

The Effect of Different Levels of Seed and Extract of Harmal (*Peganum harmala* L.) on Immune Responses of Broiler Chicks

M. Toghyani, A. Ghasemi, S. A. Tabeidian

Abstract—The present study was carried out to evaluate the effect of different levels of dietary seed and extract of Harmal (*Peganum harmala* L.) on immunity of broiler chicks. A total of 350 one-day old broiler chicks (Ross 308) were randomly allocated to five dietary treatments with four replicates per pen of 14 birds each. Dietary treatments consisted of control, 1 and 2 g/kg Harmal seed in diet, 100 and 200 mg/L Harmal seed extract in water. Broilers received dietary treatments from 1 to 42 d. Two birds from each pen were randomly weighed and sacrificed at 42 d of age, the relative weight of lymphoid organs (bursa of Fabricius and spleen) to live weight were calculated. Antibody titers against Newcastle and influenza viruses and sheep red blood cell were measured at 30 d of age. Results showed that the relative weights of lymphoid organs were not affected by dietary treatments. Furthermore, antibody titer against Newcastle and influenza viruses as well as sheep red blood cell antigen were significantly ($P < 0.05$) enhanced by feeding Harmal seed and extract. In conclusion, the results indicated that dietary inclusion of Harmal seed and extract enhanced immunological responses in broiler chicks.

Keywords—Broiler chicks, Harmal, immunity.

I. INTRODUCTION

ANTIBIOTICS have been used for years to promote the profitability of poultry production by reducing of pathogenic bacteria in the gut lumen, thereby improving performance and flock uniformity [1]. Since antibiotic usage has been forbidden due to the risk of bacterial residues in meat [2] as well as induced antibiotic-resistant bacteria [3], [4], researchers have followed some natural alternatives for antibiotics [5], [6].

Medicinal plants as natural feed additives are recently used in poultry diet to enhance the performance and immune response of chicken [7]. The use of natural feed additives as a substitute for antibiotic in poultry production has become an area of great interest [8]. Medicinal plants or herbs consist of many pharmacologically active chemical compounds which have antimicrobial activity [9], [10], antioxidant activity [11], [12], antifungal activity [13], antiviral activity [14], [15], anti-

inflammatory effects [16] as well as immunomodulatory properties [17], [18].

From ancient times, *Peganum harmala* (locally known as Harmal), a herbaceous perennial of the family *Zygophyllaceae* native to countries around the Mediterranean sea, central Sahara, the Middle East, India, Pakistan, south Australia, and western United States, has been used in traditional medicine for the treatment of variety of ailments, including cancer, depression, hallucinations, leishmaniasis, inflammation, malaria, and as an emmenagogue and abortifacient. It is a multipurpose medicinal plant and is one of the alternatives used as feed additive in poultry feeds [19]. Harmal possesses bioactive components such as alkaloid, flavonoids, steroids and saponins concentrated especially in seed and root [20]. Alkaloid includes harmaline, harmine, harmole as well as harmalole are the main beta carboline alkaloids in Harmal extracts [21]. Harmal has great variety of pharmacological and biological activities such as anti-oxidant [22], antibacterial and antifungal [23], analgesic and anti-inflammatory [24], anticancer [25], disinfectant [26], cholesterol lowering and hepatoprotective effects [27] and growth promoting [28].

Limited reports are available regarding the impact of *Peganum harmala* on immunity of broiler chicks. Therefore the present study was conducted to compare and evaluate effect of different levels of dietary seed and extract of Harmal on immune responses of broiler chicks.

II. MATERIALS AND METHODS

A total of 350 day-old Ross 308 broiler chicks were used in this experiment during a 42 d feeding trial including 1 to 14 d (starter period), 14 to 28 d (grower period) and 28 to 42 d (finisher period). The chicks were randomly assigned into five different dietary treatments with four replicate pens. Dietary treatments consisted of control, 1 and 2 g/kg Harmal seed in diet, 100 and 200 mg/L Harmal seed extract (methanolic extract) in drinking water (Table I).

For the preparation of extract, one kg of *Peganum harmala* seeds were dipped in 3 liters of 80% aqueous methanol for five days, filtered and then methanol was evaporated using rotary evaporator under low pressure.

At 42 d of experiment, two birds from each replicate were selected randomly to evaluate the relative weights of Bursa of Fabricius and spleen as lymphoid organs were precisely removed and weighed and expressed as percentage of live body weight.

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At 10 days of age, Newcastle and influenza antigens were injected to chickens with dual vaccine of Newcastle-influenza. Two chickens per pen were selected randomly for injection with a 1.0 ml of 1% SRBC suspension on day 25. Five days post immunization, the same wing-banded birds were bled to determine antibody titer against SRBC and also against influenza and Newcastle. Subsequently antibody titer against

SRBC was measured by HA method and also antibody titer against influenza and Newcastle separately were measured by HI method.

All data were subjected to ANOVA using the GLM procedure of SAS software [29] as a completely random design. The treatment means were separated by LSD tests at $P < 0.05$ statistical level.

TABLE I
COMPOSITION OF BASAL DIETS

Ingredients (%)	Starter	Grower	Finisher
Corn	54.0	56.6	63.9
Soybean meal	39.1	36.7	31
Soybean oil	2.3	3.00	1.8
Di calcium phosphate	1.9	1.6	1.5
Calcium carbonate	1.2	0.85	0.9
Salt	0.35	0.3	0.25
DL-Methionine	0.35	0.4	0.15
L-lysine HCL	0.30	0.05	0.00
Vitamin premix ^a	0.25	0.25	0.25
Mineral premix ^b	0.25	0.25	0.25
Nutrient composition			
ME _N (kcal/kg)	2950	3000	3000
Crude protein (%)	21.6	20.7	18.7
Calcium (%)	1.0	0.86	0.8
Available Phosphorus (%)	0.48	0.43	0.39
Methionine + Cysteine (%)	1.03	0.9	0.68
Lysine (%)	1.37	1.18	0.89

^a Vitamin premix provided per kg of diet: vitamin A: 2.7 mg; vitamin D₃: 0.05 mg; vitamin E: 18 mg; vitamin K₃: 2 mg; Thiamine: 1.8 mg; Riboflavin: 6.6 mg; Panthothenic acid: 10 mg; Pyridoxine: 3 mg; Cyanocobalamin: 0.015 mg; Niacin: 30 mg; Biotin: 0.1 mg; Folic acid: 1 mg; Choline chloride: 250 mg and Antioxidant: 100 mg.

^b Mineral premix provided per kg of diet: Fe (FeSO₄.7H₂O, 20.09% Fe): 50 mg; Mn (MnSO₄.H₂O, 32.49% Mn): 100 mg; Zn (ZnO, 80.35% Zn): 100 mg; Cu (CuSO₄.5H₂O): 10 mg; I (KI, 58% I): 1 mg and Se (NaSeO₃, 45.56% Se): 0.2 mg.

TABLE II
EFFECT OF HARMAL SEED AND EXTRACT ON ANTIBODY TITER AGAINST NEWCASTLE, INFLUENZA AND SRBC IN BROILER CHICKS

Treatment	Antibody titer (log2)		
	Newcastle virus	Influenza virus	Sheep red blood cell
Control	4.00 ^d	4.90 ^d	5.00 ^c
1g/kg Harmal seed	5.00 ^c	5.50 ^{cd}	5.80 ^b
2 g/kg Harmal seed	5.30 ^{bc}	6.10 ^{bc}	6.20 ^{ab}
100 mg/L Harmal extract	6.30 ^a	6.20 ^{ab}	6.90 ^a
200 mg/L Harmal extract	5.90 ^{ab}	6.80 ^a	6.60 ^a
SEM	0.035	0.031	0.033

^{a-d} values in column with different letters are significantly different ($P \leq 0.05$)

TABLE III
EFFECT OF HARMAL SEED AND EXTRACT ON LYMPHOID ORGANS WEIGHT (PERCENTAGE OF LIVE BODY WEIGHT) IN BROILER CHICKS

Treatment	Spleen	Bursa of Fabricius
Control	0.09	0.10
1g/kg Harmal seed	0.09	0.10
2 g/kg Harmal seed	0.10	0.11
100 mg/L Harmal extract	0.09	0.11
200 mg/L Harmal extract	0.10	0.12
SEM	0.02	0.02

^{a-d} values in column with different letters are significantly different ($P \leq 0.05$)

III. RESULTS AND DISCUSSION

Data regarding to the effect of dietary inclusion of Harmal seed and extract on antibody titer against Newcastle, Influenza and SRBC were outlined in Table II. Antibody titer against

Newcastle, Influenza and SRBC were significantly affected by dietary treatments ($P < 0.05$). Antibody titer against Newcastle, Influenza and SRBC in broiler chicks were fed seed and extract of Harmal were enhanced and Harmal extract was

more effective than Harmal seed. Effect of dietary treatments on lymphoid organs weight is shown in Table III. Spleen and bursa of Fabricius were not affected by Harmal seed or extract ($P>0.05$).

With respect to a higher antibody titer recorded in chicks fed harmal seed and extract, it is concluded that the active components of harmal which have antibacterial, anti-inflammatory and specially antioxidant activities [22]-[24] induced positive effects on immune responses. The two major alkaloids harmine and harmala from the seeds of *Peganum harmala* had marked high antioxidant capacity in scavenging or preventive capacity against free radicals induced by oxidation [30]. Protein isolated from the seeds of *Peganum harmala* alleviated the oxidative stress in the brain, testes and erythrocytes of ccl4 intoxicated rats [22].

Some of the pharmacological effects of *P. harmala* and extracts could result from the interaction of b-carboline alkaloids with monoamine oxidase (MAO) enzymes [31], [32]. MAO is a mitochondrial enzyme that catalyzes the oxidative deamination of biogenic amines and neurotransmitters. It appears as two isozymes, MAO-A and B, distinguished by substrate and inhibitor selectivities [19], [33]. MAO plays an important role in the central nervous system and peripheral organs. Inhibitors of this enzyme are useful as antidepressants (MAO-A inhibitors) and neuroprotectants [34]. Recent results suggest that b-carboline alkaloids may exhibit antidepressant effects probably linked to its inhibitory actions on MAO [35], [36].

In conclusion, addition of Harmal seeds to the diet or Harmal extract to drinking water of chickens were enhanced immunity and extract was more effective than seed.

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