



## RESEARCH ARTICLE

### Association of *Peganum harmala* L. Supplementation with Lipid Profile and its Economic Benefit in Broiler Production

Abdul Jabbar Tanweer, Naila Chand<sup>1</sup>, Sarzamin Khan\*<sup>1</sup>, Asad Sultan<sup>1</sup>, Muhammad Subhan Qureshi<sup>1</sup>, Amanullah Akhtar, Muhammad Inam<sup>1</sup> and Rafiullah<sup>1</sup>

Gomal College of Veterinary Sciences, Gomal University, Dera Ismail Khan; <sup>1</sup>Faculty of Animal Husbandry and Veterinary Sciences, Khyber Pakhtunkhwa Agricultural University, Peshawar, Pakistan.

\*Corresponding Author: [dr.zaminaup@gmail.com](mailto:dr.zaminaup@gmail.com)

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#### ABSTRACT

Concentration of cholesterol and other lipids in human diet has been an issue of public health concern. In present study the effects of methanolic extract of *Peganum harmala* L. (*P. harmala*) on serum lipid profile and feeding cost of broiler birds were investigated in a 28-days feeding trial. Total 240 one-week old broiler chicks were divided into four groups (Ph-0, Ph-200, Ph-250 and Ph-300) that were replicated six times (10 birds/replicate) and were randomly given methanolic extract @ 0, 200, 250 and 300 mgL<sup>-1</sup> of drinking water, respectively. Standard management practices were adopted. Feed and water were provided *ad libitum*. At the end of every week, two birds from each replicate were randomly selected to collect blood samples for serum lipid profile determination. Serum samples were analyzed for total cholesterol, high and low density lipoprotein and triglycerides. Triglycerides were measured by the enzymatic calorimetric method. Total cholesterol, triglycerides and LDL cholesterol showed gradual significant decrease and nevertheless HDL gradual increase with the increasing dose levels of *P. harmala* up to 250 mgL<sup>-1</sup> of drinking water. Feed cost was significantly ( $P < 0.05$ ) reduced by methanolic extract of *P. harmala* and was the lowest in group Ph-250. The same group had the highest ( $P < 0.05$ ) gross return compared to other treatments. It is concluded that methanolic extract of *P. harmala* @ 250 mgL<sup>-1</sup> drinking water could be effectively used in broilers to optimize serum lipid profile, to reduce feeding cost and to maximize gross return.

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#### INTRODUCTION

Medicinal plants have been used for centuries as remedies for human and animal ailments (Raziq *et al.*, 2012; Jahan *et al.*, 2012; Abbas *et al.*, 2012). They have many pharmacologically active chemical compounds which may act as anthelmintic (Chaturvedi *et al.*, 2009), antibacterial (Jung *et al.*, 2011; Shah *et al.*, 2012; Shahzad *et al.*, 2012) and antifungal (Rosina *et al.*, 2009) agents. Therefore, medicinal herbs have been reported to serve as safer alternative as growth promoter due to their suitability and preference, lower cost of production, reduced risk of toxicity, minimum health hazards and environment friendliness.

*Peganum harmala* (locally known as harmal) belongs to the family of Zygophyllaceae and have been shown a

diverse range of medicinal properties. Numerous beta carboline alkaloids like harmaline, harmine, harmalol and harmol were present in *P. harmala* extract (Herraiz *et al.*, 2010). Abdel-Fattah *et al.* (1997) reported that *P. harmala* extract exhibited great variety of pharmacological and biological activities such as antibacterial and antifungal agents as well as monoamineoxidase (MAO) inhibition and hypothermia. Similarly analgesic, anti-inflammatory (Monsef *et al.*, 2004), disinfectant (Shahverdi *et al.*, 2005), growth promoting (Qazan, 2009), cholesterol lowering and hepatoprotective effects (Hamdan *et al.*, 2008) properties have also been reported. There is dearth of information regarding the impact of feeding methanolic extract of *P. harmala* on serum lipid profile and its economic benefits in broiler chicks. Present study was designed to examine the potential benefits of methanolic

extract of *P. harmala* in terms of reducing serum cholesterol level and improving the economic output of broiler production.

### MATERIALS AND METHODS

Present study was undertaken at Khyber Pakhtunkhwa Agricultural University Peshawar and was pre-approved by the Board of Studies of the Department of Poultry Science for animal welfare issues.

**Bird husbandry and experimental procedure:** Three hundred day-old broiler chicks were purchased from commercial hatchery and were given a commercial feed during pre-experimental period of 7 days in optimum environmental conditions. On day 8<sup>th</sup>, two hundred and forty birds of similar body weight and physical appearance were transferred to 24 floor pens in an open sided house (10 birds/pen) and were grouped (n=4; 6 pens/group) as Ph-0, Ph-200, Ph-250 and Ph-300 receiving methanolic extract of *P. harmala* at the rates of 0, 200, 250 and 300 mg L<sup>-1</sup> of drinking water, respectively. Similar management practices were adopted for all experimental groups to maintain an optimum environmental condition. All the birds had free access to feed and drinking water during the experimental period that lasted for 28 days. Methanolic extract of *P. harmala* was dissolved in fresh drinking water at different doses on daily basis and were offered to birds. The dose rate was adjusted according to Abaza (2001) and infusion was prepared according to Leila (1977) method of preparation of water based infusion.

**Methanolic extraction of *P. harmala*:** Dried clean seed of *P. harmala* was obtained from Medicinal Herbs Section of Pakistan Forest Institute (PFI), Peshawar. The seed was ground to 1 mm in an electric herbs grinder (Wenzhou Arts and Crafts International Trade Co., Ltd, China). To obtain methanolic extract 1 kg ground seed was immersed in 3 liters 80 % (v/v) aqueous methanol at room temperature for five days and filtered through Wattmann Filter Paper (No.42). Extraction was performed at Hussain Ebrahim Jamal Research Institute of Chemistry, Department of Chemistry, University of Karachi, Karachi, Pakistan. The filtrate was then transferred to a rotary evaporator (BÜCHI Labortechnik, Switzerland) to evaporate the aqueous methanol under low pressure. The extract was then transferred to a glass bottle and stored in refrigerator before use.

**Serum lipid profile:** At the end of every week, two birds from each replicate were randomly selected for blood sampling and biochemical analysis. Blood samples were collected at 7:00-8:00 a.m. after fasting for a period of 2 hours. All blood samples were centrifuged at 4,000 rpm for 10 minutes to get the serum separation. Serum samples were analyzed for total cholesterol, high and low density lipoprotein and triglycerides using Bio-chemical analyzer (Micro Lab 200 Merck) and Elitech kit as described by Allain *et al.* (1974). Triglycerides were measured by the enzymatic calorimetric method of Werner *et al.* (1981). HDL (High Density Lipoprotein), VLDL (Very Low-Density Lipoproteins), and LDL (Low-Density

Lipoproteins) were precipitated from serum by adding phosphotungstic and magnesium ions, subsequently were determined as the previous methods (Lopes-Virella *et al.*, 1977).

LDL Cholesterol was calculated by the following formula: LDL Cholesterol (mg/dL) = Total cholesterol (mg/dL) - (TGR (mg/dL)/5) + HDL cholesterol (mg/dL)

**Economics of feeding *P. harmala* extract:** Data were recorded for body weight gain on weekly basis. Chicks were weighed at the start of experiment and at the end of each week. Initial weight was subtracted from final weight to obtain weight gain. Total weight gain was calculated at the end of this study. Economics of the research study was calculated in terms of total expenditure incurred and gross return. Mean feed cost per chick was calculated according to the market rate. The cost of *P. harmala* extract used was included. Mean gross return per chick was calculated according to market rate of live bird on per kg basis. Data were statistically analyzed using the completely randomized design in SAS (1998).

### RESULTS AND DISCUSSION

**Cholesterol:** The effects of different levels of *P. harmala* methanolic extract on total cholesterol were presented in Table 2. Cholesterol level was significantly altered by *P. harmala* extract on day-14, 21, 28, and 35. Group Ph-250 had the lowest serum total cholesterol value among all different treatments and days of sampling. Serum total cholesterol was decreased with the increasing level of *P. harmala* extract upto 250 mgL<sup>-1</sup> drinking water. Decrease in total cholesterol by *P. harmala* might be due to the inhibition of 3-hydroxy-3methyl-glutaryl-CoA (HMG-CoA) reductase by different alkaloids harmine, harmaline, harmol, present in *P. harmala*. These alkaloids have also been reported to have hypoglycemic properties. *P. harmala* extract has no insulin secretion activity, so the possible hypoglycemic activity is not related to pancreas and may be it affects by using or/and absorption of glucose (Singh *et al.*, 2008). *P. harmala* has antioxidant property (Jinous and Fereshteh, 2012) which reduce LDL oxidation and thereby reducing total cholesterol content. Abaza (2001) fed 2.5 kg *P. harmala* seeds and 2.5 kg chamomile flower heads per ton of broiler diet and observed reduced serum cholesterol as compared to the control group, which supported the findings of the present work. Similarly, Qazan (2009) added 10% *P. harmala* leaves to broiler ration and found the drop of serum cholesterol. The variation in findings of reducing cholesterol is probably due to the difference in experimental designs, ages and species of animal and types and parts of the plant used. Reddy and Srinivasan (2009) reported 26-31% reduction of serum cholesterol when supplemented with *Trigonella foenum-graecum* on mice. Anuja *et al.* (2012) fed an aqueous extract of *Stevia rebaudiana*, *Momordica charantia*, *Tamarindus indica*, *Gymnema sylvestre*, *Allium sativum* and *Murraya koenigii* to rats and observed decreased serum cholesterol level. The mechanism and efficacy of diverse medicinal herbs to reduce serum cholesterol level might be due to the presence of different levels and types of alkaloid.

**Table 1:** Effect of administration of different levels of methanolic extract of *Peganum harmala* on feed cost and gross return in broiler chicks

Group	Mean feed cost (Rs) per chick	Mean gross return (Rs) per chick
Ph-0	37.50 <sup>a</sup>	85.53 <sup>b</sup>
Ph-200	37.50 <sup>a</sup>	83.93 <sup>b</sup>
Ph-250	34.50 <sup>b</sup>	94.98 <sup>a</sup>
Ph-300	36.00 <sup>a</sup>	80.45 <sup>c</sup>
LSD	2.01	3.12

Ph = *Peganum harmala* levels; 0-300 = 0-300 mgL<sup>-1</sup> of drinking water; Means within a column with different superscripts are significantly different at  $\alpha = 0.05$ ; LSD= Least significant difference.

**Triglycerides:** *P. harmala* methanolic extract significantly ( $P < 0.05$ ) modulated serum triglycerides levels of broiler birds in different treated groups (Table 2). This impact was observed at day-14, 21, 28 and 35. The pattern of reducing triglycerides by different level of *P. harmala* was similar to that of serum cholesterol. Significantly ( $P < 0.05$ ) greater reduction in serum triglycerides happened in group (Ph-250) that were given extract 250 mg L<sup>-1</sup> in drinking water on day-14, 21, 28 and 35 compared to other treated and control groups. The decrease in serum triglyceride levels of birds in different treated groups could be due to the possible action of active alkaloids harmine or harmaline of the *P. harmala* on reducing the activity of lipoprotein lipase enzyme that has been reported to cause decrease in serum triglyceride level (Bertram, 2007).

No published work, to the best of our knowledge, indicates the effect of methanolic extract of *P. harmala* on serum triglycerides level in broilers. Other medicinal plants with different types of alkaloids have however, been reported to exhibit the reducing effect on the serum triglycerides levels in different species of animals. Emmanuel and James (2011) reported that an aqueous extract of garlic (*Allium sativum* L.) and onion (*Allium cepa* L.) could significantly decrease serum triglycerides in white Albino rats. Similarly, *Withania somnifera* reduced egg yolk triglycerides in laying hen (Saleem *et al.*, 2011). Of the relevant importance is the study of Shikha *et al.* (2011) who noted that medicinal plants extract can significantly ( $P < 0.05$ ) reduce serum triglycerides in broiler birds.

**High density lipoprotein (HDL) cholesterol:** Significant differences ( $P < 0.05$ ) were recorded between the control group and treated groups as well as among the treated groups at all recorded stages. The effect of different levels of *P. harmala* methanolic extract on serum HDL in broiler chicks is presented in Table 2. There was gradual increase in the HDL level with increasing level of *P. harmala* at all recorded stages in all groups. The lowest HDL values were recorded for control group and highest values were recorded for group Ph-250 and Ph-300 at all recorded stages.

No relevant literature is available regarding effects of *P. harmala* on HDL. However, other medicinal plants have been explored for their HDL increasing potential. Manan *et al.* (2012) reported that herbal infusion of *Berberis lycium*, *Allium sativum*, *Solanum nigrum* and *Terminalia arjuna* increased HDL, reduced LDL and cholesterol in broiler chicks. Findings of current study are opposed by the findings of Nasim *et al.* (2010) who worked on garlic feeding in broiler birds but did not observe any significant ( $P > 0.05$ ) effect on HDL level.

**Low density lipoprotein (LDL) cholesterol:** *P. harmala* methanolic extract had a significant influence on altering LDL cholesterol level of broiler birds at all different ages examined in present study as shown in Table 2. Significant difference between control group and treated groups as well as among the treated groups was observed at all timings of sampling except on day-14 and 21. Birds in group Ph-250 had significantly reduced serum LDL. The tendency to reduce LDL at varying stages by the alkaloids of *P. harmala*, have been reported to inhibit the mobilization of fatty acids into the liver and prevent the synthesis of very low density lipoprotein (VLDL) and LDL (Laurence *et al.*, 2005). The reduced level of LDL in present study in treated groups might also be due to the methanolic extract having potential alkaloids that might have acted physiologically to prevent the transport of fatty acids for the formation of LDL into liver.

To our knowledge, no such data has been reported regarding effects of *P. harmala* on LDL cholesterol, however, effect of other medicinal plants has been reported for LDL decreasing potentials. Our findings are also in

**Table 2:** Effect of administration of different levels of methanolic extract of *Peganum harmala* on total cholesterol, triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol in broiler chicks

Parameters	Groups	Experimental Days				
		7	14	21	28	35
Total cholesterol (mg/dL)	Ph-0	139.8±3.1	135.6±3.1a	143.7±4.6a	141.9±5.3a	141.9±5.2a
	Ph-200	135.3±5.2	117.1±1.4b	114.5±5.9c	120.7±5.6bc	126.9±3.4b
	Ph-250	126.7±6.8	103.3±5.4c	112.2±3.1c	112.1±6.6c	112.2±5.9c
	Ph-300	136.7±5.3	126.1±4.2ab	128.9±5.4b	130.9±3.3ab	128.7±3.9b
	Ph-0	67.7±4.0	80.8±3.9a	84.2±2.1a	92.2±6.8a	92.9±5.0a
Triglycerides (mg/dL)	Ph-200	80.4±4.3	74.6±3.9ab	78.4±4.9a	71.7±3.4b	69.4±4.4b
	Ph-250	76.9±4.4	51.3±4.02c	43.4±4.7c	43.3±3.1c	50.8±2.94c
	Ph-300	73.5±4.1	63.9±3.2b	65.1±4.2b	62.7±2.9b	67.2±3.6b
	Ph-0	40.9±2.3	38.1±0.70c	36.6±1.6c	37.1±1.4c	34.9±1.6c
	Ph-200	39.7±2.2	50.4±1.8b	50.8±2.9b	50.4±1.9b	47.07±1.4b
HDL (mg/dL)	Ph-250	38.0±1.7	53.9±1.7a	60.9±4.4a	57.5±2.9a	52.7±1.5a
	Ph-300	38.8±0.8	53.3±1.9ab	56.8±1.4ab	55.0±1.5ab	56.2±2.3a
	Ph-0	167.2±4.9	157.6±3.6ab	163±4.9b	160.6±5.5b	158.3±3.8b
	Ph-200	158.9±4.9	152.6±3.0bc	155.9±3.6bc	156.7±5.1b	166.2±4.4ab
	Ph-250	150.0±6.3	144.5±5.6c	149.6±4.3c	154.5±7.9b	145.4±5.9c
LDL (mg/dL)	Ph-300	160.1±5.7	159.2±5.6a	181.2±3.1a	179.8±4.1a	174.8±3.3a

Values (mean±SE) bearing different superscripts under a specific parameter in a column differ significantly ( $P < 0.05$ ).

agreement with Manan *et al.* (2012), who reported reduction in LDL cholesterol by herbal infusion of *Berberis lycium*, *Allium sativum*, *Solanum nigrum* and *Terminalia arjuna* in broiler chicks.

**Economics of the study:** Mean feed cost was significantly ( $P < 0.05$ ) affected by methanolic extract of *P. harmala*. Mean feed cost and gross return per chick are presented in Table 1. Feed cost was the lowest in group Ph-250 ( $P < 0.05$ ), and mean gross return was the highest ( $P < 0.05$ ) in group Ph-250. Lowest gross return was found in group Ph-300. Our findings are in agreement with that of Durrani *et al.* (2006), who fed Turmeric (*Curcuma longa* L.) to broiler birds and reported less feed cost per bird for the treated groups as compared to the control.

**Conclusion:** It is concluded from the results of the study that total cholesterol, triglycerides and LDL cholesterol showed gradual significant decrease with the increasing dose level of *P. harmala* up to 250 mgL<sup>-1</sup> in drinking water, while HDL cholesterol showed significant increase with increasing level of *P. harmala*. This level also had maximum return per bird.

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