

Effect of adding different levels of Lycopene to the diet on some physiological traits for Japanese quail bird exposed to thermal stress

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ABSTRACT

This study was conducted in the field of poultry birds belonging to the Department of Livestock, College of Agriculture, Al-Qasim Green University for the period from 17/10/2014 to 29/11/2014, and then followed by laboratory work. This research aims to study the best concentrations of Lycopene added with diet in reducing the thermal stress exposed to quail birds in the experiment. In the experiment, a 300 unsexed quail chicks were used, the chicks were raised on ground mattresses, with age of (1-8) and then transferred to pens. Lycopene was added to the diet from the age of 8 days to the age of 42 days. Chicks were randomly distributed into 5 treatments (T1, T2, T3, T4, T5) which represent the addition of lycopene to diets at levels of (0, 150, 200, 250, 300 mg lycopene/kg feed), respectively. The results of the experiment indicated to obtain a significant increase in the H / L ratio for the birds of the T1 treatments compared to the treatments (T2, T3, T4, T5) while no significant differences were recorded between the two treatments (T4, T5) in the same trait. As for the percentage of Packed Cell Volume (PCV) and Haemoglobin (Hb), a significant increase ($P < 0.01$) was observed in the percentage of PCV and Hb for the T3 treatment compared to the control treatment, As for the trait of the concentration of glucose and the concentration of triglycerides (g / 100 ml serum) in the serum of birds, it was noted the continued superiority of the T4 treatment on all the treatments of the experiment. while the T5 treatment recorded highly significant excelling ($P < 0.01$) in the average concentration of cholesterol on the rest of the treatments. As for the percentage of total protein, a significant ($P < 0.05$) excelling was observed for the T5 treatment on the rest of the treatments. As for the concentration of globulin, it was observed that there was a significant excelling ($P < 0.05$) for the T5 treatment on the treatments of the experiment. We conclude from this study that adding lycopene to quail diets works on increasing the percentage of the physical traits for the blood and the percentage for some blood biochemical traits.

*Research paper from MSc thesis for the first author.

تأثير أضافة مستويات مختلفة من الليكوبين (Lycopene) الى العليقة على بعض الصفات الفسلجية لطائر السمان ألياباني المعرض للإجهاد الحراري

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الملخص

أجريت هذه الدراسة في حقل الطيور الداجنة التابع لقسم الثروة الحيوانية ، كلية الزراعة / جامعة القاسم الخضراء. للمدة من 17 / 10 / 2014 ولغاية 29 / 11 / 2014، وتم تبعا العمل المختبري. ويهدف البحث الى دراسة أفضل التراكيز من الليكوبين المضاف مع العليقة في تخفيف الأجهاد الحراري المعرضة لة طيور السمان في التجربة. استخدم في التجربة 300 فرخ طائر سمان غير مجنس ربيت الافراخ على فرشة أرضية من عمر 1 – 8 يوم بعدها نقلت الى بطاريات ، وتم اضافة الليكوبين الى العليقة من عمر 8يوم الى عمر 42 يوم ، وقد وزعت الافراخ عشوائيا الى 5 معاملات T1 ، T2 ، T3 ، T4 ، T5 التي تمثل إضافة الليكوبين الى العلائق بمستويات 0 ، 150 ، 200 ، 250 ، 300 ملغم الليكوبين / كغم علف على التوالي. وأشارت نتائج التجربة الى حصول ارتفاع معنوي في نسبة H/L لطيور المعاملتين T1 مقارنة مع المعاملات T2 ، T3 ، T4 و T5 في حين لم تسجل أي فروق معنوية بين المعاملتين T4 و T5 في الصفة نفسه ، أما فيما يخص نسبة P.C.V و Hb ارتفاع معنوي ($P<0.01$) في نسبة P.C.V و Hb لمعامله T3 مقارنة بمعاملة السيطرة ، أما فيما يخص صفة تركيز الكلوكون وتركيز الكليسيريدات الثلاثية (غم / 100 مل مصل الدم) في مصل دم الطيور فنلاحظ استمرار تفوق المعاملة T4 على معاملات التجربة جميعها ، فيما سجلت المعاملة T5 تفوقاً عالياً المعنوية ($P<0.01$) في معدل تركيز الكوليسترول على بقية معاملات التجربة. فيما يخص نسبة البروتين الكلي تبين تفوق معنوي ($P<0.05$) للمعاملة T5 على بقية معاملات التجربة ، أما بالنسبة تركيز الكلوبيولين فنلاحظ حصول تفوق معنوي ($P<0.05$) للمعاملة T5 على معاملات التجربة. ونستنتج من هذه الدراسة ان اضافة الليكوبين الى علائق السمان يعمل على زيادة في النسبة المئوية لصفات الفيزيولوجية للدم والنسبة المئوية لبعض صفات الكيمياء الحيوية للدم.

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1. INTRODUCTION

Poultry has become an important economic sector that meets the worlds from meats and egg requirements. Therefore, there was a great interest by countries in urging researchers to develop this industry and work continuously to overcome production constraints and move towards commercial inbreds characterized by rapid growth and excellent feed conversion ratio and good meat quality characterized by

low-fat content (Al-Shammari, 2009). As for the economic importance of the Japanese quail (*Coturnix coturnix Japonica*), it comes from the fact that it is a dual-purpose bird and it is ranked third order after chickens and ducks, even outperform them in the economic return (Rogerio, 2009). Quail is a bird with a small body, spotty brown color, and Fast-moving that is able to fly at a low level and for long distances belonging to the Phasianidae family (Abul-Ela, 2005). These poultry birds have

been domesticated which breeding according to intensive modern breeding systems. International companies specialized in breeding and improvement have indicated to develop strains of quail abundant in their production from eggs to provide table eggs, including commercial strains known as White English quail (Attia, 2006). Among these materials used in the breeding of poultry is: Lycopene is a red carotene pigment that gives products rich with it a distinct red color, it is antioxidant material and highly effective because it helps the body fight free radicals that damage cells and cause infection with a disease (Wu et al. 2004). It is effective for its antioxidant activity, which amounts twice as active as beta-carotene, ten times as much tocopherol and it has a significant role in lowering blood lipids (Rao and Ali, 2007). Lycopene has potent as an antioxidant activity against free radicals (Suha et al., 2009). Lycopene's ability as antioxidant stems from increasing the number of reciprocal conjugated Hydrocarbons bonds, therefore it is an effective antioxidant against free radicals compared to carotenoids (Rao and Ali, 2007: Pratik and Vishal, 2007). This study aims to determine (the best concentrations of lycopene used with the diet in the relief of thermal stress exposed to birds of the experiment).

2. MATERIALS AND METHODS

This study was conducted in the field of Poultry birds belonging to the Department of Livestock, College of Agriculture, Al-Qasim Green University for the period from 17/10/2014 to 29/11/2014. In the experiment, 300 chicks (with one day age) were used which distributed over five treatments, each treatment contained 60 chicks, with three replicates, 20 chicks for each replicate. The birds were reared in locally manufactured pens, consisting of five floors with dimensions of ($1 \times 1 \text{ m}^2$), Each floor is equipped with plastic waters and a special plastic feeder. The birds were randomly distributed into five treatments:

The first treatment: Chicks were fed on a diet without the addition of lycopene and considered a control treatment, The second treatment: Chicks were fed on a diet added to it lycopene at a concentration of (150 mg/kg feed), The third treatment: Chicks were fed on a diet added to it lycopene at a concentration of (200 mg/kg feed), the fourth treatment: Chicks were fed on a diet added to it lycopene at a concentration of (250 mg/kg feed), and The fifth treatment: Chicks were fed on a diet added to it lycopene at a concentration of (300 mg/kg feed).

The temperature inside the room was measured by three thermometers distributed at the beginning and middle of the hall, The daily temperature was recorded three times at 8 am, 2 pm and 8 pm, The birds were exposed to

periodic temperatures (25 - 73 - 25 °C) as shown in Table (2).

shown in Table (1). Chicks were fed on one diet

Table 1: Average temperature (°C) recorded inside the hall during the breeding period from the age of 8 days to 49 days.

Week	Time		
	8:00 am	2:00 pm	8:00 pm
2	29.42	35.33	32.33
3	29.23	35.37	31.71
4	29.99	36.80	30.09
5	27.80	34.28	31.66
6	26.04	34.99	29.71
7	25.33	35.85	28.33

Table 2: Components and ratios of feed materials used in the quail diet with the calculated chemical composition for this diet.

Components	Percentage (%)
yellow corn	40
wheat	20
Soybeans meal (1)	31.8
Concentrated Protein (2)	5
Vegetable oil	2
Limestone	0.7
Dicalcium phosphate	0.2
Food salt	0.3
Total	100
The Calculated chemical composition	
Metabolic Energy (kcal / kg)	2954.19
Crude protein%	22.05%
Methionine%	0.474
Choline (mg / kg)	499.08
Cysteine%	0.3459
Glycine %	0.8343
Lysine%	1.1839
Calcium%	0.6772
Phosphorus%	0.3478
C / P ratio	133.97

* The chemical composition for the feeds materials included in the diet was calculated according to the recommendations (NRC 1994).

(1) The soybean meal used from an Argentina source: The percentage of crude protein 44% and 2230 kcal/kg metabolic energy.

(2) The used Concentrated protein is an Animal protein produced from Belgian company (Intraco) contains 40% crude protein, 2100 kcal / kg metabolic energy, 3.5% crude fat, 1% Raw fiber, 6% calcium, 7.5% phosphorus, 3.25% Lysine, 3.50% methionine, 3.90% methionine + cysteine. It contains a mixture of vitamins and rare minerals that meet the requirements of birds from these elements.

The studied traits:**Percentage of some physiological traits for the bird at the age of 42 days:**

Blood samples were collected at the age of 42 days from the experiment (6 weeks), where Blood was collected from 30 birds (2 from each replicate) for each treatment randomly. Blood collected from the brachial vein and placed in tubes containing anticoagulant (potassium EDTA) to prevent blood clotting, The following traits were measured:

Physical blood tests:**1- Measuring the Packed Cell Volume (PCV):**

In this test, A micro-capillary tube with open ends was used in this examination containing on the anticoagulant (Heparin), where samples were collected directly from the birds by tingling the bird with a needle in the wing area and at the flow of blood and submerge the end of the capillary tube and horizontally to help the flow of blood by capillary property until two-thirds of the length of the tube filled with blood and then close the tube immediately after collecting blood from the same end from which it was collected using a special paste (artificial clay can be used). The tubes were then placed horizontally in a special micro-hematocrit centrifuge for 15 min and the percentage of the Packed Cell Volume was then measured using a

special ruler according to the method indicated by (Archer, 1965).

2- Measuring the percentage of Heterophils / Lymphocytes:

Blood swabs were made on glass slices by placing a drop of blood from the capillary tube used in measuring the percentage of the Packed Cell Volume on the edge of a glass slide. Another glass slide was placed on the blood drop and pulled in one direction at a 45. The slide was left for 15 min in order to dry the blood. The slices were then dyed with a combination of two dyes (Wright-Giemsa) (Shen and Patterson, 1983).

The counting was conducted using an optical microscope and at a magnification of $100 \times$ by placing a drop of oil on the slide according to the method (Burton and Guion, 1968).

3- Hemoglobin (Hb):

The concentration of Blood hemoglobin was directly calculated by inferring the Packed Cell Volume, using the law mentioned (Campbell, 1995):

$$\text{The concentration of Hemoglobin} = \frac{\text{Packed Cell Volume}}{3}$$

Biochemical analyses for blood:

Total serum protein, albumin, globulins, cholesterol, glucose, and triglycerides were

measured in the blood serum at the end of the experiment at the age of 42 days, where blood samples were taken from birds immediately after slaughtering, which collected in cylindrical tubes does not contain anticoagulant. The serum was then separated from the blood using a centrifuge at a speed of (3000 rpm) for 15 min, The above traits were then measured by (Mindray Bs - 120) device. As for the concentration of globulin was calculated by the following equation:

The of concentration Globulin (g / L) = total protein concentration (g / L) - albumin concentration (g / L)

Statistical analysis:

The Statistical Analysis System program (SAS, 2012) was used to analyze the data to study the effect of different treatments on the studied traits according to a completely randomized design (CRD) and according to the mathematical model below., the significant differences between the averages were compared by Duncan's New Multiple tests (Duncan, 1955).

$$Y_{ij} = \mu + T_i + e_{ij}$$

where:

Y_{ij} = viewing value (j) for treatment (i)

μ = general mean for the studied trait

T_i = effect of treatment i

e_{ij} = Random error assumed to be a normal and independent distribution with a mean of 0 and a variation of σ^2_e .

3. RESULTS AND DISCUSSION

Table (3) shows the effect of adding different levels of lycopene to the diet on the ratio of H / L cells, the Packed Cell Volume (PCV) and the concentration of hemoglobin (Hb) for the Japanese quail bird exposed to thermal stress. where the results indicated a significant increase ($p < 0.01$) in the H / L ratio for the birds of the T1 treatments compared to the treatments (T2, T3, T4, T5) while no significant differences recorded between treatments (T4, T5) in the same trait, the T2 treatment has excelled on the two treatments (T3, T5), the T3 treatment has excelled on the T5 treatment in the same trait. As for the percentage of PCV, it is noticeable from the same table that there are significant differences ($P < 0.01$) between the treatments of the experiment in the percentage of PCV, where the T3 treatment recorded the highest percentage amounted to (47.00%) while the T4 treatment gave the lowest percentage amounted to (37.61%) while there were no significant differences between the treatments (T1, T2, T5) in the same trait which were significantly excelled ($P < 0.01$) on the T4 treatment. As for the concentration of Hb, the high significant ($P < 0.01$) excelling for the T3 treatment on the treatments (T1, T2, T4, T5),

and then the treatments (T1, T2, T5) have excelled and without significant differences over the T4 treatment for the same trait. There were no significant differences in the Hb trait for the blood of the birds' treatments (T1, T2, T5). Zulkifili et al., (2003) and Turkyilmaz, (2008) showed that the effect of thermal stress on the increase of H / L ratio, This confirmed the possibility of using this ratio as evidence of the occurrence of environmental stress on the bird, where the significant decrease in the H / L ratio for the birds of lycopene treatments is clear evidence of the role of lycopene in reducing the body temperature on the role of lycopene in reducing the body temperature for the birds compared to the hall temperature, thus reduce the thermal stress on the bird had a key role in raising blood values PCV and Hb (Sturki, 1986). Masaaki et al., (2005) reported a significant decrease in PCV and Hb values with the increase in the temperature of the environment and the exposure of birds to thermal stress, The researchers indicated to explanations for lowering these values, including increased drinking water and blood diluting, which lead to decrease blood concentrations (Al-Shukri, 2001). The percentage of PCV cells represents all red and white blood cells (Sturki, 1986), and the increase in the number of red and white blood cells when adding lycopene as a result of preventing the decomposition of blood cells by

antioxidant action, and the Protection of plasma membranes from oxidative stress damage (Surai et al., 2003). It may improve the percentage of PCV. The reason is that the consumption of foods rich in carotenoids, In particular, lycopene is associated with many health benefits because of its ability to protect tissues and cells and protect them from oxidative stress (Giuseppe et al., 2007; Feeney, 2004; Al-Nadawi, 2014). The addition of lycopene may help reduce levels of active free radicals in the body such as hydrogen peroxide H_2O_2 and nitrogen peroxide N_2O_2 by increasing the number of unsaturated bilateral hydrocarbon bonds that have a significant role in the oxidative damage for different body cells (Rao and Agarwal, 1998; Tapiero et al., 2004; Pratik and Vishal, 2007). The results of the (Giuseppe et al., 2007) study proved that Lycopene as an antioxidant is excelled on beta-carotene in protecting white blood cells, particularly lymphocytes, from the risk of free radicals, especially the radicals above nitrogen oxide, which causes cell destruction and membranes. Therefore, the addition of lycopene has contributed to enhancing the activity and vitality of the white blood cells and reducing the damage that may occur to them due to the presence of free radicals and that reducing the H / L ratio give a good impression on the health of birds. Al-Darraji, (1995) stated that the H / L ratio is the best measuring to detect the general

bird condition and the level of stress it is exposed to. The raise of this percentage on the general average indicates severe exposure to birds.

Table 3: Effect of Adding Different Levels of Lycopene to Diet on Some Physical Blood traits for Japanese Quail Birds Exposed to thermal Stress at Age (42) Days.

Treatments	(Average \pm standard error)		
	Hemoglobin Hb (g / 100 ml blood)	Packed Cell Volume (PCV) %	Percentage of heterophils/ lymphocytes (H / L) %
T1	a 0.00 ± 0.74	b 0.86 ± 42.50	b 0.29 ± 14.16
T2	b 0.02 ± 0.48	b 0.33 ± 41.66	b 0.11 ± 13.88
T3	c 0.02 ± 0.43	a 1.15 ± 47.00	a 0.38 ± 15.66
T4	d 0.00 ± 0.39	c 1.45 ± 37.61	c 0.49 ± 12.55
T5	d 0.00 ± 0.37	b 0.00 ± 41.00	b 0.00 ± 13.66
Significant level	**	**	**

Different characters within one column indicate significant differences.

** At ($p < 0.01$), treatments: T1 = Chicks were fed on a diet without the addition of lycopene and considered a control treatment, T2: Chicks were fed on a diet added to it lycopene at a concentration of (150 mg/kg feed), T3: Chicks were fed on a diet added to it lycopene at a concentration of (200 mg/kg feed), T4: Chicks were fed on a diet added to it lycopene at a concentration of (250 mg/kg feed), and T5: Chicks were fed on a diet added to it lycopene at a concentration of (300 mg/kg feed).

Biochemical traits of blood: Table (4) shows the effect of adding different levels of lycopene in some biochemical traits for Japanese quail bird exposed to thermal stress at the age of (42 days), and highly significant excelling ($P < 0.01$) for the T4 treatment is shown on all treatments, followed by the T2 treatment, then T5, then T1 and finally T3 in the concentration of glucose in serum for all the birds of the experiment. As for the concentration of triglycerides (g / 100 ml serum), it is observed the continued of excelling the T4 treatment on the treatments of the experiment, which followed by the treatments (T3, T1), which excelled on the treatments (T2, T5), which did not show significant differences between them. While the T5 treatment recorded a significant excelling ($P < 0.01$) in the

average concentration of cholesterol on the rest of the treatments and then followed by treatments (T1, T2, T3) without significant differences between them and finally the T4 treatment recorded the lowest average concentration of cholesterol in the blood serum. As for the percentage of total protein, there was no significant difference ($P < 0.05$) for the T5 treatment on the treatments (T1, T3) while there were no significant differences between them and the treatments (T2, T4) for the same trait, There were no significant differences between the treatments (T1, T2, T3, T4) for the trait of the concentration of total protein. As for the albumin concentration, there were no significant differences between all treatments. As for the concentration of globulin, it was

noticed that there was a significant superiority ($P<0.05$) for the T5 treatment on the treatments of the experiment (T1, T2, T3, T4), which does not record significant differences between them in the same trait. These results agree with (Al-Nadawi, 2014) who observed a significant improvement ($p<0.05$) in the biochemical traits for the adding treatments of lycopene compared to the T1 treatment. The decrease in the concentration of glucose in lycopene treatments did not exceed the minimum limit for the normal level of glucose in the blood plasma of birds ranging from (200 - 450 mg / 100 ml) (Al-Darraj et al., 2008). It is very important that the bird preserves on the concentration of blood sugar within range even in the most severe cases of hunger and stress because a very low level of sugar means the brain stops functioning because glucose is the only source of energy in the brain, according to (Al-Darraj et al., 2008). Whenever, the percentage of lycopene in the serum and tissues increased, The percentage of infection with diseases decreased (Takeoka et al., 2001). This is due to the role of lycopene in improving health and reducing stress resulting from oxidation of free radical (Feeney, 2004; Giuseppe et al., 2007) represented by increasing

the concentration of total protein and albumin as well as the role of lycopene in increasing immunoglobulins resulting from increasing the numbers of white lymphocytes and decrease in the H / L ratio in the treatments of adding lycopene as supported by our study. There is a direct correlation between the concentration of added lycopene in the diet and between increase immunity represented by immunoglobulins, where an increase in the level of globulins in the blood gives an indication on increasing antibodies in the blood. The reason for the high concentration of glucose in the blood plasma of birds for the treatments adding of lycopene due to the reduction effect of thermal stress exposed to these birds compared to birds of the control treatment, which may be the lowering concentration of sugar in the blood plasma is due to consumption in the resistance to stress exposed to it. This is confirmed by the results of our experiment by observing body temperature. Lycopene inhibited the activity of the enzyme controlled by the manufacture of cholesterol (Hydroxymethyl - glutamyl co A), Lycopene helps in the analysis of low-density lipoproteins-cholesterol (LDL-C) (Al-Nadawi, 2014).

Table 4: Effect of Adding Different Levels of Lycopene to Diet on Some Physical Blood traits for Japanese Quail Birds Exposed to thermal Stress at Age (42) Days.

Treatments	(Average \pm standard error)					
	Glucose (mg / 100 ml serum)	triglycerides (mg / 100 ml serum)	Cholesterol (mg / 100 ml of serum)	Total protein (mg / 100 ml serum)	Albumin (mg / 100 ml serum)	Globulin (mg / 100 ml serum)
T1	d 1.73 \pm 286.00	c 9.73 \pm 298.50	b 0.58 \pm 211.00	b 0.11 \pm 2.97	0.04 \pm 1.29	b 0.07 \pm 1.68
T2	b 2.59 \pm 384.00	d 1.73 \pm 198.00	b 1.44 \pm 227.50	ab 0.42 \pm 3.73	0.07 \pm 1.38	b 0.35 \pm 2.34
T3	e 9.23 \pm 224.00	b 7.79 \pm 463.50	b 6.63 \pm 211.50	b 0.70 \pm 3.54	0.25 \pm 1.17	b 0.44 \pm 2.33
T4	a 5.19 \pm 461.00	a 8.47 \pm 854.00	c 6.85 \pm 176.00	ab 0.12 \pm 4.02	0.06 \pm 1.50	b 0.12 \pm 2.38
T5	c 3.17 \pm 359.67	d 6.64 \pm 221.67	a 2.31 \pm 304.00	a 0.13 \pm 4.90	0.00 \pm 1.54	a 0.12 \pm 3.35
Significant level	**	**	**	*	N.S	*

Different characters within one column indicate significant differences.

** At ($p < 0.01$), treatments: T1 = Chicks were fed on a diet without the addition of lycopene and considered a control treatment, T2: Chicks were fed on a diet added to it lycopene at a concentration of (150 mg/kg feed), T3: Chicks were fed on a diet added to it lycopene at a concentration of (200 mg/kg feed), T4: Chicks were fed on a diet added to it lycopene at a concentration of (250 mg/kg feed), and T5: Chicks were fed on a diet added to it lycopene at a concentration of (300 mg/kg feed).

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