



Effect of *Peganum harmala* seeds on productive performance, immune responses and liver function in broiler chickens

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ABSTRACT

This experiment was designed to study the effects of feeding different levels of *Peganum harmala* seeds (PHS) and antibiotic on the performance, immune responses and liver function of Ross broiler chickens. A total of 240 one-d-old unsexed broiler chickens were randomly allocated to each of the 4 treatment groups, each with 4 replicate pens of 15 chicks. The dietary treatments included of control (C) - without PHS and antibiotic - the diet contains 300 mg/Kg Lincomycin 0.88% (A) and the diets contain 20g/kg (H1) and 40g/kg (H2) PHS. The performance parameters were measured during the experimental period. The chicks were raised on floor pens and received diets and water ad libitum for 6 weeks. Blood samplings were performed for determine of antibody titer against Newcastle disease virus (NDV) on 14 and 21 days and for liver function test on 42 days of age. The using of PHS at rate of 20g/kg feed improved some traits such as live body weight and FCR, but the consumption of 40g/kg had undesirable effect on these traits. Antibody titer against NDV was not affected by experimental treatments, but the relative weight of bursa and spleen increased by dietary treatments of antibiotic and H2. Broilers receiving 40g/kg PHS had a significantly higher activity of SGOT, SGPT and SALP in serum compared to control group. It can be concluded that *Peganum harmala* seeds cannot be applied as alternatives to in-feed antibiotics, but 20 g/kg inclusion of it in diet can improve production efficiency of broiler chickens.

Key word: Antibiotic, Immune system, Liver function, *Peganum harmala*, Performance

Antibiotics have been extensively used in modern livestock and poultry production to treat sick animals, but they have been also administered in sub-therapeutic doses to protect animals against disease and to stimulate growth (Belay and Teeter 1994, Swartzlander *et al.* 1995). Subtherapeutic use of antibiotics can promote growth by improving nutrient absorption and reduction the growth of organisms that compete for nutrients (Vissek 1978). There is concern that the extensive use of antibiotics in animals could promote development of drug-resistant bacteria that could pass from animals to humans and endanger human health (Burgat 1999). Therefore, animal nutritionists are trying to find an alternative for antibiotic growth promoters. In the last decade a lot of researches have been conducted on medicinal herbs as replacements for antibiotic growth promoters (Nanekarani *et al.* 2012, Goodarzi *et al.* 2013, Goodarzi *et al.* 2014). Herbs, their extracts, essential oils or the main components of the essential oil are the alternative growth promoters that have been widely used in practice (Ocak *et al.* 2008, Ayasan 2013).

Peganum harmala belongs to family Zygophyllaceae is

a traditional medicine commonly known as “Espand” in Iran. It has been employed for the treatment of a range of human diseases (Aslam *et al.* 2014). There are a variety of the beta carboline alkaloids – mainly included harmaline, harmine, harmalol and harmol in this herb. They have some of pharmacological and biological activities such as antibacterial and antifungal (Darabpour *et al.* 2011), anticoccidial (Tanweer *et al.* 2014b), disinfectant (Shahverdi *et al.* 2005), growth promoting (Tanweer *et al.* 2012), cholesterol lowering and hepatoprotective effects (Eini *et al.* 2014), Glucose lowering (Singh *et al.* 2008), monoamineoxidase inhibition (Salari *et al.* 2012), hypothermic (Abdel-Fattah *et al.* 1995), platelet aggregation inhibitory (Saeed *et al.* 1993), immuno-modulatory effects (Ghareghani Poor *et al.* 2014) and anti-inflammatory (Monsef *et al.* 2004). There are limited reports regarding the effect of *P. harmala* seed on poultry performance and liver functions. Therefore, the present study was design to compare and survey the effect of two levels of *P. harmala* and Lincomycin antibiotic on productive performance and liver function in broiler chicks.

MATERIALS AND METHODS

Animals and diets: A total of 240 one-d-old unsexed broiler chickens (mean initial weight: 37.5 ± 1 g) were randomly allocated to each of the 4 treatment groups, each

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Table 1. The ingredient and calculated composition of basal diets

Items	Starter (0-21)	Grower (22-42)
Ingredient, g/kg		
Corn	614.9	707.3
Soybean meal (43.8%)	330.6	257.3
Soybean oil	15.3	0.00
Di calcium phosphate	13.7	11.3
Oyster sell	15.1	14.1
NaCl	3.2	4.4
Mineral premix ¹	2.5	2.5
Vitamin premix ²	2.5	2.5
DL-Methionine analysis results	2.2	0.6
Metabolizable energy (kcal/kg)	3000	3000
Crude protein (g/kg)	215.7	187.4
Calcium (g/kg)	9.4	9.1
Available phosphorus (g/kg)	3.8	3.3
Methionine(g/kg)	5.5	3.6
Lysine(g/kg)	11.3	9.5
Methionine + cysteine (g/kg)	9.0	6.8

1- Ingredients per kg: Mg, 60 g; Fe, 80 g; Cu, 10 g; Zn, 50 g; Co, 2 g; I, 1 g, 2- Ingredients per kg : vitamin A, 1000,000 IU; D3, 1500000 IU; E, 15000 IU; K, 3g; B1 2g; B2, 4 g; B6, 3g; B12, 0.015 g; pantothenic acid, 10 g; nicotinic acid, 2 g; folic acid, 1 g; choline, 250g ; Se, 100 g.

with 4 replicate pens of 15 chicks. The dietary treatments included of control (C) - without *Peganum harmala* seeds (PHS) and antibiotic - the diet contains 300 mg/kg Lincomycin 0.88% (A) and the diets contain 20g/kg (H1) and 40g/kg (H2) PHS. The basal diet (Table 1) was formulated according to the nutrient requirements (NRC 1994) based on corn and soybean meal: starter (1 to 21 d) and grower (22 to 42 d). *Peganum harmala* seed powder and antibiotic were added on top of the basal diets. The *P. harmala* were supplied from a local market and the dry seeds were cleaned of foreign materials and seeds and milled to a soft powder. The chicks were raised on floor pens (0.096 m²/bird) for 6 weeks and during this period, they received diets and water ad libitum. The bird had access to feed and water through a tube feeder and a manual waterer in each pen. The chicks were reared under a lighting program which included of 23 h of light and 1 h of darkness. The ambient temperature was 32°C ± 1 during the first week, and it was reduced three degrees per week in following weeks and finally it was maintained at 22°C.

Performance: The body weight and feed intake were recorded weekly and mortality was noted as it occurred. The average daily weight gain (ADWG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated per day. The mortality was used to adjust number of birds to compute ADFI, ADWG and FCR. At the end of the experiment (day 42), 2 birds per treatment were randomly selected to study carcass characteristics. After 8 h of starvation, the birds were killed by serving the jugular vein and carotid artery on one side of the neck and allowed

to bleed. They were processed manually to specify carcass yield and relative weight of organs. The blood samples were collected from killed birds to assess of liver enzymes including serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and serum alkaline phosphatase (SALP). The activity of liver enzymes was measured by using commercial kit and auto-analyzer system.

Antibody titer: The birds were vaccinated against Newcastle disease virus (NDV) subcutaneously with 0.2 ml per bird at 7 days of age. The blood samples were collected from the brachial vein of two randomly selected birds of each replicate at 14 and 21 days of age. The samples were centrifuged at 2000 ×g for 15 min to obtain serum. Antibody titers against NDV were measured by hemagglutination Inhibition Test according to procedure described by Thayer and Beard (1998).

Statistical analysis: All data were analyzed by one-way ANOVA using the GLM procedure of SAS for Windows version 9.1 (SAS 1998). The data were analyzed base on following model:

$$Y_{ij} = \mu + T_i + \epsilon_{ij}$$

where Y_{ij} is the dependent variable, μ is the general mean, T_i is the treatment effect of the i^{th} treatment, and ϵ_{ij} is the random error. The significance of differences between means was compared by using of the Duncan's multiple range tests of SAS. Significance was declared at $P < 0.05$ for all variables measured.

RESULTS AND DISCUSSION

Performance parameters: The effect of experimental treatments on performance parameters is presented in Table 2. The impact of experimental treatment on feed intake was significant ($P < 0.05$) in grower and total period. In total period, the using of antibiotic and *P. harmal* in diet resulted in increasing and decreasing feed intake respectively.

Rahbar *et al.* (2011) reported that the inclusion of 0.25 and 40g/kg harmala seed in broilers diet resulted in a significant decrease in feed intake. They explained that this decline in feed intake may be due to β -carbolin alkaloids in harmala seed. It is believed that in rodent α -carbolin alkaloids can react with some cell surface receptor such as 5-hydroxytryptamine (5-HT; serotonin) receptor (Glennon *et al.* 2000) which through it can decrease feed intake (Halford *et al.* 1997). Unpalatability is another factor that can decrease feed intake in poultry. Despite of very low number of taste buds, they can recognize any change in taste (Gentle 1975).

The ADWG was affected significantly ($P < 0.05$) in starter, grower and total periods by using PHS in diet. Broilers receiving PHS had a lower ADWG compared to control group in starter period, but this differences were not significant in grower and total period. The using antibiotic in diet increased this parameter in grower and total period. In total period there is not significant different between antibiotic and H1 groups. The FCR was affected by experimental treatments in grower period. It decreased

Table 2. Effect of treatments on performance parameters of broilers

Performance parameters	Diets				SEM
	C	A	H1	H2	
ADFI1					
0-21 d	31.99	32.77	30.90	32.21	0.550
22-42 d	141.14 ^{ab*}	147.75 ^a	136.70 ^b	135.30 ^b	1.950
0-42d	86.57 ^b	90.26 ^a	83.78 ^c	83.77 ^c	0.285
ADWG2					
0-21d	27.53 ^a	27.81 ^a	24.85 ^b	24.70 ^b	0.519
21-42d	64.36 ^b	71.03 ^a	67.14 ^{ab}	64.35 ^b	1.047
0-42d	45.94 ^b	49.44 ^a	45.99 ^b	44.53 ^b	0.670
FCR3					
0-21 d	1.16	1.18	1.25	1.31	0.024
22-42 d	2.19 ^a	2.08 ^b	2.04 ^b	2.11 ^{ab}	0.018
0-42d	1.88	1.83	1.82	1.89	0.008
BW4(g)					
21d	615.44 ^a	622.97 ^a	558.81 ^b	555.60 ^b	11.05
42d	1963.76 ^b	2141.81 ^a	2029.30 ^{ab}	1900.26 ^b	30.26

*Values in the same row not sharing a common superscript differ significantly ($P>0.05$). SEM, Standard error of mean; 1. average daily feed intake (g per bird/day); 2. average daily weight gain (g/day); 3. feed conversion ratio (g/g); 4. body weight (g); 5. standard error of mean.

in A and H1 groups significantly ($P<0.05$). Although the differences were not significant, but numerically it decreased by using of antibiotic and 20g/kg harmala seeds in total period. In the case of BWG the differences between treatments were similar to ADWG. The mortality data were not analyzed because during the trial only 1 and 3 chickens died in the A and H2 treatments, respectively. In general, harmala seeds at the levels of 20g/kg and 40g/kg in diet resulted in improvement and deterioration of performance parameters, respectively.

Gaskins *et al.* (2002) reported that at least four mechanisms have been proposed as explanations of antibiotic mediated growth enhancement: (1) inhibition of sub-clinical infections, (2) reduction of growth-depressing microbial metabolites, (3) reduction of microbial use of nutrients, and (4) enhanced uptake and use of nutrients through the thinner intestinal wall associated with antibiotic-fed animals. Therefore, antibiotics increase gain and feed efficiency even at constant feed intake.

The better BW and FCR for 20g/kg harmala seeds in diet can be consequence of antibacterial and antiparasitic activity of them. Mashreghi and Niknia (2012) and Darabpour *et al.* (2011) showed inhibitory effect of alcoholic extract of *P. harmala* seeds on the growth of *E. coli*. Arshad *et al.* (2008) announced that the extract of *Peganum harmala* has limited antimicrobial activity against *E. coli in vivo*, but long-term continuous feeding may induce undesirable effects. Tanweer *et al.* (2014b) concluded that *P. harmala* has the anticoccidial effect in broiler chicks. They reported that the adding of a methanolic extract of *Peganum harmala* in drink water at the rate of 200, 250

and 300 mg/L decreased mean oocytes per gram (OPG) linearly ($P<0.05$) compared to control group. These effects can improve the balance of gastro-intestinal microflora and absorption properties of intestine. It is thought that the activity of the intestinal microflora in the host is an important factor that may impact gut function. Inappropriate microflora population in the alimentary canal will result in poor nutrient absorption (Partanen *et al.* 2001) and the increasing energy requirements of maintenance (Furuse *et al.* 1985). Windisch *et al.* (2008) reported that the phytogenic feed additives and antibiotics have similar effects on the gastro-intestinal tract, such as reduced bacterial colony counts, fewer fermentation products (including ammonia and biogenic amines), less activity of the gut-associated lymphatic system, and a greater prececal nutrient digestion. The improvement of immune system is another possible mechanism for increasing of performance. In the present study some immune responses (Table 4) improved by using PHS in diet.

The toxic effects of high levels of *P. harmala* could be considered the main reason for the reduction in growth performance of broilers receiving diet supplemented with 40g/kg harmala seeds. In the present study, the liver enzymes activity increased (Table 3) significantly ($P<0.05$) in broilers receiving 40g/kg harmala seeds in diet. Present findings are in agreement with those of Rahbar *et al.* (2011) who reported that *P. harmala* seeds in 0.4% level of diet had adverse effects on live performance. They attributed these results to the undesirable effects of PHS on the liver and intestinal epithelium. The results of the present experiment also are consistent with Abdel-Malak *et al.* (1995) and Tanweer *et al.* (2012).

Liver enzymes: Inclusion of 40g/kg PHS in diet resulted in a significant increase in SGOT, SGPT and ALP activity relative to the control birds (Table 3). The differences between A, H1 and control groups were not significant ($P>0.05$).

The liver is the first organ may be affected by toxic materials absorbed from the gut. Schmidt and Schmidt (1983) reported that the leaking of cellular enzymes into the plasma is a noticeable indication of hepatic damage. Song *et al.* (2002) reported that the toxic agents cause lysosomal lysis and this in turn lead to death of paranchymal cell which causes an increase in the serum levels of SGOT and SGPT. Estimating the activities of serum marker

Table 3. Effect of experimental diets on liver enzymes activity (unit/litre)

Variable	Treatments				SEM
	C	A	H1	H2	
SGOT	283.75 ^{ab*}	268.00 ^b	268.75 ^b	318.75 ^a	8.42
SGPT	3.75 ^b	4.25 ^{ab}	4.25 ^{ab}	8.25 ^a	0.24
ALP	1973.8 ^b	2765.75 ^{ab}	2531.60 ^{ab}	3306.35 ^a	180.42

*Values in the same row not sharing a common superscript differ significantly ($P<0.05$). SEM, Standard error of mean.

enzymes like AST, ALT, ALP and bilirubin can make assessment of liver function. Although ALT is present in several organs and in muscle, the highest levels are in hepatocytes, which makes this enzyme a more specific indicator of liver injury. Both AST and ALT are released into the blood in greater amounts when hepatocytes are damaged (Aragon and Younossi 2010). Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage (Mitra *et al.* 1998). According to our findings, Mohamed *et al.* (2013) reported that the repeated treatment of *peganum harmala* seeds alcoholic extract at 150 mg/kg dose caused severe destruction of hepatic cells, pyknotic of hepatic cell nuclei and vesiculation in the cytoplasm due to fatty degeneration in Albino mice. Similarly, Qazan (2009) observed hepatotoxicity, widespread congestion and hemorrhage in chicks fed diet containing 10% *P. harmala* leaves. In contrast to these reports, Diwan (2013) observed the amelioration effect of *Peganum harmala* methanol seed extract on elevated levels of liver enzymes in mice administered MTX drug. He suggested that this effect could be due to the presence of flavonoids which are known for their excellent antioxidative capacity in various model systems. Therefore, it seems that the effect of PHS on liver function depends on its consumption level.

Immunity parameters: Use of antibiotic and PHS in diet (Tables 4, 5) failed to induce any significant effects on antibody titers against NDV at 14 and 21 days of age ($P > 0.05$). Nevertheless, the relative weight of lymphoid organs was affected by experimental treatments. The relative weight of bursa and spleen was significantly ($P < 0.05$) higher for birds fed diets supplemented with antibiotic and 40g/kg harmala seeds.

It seems that the effect of PHS has been limited to the mucosal immune system and not the systemic portion of the immune system. Similar results have been reported in

some previous researches (Rahimi *et al.* 2011, Goodarzi *et al.* 2013) with other herbs. The increase of relative weight of bursa and spleen could have been due to active compounds of *P. harmala* which have some activities such as antibacterial and antifungal (Darabpour *et al.* 2011) and anticoccidial (Tanweer *et al.* 2014b). The bursa of fabricius, is one of the primary lymphoid organs of birds primarily responsible for the proliferation and differentiation of B cells (Ratcliffe 2006). The higher bursa weight can be an indicator of high immune activity. Zhang *et al.* (2006) suggested that the size of bursa of fabricius is different between poultry breeds and the production of antibody is related to bursa size. The increase of immune tissue weight makes an effect on immune cell phenotypes, immune cell proliferation, and antibody production. According to the present data, it seems the consumption of PHS did not impair the health of birds even though body weights were depressed by this herb at 40g/kg level. Our findings are in contrast with Tanweer *et al.* (2014a) who reported that the methanolic extract of *P. harmala* significantly improved antibody titer against ND at day 21 and 28 when used at the rate of 250 mg/L of drinking water. Ghareghani Poor *et al.* (2014) reported that *P. harmala* extract with optimal dose of 100 mg/kg can act as immunostimulants and enhance the immune response of cultured fish.

The results of this study showed that *Peganum harmala* seeds cannot be substituted as alternative to in-feed antibiotics, but at the level of 20 g/kg of feed can improve production efficiency of broiler chickens. The dietary inclusion of 40 g/kg PHS had unfavorable effect on performance parameters and liver function. Also, the using PHS in diet led to the larger bursa fabricius that it can be an indicator of high immune activity.

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Table 4. Effect of experimental diets on relative weight lymphoid organs at 42nd day

Lymphoid organs	Dietary treatments				SEM
	C	A	H1	H2	
Bursaa	0.199 ^{b*}	0.243 ^a	0.196 ^b	0.239 ^a	0.006
Spleena	0.095 ^{bc}	0.119 ^a	0.083 ^c	0.110 ^{ab}	0.005

*Values in the same row not sharing a common superscript differ significantly ($P < 0.05$). SEM, Standard error of mean.

Table 5. Effect of experimental diets on antibody titers against NDV at 14th and 21st days

Antibody titers (log10)	Dietary treatments				SEM
	C	A	H1	H2	
14 days	0.696	0.750	0.706	0.737	0.014
21 days	0.812	0.817	0.807	0.828	0.013

SEM, Standard error of mean.

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