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Research Article
Effects of Different Dietary Levels of Palm Pollen (*Phoenix dactylifera* L.) On the Humoral Immunity and Hematology of Broiler Blood

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Abstract

Background and Objective: In this study, we evaluated the effects of palm pollen (pp) added to the diet of broiler chicks on the immune response against both Newcastle disease virus (NDV) and infectious bursal disease (IBDV) and blood protein. **Materials and Methods:** Ninety-six 1-35-day-old chicks (Ross 308) were distributed randomly in four treatment groups, with four replicates of six chicks in each replicate. Chicks whose initial weight was 38 g were purchased from a local hatchery in the holy city of Karbala. The following treatments were used: T1 (control) birds were administered a basal diet, T2 birds were administered a 2 g kg^{-1} diet, T3 birds were administered a 4 g kg^{-1} diet and T4 birds were administered a 6 g kg^{-1} diet. **Results:** The ELISA test titer revealed significant ($p<0.05$) differences among various treatment groups at the end of the experiment. Chicks fed a 4 g kg^{-1} palm pollen diet were found to have the highest serum total protein, albumen and globulin compared to the control group. The highest values of packed cell volume, hemoglobin concentration, red blood cells and white blood cells in all treatments were found in chicks fed palm pollen. **Conclusion:** It was concluded that supplementation of palm pollen to the diets of broiler chicks improved humoral immunity and blood hematology.

Key words: Palm pollen, broiler, humoral immunity, blood protein, blood composition.

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Over the last several decades, the use of natural products as substitutes for antibiotic for improving performance and immune system in animal life has been encouraged. The use of herbal medicine has increased worldwide, with many ancient records of herbal medical plants[1]. The antioxidant activity of palm pollen (PP) is attributed to the wide range of phenolic compounds, including p-coumaric, ferulic acid, sinapic acids, flavonoids and procyanidins[2]. The pits of palm pollen contain different chemical compounds, such as saturated and unsaturated fatty acids, zinc (Zn), cadmium (Cd), calcium (Ca) and potassium (K). Saturated fatty acids include stearic and palmitic acid and unsaturated fatty acids contain linoleic and oleic acids, which can inhibit the 5α-reductase enzyme[3,4]. Therefore, palm pollen can create an appropriate situation for oogenesis and maintain efficient fertility in female mice, which may be considered useful nutraceuticals for potentiation of fertility in future human studies[5]. Suspension of *Phoenix dactylifera* date palm pollen is an herbal mixture that is widely used as a folk remedy for curing male infertility in traditional medicine. Date palm pollen contains at least six vitamins, including small amounts of vitamin C, vitamin B1 (thiamine), vitamin B2 (riboflavin), nicotinic acid (niacin) and vitamin A[6]. Studies have indicated that the aqueous extracts of dates have potent antioxidant activity[7]. The antioxidant activity is attributed to the wide range of phenolic compounds in date palm pollen, including p-coumaric, ferulic and sinapic acids; flavonoids; and procyanidins[8]. According to Shanoon *et al.*[9], palm pollen had significant effects on all characteristics and there was no side effect observed on chicken health or egg quality. A study by Al-Farsi *et al.*[10] revealed that palm pollen is a good source of natural antioxidants. Flavonoids compose the major class of phytoestrogen and they are functionally and structurally similar to estrogen, affecting spermatogenesis[11]. A study by Lotito and Frei[12] mentioned that flavonoids also act as an antioxidant. Studies have shown that antioxidants can also protect testes[13]. Date pits have been included in animal feed to enhance growth, an action that has been ascribed to increase in the plasma level of testosterone[14]. The present study was carried out to evaluate the impact of PP on the productivity of broilers.

MATERIALS AND METHODS

Ninety-six (Ross 308) 1-35-day-old chicks were distributed randomly in four treatment groups. Four replicates were completed using six chicks for each replicate. Chicks whose initial weight was 38 g were purchased from a local hatchery in the holy city of Karbala. The following treatments were used: T1 (control) birds were administered a basal diet, T2 birds were administered a 2 g kg^{-1} PP diet, T3 birds were administered a 4 g kg^{-1} PP diet and T4 birds were administered 6 g kg^{-1} PP diet. Each of them was supplied diet initiator of the chicks from the age of 1-21 days and diet growth from the age of 22-35 days (Table 1) chemical composition of feeding in the diet was calculated according to NRC[15]. The chicks were vaccinated via drinking water with ND (La Sota) on day 10, then administered a booster dose of Newcastle virus vaccine (La Sota) on day 18 and 25 and vaccinated with infectious bursal disease (IBDL strain) (Ceva, Hungary) on day 14.

This study was conducted at the poultry field of the Animal Production Department, College of Agriculture, University of Baghdad, during the period from 12/3/2016 to 16/4/2016 (35 days) to study the effect of varying dietary levels of palm pollen on humoral immunity and hematological blood of broilers.

Table 1: Composition of experimental diets prepared in this study

Constituents	Ingredients in starter diet (%)	Ingredients in final diet (%)
Plant protein (protein: 40%)	5.00	5.00
Soybean meal (protein: 48%)	25.00	24.00
Yellow corn	39.00	45.00
Wheat	28.00	22.00
Sun flower oil	1.00	2.00
Dicalcium phosphate	1.00	1.00
Minerals and vitamin mixture	1.00	1.00
Chemical composition		
Crude protein (%)	21.94	20.07
Metabolized energy (kcal kg^{-1})	2921.90	3038.20
Calcium (%)	0.84	0.84
Available phosphorus (%)	0.42	0.42
Lysine (%)	1.20	1.02
Methionine+cysteine (%)	0.82	0.78

Blood sample and analysis: Blood samples were collected (from 5 samples of blood) from each treatment of any (one bird) of each replicate in the treatments and blood samples at the age of 21 and 35 days were collected randomly for each replicate by puncturing the wings of the brachial vein zone. Blood was collected from each bird into two sets of sterilized tubes: one containing ethylenediaminetetraacetic acid (EDTA) as the anticoagulant for the determination of hematological parameters and the other without an anticoagulant. Tubes were placed in a centrifuge at a speed of 300 r min^{-1} for 5 min to separate plasma. Then, the plasma was transferred to other tubes, which were sealed and frozen under -15 to -20 degrees until testing. The antibody titers against ND and IBD[16] and the concentrations of blood protein were measured[17]. The hemoglobin concentration (Hb) was determined using the Sahli method and the value recorded in g/100 mL^{18}. RBC and WBC were recorded using the improved Neubauer hemocytometer as described by Dacie and Lewis[19]. PCV was determined by the microhematocrit method.

ELISA test (Synbiotics-USA): The procedure used in this test was performed according to the manufacturer's instructions listed in the ProFLOK ELISA Kit[20], which is a rapid serologic test for the detection of antibodies in chicken serum samples. The test was developed primarily to aid in the detection of pre and postvaccination antibody levels in chickens.

Statistical analysis: A completely randomized design (CRD) was used to investigate the effect of the studied treatments on different traits. Polynomials[21] were used to compare between means using Statistical Analysis System (SAS)[22].

RESULTS

Figure 1 and 2 show that the administration of chicken diet with different doses of palm pollen induced a highly variable antibody titer. However, the antibody titers of all treated groups (T2, T3 and T4) revealed significant differences ($p<0.05$) when compared to the control group (T1). On days 21 and 35, the mean Abs titers increased significantly ($p>0.05$) in all groups. However, T3 (palm pollen 4 g kg^{-1}) showed the highest mean Abs titer against ND and IBD. These results indicate that palm pollen improved immunity by increasing the antibody titer against ND and IBD antibodies.

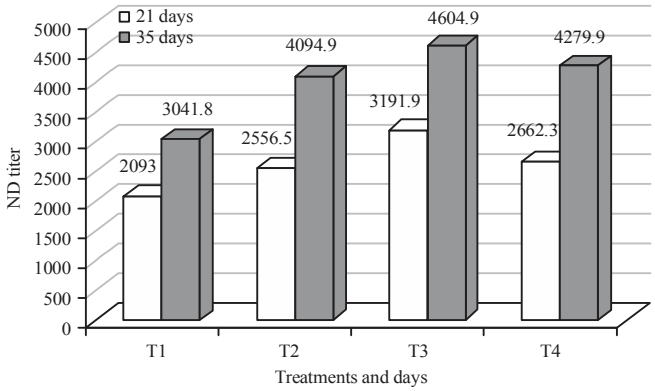

Fig. 1: Effect of treatment and day on ND titer

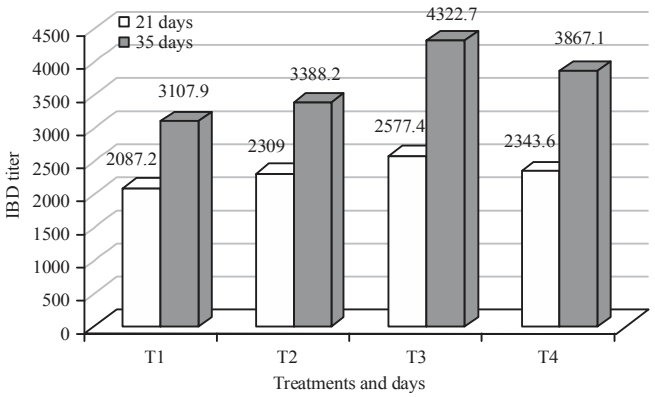

Fig. 2: Effect of treatment and day on IBD titier

Table 2: Effect of different levels of palm pollen on blood protein (mg/100 mL) ± the standard error in 3-5 week-old broiler chicks

	21 day			35 day		
Groups	Total protein	Albumin	Globulin	Total protein	Albumin	Globulin
T1	19.86±0.50^{b}	13.23±0.08	7.57±0.56^{b}	22.79±0.52^{b}	13.25±0.46^{ab}	9.16±0.21^{b}
T2	26.14±2.41^{ab}	13.24±0.61	11.33±1.20^{ab}	25.72±1.43^{ab}	13.78±0.61^{b}	11.25±0.58^{ab}
T3	30.64±1.66^{a}	19.25±3.55	12.66±2.33^{a}	30.51±2.56^{a}	16.63±0.37^{a}	14.48±1.75^{a}
T4	26.16±1.32^{ab}	13.63±0.58	10.33±0.88^{ab}	28.68±1.43^{a}	14.44±0.58^{ab}	12.28±1.31^{ab}
Significant	*	NS	*	*	*	*

The different letters appearing on the means of the same column refer to significant differences among treatment means at (p<0.05). T1: The control treatment, T2: Received palm pollen at a rate of 2 g kg^{-1} diet, T3: received palm pollen at a rate of 4 g kg^{-1} diet, T4: received palm pollen at a rate of 6 g kg^{-1} diet

Table 3: Effect of different levels of palm pollen on blood composition of ± the standard error in 3-5-week-old broiler chicks

	21 day				35 day			
Groups	RBC (×10^{6}/100 mL)	WBC (×10^{3}/100 mL)	PCV (%)	Hb (g/100 mL)	Hb (×10^{6}/100 mL)	RBC (×10^{3}/100 mL)	WBC PCV (%)	Hb (g/100 mL)
T1	1.08±0.04^{b}	21.36±0.72^{b}	27.43±1.44^{b}	9.66±0.64	1.13±0.10^{b}	21.65±0.69^{b}	28.00±1.41^{b}	10.28±0.60^{b}
T2	1.25±0.15^{ab}	21.06±0.40^{b}	27.14±1.61^{b}	10.04±0.93	1.17±0.09^{b}	21.65±0.66^{b}	28.69±0.65^{b}	10.80±0.66^{b}
T3	1.47±0.08^{a}	25.00±0.56^{a}	32.20±0.70^{a}	11.34±0.68	1.72±0.06^{a}	24.91±0.57^{a}	33.54±1.03^{a}	13.38±0.51^{a}
T4	1.36±0.13^{ab}	20.96±0.42^{b}	30.80±0.59^{ab}	10.58±0.32	1.37±0.04^{b}	23.23±0.38^{ab}	33.20±1.27^{a}	12.01±0.61^{a}
Significant	*	*	*	NS	*	*	*	*

The different letters appearing on the means of the same column refer to significant differences among treatment means at (p<0.05). T1: the control treatment, T2: Received palm pollen at a rate 2 g kg^{-1} diet, T3: Received palm pollen at a rate 4 g kg^{-1} diet, T4: Received palm pollen at a rate 6 g kg^{-1} diet, PVC: Packed cell volume, Hb: Hemoglobin, RBC: Red blood cells, WBC: White blood cells

Serum total protein, albumen and globulin of broiler chicks were significantly affected by dietary palm pollen supplementation (Table 2). Table 2 refers to the presence of significant differences (p<0.05) between the treated groups during the experimental period (21 and 35 days). Treated groups at 21 days produced higher levels of total protein and globulin than the other treatments, whereas no significant differences were observed in the albumin levels between the treated groups. Significant differences between the treated groups (p<0.05) were also observed during the 35 day. T3 produced higher total protein, globulin and albumin than did the other treatments.

Blood composition of broiler chicks were significantly affected by dietary palm pollen supplementation (Table 3). The values of PCV, Hb, RBC and WBC in chicks fed on the basal diet were lower than those of chicks fed palm pollen. Table 3 shows the presence of significant differences (p<0.05) between the treated groups during the experimental period (21 and 35 days). T3 (palm pollen 4 g kg^{-1}) showed the highest blood composition values.

DISCUSSION

There are few studies available on palm pollen supplementation in broilers. The serum antibody level is an indicator of humoral immunity. These results showed significantly higher (p<0.05) antibody titers in chicks receiving palm pollen with 2, 4 or 6 g kg^{-1} diets compared with the control treatment. Palm pollen has been reported to possess many distinct nutrients in its chemical composition, including essential and nonessential amino acids, fatty acids and proteins, carbohydrates, vitamins and minerals[23]. Pollen contains a range of vitamins, including vitamin C, vitamin B1 (thiamine), vitamin B2 (riboflavin), nicotinic acid (niacin) and vitamin A[2]. Additionally, palm pollen possesses nutritional qualities that resist inflammation and increase immunity[24]. A variety of substances that have an effect on performance levels and use of domestic birds, including flavonoids, are also antiallergic and antibacterial[25] and are resistant to lipid peroxidation[26]. These substances inhibit the activity of a group of enzymes that include hydrolase, alkaline phosphatase, lipase, phosphodiesterase, lipoxygenase, aldose reductase and α-glucosidase, which have an important role in the body's functions. High levels of enzymes can be an indicator of disease and are involved in the decomposition of fats important to the body[27]. Blood protein is composed of amino acids that play a major role in the natural balance of the body. Blood protein is the carrier of many food compounds, including lipoproteins and some carbohydrates (glycoproteins), which cannot be transmitted; they can only be linked to protein molecules[28]. According to Al-Shagrawi[29], the effect of palm pollen on fatty lipid components, total fatty lipids, triglycerides and high and low-density lipoprotein cholesterol on blood plasma, liver and rat brains showed a

significant decrease in total cholesterol and total fats. The positive effects on PCV, Hb and RBC values could be due to minerals such as Fe and Cu contained in palm pollen and due to vitamins such as folic acid and vitamin C. These minerals and vitamins are necessary for red blood cell formation and maturation[30]. Hemoglobin in red blood cells carries oxygen for energy metabolism, which may explain the relationship between palm pollen and energy. A high white blood cell count at a certain level is a good indicator of increasing immunity, suggesting that pits of palm pollen contain different chemical compounds, such as saturated and unsaturated fatty acids, zinc (Zn), cadmium (Cd), calcium (Ca) and potassium (K). Saturated fatty acids include stearic and palmitic acid and unsaturated fatty acids contain linoleic and oleic acids, which can inhibit the 5α-reductase enzyme[3].

CONCLUSION

The supplementation of 4 g kg^{-1} palm pollen to the diets of broiler chicks improved humoral immunity and blood hematology.

SIGNIFICANCE STATEMENT

The poultry industry is always searching for a new feed supplement to improve feed effectiveness and chicken health. This experiment demonstrates that supplementation of palm pollen to the diets of broiler chicks improved humoral immunity and blood hematology because the pollen contains effective natural substances and chemical compounds such as saponin, antioxidants, vitamins (A, B, C, D and E) and various mineral elements. Thus, a new theory on the use of palm pollen as a safe and nontoxic fodder additive to enhance the vitality, immunity and productive performance of these birds is presented.

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