THE IMPACT OF PALM POLLEN AND GINKGO BILOBA SUPPLEMENTATION ON PRODUCTIVE PERFORMANCE, BIOCHEMICAL PARAMETERS AND IMMUNE RESPONSE OF BROILERS

M. A.MOUSA* H. H. SAYED** A. S. ASMAN***

*Department of Nutrition and Clinical Nutrition, Faculty of Vet. Med., Sohag University, Egypt.

** Department of Microbiology, Faculty of Vet. Med., Sohag University, Egypt

***Department of Biochemistry Faculty of Vet. Med., Sohag University, Egypt

Abstract

This study was conducted to evaluate the effects of palm pollen and Gingko Biloba as feed additives, on productive performance, biochemical parameters and immune response of broiler chickens. A total of 210 one day-old chicks of Ross 308 hybrid were divided into 3 groups, (each n=70). First group (Control group), the chicks were fed on basal diet(C), second group (P), were fed on the same diet enriched with 0.5 % palm pollen, third group (G), were fed on the same diet enriched with 0.5 % Gingko Biloba (GB). During 45 days of the experimental period, the broiler performance including feed intake and body weight gain, final body weight, feed conversion ratio (FCR), carcass weight, thigh and breast weight, liver weight, biochemical parameters and antibodies titer for Newcastle and Infectious Bursal diseases vaccines were measured. Significant differences were observed between different treatments and control, at the end of the experiment (45 days), the highest body weight gain was recorded by (P) Palm Pollen enriched group (2345.45±190 g) followed by (C) control group (2170±277g) then came (G) Ginkgo Biloba (1927.27±98.4g). The impact on FCR followed the same order of live body weight. Better biochemical and immune responses were recorded in both treated groups than control one. The carcass yields were found that, thigh weight higher in P followed by C then G, while breast were higher in treated groups. From the results of this experiment, it can be concluded that addition of palm pollen to broiler diets significantly improved the body weight gain and feed conversion indices all over the period of raising while, Ginkgo Bioloba improved immune response and biochemical parameters of broilers. Finally, we could recommended the use of palm pollen as growth promoters and immune enhancer and Ginkgo Biloba as immune enhancer only, at feeding practices.

Keywords: Broilers, Palm Pollen, Ginkgo Biloba, Performance, Biochemical and Immune response.

Introduction

Broilers market is fast-growing businesses in Egypt. Latterly, broilers have been intensively selected for body weight gain. This strategy has resulted in a greater growth rate with improved feed conversion efficiency. The intensive production is challenged by many disorders, infections, and stress, which all can result in economic losses (Zhang et al., 2009). However, decrease immunity is one of the undesirable consequences of selection for elevated growth of broiler chicks. Also, the immunity of newly hatched chicks is immature and undeveloped to protect them from infectious risks found in the environment (Cox et al., 2010).

Recently, herbal science provide an alternative to immune enhancer, growth promoter and improve carcass quality, the scientist interest has been directed toward the natural feed additives such as herbs and aromatic plants that have properties of growth promoters to replace synthetic substances. These additives are given to birds to improve their performances and production, under normal or stress conditions, restrictions on the use of antibiotic as growth promoters to animals, forced scientists to found an alternative from plant source (Greathead, 2003). The anti-pathogens effect of the medicinal plants is documented with (Valero and Salmeron, 2003).

Ginkgo Biloba is a traditional herb in China and now has been used in many countries. It has been documented that extract of Ginkgo Biloba leaf can be used for the treatment of inflammations (Sochocka et al., 2010). The active components of Ginkgo Biloba leaf are mainly flavonoids, polysaccharides and terpenoids (Li et al., 2012). There were numerous reports in animals indicated that Ginkgo flavonoids posses many beneficial effects, including antioxidant and anti-inflammatory (Ding et al., 2009). Lipopolysaccharide (LPS) is found in the membrane of all gram-negative bacteria and is a stimulator of the intestinal immune system (Hu et al., 2010).

Zhang et al., (2012) found that the diets supplemented with 0.5% (in the starter phase) and 1.0% (in the grower phase) of fermented Ginkgo Biloba leaves (FG) products had favorable influences on intestinal morphology, digestion, and absorption functions without adverse effects in broiler chickens. Despite these findings, there has been a shortage of information on the possible intestinal protective effect of FG in broiler chickens.

In 2009, 7.52 million tons of date palm fruits were produced worldwide. Date palm trees were planted in 1.15 million ha and the average yield was about 6.52 t/ha (FAO, 2011). The main producers were Egypt, Iran, Saudi Arabia, the United Arab Emirates, Pakistan, Algeria,

Iraq, Sudan and Oman (FAO, 2011). Arab countries possess 70% of the world date palms (El-Juhany, 2010). Approximately, 1000 tons of DPP are produced every year by millions of palm trees grown in the Arabian region (El-Neweshy et al., 2013).

Phytochemical studies have been reported presence of estrone, α-amirin, triterpenoidal saponins and flavonoids in date palm pollen (DPP) (Hassan et al., 2012; Mahran and attia, 1976), its extract also contains cholesterol, rutin, carotenoids, oestronesgonad stimulating components that can enhance male fertility and elicit gonadotrophin activity (Hassan et al., 2012; Bahmanpour et al., 2006).

In traditional medicine, a suspension of DPP (Phoenix dactylifera L.) is a herbal therapy that is widely used as male infertility enhancer (Zaid, 1999; Zargari, 1999). DPP and male palm flowers were traditionally regarded as aphrodisiacs and fertility enhancers (Zaid, 1999). Reports have also identified microelements, such sterols and other agents that might influence male fertility, within DPP (Bajpayee, 1997). The fertility effects of DPP are not strongly supported, with the exception of one study that elucidated beneficial effect of a extract of DPP on semen quality in normal male rats (Bahmanpour et al., 2006).

It is a good source of natural antioxidants3. Flavonoid is the major class of phytoestrogen. It is functionally and structurally similar to estrogen 4 that affects Spermatogenesis. Flavonoids also act as antioxidant (Lotito and Frei, 2006). The use of DPP as feed additive to animal diet lead to more daily gain9 and the use of water extract of DPP record a significant different in production and weight of eggs compared to control group and in ovary tract weight (Arhaem, 2004).

The present study was conducted on broiler chickens to investigate the effect of using powder of palm pollen and Ginkgo Biloba leaves as individual medicinal plants on productive performance and immune response, biochemical parameters, utilization coefficients of nutrients. Besides, identifying the purpose of using such herbal in broiler production.

MATERIALS AND METHODS

Experimental chicks, housing, and management:

This experiment was conducted in accordance with the guidelines of the Department of Nutrition and Clinical Nutrition, Faculty of veterinary Med., Sohag University, Egypt. Two-hundred and ten Ross 308 hybrid chicks (n = 210) which, were fattened on conventional litter system, chopped wheat straw were used as bedding material. Room temperature and

humidity were controlled and lighting system was 24 hours light. Chicks were fed adlibitum, and health status was evaluated daily. All broilers were vaccinated for infectious bronchitis at 7days old followed by Newcastle (Zoetis-Fort Dodge) and IBD (Zoetis-Fort Dodge) at 20 days old.

Experimental design and feeding:

Two-hundred and ten, one –day old chicks were divided randomly into three groups, (70 chicks per each). The trial was conducted from day 1 to day 45 of chick's age. Chicks in the first group, control group, (C) were fed ad-libitum on the basal control diets(starter from 1 to 21 days of age, and grower-finisher from22 to 45 days of age). Birds in the second group (P) received the same basal diet enriched with DDP(0.5%) while, chicks in the third group (S) fed on basal diet enriched with Ginkgo Biloba (0.5%) during experimental period. A standard basal diet was formulated to meet nutrient requirements of broiler, as established by National Research Council (NRC, 1994) as shown in Table1.

Table 1: Ingredients and chemical composition of basal diets for broiler chicks

broller chicks	-	1	
Ingredients	Starters	Finisher	
	0-21 days	22-42 days	
	of age	of age	
Corn grain	55.5	63.45	
Soy bean meal	28	21	
Conc. mixture	10	10	
Sunflower oil	2.2	1.6	
Di-calcium phosphate	2	1.8	
Ca-carbonate	0.8	0.8	
Salt	0.3	0.3	
Na bicarbonate	0.25	0.1	
Mineral and Vitamins premix	0.3	0.3	
Methionine	0.1	0.1	
Lysine	0.05	0.05	
Slack (ADDITIVES TESTED)	0.5	0.5	
Total	100	100	
Calculated analysis			
36 (1.1) 11 (1.11)	20.00	2126	
Metabolizable energy (kcal/kg)	2968	3126	
Protein (%)	21	18	
Calcium (%)	1	0.90	
phosphorus	0.9	0.8	
Lysine (%)	1.4	1.2	
Methionine (%)	0.58	0.57	
Premix provided the following per	ka of dist: vitam	in A (witomin A	

Premix provided the following per kg of diet: vitamin A (vitamin A acetate) 7,718 IU; cholecalciferol 2,200 IU; vitamin E (source unspecified) 10 IU; menadione, 0.9 mg; B12, 11 µg; choline, 379 mg; riboflavin, 5.0 mg; niacin, 33 mg; D-biotin, 0.06 mg; pyridoxine, 0.9 mg; ethoxyquin, 28 mg; manganese, 55 mg; zinc, 50 mg; iron, 28 mg; copper,7 mg; iodine, 1 mg; selenium, 0.2 mg.

Tested parameters:

Performance measurements:

Live body weight was measured at the beginning of the experiment and recorded every 15 days till the end of experiment. The amount of feed consumed was weekly recorded in each of the different experiment groups. The average amount of feed intake of each bird was calculated by dividing the weekly consumed food by its respective number of birds in each group at this week.

Body weight gain (BWG) was calculated by subtracting the averageinitial LBW at a c ertain period from the average final LBW at the same period. Feed conversion ratio was calculated by dividing feed consumption by total BW gain. Mortality was observed and recorded. Production Efficiency Factor (PEF), Also referred to as European Production Efficiency Factor (EPEF), was calculated according the equation:

((Livability x Live Weight in kg)*100)/ Age in Days x FCR (Broiler Management Manual Ross-308, 2009)

Carcass yields

At the end of the experiment and prior to slaughter, birds were given a feed withdrawal period of 12h. twelve birds were randomly selected from each group, weighed and slaughtered. Feathers were removed and chickens were eviscerated. Carcass yield was calculated. Selected chickens were deboned and weighed breast muscle, thigh muscle, liver and abdominal fat.

Blood biochemical Parameters

Blood samples from ten chicks of each group were collected by puncturing the wing vein. Blood samples were allowed to stand for one hour and centrifuged at a speed of 3.000 rpm for 20 minutes. The clear serum was collected in sterilized tubes and stored at -20 °C for further analysis. Levels of total proteins, albumin, triglycerides and cholesterol were measured in these samples according to the manufacturer's instruction (Chema diagnostica, Italy).

Nutrients utilization.

Utilization coefficients of nutrients were calculated for dry matter (DM), crude protein (CP), crude fiber (CF), and ether extract (EE) by analyzing the diets and collecting

droplets for the last three days of the experiment; droplets were collected and weighted from 5 birds per each group. Then the samples were dried by the drying oven and grinded. The crude protein, crude fiber, and ether extracts were analyzed according to AOAC, 1990. The calculations were as follows:

Ether extract retention (%)=(total EE intake – total EE excreted)/total EE intake× 100; and the same methods were used for all nutrients.

Measurement of Antibodies (Abs) titers against ND and IBD vaccines:-

In the collected sera, Abs titer against ND vaccines was measured by Haemagglutination Inhibition (HI) test according to OIE (2012) and Abs titer against IBD vaccine was measured by Enzyme-Linked Immunosorbent Assay (ELISA) test via IBDV ELISA kits (Synbiotics Laboratories, USA) according to the manufacturer's instruction.

Statistical analysis:

The data was statistically analyzed with the standard procedures of analysis of variance (ANOVA), using SPSS Statistics 17.0 (Statistical Packages for the Social Sciences, released 23 August 2008). Differences among means were separated using Duncan's multiple range test (Duncan, 1955). a significant difference identified at a level of P < 0.05.

RESULTS AND DISCUSSION

Productive Performance:

The summary of the observed growth performance variables is listed in Table 2& 3. Obtained data revealed that addition of DPP improved significantly body weight gain from starting period (first 20 days of age) compared with both control and Gikgo biloba groups, the highest body weight gain was recorded by DPP supplemented group (266±68 g/bird) in comparison with control (235.87±58 g/bird) and Gikgo Biloba supplemented group (172.4). At the end of the experiment (45 days), the best cumulative weight gain and feed conversion recorded in the birds in (P) group (2395.45±190.34 g/bird &1.34) followed by broilers in (C) supplemented group (2170±277 g/bird &1.41) while the birds fed on Ginkgo Biloba enriched diet had the worst values (1927.27±98.4 g/bird &1.45). We could see that the difference between C and G group increased in the first period, and then decreased in finishing period. The feed intake had the same order of LBW (P>C>G groups), 3139, 2994, 2803 g/bird respectively.

European Performance Efficiency Factor index; (Table 3) Means of the EPEF index were significantly different between all the treatment of experiment whereas, the highest value was P group (397) followed by (C) group (327) where (G) group come in last (291) on day 45, end of experiment. The higher the value, the better the technical performance.

Nutrients utilization was shown in table 4, where Dry matter digestibility (DMD), crude protein utilization (CPU), extract utilization (EEU), and crude fiber utilization (CFU) were all improved by feeding DPP, while control group was higher in utilization than Ginkgo group.

Table 2: The effects of palm pollen and Ginkgo Biloba on broiler chicks live body weight (g/bird)

weight (g/ond)				
Groups	Initial	15 days old	30 days old	45 days old
	wt.			
Control group (C)	45.3	235.87±58,37	1430.68±245.95	2170±277.09
palm pollen group	45.2	266±68,799	1556.52±135.94	2395.45±190.34
(P)				
Ginkgo Biloba	45.5	172.4 ±17.04	1184±135.19	1927.27±98.40
group (G)				

Means with different alphabetical superscripts in the same column are significantly different at $P \leq 0.05$

Table 3: The effects of palm pollen and Ginkgo Biloba on broiler chicks feed intake(g/bird), final FCR and EPEF

Groups	15 days	30 days	45 days old	Total feed	FCR	EPEF
	old	old		intake		
Control group (C)	266.3	1537.5	1191	2994.8	1.41	327.6
palm pollen group (P)	303.24	1700	1136	3139.24	1.34	397.18
Ginkgo Biloba group (G)	315.57	1448	1040	2803.57	1.45	291.23

Means with different alphabetical superscripts in the same column are significantly different at $P\!\leq\!0.05$

Table 4: The effects of Palm Pollen and Ginkgo Biloba on broilers nutrients utilization

Groups	DM	CF	EE	CP
Control group (C)	70.6±6.2	63.7±3.7	55.9±4.2	72.0±3.2
palm pollen group (P)	77.1±5.3	67.9±3.8	60.2±4.2	78.6±3.2
Ginkgo Biloba group	68.6±4.6	62.6±2.4	53.6±3.4	69.6±3.7
(G)				

These results may be related to the active principles of DPP which, is considered to be an excellent food resource as it contains a wide range of biochemically and nutritionally important substances as: minerals, trace elements, wide range of carbohydrates, organic acids, lipids, sterols, nucleic acids, free amino acids, vitamins and over 100 kinds of enzymes and cofactors (Hassan, 2011). Palm pollen has anti-diarrheal activities (Campos et al., 2003), counteract the loss of mineral ions and reinstate metal ion homeostasis status(Haro et al.,

2000). Campos et al., 1997; Lee et al., 2009, reported that phenolic and flavenoid contents of palm pollen increase the resistance of tissues to toxicants and different harmful pathogens, also reported that increase number of goblet cells, that acts as a defensive barrier against chemical or mechanical damage and to trapping invading pathogens, MacDonald and Monteleone, 2005; Deplancke and Gaskins, 2001.

We could attributed the lowest LBW of (G)group to ginkgolic acid, that been recognized as hazardous compounds with suspected cytotoxic and allergenic properties (Pan, 2007; Wanwenet al., 2015). Gingko Biloba, has antinutritional factors—protein complexes might have spared more protein and starch for digestion (Hong et al. (2004), and the reduction in anti-nutritional factors—enzyme complexes may have spared digestive enzymes to enhance the digestibility of the nutrients (Mahmood et al., 2006; Wanwen et al., 2015). Our findings were similar with Zhang et al. (2012) who reported that dietary addition of G. biloba did not affect the weight gain of broilers.

Both DDP and Ginkgo Biloba have polyphenolic compounds(Graikou et al. 2011), that have growth promoter properties, which appear clearly with DPP group and not appear with Ginkgo biloba group which might be due to anti-nutritional factor and the powder has a large particle that permit the release of such active principles and this theory is strengthened by the improvement in the last weight while the bird become older.

The gastrointestinal tract morphology, such as deeper crypts and shorter villi, are parallel with ingested toxins (Xu et al., 2002), malabsorption (Perry et al., 1991). The crypt consider a villus factory, where a deeper crypt means fast tissue turnover and a high demand for maintenance(Yason et al., 1987). Broiler devotes about 12% of the newly metabolized protein to GIT(Yason et al., 1987). The shorter villus and a the poorer absorption. Besides. diarrhea, increase disease incidence, and lower production (Wanwen et al., 2015).

Biochemical Parameters: From Table 5 the biochemical data revealed that chicks in both (P) and (G) groups had significantly decreased cholesterol and triglycerides than (C) group, while there were a significant improvement of total protein and globulin values in both P & G groups than control. Our result was confirmed with (Attia et al., 2011) results, as rabbits fed with pollen showed an improved biochemical profile.

Table 5:The effects of palm pollen and Ginkgo Biloba on some Blood parameters of broiler chicks at 45 days of ages

Group	Protein	Albumin	Globulin	Cholesterol	Triglycerides
S	(g/100ml)	(g/100ml)	(g/100ml)	(mg/100ml)	(mg/100ml)
(C)	3.39±0.26 a	1.47±0.08 a	1.92±0.1 a	159.5±11 a	27.73±2.1 ^a
(p)	8.67±0.15 ^b	3.48±0.09 ^b	5.19±0.15 ^b	132.2±9 ^b	22.62±1.3 ^b
(G)	9.25±0.34 ^b	3.96 ± 0.05^{b}	5.29±0.11 ^b	141.6±7 ^b	22.71±1.4 ^b

Means with different alphabetical superscripts in the same column are significantly different at $P \le 0.05$

The pollen contents, particularly poly-unsaturated fatty acids and sterols have hypolipidemic and hypocholesterolemic effects (Wojcicki et al., 1987), They stimulate the microsomal 7 α-hydroxylation of cholesterol, step in formation the bile acids (Wojcicki et al., 1986) and the lowering effect on the β-oxidation rate of fatty acids, so decreased production of acetyl CoA, the precursor of cholesterol (Polanski et al., 1996). Lee et al., (2009) showed that pollen is an efficient inhibitor of lipid peroxidation and protein oxidation processes, in addition to its ability to inhibit production of NO and TNF-α. It exerts its action via down regulation of JNK and MMPs inflammatory pathways.

Some beneficial effects of flavonoids of Ginkgo biloba have been found in lipid metabolism apparently related to their antioxidant properties, which have included a reduction of serum lipids and cholesterol, protection against peroxidation in human platelets, and inhibition of lipoxygenase and prostaglandin synthetase activities (Kato and Tosa, 1983; Koch and Offler, 1985; Feng et al., 2011). Flavonoid upregulation of hepatic genes for β-oxidation and down regulation of those for fatty acid synthesis (Aoki et al., 2007).

Immunological Parameters: from table 6 we found an improvement of antibodies titers of both ND and IBD vaccine with DPP and G groups than c group. The titer of IBD and NDV vaccine were improved due to the presence of flavonoides, and these results confirmed Lien et al., (2013)result.

Table 6: The effects of Palm Pollen and Ginkgo Biloba on broilers immune response against ND and IBD virus vaccines (Titer±S.E)

Groups	HI of NDV	ELISA of
	(log2)	IBD
Control group (C)	10.84±1.0 ^a	1230.17±26.7 ^a
palm pollen group	12.54±1.4 ^b	2198±76.3 ^b
(P)		
Ginkgo Biloba group	13.89±1.5 ^b	3153.67±77.1 ^b
(G)		

Means with different alphabetical superscripts in the same column are significantly different at $P \le 0.05$

Some studies showed that DPP can possesses hepatoprotective activities (Polanski et al., 1996), also has protective effect upon systemic inflammatory response, via its contents of

some flavonoids, (quercerin and rutin), (Campos et al., 2003; Carpes et al., 2007), also, quercerin has scavengering properties against peroxides and free radical and a synergetic action giving protection against cytotoxicity and oxidative damage(Morales et al., 2006; Chow et al., 2005). Besides, DPP is a rich source of bioactive lipids that on contact with aqueous phase releases phytoprostanes and oxylipins that modulate innate immune response (Gutermuth et al., 2007).

Polyphenolic compounds exert a strong antimicrobial activity via interaction and disruption of microbial cell walls and membranes (Graikou et al., 2011; Dkhil et al., 2013). Also, polyphenolic compounds and Flavonoids are known by their immunomodulatory and anti-inflammatory activities and inhibiting pro-inflammatory cytokine production and their receptors (Dudov et al. 1994; Kempuraj et al. 2005). Pollen extract exerts a strong activity against oxidative damage and inflammatory response (Le Blanca et al. 2009).

Mahmoud et al., 2014 result, showed the decrease of systemic inflammatory response, after treatment of infested mice with the aqueous PP. They also inhibit histamine release from different tissues (Pearce et al. 1984) and down-regulated transcription factor NFkB/IkB involved in immune and inflammatory responses (Tsai et al. 2008). The Ginkgo supplementation reduced IFN- γ expression levels in challenged birds. The down regulation of both cytokines IL-4 and IL-13 may be part of a homeostatic mechanism of flavonoids and polysaccharides of Ginkgo for the maintenance of Th1/Th2 balance in response to extracellular pathogens. Dietary Ginkgo was favourable for the immunomodulatory effect on maintaining Th1/Th2 balance in response to extracellular pathogens (Brufau, et al., 2015). Hamalainen et al. (2007) and Yang et al. (2009) found that flavonoids are naturally inhibitors of iNOS, so beneficial to the treatment of inflammatory diseases, that explain antiinflammatory property of ginkgo biloba. Interleukin-18 is a pro-inflammatory cytokine that is primarily produced by macrophages. Its major targets are T helper type-1 (Th1) cells that subsequently produce IFN-y, which plays an role in activating macrophages (He et al., 2011). The Ginkgo supplementation reduced IFN-γ expression levels in challenged birds. He et al. (2011) found that IL-18 influence through IFN-γ function. The reason may be that flavonoids and polysaccharides modulate the action of macrophages, so normalize the expressions of IFN-γ and IL-18 (Puebla-Perez et al., 2003; Hamalainen et al., 2007; Sochocka et al., 2010). Interleukin- 4, and IL-13, a representative of T helper type-2 (Th2) cytokines, is a main player in macrophage alternative activation, in which IL-4 antagonizes IFN-γ function and suppresses inflammatory response (He et al., 2011), IL-4 and IL-13, antiinflammatory cytokines, were down regulated in challenged birds supplemented with Ginkgo Biloba.

Emma Ramiro, et al., (2005), reported that activation of T cell occurs immune response when an antigen is presented by ABCs (antigen presenting cells), to (Th) lymphocytes. After the recognition of peptides of antigen by T cell receptors, an intracellular signals steps is initiated, including the production of IL-2 (Powell et al. 1998). IL-2 binds to a receptor complex consisting of three subunits IL-2Ra (CD25), IL-2Rb (CD122) and IL-2Rg (CD132) (Nelson and Willerford, 1998). These subunits are necessary for high-affinity binding of IL-2. IL-2 by interacting with its specific cell receptor leads to the stimulation of a set of complex signal pathways resulting in cell proliferation (Hatakeyama & Taniguchi, 1990).

In addition to, flavonoids has potent T cell growth-stimulatory action, IL-2 mediates multiple processes, such as growth and differentiation of B cells (Waldmann et al. 1984). Activated Th lymphocytes divided into two distinguished subsets of defender cells based on the type of cytokines that they produce (Constant & Bottomly, 1997). The Th1 subset produced cytokines, associated with inflammation, such as IFN-g and TNF-a and induces cell-mediated immune responses. The Th2 subset produces cytokines such as IL-4 and IL-5 that induce proliferate and differentiate of B cells in associated with humoral immune responses. Th2 cells also produce IL-10 which inhibit Th1 cells (Constant & Bottomly, 1997). IL-4 is mainly produced by Th2 cells and plays an important role in regulating Th1/Th2 balance.

Also, Boligon, et al., (2012), concluded that Flavonoides show protective effects against damage caused by hydrogen peroxide in lymphocytes, possibly by decreasing oxidative stress due to their antioxidant nature.

Carcass Traits:

From table7 it can be observed that there was a significance difference (P≤0.05) between P and G groups in the liver weights than control. Carcass weights, breast weights and thigh weights the P group has a significance increase then both G and C groups, while there are significance difference between tested groups (P and G) than control.

Table 7: The effects of Palm Pollen and Ginkgo Biloba on carcass yield and parts portions of broiler chicks

portions of ordiner emens				
Carcass parameters		Control group	palm pollen group	Ginkgo Biloba
		(C)	(P)	group (G)
Carcass	Wt . mean	1405.5±581.39	1752.33±103.1 ^b	1331.0±10.39 ^a
	±SE	a		
Breast	Wt . mean	608±252.89 ^a	874.0±77.3 ^b	646±6.9a
	±SE			
Thigh	Wt . mean	697.5±328.5 ^a	782.3±49 b	642.0±30.5 ^a
_	±SE			
Liver	% of live	2.4±0.21 ^b	2.7±0.2 ^a	2.8±0.23 ^a
	body weight			
Abdominal	% of live	2±0.09 ^a	0.6±0.12 ^b	0.5 ± 0.14^{b}
fat	body weight			

Means with different alphabetical superscripts in the same column are significantly different at $P \le 0.05$

Cao et al. (2012) found that feeding broiler on Ginkgo biloba leaves enriched ration, significantly reduced abdominal fat accumulation. Previous study documented that, flavonoids exhibit unusual hormonal activities, such as estrogens when fed to livestock (Jenkins and Atwal, 1995). Flavonoids have received considerable attention for their lipolytic activity in vitro and in mammals (Nakagawa et al., 2004; Hsu and Yen, 2007; Zarrouki et al., 2010).

Zhang, et al., (2016) results, indicated that dietary addition of G. biloba leaf powder could alleviate lipid peroxidation in muscle. Some studies reported that flavonoids could inhibit the formation of oxygen radicals and scavenge superoxide radicals (Facino et al., 1990; Jia et al., 1999), and terpenoids inhibit the formation of free radical (Pietri et al., 1997).

CONCLUSION

In the current study, the use of DPP had a positive influence on growth performance, lipid metabolism, breast weight, biochemical and immune response. In addition, both Ginkgo Biloba and DPP can induce a decrease in abdominal fat and plasma TC value. Supplementation of G. biloba leaf powder had no significant effect on growth performance besides, improving immunity. Addition of Ginkgo Biloba improve similarity in body weights, which need More investigation in laying hens. Finally, it may be concluded that broiler production performance and immune response may be improved through supplying broiler with Anti-stress herbs like date palm pollen and Gingko Biloba as management practices during stress periods.

References

- AOAC. 1990. Method 960.39. Fat or ether extract in meat. Official Methods of Analysis. 15th ed. AOAC Int., Washington, DC.
- Aoki, F., S. Honda, H. Kishida, M. Kitano, N. Arai, H. Tanaka, S. Yokota, K. Nakagawa, T. Asakura, Y. Nakai, and T. Mae. 2007. Suppression by licorice flavonoids of abdominal fat accumulation and body weight gain in high-fat diet-induced obese C57BL/6Jmice. Biosci. Biotechnol. Biochem. 71:206– 214
- 3. Arhaem S.H.2004. Effects of use different level from water extract of DATE PALM POLLEN on ovary function and some production CHARACTERISTICS. Al-furat Agriculture Science 6(1):98-103.
- 4. Attia, Y. A.; AL-HANOUN, A.; EL-DIN, A. E.; BOVERA, F.; SHEWIKA, Y. E. 2011Effect of bee pollen levels on productive, reproductive and blood traits of NZW rabbits. Journal of Animal Physiology and Animal Nutrition, v. 95, n. 3, p. 294-303,
- 5. Bahmanpour ST, Talaei Z, Vojdani MR, Panjehshahin A, Poostpasand S, Zareei S, Ghaeminia M (2006) Effect of Phoenix dactylifera pollen on sperm parameters and reproductive system of adult male rats. Iran J Med Sci 31:208–212.
- 6. Bajpayee KK (1997) Ethnobotany of phoenix (Archaeae). J Econ Taxon Bot 21:155–157.
- Boligon A. A., Sagrillo M. R, Machado L.F, Filho, O. S, Machado M. M, Cruz I. B. M. and Athayde M. L.2012. Protective Effects of Extracts and Flavonoids Isolated from Scutia buxifolia Reissek against Chromosome Damage in Human Lymphocytes Exposed to Hydrogen Peroxide. Molecules, 17, 5757-5769
- 8. Broiler Management Manual Ross-308, 2009, Home page address: www.aviagen.com, pp.1-114
- 9. Brufau. J, E. Esteve, J. Tarradas, (2015): Review of immune stimulator substances/agents that are susceptible of being used as feed additives: mode of action and identification of end-points for efficacy assessment. www.efsa.europa.eu/publications, EFSA Supporting publication 2015:EN-905., pp75.
- 10. Campos M.G., Webby R.F., Markham K.R., Mitchell K.A. & Da Cunha A.P. 2003. Aged induced diminution of free radicals scavenging capacity in bee-pollens and the contribution of constituents favonoids. J. Agric. Food. Chem. 51 (3): 742–745.
- 11. Cao, F. L., X. H. Zhang, W. W. Yu, L. G. Zhao, and T. Wang. 2012. Effect of feeding *Aspergillus niger*-fermented Ginkgo biloba-leaves on growth performance, meat quality and lipid metabolism in broiler chicks. Poult. Sci. 92:1210–1221.
- 12. CHOW, J.M., SHEN, S.C., HUAN, S.K., LIN, H. AND CHEN, Y.C., 2005. Quercetin, but not rutin and quercitrin, prevention of H2O2–induced apoptosis via anti-oxidant activity and heme oxygenase 1 gene expression in macrophages. *Biochem. Pharmacol.*, 69: 1839–1851.
- 13. Constant SL & Bottomly K (1997) Induction of Th1 and Th2 CD4 responses: the alternative approaches. Annu Rev Immunol 15, 297–322.
- 14. Cox, C. M., L. H. Stuard, S. Kim, A. P. McElroy, M. R. Bedford, and R. A. Dalloul. 2010. Performance and immune responses to dietary beta-glucan in broiler chicks. Poult Sci. 89:1924–1933.
- 15. DEPLANCKE, B. AND GASKINS, H.R., 2001. Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. *Am. J. Clin. Nutr.*, 73: 1131-1141.
- 16. Ding, X.P., QI, J., Chang, Y.X., Mu, L.L., Zhu, D.N. and Yu, B.Y., 2009. Quality control of flavonoids in *Ginkgo biloba* leaves by high-performance liquid chromatography with diode array detection and on-line radical scavenging activity detection. *J. Chromatogr.*, 11: 2204-2210.
- 17. Dkhil M.A., Al-Quraishy S., Abdel Moneim A.E. & Delic D. 2013. Protective effect of Azadirachta indica extract against Eimeria papillata-induced coccidiosis. Parasitol. Res. 112 (1): 101–106.
- 18. Dudov I.A., Morenets A.A., Artyukh V.P. & Starodub N.F. 1994. [Immunomodulatory effect of honeybee flower pollen load]. Ukr. Biokhim. Zh. 66 (6): 91–93. PMID: 7785092
- 19. Duncan, D. B. (1955). Multiple range and multiple F-Test. Biometrics 11:1-42.
- 20. El-Juhany, L., 2010. Degradation of date palm trees and date production in Arab countries: causes and potential rehabilitation. Aust. J. Basic Appl. Sci., 4 (8): 3998-4010
- 21. Emma R., Angels Franch, Cristina Castellote1, Cristina Andre's-Lacueva, Maria Izquierdo-Pulido and Margarida Castell.2005. Effect of Theobroma cacao flavonoids on immune activation of a lymphoid cell line. British Journal of Nutrition, 93, 859–866.
- 22. Expression of interleukin 2 receptor on activated human B cells. J Exp Med 160, 1450-1466.
- 23. Facino, R.M., Carini, M., Franzoi, L., Pirola, O. and Bosisio, E., 1990. Phytochemical characterization and radical scavenger activity of flavonoids from Helichrysum italicum G. Don (compositae). *Pharmacol. Res.*, 22:709-721.

- 24. FAO, 2011. FAOSTAT. Food and Agriculture Organization of the United Nations
- 25. Feng, L. J., C. H. Yu, K. J. Ying, J. Hua, and X. Y. Dai. 2011. Hypolipidemic and antioxidant effects of total flavonoids of *Perilla frutescens* leaves in hyperlipidemia rats induced by high-fat diet. Food Res. Int. 44:404–409.
- 26. Graikou K., Kapeta S., Aligiannis N., Sotiroudis G., Chondrogianni N., Gonos E. & Chinou I. 2011. Chemical analysis of Greek pollen- Antioxidant, antimicrobial and proteasome activation properties. Chem. Central J. 5 (1): 33–42.
- 27. Greathead, H.(2003). Plants and plant extracts for improving animal productivity. Proc. Nutr. Soc., 62:279-290.
- 28. GUTERMUTH, J., BEWERSDORFF, M., TRAIDLHOFFMANN, C., RING, J., MUELLER, M. J., BEHRENDTH, A. AND JAKOBT, T., 2007. Immunomodulatory effects of aqueous birch pollen extracts and phytoprostanes on primary immuneresponses *in vivo. J. Allergy Clin. Immunol.*, 120: 293-299
- 29. Hamalainen, M., R. Nieminen, P. Vuorela, M. Heinonen, and E. Moilanen. 2007. Anti-inflammatory effects of flavonoids: Genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-κb activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-κb activation along with their inhibitory effect on iNOS expression and no production in activated macrophages. Mediators Inflamm. 2007:45673.
- 30. HARO, A., LÓPEZ-ALIAGA, I., LISBONA, F., BARRIONUEVO, M., ALFÉREZ, M.J. AND CAMPOS, M.S., 2000. Beneficial effect of pollenvand/or propolis on the metabolism of iron, calcium, phosphorus, and magnesium in rats with nutritional ferropenic anemia. *Agric. Fd. Chem.*, 48: 5715-5722.
- 31. Hassan H.M.M. 2011. Chemical composition and nutritional value of palm pollen grains. Global J. Biotech. Biochem. 6 (1): 01–07
- 32. Hassan WA, El-kashlan AM, Ehssan NA. 2012 Egyptian Date Palm Pollen Ameliorates Testicular Dysfunction Induced by Cadmium Chloride in Adult Male Rats. *J Am Sci* 2012; 8: 659-669.
- 33. Hatakeyama M & Taniguchi T (1990) Interleukin-2. In Handbook of Experimental Pharmacology, vol. 95/I; Peptide Growth Factors and their Receptors, pp. 523–540. [MB Spron and AB Roberts, editors] Berlin: Heidelberg: Springer-Verlag.
- 34. He, H., K. J. Genovese, and M. H. Kogut. 2011. Modulation of chicken macrophage effector function by T(H)1/T(H)2 cytokines. Cytokine 53:363–369.
- 35. HONG, K.L., LEE, C.H. AND KIM, S.W., 2004. *Aspergillus oryzae* GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. *J. Med. Fd.*, 7: 430-435.
- 36. Hsu, C. L., and G. C. Yen. 2007. Effects of flavonoids and phenolic acids on the inhibition of adipogenesis in 3T3–L1 adipocytes. J. Agric. Food Chem. 55:8404–8410.
- 37. Hu, X. F., Y. M. Guo, B. Y. Huang, L. B. Zhang, S. Bun, D. Liu, F. Y. Long, J. H. Li, X. Yang, and P. Jiao. 2010. Effect of corticosterone administration on small intestinal weight and expression of small intestinal nutrient transporter mRNA of broiler chickens. Asian-australas. J. Anim. Sci. 23:175–181.
- 38. Jenkins, K. J., and A. S. Atwal. 1995. Flavonoids increase tissue essential fatty acids in vitamin E deficient chicks. J. Nutr. Biochem. 6:97–103.
- 39. Jia, Z.S., Tang, M.C. and Wu, J.M., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Fd. Chem.*, 64: 555-559.
- 40. Kato, N., and N. Tosa. 1983. Effects of dietary quercetin on serum lipids. J. Agric. Biol. Chem. 47:2119–2120.
- 41. Kempuraj D., Madhappan. B., Christodoulou S., Boucher W., Cao J., Papadopoulou N., Cetrulo C.L. & Theoharides T.C. 2005. Flavonols inhibit proinflammatory mediator release, intracellular calcium ion levels and protein kinase C theta phosphorylation in human mast cells. British. J. Pharmacol. 145 (7): 934–944.
- 42. Koch, H. P., and E. L. Offler. 1985. Efficacy of individual flavonoids on lipid peroxidation in human platelets. Methods Find. Exp. Clin. Pharmacol. 7:13–18.
- 43. Koch, H. P., and E. L. Offler. 1985. Efficacy of individual flavonoids on lipid peroxidation in human platelets. Methods Find. Exp. Clin. Pharmacol. 7:13–18.
- 44. Leblanc B.W., Davis O.K., Boue S., Delucca A. & Deeby T. 2009. Antioxidant activity of Sonoran Desert bee pollen. Food Chem. 115 (4): 1299–1305.
- 45. LEE, K. H., KIM, A. AND CHOI, E., 2009. Antioxidant and antiinflammatory activity of pine pollen extract *in vitro*. *Phytother*. *Res.*, 23: 41–48.
- 46. Li, H., G. Y. Zhou, J. P. Xu, J. A. Liu, H. Y. Zhang, and Y. M. Tan. 2012. Research progress on polysaccharides from *Ginkgo biloba*. J. Med. Plants Res. 6:171–176.
- 47. Lien Tu-Fa, Kou-Joong Lin, Ling-Ling Yang and Lih-Geeng Chen, 2013. Effects of Supplemental Levels of Bazhen on Growth Performances, Serum Traits, Immunity, Meat Quality and Antioxidant

JOURNAL OF INTERNATIONAL ACADEMIC RESEARCH FOR MULTIDISCIPLINARY

Impact Factor 3.114, ISSN: 2320-5083, Volume 4, Issue 10, November 2016

- Activity of Taiwan Country Chickens. Asian Australas. J. Anim. Sci. Vol. 26, No. 5: 675-682 May 2013
- 48. Lotito SB, Frei B.2006. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? Free Radic Biol Med.; 41:1727-46.
- 49. M. S. El-Neweshyl, Z. K. El-Maddawyl & Y. S. El-Sayed3, 2013 Therapeutic effects of date palm (Phoenix dactylifera L.) pollen extract on cadmium-induced testicular toxicityAndrologia, 45, 369–378
- 50. MACDONALD, T. T. AND MONTELEONE, G., 2005. Immunity, inflammation, and allergy in the gut. *Science*, 307: 1920–1925.
- 51. MAHMOOD, S., KHAN, M.A., SARWAR, M. AND NISA, M., 2006. Chemical treatments to reduce antinutritional factors in Salseed (*Shorearobusta*) Meal: effect on nutrient digestibility in colostomized hens and intact broilers. *Poult. Sci.*, 85: 2207-2215.
- 52. Mahmoud S. Metwaly1, Mohamed A. Dkhil1,2 & Saleh Al-Quraishy1, 2014: Anti-coccidial and anti-apoptotic activities of palm pollen grains on Eimeria papillata-induced infection in mice. Biologia 69/2: 254—259.
- 53. Mahran GH A-WS, Attia AM. 1976 A phyto-chemical study of date palm pollen. *Planta Med* 1976; 29: 171-175.
- 54. MORALES, A. I., VICENTE-SANCHEZ, C., JERKIC, M., SANTIAGO, J. M., SANCHEZ-GONZALEZ, P. D., PEREZ-BARRIOCANAL, F. AND LOPEZ-NOVOA, J. M., 2006. Effect of quercetin on metallothionein, nitric oxide synthases and cyclooxygenase-2 expression on experimental chronic cadmium nephrotoxicity in rats. *Toxicol. appl. Pharmacol.*, 210: 128–135. Nelson BH & Willerford DM (1998) Biology of the interleukin-2 receptor. Adv Immunol 70, 1–81.
- 55. N.R.C. (1994): Nutrient requirements of poultry. 9th ed National Academy press, Washington, D.C., USA
- 56. Nakagawa, K., H. Kishida, N. Arai, T. Nishiyama, and T. Mae. 2004. Licorice flavonoids suppress abdominal fat accumulation and increase in blood glucose level in obese diabetic KK-A(y) mice. Biol. Pharm. Bull. 27:1775–1778.
- 57. OIE, 2012. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals: Mammals, Birds and Bees, Biological Standards Commission. World Organization for Animal Health, Paris, pp. 1–19.
- 58. Pan, J. (2007). Antibacterial actions of ginkgolic acids and related mechanisms. In: New biocides development. Chapter 19 pp 389-410
- Pearce F., Befus A.D. & Bienenstock J. 1984. Mucosal mast cells: III Effect of quercetin and other flavonoids on antigeninduced histamine secretion from rat intestinal mast cells. J. Allergy Clin. Immunol. 73 (6): 819–823. PMID: 6202731
- 60. PERRY, R.W., ROWLAND, G.N. AND GLISSON, J.R., 1991. Poult malabsorption syndrome. I. Malabsorption in poult enteritis. *Avian Dis.*, 35: 685-693.
- 61. Pietri S, Séguin JR, d'Arbigny P, Drieu K, Culcasi M(1997): Ginkgo biloba extract (EGb 761) pretreatment limits free radical-induced oxidative stress in patients undergoing coronary bypass surgery. *Cardiovasc Drugs Ther.* (2):121-131.
- 62. POLANSKI, M., CZARNECKI, R. AND WORON, J., 1996. The hepatoprotective and hypolipidemic effect of flower pollen lipid extract in androgenized rats. *Folia Med. Craco.*, 37: 89-95.
- 63. Powell JD, Ragheb JA, Kitagawa-Sakakida S & Schwartz RH (1998) Molecular regulation of interleukin-2 expression by CD28 costimulation and anergy. Immunol Rev 165, 287–300.
- 64. Puebla-Perez, A. M., X. Lozoya, and M. M. Villasenor-Garcia. 2003. Effect of *Ginkgo biloba* extract, EGb 761, on the cellular immune response in a hypothalamic–pituitary–adrenal axis activation model in the rat. Int. Immunopharmacol. 3:75–80.
- 65. Ramawat, K. G., 2010. Desert Plants: Biology and Biotechnology. Springer-Verlag, Berlin Heidelberg New York
- 66. SAS (1999): SAS/STAT User's Guide. Statistics. Version 6, Cary, NC, USA.
- 67. Sochocka, M., E. Zaczyńska, A. Taboł, A. Czarny, J. Leszek, and M. Sobczyński. 2010. The influence of donepezil and EGb 761 on the innate immunity of human leukocytes effect on the NF-κB system. Int. Immunopharmacol. 10:1505–1513.
- 68. Tsai P.J., Wu S.C. & Cheng Y.K. 2008. Role of polyphenols in antioxidant capacity of napiergrass from different growing seasons. Food Chem. 106 (1): 27–32.
- 69. Valero, M.; and Salmeron, M. C. 2003. Antibacterial activity of 11 essential oils against Bacillus cereus in tyndallized carrot broth. Int. J. Food Microbiol., 85: 73-81.
- 70. Waldmann TA, Goldman CK, Robb RJ, Depper JM, Leonard WJ, Sharrow SO, Bongiovanni K, Korsmeyer SJ & Greene WC (1984)
- 71. Wanwen Yu,1 Xuhui Zhang,1 Hussain Ahmad,2 Linguo Zhao,3 Tian Wang2 and Fuliang Cao1, 2015: Intestinal Absorption Function of Broiler Chicks Supplemented with *Ginkgo* Leaves Fermented with *Bacillus* Species. *Pakistan J. Zool., vol. 47*(2), pp. 479-490

- 72. WOJCICKI, J., SAMOCHOWIEC, L., BARTLOMOWICZ, B., HINEK, A., JAWORSKA, M. AND GAWRONSKA, S., 1986. Effect of pollen extract on the development of experimental atherosclerosis in rabbits. *Atherosclerosis*, 62: 39-45.
- 73. WOJCICKI, J., SAMOCHOWIEC, L., KADLUBOWSKA, O. AND KOWNACKA, A., 1987. Study on the antioxidant properties of pollen extracts. *Arch. Immunol. Therap. Exp.*, 35: 725-729.
- 74. XU, Z.R., HU, C.H. AND WANG, M.Q., 2002. Effects of fructooligosaccharide on conversion of l-tryptophan to skatol and indole by mixed populations of pig fecal bacteria. *J. Gen. Microbiol.*, 48: 83-89
- 75. Yang, S. S., G. J. Wang, S. Y. Wang, Y. Y. Lin, Y. H. Kuo, and T. H. Lee. 2009. New constituents with iNOS inhibitory activity from mycelium of *Antrodia camphorata*. Planta Med. 75:512–516.
- 76. YASON, C.V., SUMMERS, B.A. AND SCHAT, K.A., 1987. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: Pathology. *Am. J. Vet. Res.*, 48: 927-938.
- 77. Zaid AE.1999. Date palm cultivation. United Nations FAO. Plant Production and Protection Paper.
- 78. Zargari A. 1999. Medical Plants, Vol. 3. University of Tehran Press, Tehran, pp. 33–40.
- 79. Zarrouki, B., N. J. Pillon, E. Kalbacher, H. A. Soula, G. Nia N'Jomen, L. Grand, S. Chambert, A. Geloen, and C. O. Soulage. 2010. Cirsimarin, potent antilipogenic flavonoid, decreases fat deposition in mice intra-abdominal adipose tissue. Int. J. Obes (Lond). 34:1566–1575.
- 80. Zhang, X. H., F. L. Cao, Z. Y. Sun, W. W. Yu, L. G. Zhao, G. B. Wang, and T. Wang. 2012. Effect of feeding *Aspergillus niger*-fermented *Ginkgo biloba*-leaves on growth, small intestinal structure and function of broiler chicks. Livest. Sci. 47:170–180.
- 81. Zhang, X. H., X. Zhong, Y. M. Zhou, H. M. Du, and T. Wang. 2009. Effect of *RRR*-α-tocopherol succinate on the growth and immunity in broilers. Poult. Sci. 88:959–966.
- 82. Zhang, X., Peng Lu, Wenyue Xue, Dawei Wu, Chao Wen, Liren Ding and Yanmin Zhou, 2016: Effect of *Ginkgo biloba* Leaf Powder on Growth Performance, Meat Quality and Antioxidant Activity of Muscle in Growing-Finishing Pigs. *Pakistan J. Zool.*, vol. 48(5), pp. 1555-1561, 2016.