

## **EFFECT OF PROBIOTIC SUPPLEMENTATION ON SOME BIOCHEMICAL PARAMETERS AND GROWTH PERFORMANCE OF BROILER CHICKENS**

*BY*

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### **ABSTRACT**

This study was carried out on two hundred and fifty mixed sexes, one day old Hubbard broiler chickens, weighting 40 – 45 gm, obtained from local commercial hatchery (El-Mansoura Poultry Company). The experimental chickens were classified into five groups each group consists of fifty chickens as follows: The first group (G1) was a control group and fed on a ration free from prebiotics, probiotics or antibiotics. The second group (G2) was fed an experimental ration containing prebiotics (lactose 5%). The third group (G3) was fed an experimental ration containing probiotics 1%. The fourth group (G4) fed an experimental ration containing prebiotics 5 % and probiotics 1%. The fifth group (G5) was fed ration containing antibiotics (chloramphenicol 1%). Chickens were fed on the experimental ration for six weeks, at the end of the experiment the body weight was recorded and the chickens were sacrificed for collection of blood and tissue samples for biochemical analysis and histopathological examination. The obtained results revealed a significant decrease in serum triacylglycerol, total cholesterol, LDL-C and malondialdehyde (MDA) levels. There was a significant increase in body weight, total protein, globulins and HDL-C serum levels. blood SOD and catalase and GST activities showed significant increase. Moreover, Liver and kidney tissues exhibited a significant increase in MDA and a significant increase in GSH levels in all groups except for the last group in which opposite results were obtained in comparison with the control group.

So it could be concluded that the addition of prebiotics and probiotics either separate or in combination is preferable than antibiotics as growth promoter and to avoid their health hazards.

## INTRODUCTION

Probiotics are dietary supplements of live microorganisms thought to be healthy for the host organism, according to the currently adopted definition by FAO / WHO, probiotics are "live micro organisms which when administered in adequate amount confer a health benefit on the host" (FAO/ WHO, 2001). The most common types of probiotic bacteria are strains of lactobacillus and Bifidobacterium, sometimes combined with streptococcus thermophilus (Xiao *et al.*, 2003). At first, probiotics were thought to be beneficially affect the host by improving its intestinal microbial balance, thus inhibiting pathogens and toxin producing bacteria, today specific health effects are being investigated and documented including alleviation of chronic intestinal inflammatory diseases (March, 2006). Prevention and treatment of pathogen induced diarrhea (Yan and Polk, 2006), urogenital infections (Reid, 2008), and tropic diseases (Vanderhoof, 2008). Vivo and vitro experiments demonstrate that the antioxidants roles against free radicals (Nagi *et al.*, 1999). These free radicals are consumed by both enzymatic (as superoxide dismutase "SOD" and catalase enzymes) and non enzymatic (as reduced glutathione "GSH"). Probiotics has been found to increase superoxide dismutase and catalase activities and increased the level of GSH (Alessandro *et al.*, 2007) and (Mathieu *et al.*, 2009). Biosynthesis of glutathione enhanced by probiotics (Femke *et al.*, 2008).

Probiotic supplementation caused a pronounced increase in total protein and protein digestibility (Zeweil, 2003). Furthermore, they are able to increase beneficial short chain fatty acids (Moro *et al.*, 2002). And are able to lower cholesterol level and it produced a highly significant reduction in LDL-cholesterol levels and a small but significant increase in HDL-cholesterol level. This study offers the prospect of using probiotic as a side effect free alternative to drug therapy in the treatment of high cholesterol and heart disease (LaRosa *et al.*, 2003). Probiotics prevent absorption of triacylglycerol from intestine to plasma so decrease plasma triacylglycerol (Mahdavi *et al.*, 2005). Prebiotics and probiotics treatment resulted a decline in lipid peroxidation this due to either reduced amount of ROS, or enhanced antioxidative capacity. MDA levels were lowered by action of probiotics (Maite *et al.*, 2002). Supplementation of ration with probiotics improved final body weight due to increase protein digestibility ( Zeweil, 2003). So this work is aimed to throw some lights on the effects of probiotics supplementation as a growth promoter, antioxidant and lipid lowering substances and to replace antibiotics by probiotics.

## MATERIAL AND METHODS

### 1- Experimental chickens:

Two hundred and fifty mixed sexes, one day old Hubbard broiler chickens, weighting 40 – 45 gm, obtained from local commercial hatchery (El-Mansoura Poultry Company) which randomly distributed to five groups. Each group was consisted of fifty chickens these chickens were housed in well disinfected house with wheat straw as a bedding material, feed and fresh clean water was provided adlibitum.

The experimental chickens were kept in a controlled environmental and maintained under a 24 hours light, low humidity , good ventilation and maintained under temperature of 35-36 °C, then gradual decreasing in temperature to reach at the end of experiment to 20 – 24 °C. The experiment was carried out at the Faculty of Veterinary Medicine, Mansoura University on April and June , where chickens were fed the experimental diets for six weeks. chickens were fed on Al-Kahera ration from one day to 30 days (starter and growing rations) and this ration formed from (/kg): Crude protein not less than 21%. Crude fat not less than 3.26. Crude fibers not more than 3.29%. chickens were fed on finishing ration from 30 days to 45 days and this ration formed from (/kg): Crude protein not less than 17.5%. Crude fat not less than 3.8%. Crude fibers not less than 3.7%.

**Experimental groups:** The experimental chickens were classified into five groups each group consists of fifty chickens as follows: The first group (G1) was a control group and they were fed on a ration free from prebiotics, probiotics or antibiotics. The second group (G2): chickens in this group were fed an experimental ration containing prebiotics (lactose 5%). The third group (G3): chickens in this group were fed an experimental ration containing probiotics 1%. The fourth group (G4): chickens in this group were fed an experimental ration containing prebiotics 5 % and probiotics 1%. The fifth group (G5): chickens in this group were fed an experimental ration containing antibiotics (chloramphenicol 1%). Chickens were fed on the experimental ration for six weeks, at the end of the experiment the body weight was recorded and the chickens were sacrificed for collection of blood samples for biochemical analysis in blood and serum.

**Sampling:**

**A) Blood samples:** Slaughtering was carried out after 6 weeks. One blood sample was collected and divided into two parts the first one was taken on anticoagulant (heparin) and directly used for determination of the enzymatic activities in RBCs as SOD, catalase and GST and GSH level. The second part was collected in sterile clean and dry screw capped centrifuge tubes without anticoagulant and left for clotting at room temperature, then centrifuged at 3000 r.p.m. for 15 minutes for serum separation. The collected sera were used for analysis of total serum lipid (**Zollner and kirsch, 1962**), triacylglycerols (**Fassati and Precipe, 1982**), total cholesterol (**Richmond, (1973)**), HDL-Cholesterol (**Lopez, 1977**), HDL-Cholesterol (**Friedewald *et al.*, (1972)**), total protein (**Gornal *et al.*, (1949)**), albumin (**Doumas, 1971**) in serum of chicks. Serum and tissues malondialdehyde (**ohkwa *et al.*, (1979)**). Catalase activity (**Fossati, 1980**). Superoxide dismutase activity (**Nishikimi *et al.*, 1972**). Glutathione-S-transferase activity (**Habig and Pabst, 1974**) and reduced glutathione level (**Beutler *et al.*, (1963)**).

Tissues samples (liver, kidney) were collected and kept in Jars containing normal saline solution at (-20) °c to determine GSH and MDA levels in the collected tissue. Liver and kidney specimen of chickens were carefully examined by naked eye for detection of any abnormalities and then fixed in neutral formalin 10%. Paraffin sections of 5µ thick were prepared and stained with hematoxylin and eosin and examined microscopically (**Wood and Ellis, 1994**). Statistical Analysis was carried out (**Snedecor and Cochran, 1989**).

## RESULTS

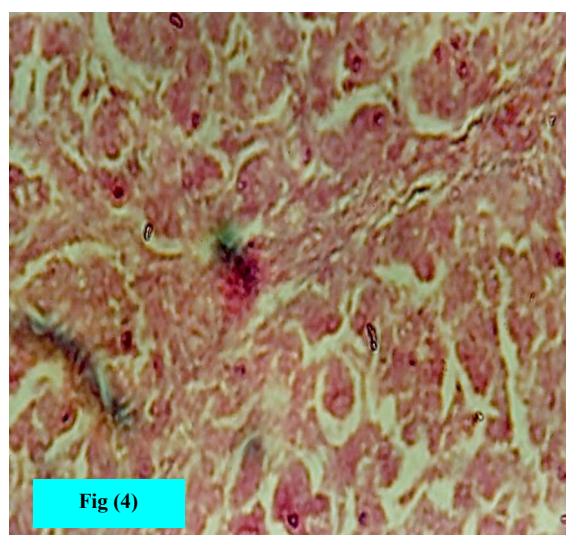
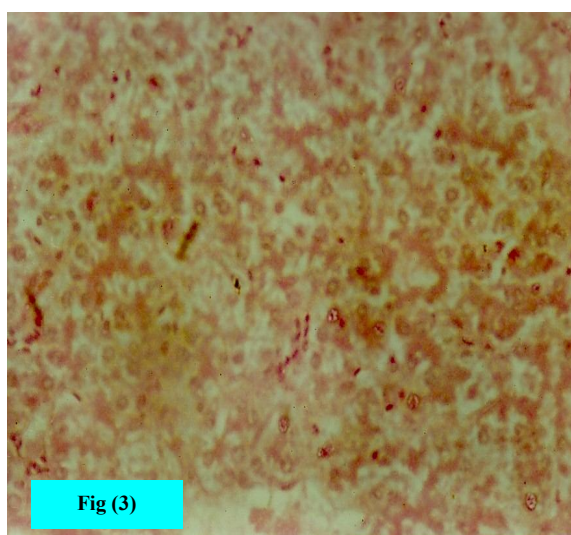
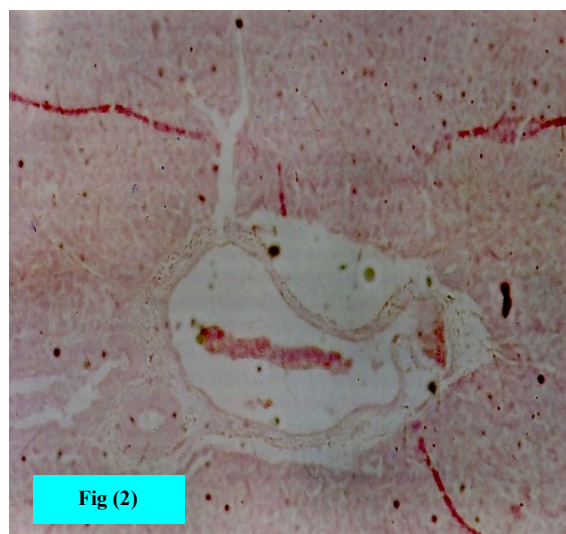
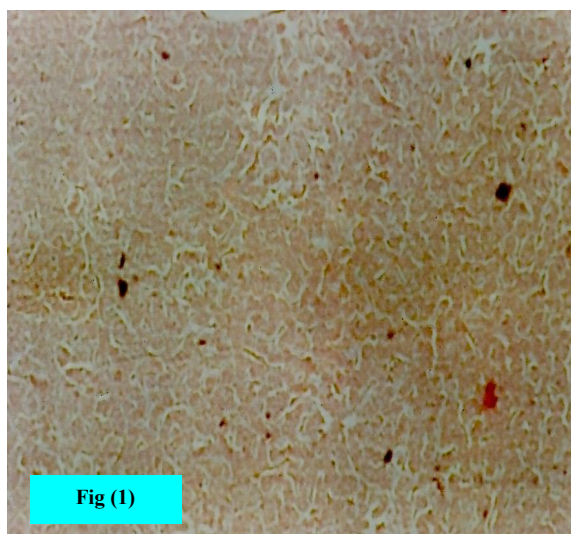
**Table (1):** Effect of prebiotics, probiotics and antibiotics on body weight and some serum and tissues parameters in broiler chickens (M ± SE).

	G1	G2	G3	G4	G5
Body weight( kg)	1.425 ±0.025 <sup>d</sup>	1.525 ±0.025 <sup>c</sup>	1.7 ±0.033 <sup>b</sup>	1.8 ±0.05 <sup>a</sup>	1.37 ±0.02 <sup>de</sup>
Tr lipids (mg/dl)	771.815±28.2 <sup>abcd</sup>	767.72±19.176 <sup>cd</sup>	742.035±21.23 <sup>d</sup>	682.13±3.94 <sup>e</sup>	819.395±2.431 <sup>a</sup>
Triacylglycerol (mg/dl)	147.595±1.198 <sup>b</sup>	142.652±1.25 <sup>c</sup>	131.665±1.428 <sup>d</sup>	121.285±1.38 <sup>e</sup>	167.89±0.753 <sup>a</sup>
T. cholesterol (mg/dl)	229.135±0.361 <sup>b</sup>	221.785±2.2616 <sup>bc</sup>	209.245±3.0816 <sup>de</sup>	204.488±0.607 <sup>e</sup>	244.535±5.3216 <sup>A</sup>
HDL-cholesterol (mg/dl)	23.1834±1.11 <sup>cd</sup>	24.3572±0.115 <sup>c</sup>	27.506±0.549 <sup>b</sup>	27.6622±1.13 <sup>a</sup>	17.781±0.100 <sup>e</sup>
LDL-cholesterol (mg/dl)	183.9±1.483 <sup>b</sup>	178.82±8.0132 <sup>bc</sup>	168.41±0.786 <sup>de</sup>	167.396±0.6706 <sup>e</sup>	235.066±1.272 <sup>A</sup>
Total protein (gm/dl)	6.604±0.444 <sup>d</sup>	6.898±0.148 <sup>c</sup>	7.92±0.301 <sup>b</sup>	8.666±0.129 <sup>a</sup>	4.961±0.117 <sup>E</sup>
Albumin (gm/dl)	5.407±0.329 <sup>abcd</sup>	5.525±0.241 <sup>abcd</sup>	5.604±0.085 <sup>abcd</sup>	5.748±0.192 <sup>abcd</sup>	3.674±0.137 <sup>E</sup>
Globulin (gm /dl)	1.75±0.05 <sup>d</sup>	1.999±0.011 <sup>c</sup>	2.17±0.03 <sup>b</sup>	2.22±0.08 <sup>a</sup>	2.22±0.08 <sup>A</sup>
(nmol/ml)	7.898±0.0783 <sup>b</sup>	6.744±0.263 <sup>c</sup>	5.077±0.101 <sup>de</sup>	4.52±0.11 <sup>e</sup>	10.25±0.64 <sup>A</sup>
Liver MDA (nmol/g tissue)	20.275 ±0.558 <sup>b</sup>	17.525 ±0.158 <sup>c</sup>	16.845 ±0.2716 <sup>cd</sup>	16.505 ±0.168 <sup>ce</sup>	23.525 ±0.158 <sup>a</sup>
Kidney MDA (nmol/gm. tissue)	4.348 ±0.099 <sup>b</sup>	3.28 ±0.212 <sup>cde</sup>	2.82 ±0.061 <sup>de</sup>	2.75 ±0.25 <sup>e</sup>	5.509 ±0.375 <sup>a</sup>

The different superscript letters in the same row means significant difference

**Table (2):** Effect of prebiotics, probiotics and on some RBCs and tissues enzyme activities in broiler chickens(M ± SE).

	G1	G2	G3	G4	G5
Catalase activity (u/l)	225.07±5.02 <sup>d</sup>	253.625±4.365 <sup>c</sup>	315.061±4.982 <sup>b</sup>	363.575±9.525 <sup>a</sup>	213.125±0.931 <sup>e</sup>
SOD activity (u/ml)	179.022±4.365 <sup>e</sup>	228.185±0.188 <sup>d</sup>	264.9±0.4 <sup>b</sup>	269.516±0.266 <sup>a</sup>	251.27±0.727 <sup>c</sup>
GST activity (u/l)	15.46±1.4 <sup>d</sup>	15.612±0.258 <sup>c</sup>	28.39±0.137 <sup>b</sup>	35.428±1.125 <sup>a</sup>	12.26±0.2 <sup>E</sup>
Serum GSH (mg/dl)	15.46±1.4 <sup>d</sup>	15.612±0.258 <sup>c</sup>	28.39±0.137 <sup>b</sup>	35.428±1.125 <sup>a</sup>	12.26±0.2 <sup>E</sup>
liver GSH (mg /gm. tissue)	13.372±0.339 <sup>d</sup>	14.642±0.0613 <sup>c</sup>	15.895±0.1216 <sup>b</sup>	23.814±0.427 <sup>a</sup>	9.065±0.311 <sup>e</sup>
Kidney GSH (mg/gm. tissue)	11.377±0.365 <sup>cd</sup>	12.216±0.605 <sup>c</sup>	14.177±0.245 <sup>b</sup>	18.337±0.342 <sup>a</sup>	9.294±0.284 <sup>E</sup>



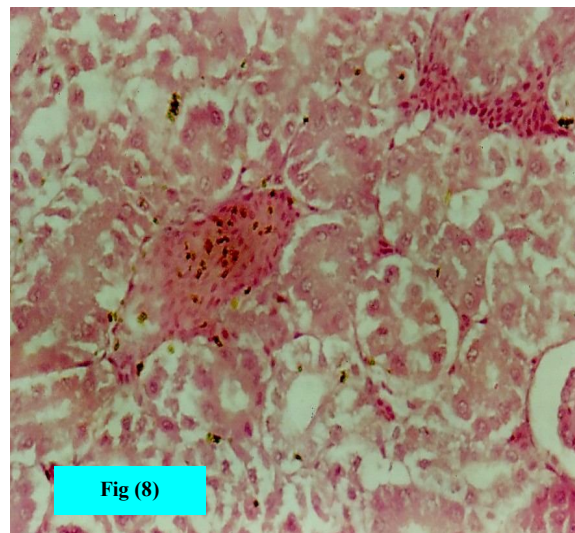
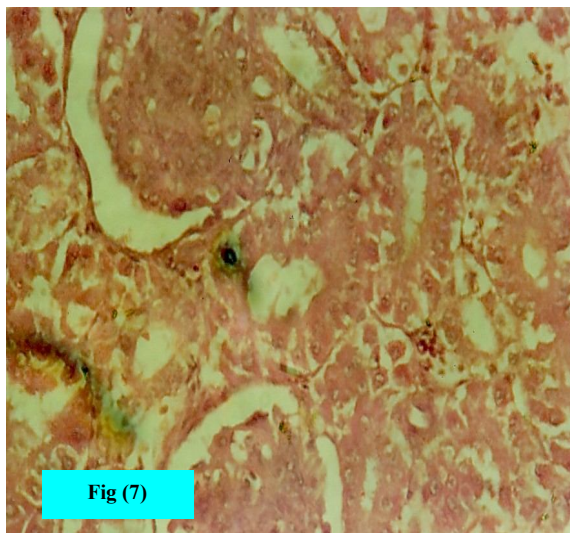
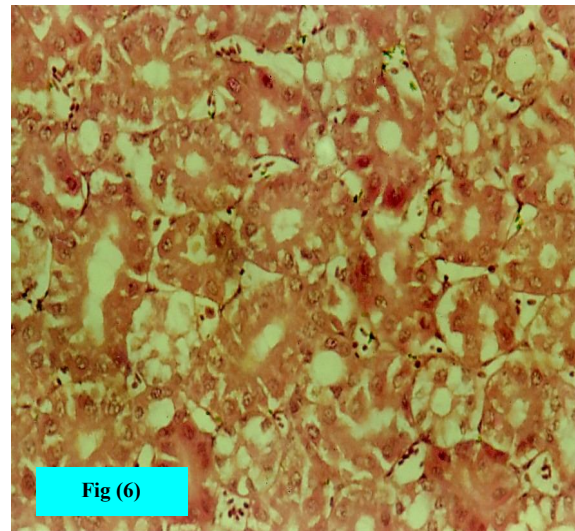
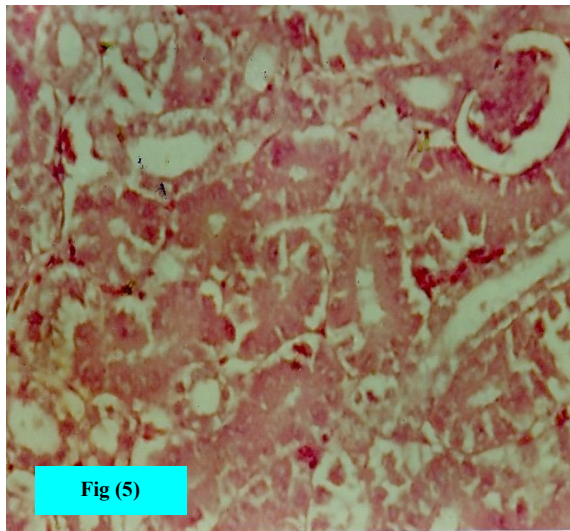
**Fig. (1) of group (2):** liver showing normal hepatic histological structure, without any necrosis or degenerative changes. (H&E., 120x).

**Fig. (2) of group (3):** liver showing normal hepatic cords around portal area without any inflammatory reaction in portal area. (H&E., 120x).

**Fig. (3) of group (4):** liver showing normal histological arrangement of hepatic cords with normal hepatocytes, only minimal degenerative change. (H&E., 120x).

**Fig. (4) of group (5):** liver showing intralobular fibrosis, also degenerative changes and necrosis in hepatocytes. (H&E., 430x)





**Fig. (5) of group (2):** kidney showing normal renal tubule and glomeruli. (H&E., 420x).

**Fig. (6) of group (3):** kidney showing normal renal tubules with only slight or minimal hydropic degeneration in renal tubules. (H&E., 120x).

**Fig. (7) of group (4):** kidney showing normal glomeruli and renal tubules with normal renal tubular epithelium. (H&E., 420x).

**Fig. (8) of group (5):** kidney showing marked congestion and hemorrhage in interstitial capillaries. (H&E., 420x).

## DISCUSSION

There was a significant increase in body weights in all groups receiving prebiotic (G1) , probiotic (G3) Or their mix (G4) except for the last group (G 5) received antibiotic exhibited an opposite effect when compared with the control group (G1). The obtained results are confirmed by the results of **Ashayerizadeh *et al.*, (2009)**. Probiotics increase body weight through enhancing broiler performance parameters such as body weight, feed intake and feed conversion ratio ( **Mead, 2000**). Supplementation of probiotics improved final body weight due to increase protein digestibility (**Zeweil, 2003**).

There was a significant decrease in serum triacylglycerol, total cholesterol and LDL-C. A significant increase in HDL-C in all groups receiving prebiotic (G2) , probiotic (G3) or their mix (G4) except for the last group (G 5) received antibiotic showed an opposite effect when compared with the control group (G1). The obtained results are confirmed by the results of **Xiao *et al.*, (2003) and Daniela *et al.*, (2009)**.

Abnormal high TG levels are associated with a number of diseases and conditions such as cirrhosis, hypothyroidism, diabetes and pancreatitis TG may contribute to a type of thickening of artery wall lead to atherosclerosis (**Susan *et al.*, 2004**). Probiotics are able to synthesize esterase enzymes alongside with lipase enzymes, which the former converts free fatty acids to esterified form differed from triacylglycerol of intestinal content and finally less enhances for triacylglycerol absorption into the plasma (**Genedy and Zewil, 2003**).

There was a significant increase total proteins and globulins in all groups receiving prebiotic (G2) , probiotic (G3) or their mix (G4) except for the last group (G 5) received antibiotic revealed an opposite effect when compared with the control group (G1). The obtained results are confirmed by the results of **Gordon *et al.*, (1993), Zeweil, 2003) and Monari *et al.*, (2008)**. Supplementation of ration with probiotics increase protein digestibility and absorption ( **Zeweil, 2003**). Probiotics increase efficiency of digestion and assimilation of food materials leading to increase protein synthesis and subsequent accumulation of storage proteins in the body (**Abdulrahim *et al.*, 1996 & Singj *et.al.*, 2005**).

MDA is the end product of lipid peroxidation (**Freeman and Cropo, 1982**). There was a significant decrease in serum and tissue MDA level in all groups receiving prebiotic (G1) , probiotic (G3) Or their mix (G4) except for the last group (G 5) received antibiotic exhibited



an opposite effect when compared with the control group. These results agree with those of **Maite *et al.*, (2002)** and **Osman *et al.*, (2006)**.

The antioxidant enzyme catalase, SOD, and GST activities were significantly increased in all groups except the last group relieving antibiotics showed a significant decrease when compared with the control group. Similar results were recorded in serum and tissues GSH levels.

Catalases are enzymes that catalyse the conversion of hydrogen peroxide to water and oxygen so protect the cell from H<sub>2</sub>O<sub>2</sub> accumulation (**Chelikani *et al.*, (2004)**). The obtained results were confirmed by the results obtained by **Mathieu *et al.*, (2009)**. The increase in catalase activity was due to radical scavenging activity as probiotic had antioxidant properties so probiotics increase antioxidant enzymes like catalase, SOD and GST as a defense mechanism (**Mathieu *et al.*, 2009**). Administration of probiotics stimulate intracellular accumulation of short chain fatty acids produced by bacteria, thereby inducing increased capacity of antioxidant enzymes like catalase and SOD (**Lutgendorff *et al.*, 2008**).

Superoxide dismutase is an enzyme that catalyses the breakdown of harmful Superoxide anion into oxygen and hydrogen peroxide (**Zelko, *et al.*, 2002 & Lutgendorff *et al.*, (2008), and Mathieu *et al.*, (2009)**).

GST represent a major group of detoxification enzymes (**Hayes and Pulford, 1995 & Ingrid *et al.*, (2001)**). GSH is essential for regulation of enzymes and protect cells against ROS and free radicals produced in normal metabolism (**Sen, 1997**). These results confirmed by the results of **Lutgendorff, (2008)**. Reduced glutathione is essential for metabolic and cell cycle related functions in all cells and has ability to directly scavenge free radicals so GSH as found by **Alessandro *et al.*, (2007)**, **Cetin, (2008)**, **Paez *et al.*, (2008)** and **Lutgendorff, (2008)**.

Supplementation of prebiotics and probiotics together enhancing the microbial fermentation, the anaerobic breakdown of undigested poly saccharides and fibers which enhancing the formation of LAB (lactic acid bacteria) and short chain fatty acids as fermentation products, increased production of shortchain fatty acids (especially butyrate) enhancing glutathione –S- transferase expression in tissues (**Ingrid *et al.*, 2001**).

Kidney showed normal renal tubule and glomeruli in figures (5, 6) of groups (2, 4), but in figure (7) of group (3) kidney showed normal renal tubule with minimal hydropic

degeneration, and in figure (8) of group (5) kidney showed marked congestion and hemorrhage in interstitial capillaries.

Liver showed a normal histological structure without any necrosis, degenerative changes or any inflammatory reaction in portal area in figures (1, 2) of group (2, 3), but in figure(3) of group (4) there was minimal degenerative change with normal hepatic cords and hepatocytes. And in figure (4) of group (5) liver showed intralobular fibrosis, also there were a degenerative changes and necrosis in hepatocytes.

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## المخلص العربي

### تأثير إضافة البروبيوتك على بعض القياسات الكيميائية الحيوية ومعدل النمو فى بدارى التسمين

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تهدف هذه الدراسة لاستبيان تأثير إضافة البروبيوتك والبروبيوتك كل على حدة او معا ومقرنتها بالمضادات الحيوية فى علائق بدارى التسمين على بعض القياسات الكيميائية الحيوية ومعدل النمو. وقد أجريت هذه الدراسة على عدد مائتين وخمسين كتكوت تسمين عمريوم من سلالة هبرد والتي تم تقسيمها عشوائيا إلى خمسة مجموعات متساوية كل مجموعة تحتوى على خمسين كتوتا كانت المجموعة الأولى هى الضابطة وتم تغذيتها على عليقة خالية من أى إضافات ، والمجموعة الثانية تحتوى على لاكتوز بنسبة 5% والمجموعة الثالثة تحتوى على بروبيوتك بنسبة 1% والمجموعة الرابعة تحتوى على خليط من اللاكتوز 5% والبروبيوتك 1% والمجموعة الخامسة تحتوى على كلورامفينكول 1% وقد تم تغذية الكتاكيت على هذه العلائق لمدة ستة أسابيع وفى نهاية التجربة تم وزن بدارى التسمين وتم تجميع عينات الدم والأنسجة ( الكبد والكلى ) لقياس مستويات المألون داي ألدهيد ، البروتينات الكلية ، الالبومين ، الجلوبيولين ، الدهون الكلية ، الجلوسريدات الثلاثية ، الكوليسترول الكلى ، البروتينات الدهنية (الليبوبروتينات) عالية وقليلة الكثافة فى مصل الدم بالإضافة إلى قياس نسبة المألون داي ألدهيد والجلوتاثيون المختزل فى أنسجة الكبد والكلى وأيضا نشاط انزيم السوبرأكسيد ديسميوتيز.. وقد أظهرت النتائج ان هدة الاضافات تحدث تغيرات بيوكيميائية فى هدة المركبات.

## الخلاصة

يتضح من هذه الدراسة أن إضافة البروبيوتك كان له تأثيرا إيجابيا علي كلا من وزن الجسم وبعض القياسات البيوكيميائية مثل زيادة البروتين وأيضا زيادة مناعة الجسم لدوره الفعال كمضاد للأكسدة وخصوصا عند إضافة اللاكتوز مع البروبيوتك والذي يزيد من فاعلية البروبيوتك ، وعلي العكس إضافة الكلورامفينكول كان له تأثيرا سلبيا علي كل من وزن الجسم وبعض القياسات البيوكيميائية مثل تقليل البروتين وتقليل مناعة الجسم وتأثيره السيئ علي الكبد لزيادة نسبة الدهون والتليف داخله . لذلك توصي هذه الدراسة بإضافة البروبيوتك واللاكتوز علي العلائق والتحذير من إضافة الكلورامفينكول علي العلائق..