INFLUENCE OF QUERCETIN ON SOME PHYSIOLOGICAL MEASUREMENTS OF LAYER HENS

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Abstract

This study was conducted to investigate the influence of different levels of quercetin on physiological status of layer hens. Hens were reared in temperature controlled and well ventilated private hall for 20 weeks, five periods (28day/period). A total of 120 hens (Isa Brown) 40 weeks of age, supplied by Hendrix Genetics Company, were distributed randomly into four treatments, each treatment contained three equal replicates (10 hens/replicate). Hens were fed on the same basal diet during the adaptation period for one week which contained 17% crude protein with 2750 Kcal/kg diet energy. Experimental treatments were as follow: T1 (Control): basal diet without any addition, T2: basal diet supplemented with 400mg quercetin/kg diet, T3: basal diet supplemented with 800mg quercetin/kg diet, and T4: basal diet supplemented with 1200mg quercetin/kg diet.

Physiological parameters findings, showed a significant ($p \leq 0.05$) increasing in packed cell volume and hemoglobin, were improved significantly in T2 which supplemented with 400mg quercetin/kg diet. Total cholesterol, triglycerides, HDL, LDL and VLDL improved high significantly ($p \leq 0.01$) with additive treatments (T2, T3 and T4). Total protein and globulin were differed high significantly ($p \leq 0.01$) in T3 and T4 as compared with control. ALT and AST also differed high significantly ($p \leq 0.01$) in T2 and T4 groups.

In a ward, there is consideration of quercetin addition on physiological status which directly reflected on the performance and health of hens.

Key words: physiological measurements, Experimental treatments

Introduction

Performance and egg quality such as egg production, eggshell quality and nutrients in eggs will decrease with increasing age specially after the end of laying peak period. Genetics, feed quality, and environment are involved in the performance and egg quality in laying hens, decline of performance is mainly due to changes in hormone concentrations, thereby resulting in restriction of growth and development of follicles, thus decreases of ovulation, egg quality and components (Ying *et al.*, 2015).

Quercetin (generally recognized as safe) is a flavonoid that widely distributed in vegetables and plants. It has been demonstrated to possess a wide array of biological effects that are considered beneficial to health, including antioxidative, anticancer, and antiviral activities. (Formica and Regelson, 1995).

Many studies proved the quercetin has ability to prevent the oxidation of low-density lipoproteins (LDL) by scavenging free radicals and chelating transition metal ions. Consequently prevention of certain diseases, such as cancer, atherosclerosis, and chronic inflammation (Kim *et al.*, 2006).

Xu *et al.*, (2009) reported that there were increases in high density lipoprotein and insulin in addition to T and B lymphocytes. Total cholesterol, triglycerides and yolk cholesterol are decreased as flavonoids supplementation in hens.

Results of Kim *et al.*, (2015) who showed that compared with control, serum triglyceride was significantly decreased by 0.04% quercetin, also yolk cholesterol were significantly decreased by 0.02% and 0.04% quercetin, liver cholesterol were significantly decreased by 0.02%, 0.04% and 0.06% quercetin, There were no significant effect of quercetin on triglyceride in eggs and liver of hen.

Provision of hens diet with quercetin at 0.5g/kg feed reduced egg yolk cholesterol content as well as the cholesterol ester, free fatty acids and phospholipids fraction increased (Iskender *et al.,* 2017). Also, It has been shown that improve oxidative status of broiler meat when added to the birds' feed and prolong the shelf-life of poultry meat (Rupasinghe *et al.,* 2010).

The objective of the present study was to investigate the quercetin Csimpacts on some physiological measurements of layer hens.

Materials and Methods

Experimental animals and design

A total of 120 Isa Brown laying hens from Hendrix Genetics Company at 40 weeks of age with a mean live weight of 1825±50gm. All hens were reared on letter floor with clean litter in well ventilated and temperature-controlled house room at average of 22.5°C, Clean water was presented *ad libitum*. Layers were fed and cared under the guidelines stated in the guide of productive company. The experimental birds were reared up to 20 weeks (w) for five periods (28day/period). Basal diet was formulated to meet nutrient requirements (Table 1). All diets were in mash form in order to uniformly mix quercetin with the basal diet. Lighting program was applied include 16h light followed by 8h dark during all trait weeks. After one week adaptation period, hens were randomly distributed into four treatments with three replicates per each (10hens/pen) and placed into 12pins with dimensions (200×175×150cm) length, width and height, respectively of each cage. The treatments as follow:

1. T1(Control): basal diet without any addition.

2. T2: basal diet supplemented with 400mg quercetin/ kg diet.

3. T3: basal diet supplemented with 800mg quercetin / kg diet.

4. T4:basal diet supplemented with 1200mg quercetin / kg diet.

Studied Parameters

Blood samples

At 50 and 60 w of the experiment, two hens per replicate were selected (*n*=6 per group) for blood collection. blood samples was collected from wing vein (4ml) from each hen, collected samples were divided into two parts: whole blood was immediately collected in sterile tubes contains anticoagulant (K-EDTA) to estimate packed cell volume and hemoglobin concentration. Another part was collected in tube without anticoagulant,

Table 1: Composition and Chemical Analysis of Experimental Diet

N	Ingredients	Weight
1	Yellow corn	37
2	Wheat	20
3	Barley	5
4	Wheat bran	5
5	Soya bean meal	18
6	Concentrate protein(40%) *	5
7	Oil	1
8	Limonstone	7.5
9	Di-Calcium phosphate	1
10	Salt	0.3
11	Premix**	0.2
12	Total	100
Chemical analysis(NRC,1994)		
1	Crude protein	17%
2	M.E (Kcal/kg)	2750 kcal/kg
3	Ca	4.6%
4	Available phosphorous	3.5%
5	Methionine	0.32%
6	Methionine +cysteine	0.68%

*Al-wafi: each kg contain:- metabolisable energy 2100kcal/ kg, crude protein 40%, crude fat 5%, fibers 2%, Ca 8%, P 2%, Methionine 2.85%, Metionine+cycteine 3.20%, Lycine 2.85%, Na 2.20%.

**Provimi: each kg contain metabolisable energy 660kcal/kg, crude protein 8%, Fat 1%, Ash 85%, Ca 15-18%, Ava P 12%, Na 5.2-5.6%, Cl 6%, Meth 8.5, Lys 2.3%, vit A 40000 iu, vit D3 80000, B1 140 mg, B2 24 mg, B6 1000mg, B12 72 mg, K3 800 mg, Niacin 280 mg, Biotin 20 mg, Pantothenic acid 200 mg, Folic acid 800 mg, Choline 2000 mg, Mn 4000 mg, Mn 4000 mg, Zinc 2000mg, Mn 4000 mg, Se 200 mg, I 1760 mg, Antioxidant 2000 mg.

to obtained serum by centrifugation of blood sample at 4000 rpm for 10 minutes, then serum was infused into sterilized tube, closed tightly and stored in deep freezing (-18ºC) until conduction of biochemical measurements. The methods of measurement was photometrical method with spectrophotometer (Apel 310 Spectrophotometer, Japan).

Packed cell volume (PCV)(%):

Packed cells volume was measured by using micro hematocrit capillary tubes after being filled to 2/3 up to their length with blood. The other side of the tube was blocked by artificial clay and been set in micro hematocrit centrifuge for 5min. The reading was taken using micro-hematocrit reader according to the method mentioned by Archer, (1965).

Hemoglobin concentration (Hb)(mg/dL):

In the presence of alkaline potassium ferricyanide,

hemoglobin was oxidized to methemoglobin , that reacts with potassium cyanide to form cyanomethemoglobin which was absorbed at 540nm wavelength. The intensity of this absorbance was direct to total Hb concentration (Varley *et al*., 1980).

Total cholesterol (mg/dL):

Serum cholesterol was measured by using of a commercial cholesterol reagent kit (Spinreact commercial kit. Spain) according to Naito, (1985) by photometrical method at 505nm wavelength.

Triglycerides (mg/dL):

Serum Triglyceride was calorimetrically measured at 505nm in a spectrophotometer by using of triglycerides reagent kit (Spinreact commercial kit. Spain) according to Fossati *et al*., (1982).

High Density Lipoprotein (HDL)(mg/dL):

This test was photometrically measured according to Friedewald *et al*., (1972) by using a commercial reagent kit (Randox Laboratories, Ltd .United Kingdom) at 500nm wavelength.

Low Density Lipoprotein (LDL)(mg/dL):

This value was calculated by the following formula (Friedewald *et al*., 1972):

$$LDL \, (mg/dL) = Total \, cholesterol - \frac{Triglycerides}{5} - HDL$$

Very Low Density Lipoprotein (VLDL)(mg/dL):

This value was calculated according to Friedwald *et al*., (1972) by the following formula:

$$VLDL \, (mg/dL) = \frac{Triglycerides}{5}$$

Total serum Protein (gm/dL):

Total Serum protein was estimated with Biuret method according to Cornall *et al*., (1949), at wavelength 545nm by using a commercial reagent kit (Biosystem. S.A. Spain).

Albumin (gm/dL):

Serum albumin was estimated with reagent kit (Vitro Science. Germany), in spectrophotometer at 580nm (Rodkey, 1964).

Globulin (gm/dL):

These partition of serum protein was calculated indirectly according to following formula:

Globulin (gm/dL) = Total protein - Albumin

Alanine amino transferase (ALT)(U/L):

Serum ALT was measured photometrical method according to Reitman and Frankel, (1957)**,** by using of a commercial reagent kit (Randox Laboratories, Ltd. United Kingdom), at 546nm wavelength.

Aspartate amino transferase (AST)(U/L):

Serum AST was photometrically measured at 546nm in a spectrophotometer by using a commercial reagent kit (Randox Laboratories, Ltd. United Kingdom), (Reitman and Frankel, 1957).

Statistical analysis:

All data were expressed as mean±standard error. The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SAS-2010, The individual comparisons were obtained by Duncan's multiple range test (Duncan, 1955). values were considered statistically significant when $p \leq 0.05$ and p ≤ 0.01.

Results and Discussion

Packed cell volume and hemoglobin concentration:

Table 2 showed the effect of dietary quercetin on blood traits at two ages (50 and 60w), including include packed cell volume (PVC) and hemoglobin concentration (Hb).

About packed cell volume of hen blood at fifty weeks of age, there were significant differences (p $\leq$ 0.05) among treatments. No difference has been presented among T1, T2 and T3, also no difference was presented between T3 and T4, the better additive treatment was T2 but, didn't differed significantly than T1. The results of sixty weeks of experiment indicated that no significant differences among treatments, in spite of, the fourth treatment has been taken higher percent as compared with others.

As for, values of hemoglobin concentration, the data indicated that there were significant differences among treatments at fifty weeks, T2 was significantly (p $\leq$ 0.05) increased as compared with T4, there was no differences among T1, T2 and T3, there was no differences between T3 and T4 also.

As the same manner of PCV, findings of 60 weeks proved there was no significant differences take place among experimental groups among each other and between them and control group. From these findings, we can find similarity between the results of PCV and Hb at two ages and that similarities are due to closed relation between them.

Total cholesterol and triglycerides (mg/dL):

Table 3 included the influence of dietary quercetin on total cholesterol, triglyceride and glucose in serum of

laying hens at 50 and 60w. With respect of total cholesterol at fifty weeks age, the data showed presence of high significant differences (p ≤ 0.01) among treatments, so T2 was significantly (p ≤ 0.05) higher than control and other supplemented groups while, there was no significant differences between T1, T3 and T4 which, differed significantly than T2. We suggested that there was decrease in cholesterol concentration with increased quercetin level in diet.

Total cholesterol was not differed significantly among treatments at sixty weeks of experiment. The T4 and T3 achieved the better treatment at 50 and 60 w, respectively.

There were significant differences (p ≤ 0.05) among treatments concerning of triglyceride at sixty weeks, so there was no considerable difference among supplementary treatments T2, T3 and T4 also between T1 and T2 treatments. T4 achieved higher concentration and T1 recorded lower one. While at fifty weeks age, the triglyceride values hasn't been differed significantly, in spite of, there were numerical differences among treatments. Triglycerides are fats that provide energy for

the cell like cholesterol, they are delivered to the body's cells by lipoproteins present in the blood. A diet with a lot of saturated fats or carbohydrates will raise the triglyceride level. Increased serum triglycerides are relatively nonspecific (Robertson *et al.*, 2004).

We conclude from results, that manner of quercetin effect on the two measurements is the same except, the values of triglyceride at 60w age was different.

Concentrations of lipoproteins in hen Cs serum (mg/dL):

Data of table 4 that implied values of high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) at two ages (50 and 60w).

HDL (good cholesterol) results showed that there were high significant differences (p<0.01) among treatments during sixty weeks of experiment. T2 didn't differed than T4, while it differed than T1 and T3. The best supplementary treatment was T3 which increased significantly HDL comparing with control and other additive groups.

In spite of great numerical differences of HDL among treatments, the results did not differed significantly at 50w, T2 has been taken superior value(34.83mg/dL).

As for LDL (bad cholesterol) statistical analysis, the findings proved that there were high significant differences (p<0.01) at 50 and 60w of hens age. At 50w, the treatments T1, T3 and T4 did not differed significantly among them but they differed significantly than T2 which record high value but, T4 recorded better value, these values refer to significant ameliorative effect of quercetin on LDL of supplementary groups especially at T3 and T4.

At 60w, the treatments T2, T3 and T4 were not differed significantly among them, but they differed significantly from T1 which record high value. The additive treatments T4 and T3 achieved the better record at 50 and 60w respectively. As the same manner, the supplementary groups decreased the LDL concentration as compared with control.

As for VLDL values, the differences were significant between the treatments during 60w, the supplementary treatments T2, T3 and T4 didn't differed significantly among each other but, differed significantly than T1 which also not differed than T2. While the results of 50w did not give any significance although, there were

Table 2: Effect of supplementing quercetin on PCV and Hb at two ages (mean±standard error).

TRT	PCV(%)		Hb(gm/dL)	
	Age(w)			
	50w	50w	50w	50w
T1	a 28.08±0.53	27.16±2.70	a 10.03±0.17	9.72±0.90
T2	a 28.08±0.85	25.50±1.96	a 10.02± 0.28	9.16±0.65
T3	ab 26.76±1.43	25.00±1.21	ab 9.59±0.47	9.00±0.40
T4	b 24.85±0.72	28.00±1.94	b 8.95±0.24	10.00±0.64
Significance	*	N.S	*	N.S

T1=control, T2, T3 and T4 supplemented with 400, 800 and 1200 mg quercetin/Kg diet respectively, N.S = No Significant differences between treatments, *significant differences between treatments at (p ≤ 0.05) in the same column.

Table 3: Effect of supplementing quercetin on total cholesteroland triglycerides at different ages (mean±standard error).

TRT	Total cholesterol(mg/dL)		Triglycerides(mg/dL)	
	Age(w)			
	50w	50w	50w	50w
T1	b 179.42±3.81	136.17±15.22	131.60±7.31	b115.06±4.37
T2	a211.35±17.08	232.91±6.61	124.83±8.12	b114.56±9.27
T3	b159.28±1.81	260.43±10.94	118.90±2.22	b134.59±6.85
T4	b161.48±6.95	222.62±10.74	114.13±4.06	a163.28±9.84
Significance	**	N.S	N.S	**

T1=control, T2, T3 and T4 supplemented with 400, 800 and 1200mg quercetin/Kg diet respectively, N.S = No Significant differences between treatments, *significant differences between treatments at (p ≤ 0.05) in the same column. **significant differences between treatments at (p ≤ 0.01) in the same column.

numerical differences among them, where the T4 recorded less value (115.0mg/dL) versus T1 which recorded higher value (125.66mg/dL).

Interestingly, there were noticeable increase in HDL and decreased values of LDL with increasing levels of quercetin at dose-dependent manner, which provide good evidence of positive effect of quercetin on lipid profile of layers at certain levels.

Serum proteins (gm/dL):

Table 5 which involve values of serum proteins (total protein, albumin and globulin) that showed there were high significant differences (p ≤ 0.01) among treatments during 50 and 60w considering total serum protein.

The findings of total protein at 50w, refer to there was high significant differences (p ≤ 0.01) between additive treatments and control, in spite of, there was no significant difference among additive treatments. T3 scored high one.

As for values of total protein at sixty weeks, there were also high significant differences (p ≤ 0.01) among treatments, so the T2 and T3 were not differed significantly also T2 and T4 didn't differed. T4 has been achieved higher score, whilst, the control achieved lower score.

For albumin values, there were no significant differences among treatments during 50 and 60 weeks of age. As for, globulin findings during 60w, the statistics

proved high significant differences between control and supplementary treatments, there were no differences among T2, T3 and T4, same thing occurs between T1 and T3. There was no significant difference among treatments at 50 w. We can noticed from obvious results, the globulin concentration in serum was significantly high in T2 and T4 as compared with control, in spite of, dominance of additive treatments on control, but no significance between T3 and control.

Liver enzymes (AST and ALT)(U/L):

Findings of table 6 showed the values of liver enzymes (ALT and AST) in hen serum at 50 and 60w of age. As for ALT, the results of sixty weeks stated that high significant differences (p ≤ 0.01) among treatments, T2 significantly decreased the ALT value as compared with control, in addition to, T4 significantly decreased the ALT comparing with T2, correspondingly, there was no differences between T1 and T4 which they differed from T2 and T3, which differed at each other. T2 recorded higher value in contrast of control which record low value, in contrast, the results of 50w, there were no differences among treatments although, there were numerical differences among treatments.

With regard of data of AST at 50w, the experimental groups were differed significantly (p ≤ 0.05), the T1, T2 and T3 did not differed significantly among each other but, differed significantly than T4, which is same letter with T3. T4 achieved upper record whilst, T2 has the

Table 4: Effect of supplementing quercetin on lipid profile of laying hen at different ages (mean±standard error).

TRT	HDL(mg/dL)		LDL(mg/dL)		VLDL(mg/dL)	
	50w	60w	50w	60w	50w	60w
T1	88.00±6.46	c46.00±2.96	b65.10±1.27	a67.15±7.84	26.32±1.46	B23.01±0.87
T2	104.50±13.29	b178.50±13.85	a81.88±8.63	b31.49±4.58	24.96±1.62	B22.91±1.85
T3	70.00±14.08	a212.50±8.21	b65.50±4.76	b21.01±4.97	23.78±0.44	B26.92±1.37
T4	78.50±12.59	b157.00±9.81	b60.15±2.83	b32.95±3.59	22.82±0.81	A32.65±1.97
Significance	N.S	**	*	**	N.S	**

T1 = control, T2 = 400 mg quercetin/Kg diet, T3 = 800 mg quercetin/Kg diet, T4 = 1200 mg quercetin/Kg diet, N.S = No Significant differences between treatments, *significant differences between treatments at (p ≤ 0.05) in the same column. **significant differences between treatments at (p ≤ 0.01) in the same column.

Table 5: Effect of supplementing quercetin on serum proteins at different ages (mean±standard error).

TRT	Total protein (gm/dL)		Albumin(gm/dL)		Globulin(gm/dL)	
	50w	60w	50w	60w	50w	60w
T1	b4.65±0.16	c4.99±0.37	2.16±0.36	2.42±0.16	2.49±0.32	b2.56±0.47
T2	a5.98±0.26	ab7.12±0.22	2.28±0.36	2.29±0.10	3.70±0.23	a4.82±0.32
T3	a6.61±0.24	bc6.44±0.68	2.38±0.41	2.27±0.07	4.22±0.59	ab4.17±0.70
T4	a6.56±0.36	a8.50±0.84	3.21±0.65	2.39±0.05	3.35±0.87	a6.10±0.87
Significance	**	**	N.S	N.S	N.S	**

T1 = Control, T2, T3 and T4 supplemented with 400, 800 and 1200 mg quercetin/Kg diet respectively, N.S = No Significant differences between treatments, **significant differences between treatments at (p ≤ 0.01) in the same column.

Table 6: Effect of supplementing quercetin on liver enzymes at different ages (mean±standard error).

TRT	ALT(U/l)		AST(U/l)	
	50w	60w	50w	60w
T1	6.33±0.66	b 6.16±0.74	b 147.33±7.55	a 175.83±6.88
T2	4.66±0.55	a 8.66±0.33	b 140.66±14.90	b 135.00±5.32
T3	5.66±0.42	c 4.16±0.16	ab 161.33±20.62	c 113.33±9.88
T4	5.83±0.90	b 6.50±0.88	a 204.83±13.98	b 155.00±5.00
Significance	N.S	**	*	**

T1=control, T2, T3 and T4 supplemented with 400, 800 and 1200 mg quercetin/Kg diet respectively, N.S= No Significant differences between treatments, * significant differences between treatments at (p"0.05) in the same column.** significant differences between treatments at (p" 0.01) in the same column.

lower value.

AST values at 60w cleared a presence of high significant differences (p ≤ 0.01) among treatments, the additive treatments (T2, T3 and T4) significantly differed and decreased than control, however, there was no significant difference between T2 and T4.

Discussion

The findings of current study as for physiological traits, indicated there were significant differences, so, it has been proved the ameliorative potential of quercetin on chicken physiological traits accordingly, reflected on performance and health.

With regard of whole blood measurements (PCV and Hb) there are close correlation between these two traits, because these two traits are accompanied with each other, The manner of statistics refer to effect of low levels of dietary quercetin on lowering hemolysis which happen normally in hot climate at summer season, consequently, improving of blood status. The findings of research at late periods are in line with Abimbola, (2017) who noticed that no significant differences concerning PCV and Hb in addition to red blood cells in broilers which fed on 50mg quercetin/kg diet at 28, 35 and 42 day of age.

Our results are consistent with Selvakumar et al., (2013) who indicated that quercetin didn't affect on PCV, Hb, RBC, WBC, glucose, creatinine, but changed significantly AST, ALT, AlP, in addition to cholesterol, triglycerides, HDL, VLDL, and decreased total protein, albumin and globulins in the serum of rats.

Concerning the lipid profile of layers serum at 50 and 60w of age, the quercetin was decreased the levels of lipid parameters except levels of good cholesterol (HDL) at 60w age which raised. In addition to, the significant differences have been appeared at 60w which is the positive thing that has been happened at advanced age of hens. Quercetin also has activity more potently for lowering serum cholesterol levels in poultry (Hooper et al., 1983; Arai et al., 2000; Egert et al., 2010) as well as cholesterol content in yolk (Liu et al., 2013).

Our findings are in agreement with Iskender et al., (2017) who stated that quercetin decreased total cholesterol concentration, lipid profile varied by the dietary treatments remained unchanged in response to dietary supplementation with quercetin at 0.5gm/kg. Qureshi et al., (2011) noticed that combined treatment of quercetin and δ-tocotrienol in poultry diet at 25 and 50 ppm were generally decreased the serum total cholesterol, low density lipoprotein cholesterol and triglyceride levels.

The results of Kim et al., (2015) showed that compared with control, serum triglyceride was significantly decreased by 0.04% quercetin, also yolk cholesterol were significantly (P ≤ 0.01) decreased by 0.02% and 0.04% quercetin, liver cholesterol were significantly decreased by 0.02%, 0.04% and 0.06% quercetin (P ≤ 0.05), There were no significant effect of quercetin on triglyceride in eggs and liver (P ≤ 0.05). Chun-Yan et al., (2017) also found that quercetin decreased the crude fat and total cholesterol of livers and yolks, attributing that outcome to affecting on serum hormones levels in hen layers.

In the other hand, Yugarani et al., (1992) found the quercetin didn't cause any significant changes on the plasma total cholesterol, triglycerides and liver fat at weeks 4, 7 and 10 in the rats fed high fat diet, also Nakamura et al., (2000) indicated that oral quercetin with 1.0g/kg had no remarkable influence on lipid concentrations of serum.

Current results may be resulted from effectively regulative effect of quercetin on LDL receptors through by quercetin could regulation of LDL receptors gene expression by activating c-jun-N-terminal kinase and extracellular signal-regulated kinase signaling pathways and increasing nuclear sterol regulatory element binding protein-2 levels, which might obtain hypolipidemic effects by increasing the clearance of circulating LDL cholesterol levels from the blood (Moon et al., 2012).

Another mechanism was explained by Lee et al., (2003) that supposed the cholesterol decreasing effect of quercetin was related inhibition of HMG–CoA reductase enzyme activity: the first step enzyme in cholesterol synthesis process, or modulates cholesterol metabolism (Zhao et al., 2011).

Quercetin has ability for regulating fat anabolism and

catabolism viainhibiting gene expression in lipocytes of broiler (Li *et al*., 2013) and fat anabolism *via* regulating cAMP signal pathways (Ouyang *et al*., 2013), thereby reducing fat deposition in broiler meat.

Concerning the data of proteins in serum, data provided the evidence that augmentative effect of quercetin on total protein, albumin and globulin during 50 and 60w even with non significant results, there were mathematical differences. The increased total protein of quercetin additive groups may be connected with the poor cellular availability of quercetin because of its extensive binding to plasma proteins as reported by Boulton *et al*., (1998). Data of albumin are agree with Kim *et al*., (2015) who didn't find a significant differences between supplementary and control groups. Williams and Iatropoulos, (2002) who reported that the decrease of protein may be because of reduction of serum globulin level and change the production of immunoglobulin.

With concerning results of liver enzymes (ALT and AST) are conflicting, however, there was noticeable decrease of activity of these enzymes that related with supplementary groups at two ages. Liver enzymes were considered as indication of hepatic function, so it increased during liver damage (Al-Daraji *et al*., 2008).

Previous study indicated that statistically significant differences were exist among the groups that supplemented with quercetin according to aspartate amino transferase, alanine amino transferase, alkaline phosphatase and amylase enzyme levels compared to the control group (p ≤ 0.05), despite no statistically significant differences among the groups according to lactate dehydrogenase (Kim *et al*., 2015).

Form our findings, we can say that there were usefulness of additive quercetin through regulating of some biomarkersof layer hens.

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