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Effect of effective microorganisms on some biochemical parameters in broiler chicks

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Abstract

This study was conducted to investigate the potential effect of effective microorganisms (EM) on some biochemical parameters which includes; glucose, cholesterol and triglycerides levels in addition to the levels of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) in the serum of broilers. 50 One day-old unsexed broiler chicks were randomly assigned in to two treatments (25 chick/pen); control group (T1) and treatment group (T2) which supplied with 1% EM (10 ml/litre) to the drinking water for 5 weeks of age. Results showed that inclusion of 1% EM significantly increase blood glucose ($P<0.05$) and lower cholesterol but the triglycerides decrease was insignificant in (T2) than those of (T1). On the other hand (AST) increased significantly ($P<0.05$) in (T2) as compared to the (T1). It can be concluded from this study that EM supplementation may have some beneficial effect by lowering cholesterol and triglycerides on the other hand it may cause some tissue damage as indicated by increasing levels of (AST), these results need to be studied using different types and doses of EM.

Keywords: Effective Microorganism; glucose, cholesterol; triglycerides; ALT; AST
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تأثير المتعضيات الفعالة في بعض المعايير الكيموحيوية لفروج اللحم

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فرع الأدوية والسموم، كلية الصيدلة، جامعة الموصل، الموصل، العراق

الخلاصة

الهدف من هذه الدراسة هو لتقييم تأثير إضافة المتعضيات الفعالة على بعض المعايير الكيموحيوية لأفراخ فروج اللحم وشملت؛ مستوى السكر، الكولسترول والشحوم الثلاثية كما تم قياس مستوى أنزيمات الكبد; إنزيم اسبارتيت امينو ترانس فيريز (AST)، إنزيم الالنين امينو ترانس فيريز (ALT) في مصل الدم. استخدمت ٥٠ فرخة من نوع روز غير مجنسة وبعمر يوم واحد، حيث وزعت عشوائيا على مجموعتين متساويتين (مجموعة سيطرةT1 ومجموعة معاملة T2) بواقع ٢٥ فرخة لكل مجموعة حيث تم اضافة ١٠مل من محلول ال EM الى ماء الشرب لـ (T2) ولمدة خمسة اسابيع. اظهرت النتائج وجود تأثير معنوي ($P<0.05$) للـ EM حيث أرتفع مستوى السكر وأنخفض الكولسترول بينما كان انخفاض الشحوم الثلاثية غير معنويا لمجموعة (T2) مقارنة بـ (T1) من جهة أخرى كان هنالك أرتفاعا معنويا ($P<0.05$) لانزيم اسبارتيت امينو ترانس فيريز (AST) في مصل الدم لمجموعة (T2) مقارنة ب (T1). يمكن الاستنتاج من نتائج الدراسة الحالية أن أضافة المتعضيات الفعالة قد يكون لها تأثيرات مفيدة بخفض مستوى الكولسترول والشحوم الثلاثية ومن جهة أخرى قد تسبب أضرارا في الانسجة كما تبين من خلال ارتفاع مستويا (AST). هذه النتائج تحتاج الى دراسات أخرى باستخدام أنواع وجرع مختلفة من المتعضيات الفعالة.

Introduction

Over the last several years considerable attention has been given to the use of probiotics, yeast and other natural feed additives in poultry feeds. Much of this interest has been generated because of increased public awareness and objection to use antibiotic as growth promoter (1).

Probiotics which beneficially affects the host animal by improving its intestinal balance, it has been used as a substitute for antibiotics that is being used in considerable amounts as growth promoters in broilers production and is, associated with incalculable risks for human health resulting from the use of particular feed additives (2).

Probiotics are live microbial supplements that, when administered in adequate amounts, confer a beneficial effect on the health of the host by improving its intestinal microbial balance (3) sustain of the immune system, prevention of cancer, and reduction of triglycerides, cholesterol, and odor compounds associated with probiotic, and prebiotic use (4,5).

EM was successfully used in poultry units as feed constituent (6) to increase productivity in integrated animal units and poultry farms (7). EM may offer potential benefits to the poultry industry, such as improvements in body weight gain and feed conversion ratio (8).

EM is a combination of 70 to 80 different types of "good" and beneficial microorganisms contributing to the wide range of applications. The principal organisms of are usually five; photosynthetic bacteria (phototrophic bacteria), lactic acid bacteria, yeasts, actinomycets and fermenting fungi (9). Lactic acid bacteria produce lactic acid from sugars, and other carbohydrates produced by photosynthetic bacteria and yeast (10).

The liver plays a key role in cholesterol homeostasis, involving the metabolism of low density lipoproteins (LDL). In the liver, the conversion of cholesterol to bile acids is a principle pathway of cholesterol catabolism, providing sufficient amounts of bile acids as detergents for the digestion and absorption of lipid nutrients and removing excess cholesterol from the body (11).

It seems that probiotics have beneficial influence on blood cholesterol and triglycerides content in blood (12-14).

AST is an enzyme that increases in activity in some diseases such as severe bacterial infections, malaria, pneumonia, pulmonary infarcts, and tumors of organs such as heart and muscle (15). ALT is principally found in the liver and is regarded as being more specific than AST for detecting liver cell damage (15,16).

Material and methods

This study was conducted at farms of college of Veterinary Medicine in Mosul, during Autumn months to study the effect of inclusion of EM as a additive to the drinking water on glucose, cholesterol and triglycerides levels in broilers. ALT and AST levels also determined.

A total of 50 Rose one day-old, were assigned randomly in two equal groups named (T1) control group and (T2) treatment group (25 chick/pen). The chicks were reared on deep litter system. Feed and water were provided *ad libitum* throughout the experimental period form 1-5 weeks of age.

Liquid forms of EM used, EM stock solution provided by the (EM1 Natural probiotic contains more than 60 type of live microorganisms includes; Photosynthetic bacteria, Lactic acid bacteria, Yeast, Fungi), a product of Alannam company for natural agriculture, Tortuous–Syria under the supervision of Emro Japanese institute – Okinawa - Japan.

EM were administered in solution form through drinking water at the rate of 10 ml. per liter of drinking water five days/week to (T2).

Artificial lighting was set to provide 24 h of light day. Environmental temperature was kept between 22-25 °C, till the end of experiment.

Chicks were vaccinated against New Castle and Gumboro disease according to their age.

At 35 days of age, chickens were slaughtered. The blood was collected in non-heparanized collecting tubes to obtain the serum. Blood samples were centrifuged at 3500 rpm for 15 min and the serum was transferred using individual pasteur pipettes into vials and stored at -20°C until used. Serum samples were analyzed for glucose, total cholesterol, and triglycerides by enzymatic diagnostic kits (17).

Glucose determined after enzymatic oxidation in presence of glucose oxidase using kits from Randox. Cholesterol determination according to the enzymatic end point method by using kits from Randox. Triglycerides determination according to the colorimetric method using kits from Randox, following the same steps as described by manufactures (Randox laboratories limited, United Kingdom).

On the other hand transaminases (ALT and AST) were determined calorimetrically using transaminases-kit from (biomérieux® sa) France, following the same steps as described by the manufactures.

Analysis of variance (ANOVA) through General Linear Model procedure of SPSS (10.0) software considering replicates as experimental units, and the values were expressed as means ± standard error. Duncan's multiple range test was used to test the significance of difference between means by considering the differences significant at ($P < 0.05$) (18).

Results

Our results shows that there are a significant ($P<0.05$) increase in glucose level of (T2) compared to (T1).

Concerning cholesterol levels, there was a significant ($P<0.05$) decrease in concentration of serum total cholesterol in (T2) compared to (T1), on the other hand, triglycerides levels don't affected significantly by the treatment, table (1). The results indicated that EM had a hypocholesterolaemic effect on broilers.

Table 1: Effect of EM on serum glucose, cholesterol and triglyceride levels (mg/dl) of chickens

	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
T1	130±2.4	235.9±18.2	93±3.4
T2	*177.4±34.3	*196.8±3.7	90.2±32.9

Mean ±SE for 25 chick/group, *significant ($P<0.05$).

Concerning to serum liver enzymes, our result revealed that there was a significant increase ($P<0.05$) in AST levels of (T2) compared to (T1), while the increase in ALT of (T2) was not significant compared to (T1) as shown in Table (2).

Table 2: Effect of EM on serum ALT and AST levels (IU/L) of chickens

	ALT (IU/L)	AST (IU/L)
T1	12.61±1.06	44.82±7.28
T2	14.41±5.75	*63.44±8.18

Mean ±SE for 25 chick/group, * significant ($P<0.05$).

Discussion

Our results disagree with (19,20) who reported no change in glucose level and (21) who shows significant reduction in glucose level in broiler treated with probiotic, and with (22) who indicate that administration of EM produced a significant decreased in glucose levels of alloxan-induced diabetic rats.

We suggest that the increase of glucose levels may be due to the effect of EM on gluconeogenisis and may be due to the lowering effect of EM on insulin secretions from the pancreas, as the theories of the substances that have abilities to decrease glucose levels in the body may be due to; stimulation of B cells of pancreas to secret more insulin, increasing muscles and other tissues sensitivity for insulin, inhibition of gluconeogenesis in the liver and decreasing carbohydrate absorption from the gastrointestinal tract (23).

The beneficial effects of probiotics in lowering cholesterol in serum (24) have been reported. While (14,25,26) has shown no benefit of using microbial preparation or probiotics on blood cholesterol and triglycerides. EM application both in feed and water combined being most effective in lowering the total blood cholesterol than the other application methods (8).

Our observations corroborated data published by some authors who stated that there was a decrease in plasma cholesterol of chicks fed diets contain EM. The level of serum cholesterol at 42 days was lower though not significantly different ($P<0.05$), for the EM supplemented chicks than for the control (27). Administration of (EM) produced a significant decreased in cholesterol, triglycerides, levels of alloxan-induced diabetic rats (22).

Low cholesterol levels may be due to the flavonids which present in it and acts as antioxidant supporting cholesterol metabolism or by increasing 7-α hydroxyase enzyme which convert cholesterol to bile acid (28). The decrease in cholesterol levels could be associated with both a reduction in cholesterol biosynthesis in the liver and an increase in degradation of bile acids by Lactobacillus species (29). We suggest that the low cholesterol levels could be due to the binding of cholesterol to the cellular membrane of bacterial cells and also through deconjugation of bile salts which may interfere with the enterohepatic cycle as there are elevated levels of liver transasminases.

Diets supplemented with probiotics can also significantly reduce plasma triglycerides in hens (30). On the other hand Probiotic lead to insignificant ($P>0.05$) decreased in triglycerides content (14). Continuous supplementation of *L. acidophilus* to chickens did not reduce the triglyceride levels in plasma (31).

Hepatocytes play a major role in absorbing and metabolising many toxic chemicals they are therefore liable to injury by various chemicals, including food (32).

AST, ALT activities of the serum may indicate the liver function and health. Cellular injury in liver may increase the level of these enzymes in serum. ALT is principally found in the liver and is regarded as being more specific than AST for detecting liver cell damage (15,16).

The elevation in the hepatic enzyme levels (AST,ALT) in T2 serum reflect the hepatic damage and leakage of enzymes in the blood stream. Similar data have been reported previously (33-35) revealed a significant increase in AST and ALT enzymes as a result of challenge with *salmonella typhimurium* which regard as hepatocellular damage indicator.

Animal studies have shown that translocation of bacterial products from the intestinal lumen to the mesenteric lymphatic circulation and activates the Kupffer cells in the liver, induces regional and systemic production of proinflammatory cytokines, and enhances production of free radical species in the splachnic area (36). Lactobacilli can translocate (37) and survive in the spleen, liver, and lungs (38). In the course of their translocation, they can cause cellular injury that may increase AST level in the serum. This may account for increase in AST observed.

We can conclude from this study that EM supplementation may have some beneficial effect by lowering cholesterol and triglycerides on the other hand it may cause some tissue damage as indicated by increasing levels of AST, these results need further studies using different types and doses of EM.

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