractioning faeces and thus reduction of cholesterol accretion in the body [25]. Several studies done by (Taherpour et al., 2009) [26], (Jang, 2011) [27], (Mansoub, 2011a) [28], (Kamal and Ragaa, 2014) [29] and (Deepa et al., 2017) [30] was also showed the reduced levels of serum total cholesterol due to addition of various forms of organic acids. In the present study, the addition of formic acid to diets was significantly lowered serum triglycerides concentration and this decline may be due to secretion of the inulin where the inulin, in turn, has an Inhibitory effect on the triglycerides synthesis processes [31]. Contrary to our findings, studies were done by (Jang, 2011) (27) (Mansoub, 2011a) (28) and (Dehghani-Tafti and Jahanian, 2016) [32] was also found that the serum triglyceride level was reduced when the diet is supplemented with different forms of organic acids. This experiment showed a significant age-related difference in the levels of the serum total protein, albumin and serum glucose. These age-related changes have also been observed in broilers chickens that reported by (Sribhen C. et al, 2003) [33]. The significant differences in the total protein and albumin levels may be attributed to that organic acids raised the gastric proteolysis and improved protein and amino acids digestibility as reported by (Samanta et al., 2010) [34]. It was thought that the organic acids supplementation lowers the pH of the chime which might increase the pepsin activity and thus enhance the digestibility of protein [35]. According to (Van Der Sluis, 2002) [36], the positive effect of organic acids on digestion was related to a slower passage of feed in the intestinal tract, a better absorption of the necessary nutrients and less wet droppings.

## Conclusion:

This study concluded that using of organic formic acid had a role in the improvement of some serum biochemical parameters in broiler chickens.

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# تأثير إضافة حمض الفورميك على بعض المؤشرات البيوكيميائية في فروج اللحم

**الملخص:** هدفت هذه الدراسة إلى معرفة أثر إضافة تراكيز مختلفة من حمض الفورميك العضوي إلى ماء الشرب على بعض المؤشرات البيوكيميائية لمصل الدم في فروج اللحم والتي تراوحت أعمارها مابين 21 إلى 42 يوم. تمت اضافة حمض الفورميك العضوي إلى ماء الشرب بالتراكيز التالية (0.1 و 0.2 و 0.3 %) ممثلةً مجاميع العلاج (T2وT3 وT4) على التوالي، بينما خلا ماء الشرب لمجموعة السيطرة (T1) من إضافة حمض الفورميك العضوي. تم جمع عينات الدم بعمر 21 و 42 يوم. أظهرت النتائج بعمر 21 يوم وجود ارتفاع معنوي ($P\leq0.05$) في مستوى كل من تركيز البروتين الكلي وتركيز الالبومين ووجود انخفاض معنوي ($P\leq0.05$) في تركيز كل من الكوليسترول، الدهون الثلاثية، والسكر في مصل الدم لمجموعة المعاملة الاولى (T2). وأظهرت النتائج في مجموعة المعالجة الثانية (T3) كذلك ارتفاعاً معنوياً ($P\leq0.05$) في تركيز كل من البروتين الكلي والالبومين بالإضافة إلى تركيز الكوليسترول في مصل الدم ووجود انخفاض معنوي ($P\leq0.05$) في تركيز كل من الدهون الثلاثية والسكر في مصل الدم. بينما أظهرت النتائج في مجموعة المعاملة الثالثة (T4) ارتفاعا معنويا ($P\leq0.05$) في تركيز كل من البروتين الكلي، الالبومين، الكوليسترول، والدهون الثلاثية لمصل الدم وفرق غير معنوي ($P>0.05$) في تركيز السكر لمصل الدم. بينما بينت النتائج لمجموعة المعالجة الاولى (T2) بعمر 42 يوم وجود انخفاض معنوي ($P\leq0.05$) في تركيز البروتين الكلي وفرق غير معنوي ($P>0.05$) في تركيز كل من الالبومين والكوليسترول في مصل الدم، بينما أظهرت النتائج لنفس المجموعة وجود ارتفاع معنوي ($P\leq0.05$) في تركيز كل من الدهون الثلاثية والسكر في مصل الدم. وأظهرت النتائج لمجموعة المعاملة الثانية (T3) بعمر 42 يوماً ارتفاعاً معنوياً ($P\leq0.05$) في تركيز كل من البروتين الكلي والدهون الثلاثية والكوليسترول في مصل الدم ووجود فرق غير معنوي ($P>0.05$) في تركيز الالبومين مع وجود انخفاض غير معنوي ($P>0.05$) في تركيز السكر لمصل الدم. في حين أظهرت النتائج لمجموعة المعاملة الثالثة (T4) فروقاً غير معنوية ($P>0.05$) في تركيز كل من البروتين الكلي، الالبومين، والكوليسترول لمصل الدم مع وجود انخفاض غير معنوي ($P>0.05$) في تركيز السكر لمصل الدم. من النتائج المذكورة أعلاه، تتضح أهمية إضافة حمض الفورميك العضوي إلى ماء الشرب على تحسين بعض الصفات البيوكيميائية لمصل الدم في فروج اللحم.

**الكلمات المفتاحية:** حمض الفورميك، المؤشرات البيوكيميائية لمصل الدم، فروج اللحم.