Effect of adding locally probiotic and Cupric Sulphate to Laying Hens Diets on the Performance , Egg Yolk Cholesterol and Blood biochemical Parameters

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Abstract

The present experiment aimed to study the effect of diets supplementation with local probiotic (IRAQI PROBIOTIC ™), and cupric sulphate (CuSo4) on performance of shaver laying hens. A locally prepared probiotic were used each one gram of this probiotic contain $(10)^{10}$ *Lactobacilli* , $(1)^{10}$ *Lactobacillus acidophilus,* $(100)^{10}$ *Bacillus subtilus* and $(10)^{10}$ *Saccharomyces cerevisia*e yeast. A total of 112, 42 week old, Shaver laying hens were divided into 7 treatments with 4 replicates per treatment (4 laying hens per replicate). Laying hens in T1 were fed a basal diet used as control. Laying hens in T2, T3 and T4 were fed diets supplemented with 5 ,10 and 15gm/kg ration of local probiotic respectively, Laying hens in T5,T6 and T7 were fed diets supplemented with 100,200 and 300ppm of cupric sulphate respectively. The experiment was conducted at the poultry farm belong to Animal Resources, College of Agriculture, University of AL- Anbar . During the experimental periods hens performance traits included egg production, egg weights, egg mass, feed consumption, and feed conversion ratio were recorded, At the end of the experimental periods blood serum and egg yolk samples were collected for cholesterol analysis and biochemical which including glucose, protein, albumin, globulin, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), Liver weights, GOT and GPT enzymes. A significant increase ($p<0.05$) of record the laying hens supplemented with local probiotic T2 (adding local probiotic 5 gm/kg) , T3 (adding local probiotic 10 gm/kg) ,T4 (adding local probiotic 15 gm/kg) in egg production and egg mass as compare with other treatments. In general the study revealed that there were a significant improvement ($P<0.05$) in feed conversion ratio of T2(adding local probiotic 5 gm/kg) ,T3 (adding local probiotic 10 gm/kg) ,T4 (adding local probiotic 15gm/kg) ration compared with T1(control). Serum cholesterol concentration, triglycerides, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were decreased markedly by both local probiotic and cupric sulphate supplementation, while high density lipoprotein (HDL) increased in both local probiotic and cupric sulphate treatments. There were no differences ($p>0.05$) regarding in feed intake, total glucose ,total protein, total albumin, total globulin ,liver weight, GOT and GPT enzymes.

Key words: (probiotic, Cupric sulphate, laying hen, performance, egg yolk cholesterol)

تأثير إضافة المعزز الحيوي المحلي (بروبايوتيك العراق) وكبريتات النحاس إلى علائق الدجاج البياض في الأداء الإنتاجي وكوليسترول صفار البيض وبعض الصفات الكيموحيوية

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المستخلص

هدفت الدراسة الحالية إلى معرفة دراسة تأثير إضافة المعزز الحيوي المحضر محلياً (بروبايوتيك العراق) وكبريتات النحاس Cuso4 في الأداء الإنتاجي للدجاج البياض(شيفر). استخدم في الدراسة المعزز الحيوي المحضر محلياً (بروبايوتيك العراق) إذ يحوي الغرام الواحد من المعزز الحيوي على مالا يقل عن 1010 خلية من بكتريا *Lactobacilli* وما لا يقل عن 1010 خلية من خميرة S.cerevisiae وما لا يقل عن 101 خلية من بكتريا *Lactobacillus acidophilus* وما لا يقل عن 10100 خلية من بكتريا *Bacillus subtilus*. استخدم في هذه الدراسة 112 دجاجة بياضة من نوع شيفر بعمر 42 أسبوع وزعت عشوائياً على سبعة معاملات بواقع أربعة مكررات (4 دجاجات/مكرر). غذيت الدجاجات في المعاملة T1 على عليقة دجاج بياض خالية من أية إضافة (السيطرة)، بينما أضيف إلى المعاملات T2 ، T3 وT4 المعزز الحيوي المحضر محلياً (بروبايوتيك العراق) وبالنسب 5، 10 و15 غم/ كغم علف على التوالي . أضيف إلى المعاملات T5 ،T6 وT7 كبريتات النحاس وبتركيز 100 ،200 و300 ملغم / كغم علف على التوالي . أجريت الدراسة في حقل الطيور الداجنة التابع إلى قسم الثروة الحيوانية في كلية الزراعة / جامعة الانبار ، خلال فترات التجربة تم دراسة الصفات التالية : إنتاج البيض ، وزن البيض ، كتلة البيض ،استهلاك العلف ومعامل التحويل الغذائي . في نهاية مدة التجربة تم جمع عينات الدم من الدجاج لتقدير كوليسترول الدم وكوليسترول الصفار بالإضافة إلى دراسة الصفات الكيموحيوية والتي تضمنت : تقدير الكلوكوز الكلي، تقدير البروتين الكلي ، تقدير الألبومين ، تقدير الكلوبيولين ، الكليسيريدات الثلاثية ، البروتين الدهني عالي الكثافة (HDL) ، البروتين الدهني واطئ الكثافة (LDL) ، البروتين الدهني واطئ الكثافة جداً (VLDL) بالإضافة إلى وزن الكبد ، تركيز إنزيم GOT وتركيز إنزيم GPT. أشارت النتائج إلى زيادة معنوية ($P<0.05$) في إنتاج البيض وكتلة البيض المنتج لمعاملات إضافة المعزز الحيوي المحلي (بروبايوتيك العراق) T2 (إضافة المعزز الحيوي 5غم /كغم علف) ، T3 (إضافة المعزز الحيوي 10 غم /كغم علف) وT4 (إضافة المعزز الحيوي 15 غم /كغم علف) مقارنة بباقي معاملات التجربة . أشارت النتائج إلى تفوق معنوي لمعاملات إضافة المعزز الحيوي المحلي (بروبايوتيك العراق) T2، T3 وT4 في معدلات معامل التحويل الغذائي مقارنة مع معاملات التجربة المختلفة . سجلت جميع معاملات إضافة المعزز الحيوي المحلي (بروبايوتيك العراق) ومعاملات إضافة كبريتات النحاس انخفاض معنوي ($P>0.05$) في تركيز كوليسترول الدم وكوليسترول صفار البيض ،

الكليسيريدات الثلاثية، البروتين الدهني واطئ الكثافة (LDL) وتركيز البروتين الدهني واطئ الكثافة جداً(VLDL) مقارنة بمعاملة السيطرة الخالية من أي إضافة . لوحظ ارتفاع معنوي($P<0.05$) في تركيز البروتين الدهني عالي الكثافة (HDL) لمعاملات إضافة المعزز الحيوي المحلي (بروبايوتيك العراق) ومعاملات إضافة كبريتات النحاس مقارنة بمعاملة السيطرة . لم يلاحظ وجود أي فروق معنوية ($P>0.05$) بين معاملات التجربة المختلفة في معدلات استهلاك العلف ، تركيز الكلوكوز الكلي، تركيز البروتين الكلي ، تركيز الألبومين الكلي ، تركيز الكلوبيولين الكلي، وزن الكبد ، تركيز إنزيم GOT وتركيز إنزيم GPT .
الكلمات المفتاحية : (معزز حيوي ، كبريتات النحاس، دجاج بياض، الأداء الإنتاجي ، كوليسترول الصفار)

Introduction

Although antibiotics achieved good performance, their potential side effects became a real public health concern globally (1).Probiotic is a Greek word, which means for life". Probiotics are microbic food supplements, which have beneficial effects on the host by bowel microbic balance optimizing (2). Many attempts have been undertaken to improve the growth rate and feed conversion ratio to reduce the cost of diets by addition of dietary supplementation such as probiotics (3), In recent years, using probiotics in flocks' nutrition impressed scientists due to its good effects on growth, food conversion coefficient and flocks health (4). Probiotics are bacteria or yeast in origin, could be fed either alone or in combination, Probiotics regulate microbial environment of the intestine, decrease the digestive disturbances, inhibit pathogenic intestinal microorganisms and improve feed conversion ratio (5). Some investigators demonstrated that growth performance and nutrient digestibility of birds had improved by feeding diets containing probiotics (6). Besides ,these microorganisms are responsible for production of vitamin B complex and digestive enzymes and for stimulation of intestinal mucosal immunity, increasing protection against toxins produced by pathogenic microorganisms (7). Copper is the third most abundant essential trace element in animals, after Iron and Zinc. Copper deficiency was shown hypercholesterolemia in rats (8). Feeding chickens different levels of copper for 35 and 42 day resulted in decreases of plasma and breast muscle cholesterol and plasma triglycerides (9),(10) and (11). Copper is an essential nutrient and it has been reported that sulfate form (CuSo4) is more effective than oxide (CuO) (12). Copper is often added to poultry diets at prophylactic concentrations for its growth promoting effects (11). Traditionally, the source of copper has been cupric sulfate pentahydrate due to cost and commercial availability. Copper is usually fed commercially at much higher pharmacological levels (100-300 mg/kg diet) because of its growth promoting properties (10). Egg production and feed conversion of laying hens were improved by addition of 300 mg Cu/kg of diet(13). Copper deficiency in laying hens resulted in hypercholesterolemia (8),(14) demonstrated that pharmacological levels of cupric sulphate (250 mg/kg diet) caused changes in 17

beta-estradiol and enzymes involved in carbohydrate, lipid and amino acids metabolism in mature laying hens and suggested that copper supplement can affect reproductive physiology and lipid metabolism. (15) demonstrated that high cupric sulphate (500 mg/kg) in diet have damaged duodenal villi, without disturb normal protein metabolism of broiler chicken therefore, have impact on nutrient absorption, depress food intake resulting in poor growth performance of broiler chickens. Addition of different amounts of copper to the ration of laying hens resulted in decreases in yolk cholesterol concentrations and blood plasma lipid concentrations (14) ,(16) ,(17). The purpose of this work is to study the effect of different levels of local probiotic and cupric sulphate supplementation to the basal ration on the performance and blood biochemical parameters of shaver laying hens .

Materials and Methods

This study was carried out at the poultry farm belong to animal resources, College of Agriculture, University of AL- Anbar .The object of this study was to compare the effect of supplementation different levels of local probiotic (IRAQI PROBIOTIC ™) shown in (Table 1), and cupric sulphate to the basal diets on the performance and blood biochemical parameters of shaver laying hens , during the period 20th of July to 11th of Nov 2010. A total number of 112, at 42 week old shaver laying hens were randomly divided into seven treatments (each treatment contain four replicates with 4 hens) The diets were formulated to meet the requirements of birds established by the (18) for laying hen (Table 2). The treatments were as follow: first treatment Control (basal diet only) ,second treatment: (basal diet+5gm/kg local probiotic), third treatment: (basal diet+10 gm/kg local probiotic), fourth treatment: (basal diet+15 gm/kg local probiotic) , fifth treatment: (basal diet+ 100ppm cupric sulphate), sixth treatment: (basal diet + 200ppm cupric sulphate) and seventh treatment: (basal diet + 300ppm cupric sulphate). Feed and water were provided , The chickens were reared in twenty-eight cages (40×50×45 cm) each replicate placed in pen .The study continued for four periods (28day/ period). House system was whole controlled,16 hours light was provided per day. Feed and water were available throughout the experiment. Egg production percentage, egg mass, egg weight ,feed intake and feed conversion ratio were determined weekly (19). Egg mass was measured by multiplying of egg production percentage to egg weight and feed conversion ratio was measured by dividing amount of feed intake to egg mass (20). At the end of the experiment period, two birds from each replicate were randomly chosen for blood collection and approximately 5 ml blood samples were collected from the right brachial vein, Serum was isolated by centrifugation at 3000 rpm for 15 minute for determining the blood biochemical parameters which included total glucose was determined according to (21) ,total protein was determined as (22). The concentration of GOT and GPT were determined as (23). Triglyceride, cholesterol,

high density lipoprotein (HDL) were determined by enzymatic method as reported by (24) by using the commercial kit (Biolabo SA,France), and low density lipoprotein (LDL) was determined as mathematical equation LDL= [Cholesterol - (Triglycerides /5) - HDL] , The serum very low density lipoprotein (VLDL) fraction were calculated mathematically by applying the following equation : VLDL= Triglyceride /5 according to (25). For measuring the egg cholesterol, at the end of the experiment, 8 eggs were collected from each treatment to measure egg yolk cholesterol, using commercial kit (Biolabo SA, France), according to (26). Data obtained from the study were analyzed using computer software by statistical analysis system (27) and significant differences among means were determined by using Duncan´s multiple range test (28).

Table (1). Local probiotics (IRAQI PROBIOTIC ™) compound mixed of bacteria and *Saccharomyces* useful (cfu/gm). Each kilogram of Iraqi probiotic prepared locally content of

Microbial Strains	Bacteria total count Per gram
Lactobacilli	10 billion cells
Lactobacillus acidophilus	1 billion cells
Bacillus subtilus	100 billion cells
Saccharomyces cervisia	10 billion cells

Table (2). Composition of the experimental laying hen basal diet and calculated chemical analysis.

Ingredient	Quantity %
Yellow Corn	36
Wheat	28.5
Protein*	10
Soy bean meal (44%)	16
Oil	1.5
Limestone	7.7
Salt	0.3
Total	100
Chemical Analysis**	
Metabolizable Energy kcal/kg	2759
Crude Protein %	17.75
ME: C.P	155
Calcium%	3.60
Available Phosphor%	0.5
Lysine	0.86
Methionine	0.41
Methionine+ Cystein	0.68

***Jordan origin:(45%crude protein , 2200 kcal metabolizable energy, 6% fat, 2.5% ash, 6-7% Ca, 3.3% P ,2.0% Methionine+ Cystin, 2.5% Lysine. ** Chemical analysis according to (18)**

Results and Discussion

Data presented in table (3) showed that there is significant difference among the treatments of adding locally prepared probiotic and cupric sulphate to laying hens diets on egg production percentage. Significant difference among the treatments during first period from the research T3 (local probiotic 10 gm/kg) increase significant is achieved highest value in egg production percentage followed by T2 (local probiotic 5 gm/kg) which there are no significant difference between them. T5(cupric sulphate 100ppm) and T6 (cupric sulphate 200ppm) are achieved lightest values in egg production percentage. During second period (46-50 week), T1(control) , T2 (local probiotic 5 gm/kg) ,T3 (local probiotic 10 gm/kg), T4 (local probiotic 15 gm/kg) which there are no significant difference between them achieved highest values in egg production percentage while the treatment T6 (cupric sulphate 200ppm) record lightest values in egg production percentage. During the third and fourth periods the treatments including supplementation local probiotic keep highest values in egg production when treatments including supplementation cupric sulphate at levels 200ppm and 300ppm significant decreased ($P<0.05$) in egg production percentage .These results agree with the finding of (29) who observed adding probiotic to the control diet at levels 0.25% and 0.5% improved the performance of laying hens which include egg production. Also the results of our study agree with the finding of (30) who observed adding of local probiotic to the control diet at level 5kg/ton improved egg production percentage.(31) reported that probiotics improved digestion, absorption and availability of nutrition accompanying with a positive effect on intestine activity and increasing digestive enzymes. These data are against the results of (32) and (33) they found that there is no difference between control group and laying hens fed with *lactobacillus* in egg production, but on the other hand (34) achieved the same results as we did, he reported the positive effect of probiotic on laying hens performance and egg production (H.D.%). Actually many researchers found that egg production was significantly improved by adding copper, (17) reported that layer hens that have been given diets supplemented with 0, 125 and 250 mg/kg cupric sulphate for 28 day revealed 86.8, 89.6 and 91.8% hen day (H.D%) egg production values and the only statistically significant difference was observed between 250 mg/kg cupric sulphate and control groups ($P<0.05$),whereas(16) showed a significant reduction in egg production. (35) also showed that chromium or copper supplementation did not influence in egg production.

Table (3) .Effect of adding locally probiotic and Cupric Sulphate to Laying Hens Diets on Egg production%

Treatments	42-46 week Period 1	46-50 week Period2	50-54 week Period 3	54-58 week Period4	Accumulative 42-58week
T1 Control	79.91±3.04 B	80.55±1.97 Ab	80.38±1.99 Ab	78.49±2.26 B	79.83±1.37 ab
T2 Local probiotic 5gm/kg	80.05±3.17 Ab	82.87±0.56 A	81.63±1.00 A	80.30±1.26 Ab	81.21±0.88 a
T3 Local probiotic 10gm/kg	81.72±1.58 A	80.65±3.97 Ab	82.54±1.07 A	81.73±0.92 A	81.66±0.13 a
T4 Local probiotic 15gm/kg	79.80±1.63 B	80.99±2.59 Ab	80.74±2.28 Ab	79.11±1.98 B	80.16±1.54 a
T5 Cupric sulphate 100ppm	77.29±2.89 c	79.98±1.10 B	80.53±0.49 Ab	80.71±2.44 A	79.63±0.89 ab
T6 Cupric sulphate 200ppm	78.10±3.63 c	76.72±2.93 C	77.56±1.64 B	78.31±2.11 B	77.67±0.86 b
T7 Cupric sulphate 300ppm	79.92±1.62 b	78.44±1.52 B	76.12±0.85 B	79.52±2.47 Ab	78.50±0.85 b

*The different letters within the same columns are significant differences at ($P<0.05$).

Table (4) shows the effect of adding locally prepared probiotic and cupric sulphate on egg weight. Data indicated that supplementation of local probiotic 10 ,15 gm/kg ration and cupric sulphate (300ppm**)** to laying hens diets increased significant egg weight compared with control. According to data from table (4), No significant difference ($P<0.05$) in accumulative egg weight between control treatment and T2 (local probiotic 5gm/kg ration) ,T5 (cupric sulphate 100ppm) and T6 (cupric sulphate 200ppm) which recorded lowest values in egg weight. In agreement with the present study,(30) reported that there were significantly increased ($P<0.05$) in egg weight as compared with that of control treatment. Also (29) observed that there were significantly increased ($P<0.05$) in egg weight for laying hens fed diets containing probiotic (Biomin Imbo) at levels 0.25% and 0.5%. Egg weight did not show any statistically significant variation between treatments containing cupric sulphate and control diet at 42-58 week, However, (36) have also reported that egg weight significantly increased in birds given a diet containing 200 mg/kg copper. At the end of the experiment, egg weight increased insignificantly, when the diets was supplemented with cupric sulphate ($P>0.05$).

Table (4). Effect of adding locally probiotic and Cupric Sulphate to Laying Hens Diets on Egg weight (gm).

Treatments	42-46 week Period 1	46-50 week Period2	50-54 week Period 3	54-58 week Period4	Accumulative 42-58week
T1 Control	58.44±1.14 b	59.06±1.07 b	59.39±0.61 b	60.93±0.42 B	59.46±0.41 b
T2 Local probiotic 5gm/kg	57.40±1.03 c	59.92±1.05 ab	60.07±0.42 ab	61.59±1.36 Ab	59.75±0.13 b
T3 Local probiotic 10gm/kg	58.45±0.66 b	61.01±2.06 a	60.39±0.33 a	62.89±0.34 A	60.69±0.14 a
T4 Local probiotic 15gm/kg	59.72±1.64 a	58.47±1.22 b	61.16±0.39 a	62.87±1.48 A	60.56±0.24 a
T5 Cupric sulphate 100ppm	58.89±0.82 ab	59.82±0.27 b	59.89±0.58 b	59.77±0.45 B	59.59±0.45 b
T6 Cupric sulphate 200ppm	57.99±0.37 b	60.59±0.37 a	60.85±0.45 a	59.86±1.42 B	59.82±0.13 b
T7 Cupric sulphate 300ppm	59.78±0.92 a	59.96±0.26 ab	60.21±0.41 ab	60.33±0.55 B	60.07±0.18 a

*The different letters within the same columns are significant differences at ($P<0.05$).

Data presented in table (5) showed that there is significant difference ($P<0.05$) among the treatments in egg mass. The data in Table 5 shows that layer hens fed basal diet added local probiotic 10 gm/kg ration (T3) had the highest average of egg mass at the end of this study followed by T2 (local probiotic 5 gm/kg ration) and T4 (local probiotic 15 gm/kg ration) which increased insignificantly as compare with control and T5 (cupric sulphate 100ppm) , T6 (cupric sulphate 200ppm) and T7(cupric sulphate 300ppm). The results of our study agree with the finding of (37) who reported that probiotic have useful microbial activity of digestive system which lead to better performance for laying hens. In agreement with the current study,(38) reported that egg mass didn't affect ($p>0.05$) by adding cupric sulphate to the basal diet during 6 weeks of experiment .

Table (5). Effect of adding locally probiotic and Cupric Sulphate to Laying Hens Diets on Egg mass (gm).

Treatments	42-46 week Period 1	46-50 week Period2	50-54 week Period 3	54-58 week Period4	Accumulative 42-58week
T1 Control	46.70±1.16 b	47.57±1.56 b	47.74±1.22 B	47.82±1.54 C	47.46±6.31 c
T2 Local probiotic 5gm/kg	45.95±5.83 b	49.66±6.52 a	49.01±4.34 A	49.46±2.06 B	48.52±8.02 b
T3 Local probiotic 10gm/kg	47.78±1.03 a	49.20±2.50 A	49.85±1.20 a	51.40±1.17 A	49.56±2.71 a
T4 Local probiotic 15gm/kg	47.66±1.26 a	47.35±1.19 B	49.38±1.44 a	49.74±1.38 B	48.53±2.84 b
T5 Cupric sulphate 100ppm	45.52±5.58 c	47.84±6.28 B	48.23±3.99 b	48.24±2.79 C	47.46±7.22 c
T6 Cupric sulphate 200ppm	45.29±1.36 c	46.48±3.38 C	47.20±1.43 c	46.88±2.24 D	46.46±2.20 d
T7 Cupric sulphate 300ppm	47.78±3.82 a	47.03±2.65 B	45.83±2.04 d	47.97±1.52 Cd	47.15±5.56 c

*The different letters within the same columns are significant differences at ($P<0.05$).

Food intake for all the periods of the study are shown in Table (6). Supplementary dietary local probiotic and cupric sulphate had no statistically significant effects on this parameter. The results of another report (30) feeding local probiotic in laying hens not in agree of these results obtained. On the other hand,(29) reported that there were no statistical differences among the groups in food intake with diets containing 0.25 % and 0.5 % probiotic (Biomin Imbo) and these results were similar to the present findings. The researchers (34),(37) reported similar results to the results of this study in laying hens . (39) reported that laying hens fed standard feed enriched by copper and iron reduced daily consumption of feed from 122 to 120g. The results shown in table (6) may be due to (40) who found no statistically significant effects of supplementary copper on food intake.

Table (6). Effect of adding locally prepared probiotic and Cupric Sulphate to Laying Hens Diets on feed intake (gm/hen/day)

Treatments	42-46 week Period 1	46-50 week Period2	50-54 week Period 3	54-58 week Period4	Accumulative 42-58week
T1 Control	99.08±0.27	102.98±0.55	110.83±0.93	113.27±0.37	106.54±1.81
T2 Local probiotic 5gm/kg	98.11±1.05	100.89±0.51	101.83±1.88	114.43±0.23	103.82±1.79
T3 Local probiotic 10gm/kg	96.97±0.80	106.57±0.29	112.94±0.47	116.75±0.42	108.31±1.71
T4 Local probiotic 15gm/kg	97.96±1.09	105.73±1.02	109.97±2.13	113.82±0.59	106.87±1.59
T5 Cupric sulphate 100ppm	96.31±1.23	102.47±0.85	114.03±0.81	118.19±0.32	107.75±1.88
T6 Cupric sulphate 200ppm	94.35±0.63	107.81±0.46	113.17±0.85	117.28±0.83	108.15±1.47
T7 Cupric sulphate 300ppm	98.68±1.25	106.29±0.73	111.21±0.49	114.96±0.46	107.79±1.69

*The different letters within the same columns are significant differences at ($P<0.05$).

As shown in table (7) the data showed that there were significant effect of adding locally probiotic and cupric sulphate to laying hens diets on feed conversion ratio . The data in Tables (7) shows that adding local probiotic at levels 10 gm/kg ration and 15 gm/kg ration and the supplementation of cupric sulphate at levels 200ppm and 300ppm were significantly improved ($p<0.05$) through first period (42-46week) of age ,while T2 (adding 5 gm/kg ration of local probiotic) had the best average of feed conversion ratio (gm feed/gm egg) through second period (46-50week) of age. The results show that T2,T3 and T4 improved in averages of feed conversion ratio and T2 (adding 5 gm/kg ration of local probiotic) recorded the better value of averages through third period (50-54week) of age . At the last period (54-58week) of age T3(adding 10% local probiotic) and T4(added 15 gm/kg ration of local probiotic) have significantly improved ($p<0.05$) in feed conversion ratio as compared with all treatments. The data in Table 7 shows that laying hens fed diets supplemented with local probiotic 5, 10 and 15 gm/kg ration, have the better values in accumulative average (42-58week) in feed conversion ratio (gm feed/gm egg) and

T2 (local probiotic 5 gm/kg ration) had the lowest average as a compare with all treatments. The results are agreement with (30) who report that increase significant in feed conversion ratio in laying hens diets supplementation with local probiotic at level 5 kg/ton as compare with control , Also these results with agreement the findings of (29),(41)and (42) that laying hen fed diets added probiotic have best values of feed conversion ratio as compared with control groups. Improvement in feed conversion ratio could be due to ability of *lactobacilli* in probiotic to produce lactic acid which reduce gastrointestinal PH that increase nutrient absorption from intestine, also *lactobacilli* and *bifidobacterium* in probiotic can produce lactic acid that kill harmful bacteria (41). Bacteria could produce catalytic enzyme like phytase which increase phosphor availability that lead to positive effect on nutrient availability (43).

Table (7). Effect of adding locally probiotic and Cupric Sulphate to Laying Hens Diets on feed conversion ratio (gm feed/gm egg)

Treatments	42-46 week Period 1	46-50 week Period2	50-54 week Period 3	54-58 week Period4	Accumulative 42-58week
T1 Control	2.12±0.61 a	2.16±0.36 b	2.32±0.86 b	2.37±0.47 C	2.24±0.52 ab
T2 Local probiotic 5gm/kg	2.14±0.29 a	2.03±0.25 c	2.08±0.39 d	2.31±0.24 Cd	2.14±0.40 c
T3 Local probiotic 10gm/kg	2.03±0.32 b	2.17±0.39 b	2.27±0.28 c	2.27±0.30 D	2.19±0.37 b
T4 Local probiotic 15gm/kg	2.05±0.28 b	2.23±0.48 b	2.23±0.34 c	2.29±0.21 D	2.20±0.29 b
T5 Cupric sulphate 100ppm	2.12±0.83 a	2.14±0.59 b	2.36±0.68 b	2.45±0.96 A	2.27±0.78 a
T6 Cupric sulphate 200ppm	2.08±0.75 b	2.32±0.79 a	2.40±0.71 a	2.50±0.55 A	2.33±0.81 a
T7 Cupric sulphate 300ppm	2.07±0.38 b	2.26±0.37 ab	2.43±0.43 a	2.40±0.32 B	2.29±0.28 a

*The different letters within the same columns are significant differences at ($P<0.05$).

Results presented in Table (8), shows the effects of adding local probiotic and cupric sulphate to laying hens diets on total glucose , total protein ,total albumin and total globulin, the results indicated that there were no significant differences among treatments during the experimental periods, These results agreed with (44) who reported that broiler breeders diets containing 10 and 20 gm of local probiotic per

kilogram of ration for 4 weeks don't effect on glucose concentration as compared with control treatment at ($p<0.05$) (45),(46) and (47).

Table (8). Effect of Adding Locally Probiotic and Cupric Sulphate to Laying Hens Diets on Serum Biochemical Parameters.

Treatments	Glucose (mg/100ml)	Total Protein (gm/100ml)	Total Albumin (gm/100ml)	Total Globulin (gm/100ml)
T1 Control	216.02±0.02	6.42±0.03	3.52±0.03	2.90±0.017
T2 Local probiotic 5gm/kg	216.12±0.03	6.72±0.01	3.54±0.06	3.18±0.026
T3 Local probiotic 10gm/kg	215.95±0.01	6.19±0.04	3.37±0.27	2.82±0.061
T4 Local probiotic 15gm/kg	216.03±0.05	6.52±0.01	3.68±0.25	2.84±0.046
T5 Cupric sulphate 100ppm	216.16±0.02	7.09±0.05	3.80±0.25	3.29±0.014
T6 Cupric sulphate 200ppm	215.98±0.02	6.95±0.07	4.39±0.14	2.56±0.021
T7 Cupric sulphate 300ppm	216.17±0.01	6.66±0.01	4.35±0.18	2.31±0.024

*The different letters within the same columns are significant differences at ($P<0.05$).

The results of lipid parameters studied in treatments additives and control are presented in table (9). Among biochemical parameters, significant differences ($P<0.05$) were observed in triglycerides, cholesterol, High density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL). Triglycerides values were lower in birds of all treatments as compared to the values by the chickens kept on basal diet, and T4 (local probiotic 15 gm/kg ration) recorded the lowest value. As show data in table (9) the addition treatments decreased significant ($P<0.05$) in triglycerides levels while T1 (control) had highest level in triglycerides values. The table reveals that serum cholesterol concentration was reduced by local probiotic and cupric sulphate supplementation. Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) were reduced while high density lipoprotein (HDL) was increased by dietary probiotic and cupric sulphate, T1 (control) had the highest values in low density lipoprotein (LDL) and Very low

density lipoprotein (VLDL) and had lowest values in high density lipoprotein (HDL). The beneficial effects of probiotics in lowering cholesterol in serum have been reported (48) and(49) who reported rats fed diets containing probiotic 0.2% for 4 weeks decreased triglycerides in the liver, the results agreed with (29) who reported that triglycerides, cholesterol and low density lipoprotein(LDL) decrease in laying hens fed diets containing probiotic at 0.25% and 0.5% . 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 (50). The decrease in cholesterol may be refer to 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 transaminases (51). The present study showed that copper supplementation reduced serum cholesterol as in the reports of (16) and(17). Studies with broilers have also shown a similar effect of copper reducing serum cholesterol concentrations (10) and (52). This reduction occurred because copper decreased hepatic glutathione formation (10) and (53). Glutathione is known to regulate cholesterol biosynthesis through the stimulation of 3-hydroxy methyl glutaryl coenzyme A (HMG-CoA) reductase, the key enzyme of cholesterol biosynthesis (51). Lipid metabolism may also be affected by added copper. The change of lipid metabolism in the present study was observed to decrease serum cholesterol and very low density lipoprotein(VLDL), and tended to reduce triglycerides.(14) demonstrated that copper supplementation decreased plasma lipid, 17-estradiol and hepatic lipogenic enzyme activity. Moreover, (51) also indicated that the addition of copper decreased fatty acid synthesis activity. Estradiol can stimulated lipid synthesis, thus decreased estradiol concentrations decrease triglycerides synthesis and the major triglycerides transport very low density lipoprotein (VLDL) decreased as well, while high density lipoprotein (HDL) concentration increased.

Table (9).Effect of Adding Locally Probiotic and Cupric Sulphate to Laying Hens Diets on Lipid profile.

Treatments	Triglycerides (mg/100ml)	Cholesterol (mg/100ml)	High density lipoprotein (HDL) (mg/100ml)	Low density lipoprotein (LDL) (mg/100ml)	Very low density lipoprotein (VLDL) (mg/100ml)
T1 Control	265.62±2.97 a	179.00±2.74 A	27.22±1.69 C	98.66±0.64 a	53.12±1.21 a
T2 Local probiotic 5gm/kg	243.87±5.04 b	165.57±2.21 B	29.48±1.86 Bc	87.32±0.76 b	48.77±0.59 b
T3 Local probiotic 10gm/kg	221.12±4.56 d	159.35±3.33 bc	30.35±1.93 B	84.78±0.43 bc	44.22±0.61 d
T4 Local probiotic 15gm/kg	197.63±5.72 f	148.25±1.59 d	35.50±1.61 A	73.22±0.94 d	39.53±1.17 f
T5 Cupric sulphate 100ppm	234.75±6.61 c	161.12±1.18 b	33.24±2.37 Ab	80.93±0.53 c	46.95±0.88 c
T6 Cupric sulphate 200ppm	232.16±2.80 c	154.33±2.75 c	34.60±2.29 A	73.30±0.63 d	46.43±0.55 c
T7 Cupric sulphate 300ppm	213.00±3.01 e	144.65±3.39 d	36.85±2.20 A	65.20±0.79 e	42.60±1.53 e

*The different letters within the same columns are significant differences at ($P<0.05$).

Our results shows in table (10). There are significant ($P<0.05$) decrease in egg yolk cholesterol in treatments additives as compared to (T1). Present study showed that the cholesterol concentration per gram of egg yolk in laying hens fed diets containing 5 , 10 and 15 gm/kg ration of local probiotic and diets containing 100ppm,200ppm and 300ppm cupric sulphate were lower than those fed control diet (T1). Also (40) reported that cholesterol concentrations per gram of yolk decreased linearly with increasing levels of probiotic. (17) reported that after 8 weeks of copper supplementation in two experiments, egg yolk cholesterol levels were reduced by 20

and 28% in first experiment, and by 30 and 35%, in second experiment from 125 and 250 mg/kg copper, respectively, he reported that contraction may have been related to the differences in age or strain of the hens, or differences in the percentage of copper in cupric sulphate that was used in the present study. Our results in agreement with (40) who reported that the use of supplementary copper to provide 150 mg/kg in poultry diets decreased yolk cholesterol concentrations without any adverse effect on production performance. Similarly, laying hens given diets supplemented with 0, 125 and 250 mg/kg copper for 28 day revealed yolk cholesterol concentrations of 11.6, 9.0 and 8.0 mg/g respectively (17). Supplemental local probiotic and cupric sulphate decreased linearly yolk cholesterol concentration without having significant effect on laying hens performance. It was concluded that local probiotic and cupric sulphate supplement at higher level can be used as hypocholesterolemic materials in laying hens diets without having significant effects on layer performances .

Table (10). Effect of Adding Locally Probiotic and Cupric Sulphate to Laying Hens Diets on Egg yolk cholesterol (mg/gm yolk)

Treatments	Egg yolk cholesterol (mg/gm yolk)
T1 Control	12.39±0.07 a
T2 Local probiotic 5gm/kg	11.29±0.18 b
T3 Local probiotic 10gm/kg	11.15±0.15 b
T4 Local probiotic 15gm/kg	11.17±0.14 B
T5 Cupric sulphate 100ppm	11.12±0.08 B
T6 Cupric sulphate 200ppm	10.82±0.12 C
T7 Cupric sulphate 300ppm	10.77±0.32 c

*The different letters within the same columns are significant differences at ($P<0.05$).

Looking at the results in Table (11) ,The serum GOT and GPT enzymes are good indicator of liver and activity, whether the increase or decrease of serum GOT and GPT levels are associated with the possible negative effects by dietary local probiotic or cupric sulphate (54). The data shows that the average concentration of the enzymes GOT and GPT and liver weight were not significantly affected by the

diet supplemented with different levels of local probiotic and cupric sulphate. These results agree with founding obtained by (29) who observe that the concentration of serum GOT and GPT enzymes did not affected by addition probiotic (Biomin Imbo) in laying hens diets at 0.25% and 0.5%.

Table (11).Effect of Adding Locally Probiotic and Cupric Sulphate to Laying Hens Diets on liver weight and plasma enzymes concentration.

Treatments	Liver weight (gm)	GOT (U/L)	GPT(U/L)
T1 Control	2.68±0.69	151.00±0.10	18.08±0.05
T2 Local probiotic 5gm/kg	2.58±0.35	151.10±0.20	18.07±0.05
T3 Local probiotic 10gm/kg	2.75±0.18	151.04±0.17	18.07±0.05
T4 Local probiotic 15gm/kg	2.61±0.76	151.06±0.57	18.09±0.05
T5 Cupric sulphate 100ppm	2.65±0.36	150.00±0.14	18.06±0.05
T6 Cupric sulphate 200ppm	2.64±0.37	149.88±0.73	18.04±0.05
T7 Cupric sulphate 300ppm	2.66±0.23	149.86±0.20	18.05±0.05

*The different letters within the same columns are significant differences at ($P<0.05$).

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