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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

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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 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 Fabercius 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 residua 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 consists 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, flavonoide, steroids and saponine 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 (methalonic extract) in drinking water (Table I).

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

At d 42 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.

M. Toghyani is with the Department of Animal Sciences, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran (Corresponding: phone: +98-31-35354001; fax: +98-031-35354033; e-mail: toghiani@hotmail.com).

A. Ghasemi is with the Department of Animal Sciences, Isfahan (Khorasgan) Branch, IAU, Isfahan, Iran (e-mail: anaaghasemi67@yahoo.com).

S.A. Tabeidian is with the Department of Animal Sciences, College of Agriculture, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran (e-mail: tabeidian@yahoo.com).

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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			
MEn (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 D_3: 0.05 mg; vitamin E: 18 mg; vitamin k_3: 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

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more effective than Harmal seed. Effect of dietary treatments on lymphoid organs weight is shown in Table III. Spleen and bursa of Fabercius 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.

REFERENCES

[1] H.C. Wegener, F.M. Aarestrup, L.B. Jensen, A.M. Hammerum, F. Bager, "The association between the use of antimicrobial growth promoters and development of resistance in pathogenic bacteria towards growth promoting and therapeutic antimicrobials," *J. Anim. Feed Sci.,* vol. 7, pp.7-1, 1998.

[2] I. Phillips, M. Casewell, T. Cox, B. De Groot, C. Friis, R. Jones, C. Nightingale, R. Preston, J. Waddell, "Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data" *J. Antimicrobial Chemotherapy,* vol. 53, pp. 28–52, 2004.

[3] M. K. Glynn, C. Bopp, W. Dewitt, P. Dabney, M. Mokhtar, F.J. Angulo, "Emergence of multidrug-resistant salmonella enterica serotype typhimurium DT104 infections in the United States," *New England J. Med.,* vol. 338, pp. 1333-1339, 1998.

[4] P. D. Fey, T. J. Safranek, M. E. Rupp, E.F. Dunne, E. Ribot, "Cefriaxone-resistant salmonella infection acquired by a child from cattle," *New England J. Med.,* vol. 432, pp. 1242-1249, 2000.

[5] H. J. D. Dorman, S. G. Deans, "Antimicrobial agents from plants: antimicrobial activity of plant volatile oils," *J. Appl. Microbiol.,* vol. 83, pp. 308-316, 2000.

[6] B.C. Arldogan, H. Baydar, S. Kaya, M. Demirci, D. Ozbasar, E.Mumcu, "Antimicrobial activity and chemical composition of some essential oils," *Arch. Pharmacol. Res.,* Vol 25, pp. 860-864, 2002.

[7] I.M.K. Abaza, M.A. Asar, G.E. Elshaarrawi, M.F. Hassan, "Effect of using nigella seeds,chamomile flowers, thyme flowers and harmal seeds as feed additives on performance of broiler," *Egypt. J. Agric. Res.,* vol. 81, pp. 735-750, 2003.

[8] V.F. Samanidou, E.N. Evaggelopoulou, "Chromatographic analysis of banned antibacterial growth promoters in animal feed," *J. Sep. Sci.,* vol. 31, pp. 2091-2112, 2008.

[9] M.M. Cowan, "Plant products as antimicrobial agents" *Clinic. Microbiol. Rev.* vol.12,pp. 564-582, 1999.

[10] M. Charai, M. Mosaddak, M. Faid, "Chemical composition and antimicrobial activities of two aromatic plants: Origanum majorana L. and O. compactum Benth," *J. Essential Oil Res.,* vol. 8, pp. 657–664, 1996.

[11] N. A. Botsoglou, P. Florou-Paneri, E. Christaki, D. J. Fletouris, A. B. Spais, "Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues," *Brit. Poult. Sci.,* vol. 43, pp. 223-230, 2002.

[12] L. Korimova, J. Nagy, D. Mate, P. Korim, P. Turek, "Influence of rosemary and ascorbic acid on stability of fats in, Cingovska salama, stored in nonstandard conditions," *Slovenska Vet. J.* vol. 28, pp. 35-36, 2003.

[13] K.H. Bang, D. W. Lee, H. M. Park, Y. H. Rhee, "Inhibition of fungal cell wall synthesizing enzymes by trans-cinnamaldehyde," *Biosci. Biotechnol. Biochem.,* vol 64,pp. 1061-1063, 2000.

[14] C. Bodinet, R. Mentel, U. Wegner, U. Lindequist, E. Teuscher, J. Freudenstine, "Effect of oral application of an immunomodulating plant extract on influenza type A infection in mice," *Planta Med.,* vol 68, pp. 896-900, 2002.

[15] K.R. Sharma, M. Agrawal, M.F. Marshall, "Heavy metals in vegetables collected from production and market sites of a tropical urban area of India," *Food Chem. Toxicol.,* vol. 47, pp. 583-591, 2009.

[16] P. Arumugam, N. Gayatri Priya, M. Subathra, A. Ramesh, "Anti-inflammatory activity of four solvent fractions of ethanol extract of menthe spicata l. investigated on acute and chronic inflammation induced rats," *Environ. Toxicol. Pharmacol.,* Vol. 26, pp. 92-95, 2008.

[17] M. Toghyani, M. Toghyani, A. Gheisari, G. Ghalamkari, M. Mohammadrezaei, "Growth performance, serum biochemistry, and blood hematology of broiler chicks fed different levels of black seed (Nigella sativa) and peppermint (Mentha piperita)," *Livest. Sci.,* vol. 129, pp. 173-178, 2010.

[18] N. Landy, G. Ghalamkari, M. Toghyani, "Performance, carcass characteristics, and immunity in broiler chickens fed dietary Neem (Azadirachta indica) as alternative for an antibiotic growth promoter," *Livest. Sci.,* vol. 142, pp. 305-309, 2011.

[19] T. Herraiz, D. Gonzalez, C. Ancin-Apilicueta, V. J. Aran, H. Guillen, "Beta-Carboline alkaloids in *Pegannum harmala* and inhibition of human monoamine oxidase (MAO)," *Food Chem. Toxicol.,* vol. 48, pp. 839-45, 2010.

[20] L. Farouk, A. Laroubl, R. Aboufatima, A. Benharref, A. Chait, "Antinociceptive activity of various extracts of *peganum harmala L.* and possible mechanism of action," *Iran. J. Pharmacol. Therapeutics,* vol. 8, pp. 29-35, 2008.

[21] G. Frison, D. Favretto, F. Zancanaro, G. Fazzin, S.D. Ferrara, "A case of bcarboline alkaloid intoxication following ingestion of Peganum harmala seed extract," *For. Sci. Int.,* vol. 179, pp. e37–e43, 2009.

[22] A.M. Soliman, S.R. Fahmy, "Protective and curative effects of the 15KD isolated protein from the *Peganum harmala* L.seeds against carbon tetrachloride oxidative stress in brain, tests and erythrocytes of rats," *Eur. Rev. Med. Pharmacol. Sci.,* vol. 15, pp. 888-90, 2011.

[23] A.F. Abdel-Fattah, K. Matsumoto, H.A. Gammaz, H. Watanabe, "Hypothermic effect of harmala alkaloid in rats: involvement of serotonergic mechanism," *Pharmacol. Biochem. Behav.,* vol. 52, pp. 421 – 6, 1995.

[24] H.R. Monsef, A. Ghobadi, M. Iranshahi, A. Abdollahi, "Antinociceptive effects of *Peganum harmala* L. alkaloid extract on mouse formalin test," *J. Pharmaceut. Sci.,* vol. 19, pp. 221-222, 2004.

[25] S.M. Adams, "The antineoplastic effects of prunusarmeniaca and *Peganum harmala*," *Dis. Abstr. Int. Sci.,* vol. 44, pp. 1052- 1055, 1983.

[26] A.R. Shahverdi, H. R. Monsef-Esfahani, B. Nickavar, L. Bitarafan, S. Khodaee, N. Khoshakhlagh, "Antimicrobial activity and main chemical composition of two smoke condensates from *Peganum harmala* seeds. Z. Naturforsch," *Chinese J. Biosci.,* vol. 60, pp.707–710, 2005.

[27] K. Hamdan, H. Masmoudi, F. Ellouz, A. ElFeki, S. Carreau, "Protective effects of *Peganum harmala* extracts on thiourea-induced diseases in adult male rat" *J. Env. Bio.,* vol. 29, pp. 73-77, 2008.

[28] S.Q. Walid, "The effect of low levels of dietary *Peganum harmala* L. and *Ballota undulata* or their mixture on chicks," *J. Anim. Vet. Adv.,* vol. 8, 1535-1538, 2009.

[29] SAS Institute, *"SAS User's Guide*," Version 8.02 ed. SAS Institute Inc., Cary, NC. 2001.

[30] H. Berrougui, M. Isabelle, M. Cloutier, M. Hmamouchi, A. Khalil, "Protective effects of *Peganum harmala* L. extract, harmine and harmaline against human low–desity lipoprotein oxidation," *J. Pharmacy & Pharamacol.,* vol. 58, pp. 967-974, 2006.

[31] J.C. Callaway, D.J. McKenna, C.S. Grob, G.S. Brito, L.P. Raymon, R.E.Poland, E.N. Andrade, E.O. Andrade, D.C. Mash, "Pharmacokinetics of hoasca alkaloids in healthy humans," *J. Ethnopharmacol.* vol. 65, pp. 243–256, 1999.
[32] D.J. McKenna, J.C. Callaway, C.S. Grob, "The sciencific investigation of Ayahuasca: a review of past and current research," *Heffter Rev. Psychedelic Res.* Vol. 1, pp. 65–76, 1998.
[33] M.B.H. Youdim, D. Edmondson, K.F. Tipton, "The therapeutic potential of monoamine oxidase inhibitors," *Nat. Rev. Neurosci.* Vol. 7, pp. 295–309, 2006.
[34] T. Herraiz, C. Chaparro, "Human monoamine oxidase is inhibited by tobacco smoke: b-carboline alkaloids act as potent and reversible inhibitors," *Biochem. Biophys. Res. Commun.* Vol. 326, pp. 378–386, 2005.
[35] T. Herraiz, C. Chaparro, "Analysis of monoamine oxidase enzymatic activity by reversed-phase high performance liquid chromatography and inhibition by b-carboline alkaloids occurring in foods and plants," *J. Chromatogr.* Vol. 1120, pp. 237– 243, 2006a.
[36] T. Herraiz, C. Chaparro, "Human monoamine oxidase enzyme inhibition by coffee and b-carbolines norharman and harman isolated from coffee," *Life Sci.* vol.78, pp. 795–802, 2006b.

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