(SPARING EFFECTS OF NATURAL ANTIOXIDANT DERIVED FROM TWO LEVELS OF DATE PALM POLLEN (PHOENIX DACTYLIFERA) EXTRACT ON ANTIOXIDANT ENZYMES, PERFORMANCE, DIGESTIBILITY, BIOCHEMICAL PARAMETERS AND IMMUNITY OF EGYPTIAN *FAYOUMI* LAYING CHICKENS)

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ABSTRACT

One-hundred and fifty, 42 wk-old Egyptian Fyoumi laying chickens were used to clarify the sparing effect of natural antioxidant derived from two different amounts of DPP extract on body antioxidant enzymes of Egyptian Fayoumi laying hens and assessment its implication on performance, digestibility, biochemical, and immunity parameters. Extraction of date palm pollen (30 %weight per volume) was carried out with water plus acetone. The study time was 12-wk, where it divided into three successive equal period (4-wk per each). The serum samples were used to evaluate total serum protein, glutathione reductase activities, malondialdehyde, glutathione-*S*-transferase, glutathione peroxidise and superoxide dismutase. Also, the digestibility were estimated at the end of the experiment, besides, the performance efficiency and effectiveness were noticed. Both DPP extract amount supplementations had a positive effects on all serum biochemical parameters, where the higher concentration are more effective than both lower and control groups. DPP extract decreased malondialdehyde concentration, where increased all antioxidant activities and glutathione level. The digestibility are improved with extract amount increased. Finally we could conclude that, DPP extract supplementations, have a potent antioxidant activity and a positive impact on productivity and egg quality, and consequently, it may be considered fundamental at production and any other stress conditions.

Keywords: Layers, antioxidant, productivity, immunity.

INTRODUCTION

The Egyptian people prefer the native breeds of poultry than global breeds for consumption both meat and eggs. These products demand increases as a consequence for our population increase as well as the world population increase (Florou-Paneri *et al.*, 2005). No one ignore that, health of the birds is the key to improve production decrease stressors and treatment costs, as well as maximize feed efficiency. Whereas, bacterial resistance to antibiotics and tissue residue becomes a critical issue in the poultry production with public health concerns, so the addition of growth promoters such as antimicrobial materials have been illicit (Ertas *et al.*, 2005). Many feed supplementations were evaluated to maximize feed utilization besides, improving the immune response of bird, these additives may contain ingredient with immune modulating, antioxidant, and antimicrobial activities (Mousa et al., 2016 and 2017 and Kamboh *et al.*, 2015).

The antioxidant activity consider a self defence mechanism of the body cells, that prevent destructive action of free radicals and peroxides (reactive oxygen, RO) that produced during diseases and stressors. Also, antioxidants decrease lipid peroxidation by preventing peroxidation chain reactions and cleaning RO (Valko *et al.*, 2007).

Many herbal plants contain flavonoides and polyphenols (Pikulski et al., 2003), that have antioxidant activity (Urso et al., 2003). In poultry, the appetite and microflora population was positively affected with herbal Polyphenols when ingested, moreover it had an antioxidant and anti-inflammatory properties (Goliomytis et al., 2014).

In addition, Mousa et al., (2016) reported a positive effects of DPP extract on both digestibility and productivity of boilers, as well as improving health status of bird, where Ali et al., (2014) recorded long shelf life of poultry products.

This study was carried out to assessment the sparing effects of DPP extract on body antioxidant systems and determine its implication on digestibility, immunity, biochemical parameters and egg production and quality.

METHODOLOGY

Birds, housing, and Experimental Design

This study was carried out according to the guidelines of Nutrition and Clinical Nutrition Dept., Faculty of veterinary Med., Sohag University, Egypt. One-hundred and fifty, 42 wk-old Fayoumi laying birds, body weight average was 1.35 kg, hens were kept on conventional litter system (chopped wheat straw) as bedding material. Lighting system was 16 hours lighted per day. Hens were fed adlibitum, and the residues were collected to estimate the daily intake, besides health status was evaluated daily. The adaptation period was 1-wk, where birds were randomly divided into three experimental groups (50 birds per each), with five replicates (10 per each). Experimental design is illustrated in table 1.

Table 1. The research design

Parameters	Control groups (C) (No supplements)	Treated group (TG1) (supplemented with DPP extract 2ml / litre of drinking water)	Treated group (TG1) (supplemented with DPP extract 4ml / litre of drinking water))
No. Of birds	50 Fayoumi-laying hens	50 Fayoumi laying hens	50 Fayoumi-laying hens
No. Of replicates	5	5	5

Date palm pollen extract preparation

Date palm pollen (phoenix dactylifera L.) was collected from Sohag governorate, Egypt, and separated from the kernels by fine gauze sieve and left in an incubator at 35 C for 3 hours.
The 200 g date palm pollen was purified from foreign materials, minced then merged in 600 ml hot distilled water (60 ºC), 10g EDITA, and 20ml acetone, with continuous stirring for 2 hrs, then incubated at 5 ºC for one week. After incubation, the whole substances were filtered in a piece of gauze (20 cm X 20 cm) to remove the bulky materials then filtered in Whatman paper No. 5 to remove any debris. The filtrated date palm pollen extract was segregated into aliquots of 05 and 100 mL capped bottles and kept in refrigerator to be used daily along the experimental period. This method is according to Khalifa et al., (2018) with some modifications.

Feeding

Fayoumi laying hens in all groups, control group, (CG); treated group (TG1); treated group (TG2) were fed free choice on the same basal control diets. Birds in the second group (TG1) supplemented with DDP extract (33.3% weight per volume) in drinking water by 2ml/litre, while birds in the third group (TG2) supplemented with DPP extract by 4ml/litre along the experimental time. A basal diet was balanced to cover the nutritional requirements of layers, regarding the standard of NRC, (1994) as shown in Table2.

Table 2. Diet composition and chemical analysis

Ingredients	%
Corn	65.1
Soybean meal	20
Wheat bran	39.1
Lime stone	9.2
Premix [1]	0.3
Common salt	0.3
Mono-calcium phosphate	1.5
Slack (sand or DPP)	0.5
Total	100
Chemical analysis	
Crude protein	16.14
Ether extract	2.68
Crude fiber	2.59
Ash	13.3
Calcium	3.93
Available Phosphorus	0.29
Lysine	0.9
Methionine	0.26
ME (Kcal/kg) [2]	2713

[1] premix provides the mineral and vitamins according to the recommended levels of **NRC, 1994**.
[2] ME (Metabolizable Energy) was estimated according to **NRC, 1994**.
Data percentage expressed on dry matter basis.

Sampling and Laboratory Analyses

Blood Sample

After the experimental period, wing vein was used to collect blood samples into test tubes. After coagulation of blood samples in test tubes, we obtain serum by centrifugation 1,500 *rpm* for 10 min, then it was freeze at −80°C in Eppendorf tubes for analysis (serum protein, cholesterol, and MDA levels).

A heparinised-blood were stored to estimate the activities of GR, GST, GSH-Px and SOD and the level of GSH. Samples was centrifuged at 1000 *rpm* for 18 minutes, the erythrocytes were collected and washed three times with normal saline solution.

Serum protein was estimated regarding Lowry et al., (1951), while cholesterol concentration was determined by Allain et al., (1974) method.

Antioxidant indices

Serum MDA level (nmol/ mL) is estimated at spectrophotometer wave length 532 nm, according to Ohkawa *et al.*, (1979) method. Activity of erythrocyte SOD is estimated with spectrophotometer according to Sun *et al.*, (1988) method. The level of Erythrocyte GSH (µmol g/hemoglobin) is estimated according to Beutler *et al.*, (1975), with spectrophotometer at wave length 412 nm. The activity of Erythrocyte GST (U/g hemoglobin) is estimated according to Habig *et al.*, (1974) by recording the absorbance increase at wave length 340 nm. In the GSH-Px expressed as U/g haemoglobin, and was measured at 340 nm (Beutler, 1984). The activity of GR (U/g hemoglobin) is estimated according to Andersen *et al.*, (1997) at wavelength 340 nm.

Total phenolic contents measurements

The total phenolic compounds of Date Palm pollen Grain was determined using Folin–Ciocalteu reagent according to Singleton et al., (1999) method, where gallic acid used as the standard. The calibration equation was:

$y=0.000761x+0.046313$ ($R^2 = 0.944689$)

Where absorbance is y value and gallic acid concentration is x in mg mL-1.

Determination of the total flavonoid contents

Total flavonoid content of Date Palm pollen Grain was determined using quercetin dehydrate as the standard according to Da Silva et al., (2015).

Antioxidant activity of DPP extract (Free radical scavenging activity (DPPH))

It was measured according to Barros et al. (2007) method, where we dissolve 1 ml DPPH (0.1 mM) in methanol then added to 0.5 ml solution of DPP extract at different levels. Samples were stored in dark place for 30 min, and absorption decrease was estimated with spectrophotometer at 517 nm. The blank was prepared without DPPH solution. BHT was used as positive controls. This test expressed as IC50 (µg/ml) values, referring the amount of extract necessary to decrease the absorbance of DPPH radicals by 50%.

Blood biochemical parameters

For biochemical analysis, serum was stored at -20°C in sterilized tubes. Concentrations of serum protein, albumin, cholesterol and triglycerides were estimated in these samples according to Chema Diagnostica, Italy manufacturer's instruction. Urea, creatinine, AST, ALT, calcium and phosphorous were measured according to AOAC, (2004) procedures.

Measurement of antibodies

Hens 52 wks-old were injected with 3% suspension of washed sheep red blood cells (SRBC), that is non-pathogenic antigen, to stimulate T-cell (Saxena et al., 1997). Hens were injected with 1 ml of SRBC twice, the first time is the primer dose followed by booster dose after 14 days. Blood were collected at 7th and 14th days after each dose. The serum was kept at –20°C to be tested later. Hemaglutination test was used to determine antibodies levels. Serum was incubated for 30 min at 56°C, and then was analyzed for total antibodies, mercaptoethanol-sensitive (IgM) and mercaptoethanol-resistant (IgG) against SRBCs according to (Ramadan et al., 2011).

Feed and fecal samples

The feed and fecal samples were collected and kept in refrigerator at 5ºC for chemical analysis. Feed composition and digestibility were measured to evaluate impact of DPP extract on laying hens, where crude protein, ether extract, and crude fiber were analyzed according to AOAC, 2004.

Performance measurements

Productive performance: Daily egg production% was estimated with this calculation: (daily number of eggs / number of hens in each replicate)*100.
Egg weight was determined weekly, egg mass was determined as egg weight g/hen/day.

Egg Quality: the assessment of quality was carried out weekly, on the fourth day.
Quality parameters included dry shell weight, shell %, thickness and dry shell weight per unit of surface area (SWUSA).

Internal qualities Measurements were done in the first 24 h after laying. The collected data were yolk weight, yolk percentage, albumen height, albumen weight, albumen percentage and Haugh units. All these parameters were documented according to Alsaffar et al. (2013) and Abd El-Rahman and Attia (2002).

Data Statistics

ANOVA (One-way) was used to assessment the influence of DPP extract supplementations. Bonferroni t statistics test was used to determine mean values significance by (SPSS 10.01, SPSS Inc., Chiago, IL). Significance difference was found at $P < 0.05$. All data of the work were presented as mean value ± SD.

RESULTS

Extract analysis

The extract was analysed to evaluate the concentrations of total polyphenolic compounds and flavonoides and determine its antioxidant ability against BHT, shown in Table 3.

Table 3. Total phenolic compounds, flavonoides content and IC50 of the DPPH free radical scavenging of date palm pollen extract

Parameters	Total phenolic (mg GDE/g)	Total flavonoids (mg QE/g)	IC_{50} (µg/ml) DPPH
Aqueous Extraction	237.53±25	72.59± 11	230.72±19
BHT	-	-	39.51±5

(mg GAE/g): mg of gallic acid equivalent per g of dry extract.
(mg QE/g): mg of quercitin equivalent per g of dry extract.
IC50 (lg/ml) values corresponding to the amount of extract required to scavenge 50% of radicals present in the reaction mixture.

Layer performance

The implications of DPP extract supplementation on Fayoumi laying chickens performance during the experimental period is illustrated in Table 4. Body weight were improved in spite it was insignificant. Feed consumption had no differences among groups along the experimental period. Water palatability problems was not an issue with added DPP extract to it. The egg production and weight were significantly increased in positive relationship with dietary DPP extract supplementation, ($P < 0.05$; Table 4).

In the current work, our results confirm the previous findings of **Mousa et al., (2018),** who recorded that feed consumption not affected by DPP extract supplementation but had a positive impact on feed conversion ratio compared with the control group.
The egg weight improvement with supplementations birds with DPP extract, that could be explained as a result of the presence of active ingredients that enhance digestion and

absorption in poultry digestive tract. Moreover, it could be also related to total phenolic compounds and flavonoides that are estimated in DPP, that increase efficiency and utilization of feed.

In the current work, the higher eggs number was achieved by birds supplemented with DPP extract TG2 (4ml/l drinking water) followed by TG2 (2ml/l drinking water). Whereas, the lower eggs number was recorded for the control group.

Fayoumi hens allotted on TG2 (4ml/l drinking water) had better ($P<0.05$) egg mass values than other groups. Egg mass was significantly dose dependant improved with DPP extract supplements at different phases of study ($P<0.05$).

Egg quality

The addition of DPP extract influence on egg quality items of Fayoumi chickens is shown in Table 9. Generally, criteria of egg quality were significantly ($P < 0.05$) affected by DPP extract supplementation. The better means of shell index and yolk index were recorded by hens supplemented with DPP extract (4ml/l drinking water). Haugh unit, yolk and shell %, and shell thickness were not significantly affected with DPP extract supplementation, Table 9.

Blood metabolites

Birds health status are usually related to biochemical blood parameters, that considered a vital indicators of the nutritional and metabolic status of livestock. The influence of DPP supplementation on blood biochemical parameters of Fayoumi laying chickens are illustrated in Table 6. Serum protein, albumin, cholesterol and urea were affected ($P<0.05$) by DPP extract addition, whereas calcium and phosphorous were not affected. Addition of DPP extract have a significant positive effect on blood metabolites related to immunity and lipid profile.

In this study, addition DPP extract to drinking water reduced both serum cholesterol and triglyceride concentrations.

Antioxidant indices

Table 7 illustrates the influence of DPP extract supplementation on the antioxidant ability and antioxidant enzymes of laying chickens. DPP extract supplementation reduce serum MDA concentration compared with the control group, and increase the activities of GSH, GSH-Px, GR, GST and SOD activity.

Immunity parameter

In table 8, the response of immune system of laying hens against sheep RBCs injection was illustrated, the immunoglobulins (IgG and IgM) were measured and we noticed an improvement in all phases of our study to groups supplemented with DPP extract than control one.

The digestibility parameters

The digestibility parameters also($P<0.05$) improved with the supplementation of DPP extract. As the efficiency of digestive system improved, the digestive process improved. We found that the improvement of digestibility parameters were dose related to DPP extract concentration added to birds, table 5.

Table 4. The impact of date palm pollen extract supplementation on production effectiveness and efficiency of fayoumi laying hens

parameters	period	Control G	T1 G	T2G
Egg weight, g	1st	42.82±0.55^{b}	43.28±0.23^{b}	44.67±0.34^{a}
	2nd	41.26±0.65 ^{c}	43.18±0.25^{b}	45.39±0.29^{a}
	3rd	42.82±0.21^{c}	42.93±0.25^{b}	45.87±0.31^{a}
	Collective	42.3±0.31^{c}	43.1±0.22^{b}	45.34±0.33^{a}
Egg No.	1st	214±3.86^{c}	219±0.82^{b}	227±3.22^{a}
	2nd	213±5.53^{c}	221 ±0.5^{b}	232 ±2.29^{a}
	3rd	216±4.94^{c}	223±0.82^{b}	233±0.34^{a}
	Collective	214.33±4.22^{c}	221 ±1.87^{b}	230.6±3.08^{a}
Egg production %	1st	61.73±1.35^{b}	65.57±0.23^{a}	65.54±0.32^{a}
	2nd	62.13±0.86^{c}	63.36±0.14^{b}	66.57±0.42^{a}
	3rd	62.34±0.94^{c}	63.71±0.23^{b}	66.83±0.36^{a}
	Collective	62.07±0.99^{c}	63.21±0.53^{b}	66.37±0.55^{a}
Feed intake (g/d/hen)	1st	99.5±3.16	98.25±0.5	98.5±2.96
	2nd	102.5±2.73	99.75±1.71	100.75±2.5
	3rd	104.5±1.7	102±0.82	102.25±1.5
	Collective	102.17±3.06	100±1.83	100.5±1.43
Feed consumption per egg (g)	1st	161.19±5.13^{a}	157.02±0.99^{b}	150.28±1.53^{c}
	2nd	164.98±4.45^{a}	157.44±2.75^{b}	150.94±1.53^{c}
	3rd	167.63±4.49^{a}	160.41±1.73^{b}	150.38±1.6^{c}
	Collective	164.6±8.19^{a}	158.29±2.37^{b}	150.57±1.59^{c}
Average No. of egg/hen/week	1st	4.28±0.08^{c}	4.38±0.02^{b}	4.54±0.02^{a}
	2nd	4.26±0.07^{c}	4.44±0.01^{b}	4.64±0.04^{a}
	3rd	4.32±0.06^{c}	4.46±0.02^{b}	4.66±0.01^{a}
	Collective	4.29±0.07^{c}	4.43±0.04^{b}	4.61±0.05^{a}
Egg mass g/hen/day	1st	26.43±0.57^{c}	27.09±0.16^{b}	29.28±0.19^{a}
	2nd	25.64±0.3^{c}	27.21±0.09^{b}	30.22±0.2^{a}
	3rd	26.69±0.25^{c}	27.36±0.15^{b}	30.65±0.12^{a}
	Collective	25.25±0.37^{c}	27.22±0.17^{b}	30.05±0.26^{a}
FCR (g feed/egg mass)	1st	3.76±0.16^{a}	3.6±0.03^{b}	3.36±0.03^{c}
	2nd	3.99±0.07^{a}	3.67±0.06^{b}	3.33±0.02^{c}
	3rd	3.92±0.05^{a}	3.73±0.04^{b}	3.34±0.03^{c}
	Collective	3.89±0.15^{a}	3.67±0.06^{b}	3.34±0.04^{c}
Body Wt.	Initial	1.34±0.12	1.34±0.11	1.34±0.09
	Final	1.52±0.08	1.53±0.05	1.52±0.03
Mortality No.	Collective	2	0	0

Mean values within same row with different superscripts differ significantly ($p<0.05$

Table 5. The influence of supplementation Fayoumi laying hens with date palm pollen extract on nutrients digestibility

parameters	Control G	T1 G	T2G
DM	72.9±1.25^{c}	74.4±1.33^{b}	76.81±1.12 ^{a}
CP %	84.55±0.55 ^{c}	87.3±0.38 ^{b}	88.5±0.52^{a}
EE %	81.15±1. 2 ^{c}	82.5±0.95 ^{b}	85.12±1.21^{a}
CF %	21.52±0.55 ^{c}	23±0.44 ^{b}	24.38±0.68^{a}
NFE %	80.35±0.75 ^{c}	82±0.95 ^{b}	83±1.23^{a}

Mean values within same row with different superscripts differ significantly ($p<0.05$)

Table 6. The effect f Date palm pollen extract supplementation on blood biochemical parameters of Fayoumi laying hens.

parameters	Control G	T1 G	T2G
Total protein, mg/dl	3.88±0.25 [c]	4.26±0.16 [b]	4.45±0.22 [a]
Albumin, mg/dl	3.29±0.09 [c]	3.39±0.08 [b]	3.58±0.04 [a]
Urea, mg/dl	31.1±2.3	29.7±2.6	28.3±3.1
Creatinine, mg/dL	0.52±0.09[a]	0.46±0.1b	0.47±0.1 [b]
Cholesterol, mg/dL	118.2±6.2 [a]	112.52±4.9[b]	105.32±6.08 [c]
AST, µl	123.3±1.4 [a]	114.7±1.2 [c]	118.7±2.1 [b]
ALT, µl	129.9±0.03 [a]	117.3±0.02 [c]	119.3±0.02 [b]
Ca, mg/dl	17.93±0.74	18.74±0.81	17±0.82
P, mg/dl	6.46±0.34	6.54±0.28	5.98±0.18
Alp mU./ml	181.7±2.3 [a]	174.4±1.5 [b]	164.8±2.5 [c]

Mean values within same row with different superscripts differ significantly ($p<0.05$).
AST- Aspartate aminotransferase, ALT- Alanine aminotransferase, Alp- alkaline phosphatise.

Table 7. The impact of dietary date palm pollen extract supplementation on the antioxidant status of Fayoumi laying hens

Parameters	Control	T1	T2
MDA, nmol/ml	119±5.2 [a]	96.9±4.6 [b]	100.5±5.4 [b]
GSH, µg/g Hb	1.48±0.02[c]	3.11±0.02 [a]	2.1±0.01 [b]
GSH PX, U/g Hb	29.62±4.2 [c]	136±12.5 [a]	105±14.8 [b]
GR, U/g Hb	5.95±0.5 [c]	17.7±0.3 [a]	11.8±0.2 [b]
GST, U/g Hb	2.23±0.3 [c]	5.63±0.2 [a]	4.7±0.2 [b]
SOD, U/g protein	247±31.4 [c]	329.6±28.5 [a]	277±29.4[b]

Mean values within the same row with different superscripts differ significantly ($p<0.05$)

Table 8. Total antibody, IgG and IgM levels for Fayoumi laying hens at 52 wks-old.

items	Groups	Day 7ppi	Day 14 ppi	Day 7 psi	d ay14 psi
Total antibodies	control	3.8±0.23 [c]	3.4±0.09 [c]	5.3±0.08 [c]	3.6±0.04 [c]
	T1	4.5±0.33 [a]	4.1±0.08 [a]	7.4±0.04 [a]	4.3±0.07 [a]
	T2	4.1±0.12 [b]	3.6±0.9 [b]	5.9±0.02 [b]	3.8±0.06 [b]
IgG	control	2.2±0.12 [c]	1.8±0.10 [c]	2.1±0.11 [c]	2.2±0.04 [a]
	T1	2.8±0.11 [a]	2.4±0.10 [a]	4.1±0.08 [a]	2.1±0.05 [b]
	T2	2.4±0.13 [b]	1.9±0.12 [b]	2.6±0.09 [b]	2.1±0.05 [b]
IgM	control	1.5±0.08 [c]	1.6±0.10 [b]	3.22±0.06	1.4±0.05 [c]
	T1	1.7±0.12[b]	1.7±0.11 [a]	3.3±0.07	2.12±0.07[a]
	T2	1.8±0.11 [a]	1.7±0.04 [a]	3.3±0.09	1.7±0.10 [b]

Mean values within the column, for same item, with different superscripts differ significantly ($p<0.05$)

Table 9. The impact of date palm pollen extract supplementation on some egg quality trait of fayoumi laying hens.

	Parameters	Control G	T1 G	T2G
Egg content	Shell weight%	10.45±0.87	10.78±0.61	10.54±0.37
	Yolk weight%	32.48±0.24	31.96±0.31	31.59±0.29
	Albumin weight%	57.07±0.52	57.26±0.61	57.87±0.54
External quality	Shell thickness, mm	0.46±0.07 [c]	0.48±0.08[a]	0.47±0.09 [b]
	SWUSA, mg/cm[1]	78.97±6.45 [b]	82.1±4.68 [a]	81.62±2.86 [a]
	Shell index	0.76±0.03a	0.74±0.03 [b]	0.74±0.04 [b]
Internal quality	Yolk index	45.75±1.73 [b]	46.25±1.69 [ab]	47.06±2.01a
	Haugh unit	95.15±3	95.48±2.4	94.78±3.11
	Albumin hight, mm	8.14±0.79 [b]	9.54±0.41 [a]	9.48±0.51 [a]
	Yolk pH	6.58±0.1	6.51±0.07	6.46±0.09
	Albumin pH	8.95±0.12	8.92±0.23	8.9±0.18

Mean values within the same row with different superscripts differ significantly ($p<0.05$)
[1]SWUSA shell weight per unit of surface area.

DISCUSSION

Productivity

The significant improvement in egg production for DPP extract supplementation for all experimental period for TG2 and TG1 groups compared with control group, this may be caused by improvement of reproductive hormones, FSH and LH, as a response to high concentration of estradiol and estrogen hormonein DPP, also it has the ability to increase ovary growth, oviducts and their functions. (Arhaem, 2004, and Walzem, et al., 1999).

Mousa et al., (2018) reported such improvement in egg production and in body weight as well, where they explained it as consequences to improve in liver function and morphological improvements in intestinal villi and the Payers patches. In the current work, the digestibility of the feed was improved as illusterated in table 5 and this finding also explan the increased production and improvement of egg quality.
The body weight not affected but the egg weight was improved, that can be explained as the intake energy are directed toward production mainly

Immunity

In the poultry industry, everyone know that immune system stimulation is the answer key to reduce or prevent infectious diseases. Immunodeficiency are related to many issues like miss use of antibiotics, vaccination failure and other stress condition. In order to improve immune response, poultry should supplemented with immune enhancers. Herbal extracts, which are rich in flavonoids, total phenolic compounds act as natural antioxidants and immune modulators (Acamovic and Brooker, 2005, Mousa et al., 2018). In contrast to Karimi, (2014) finding, Mousa et al., (2018) reported an improvement in such parameters.

The current result could help in explaining the nutritional and biological effects on immunity parameters of tested treatments (Table 6). The DPP extract supplementation were significantly ($P < 0.05$) influence on immunoglobulin G and M, where they were increased in serum for chickens supplemented with 4ml /l drinking water, whereas results of Karimi (2014), recorded that herbs supplementation layer feeds had no significant effects on IgM.

Antioxidant indices

As the oxidation process causes undesirable and unaccepted transformation in products (meat and egg) quality, so the antioxidant activities of herbs may become an important issue (Bozkurt et al., 2014). The assessment of supplementation of DPP extract on antioxidant system, including serum activity of SOD and the concentrations of GSH and MDA, of Fayoumi laying hens is presented in Table 7. The groups supplemented with DPP extract, shows a significant elevation in serum SOD activity and GSH concentrations, whereas MDA concentration was declined by extract supplementation, where serum MDA concentration used as indicator for antioxidant systems activity.

These findings were supported by Alagawany et al. (2015) findings, who noticed that herbs supplementation or their constituents leads to elevate antioxidant enzyme activities, like SOD and GSH-Px , where decline of MDA concentration. SOD is an important metallo-protein enzyme that engages to antioxidant defence mechanism, so high levels of it may participate in improving antioxidant system activities of birds. In general, the improvement in bird health would be expected as a result of antioxidant activity.

We could expect that presence of total phenolic compounds in DPP extract have good biological activity as a natural antioxidant, where it is considered a hydrogen donor to proxy

radicals formed in oxidation of lipid, so preventing the production of hydroxyl peroxide (Hashemipour et al., 2013).

DPP extract are rich with phenolic compound and flavonoides that have strong antioxidant activity table 7. We could expect that low mortality are a result of effective and efficient antioxidant activities of the bird.

Goliomytis *et al.*, (2014) were used flavonoids as additive to improve the antioxidant ability and boost immune response. Generally free radicals elevated with high oxygen consumption, where antioxidant systems (enzymatic and non-enzymatic) neutralize it (Goktepe *et al.*, 2014). The enzymatic system participate at cellular level and includes SOD, GSH-Px, GST, and GR, whereas intracellular redox buffer considered non-enzymatic antioxidants, as GSH that is free radical scavenger, a co-factor for GPx activity and many other enzymes. Rajadurai and Prince, (2009) noticed that susceptibility to oxidative stress increased with decline in cellular non-enzymatic antioxidant.

Some researchers have assessment the activity of flavonoids as antioxidant and radical scavenging , (Tirkey *et al.*, 2005 and Mahmoud *et al.*, 2012 and Sahu *et al.* (2013) found that supplementation significantly elevate levels of GSH, GST, GR and GSH-Px, whereas decline lipid peroxidation.
In current work, levels of GSH elevated, as well as SOD, GR, GPx, and GST activities, in the supplemented groups with DPP extract. In response to supplemental DPP extract, elevated enzyme abilities might suggest that capacity to clear free radicals in chickens become better, causing lower MDA level (Goliomytis *et al.*, 2014).

Generally, estimated items, flavonoids and total phenolics have antioxidant ability, where levels of enzymatic and non-enzymatic defence systems increased and lipid peroxidation level reduced in laying birds.. Therefore, supplementation as an additive for laying chickens, may assist to obtain an efficient productivity and better health status.

Digestibility
The digestion process was improved and resulted in improved digestibility parameters, that could be explained with many point of view, the first one as shown in Mousa et al., (2018) the morphology of intestine was improved via increased villi length, blood supply and increased size of Payers patches, the second explanation could be related to improved antioxidant systems and reduced free radicals that destruct cell wall, so the cells of digestive system stay much longer time, finally its effect on endocrine and metabolism processes of nutrient and its content of vitamins and minerals that help bird growth (Wolfe and Liu, 2007).

Biochemical
Serum metabolites are shown in Table 6. The control group, has the lowest serum protein concentration compared with the supplemented groups. Dietary DPP supplementation decreases serum cholesterol concentration.

The beneficial effects of herbal additives might have many mechanism, such as efficient feed consumption and conversion, digestion and gastrointestinal morphology, as well as immune actions, besides, nutrients metabolism, antioxidant, antimicrobial (viral, bacterial, protozoa) activities (Basmacioğlu et al., 2010).

CONCLUSIONS

From current result, we could concluded that the use of DPP extract as feed additive could be beneficial to improve nutritional and economical aspects of laying hens, as it has a sparing effects on defence systems during production and other strsess condition.

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