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Effects of Hogweed (*Heracleum persicum*) Powder, Flavophospholipol, and Probiotics as Feed Supplements on the Performance, Carcass and Blood Characteristics, Intestinal Microflora, and Immune Response in Broilers

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The effect of different levels of hogweed powder (HP; *Heracleum persicum*), flavophospholipol (antibiotic), and probiotics in diet on the performance, carcass quality, blood biochemical parameters, immunity, and intestinal flora of broiler chickens was investigated. In total, 270-day-old male broilers were randomly assigned to six treatment groups as follows: control basal-diet and diet supplemented with flavophospholipol, probiotics, or 0.25, 0.5, and 0.75% HP. Birds in each group were divided into three subgroups with 15 chicks each. Results indicated that the treatment groups did not vary with respect to feed intake (FI), whereas those supplemented with the antibiotic or 0.5% HP showed significantly higher body weight gain (BWG) and improved feed conversion ratio (FCR). Carcass characteristics did not vary among treatments, with the exception of abdominal fat percentage, which was the lowest in broilers fed 0.5% and 0.75% HP. Supplementation of 0.5% and 0.75% HP decreased plasma cholesterol and triglyceride levels. Furthermore, dietary HP significantly reduced serum low density lipoprotein (LDL) levels compared to that in the other groups. Antibody titers against Newcastle disease vaccine were not markedly affected by the treatments, whereas titers against avian influenza vaccine were significantly higher in probiotic- and 0.75% HP-supplemented groups. Antibody production against sheep red blood cells (SRBC) and IgM and IgG levels were not significantly different among groups. The ileum *Lactobacillus* counts in broilers fed 0.5% or 0.75% HP were significantly higher than those in the other treatment groups, whereas *Escherichia coli* counts in all treatments were significantly lower than that in the control. Therefore, our observations indicated that HP positively affected the gut microbiota and enhanced feed digestion. In conclusion, supplementation of 0.50−0.75% HP in broiler diet during the entire rearing period improved BWG and decreased abdominal fat deposition.

Key words: blood, broiler, *Heracleum persicum*, immune system, intestinal microflora, probiotics

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Introduction

Currently, one of the major concerns regarding the consumption of commercially manufactured food is the presence of excess antibiotics and other medical products, especially in poultry items, as this may lead to the development of antibiotic-resistant bacteria and transmission of antibiotic residues into the human food chain (Sanjyal and Sapkota 2011; Dhama *et al*., 2015). Spices, plant extracts, and various phytobiotics derived from the leaves, roots, tubers, or fruits of herbs have shown excellent growth enhancement in poultry (Wallace *et al*., 2010; Khan *et al*., 2012a; Dhama *et al*., 2015). This may be due to the synergistic action of various active molecules and higher feed utilization efficiency, resulting in enhanced productive performance (Hashemi and Davoodi, 2010; Khan *et al*., 2012b).

Heracleum persicum (HP) (family: Apiaceae), commonly known as Persian hogweed or golpar, is a flowering plant native to Iran, the seeds of which are used as spice in Persian

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cooking and in traditional and folk medicine (Hasani *et al*., 2016). Hogweed contains many secondary substances with antioxidant, anticonvulsive, antibiotic, antifungal, and immunestimulatory activity (Nazemi *et al*., 2005; Sayyah *et al*., 2005; Shahrani *et al*., 2006; Asgarpanah *et al*., 2012). The chemical composition of different parts of hogweed has been investigated and results indicate the presence of six furanocoumarins and flavonoids in the fruits, leaves, flowers, and roots (Sefidkon *et al*., 2004; Hajhashemi *et al*., 2014). Previous studies have shown that certain furanocoumarins isolated from hogweed possess antioxidant property against lipid peroxidation (Souri *et al*., 2004), as well as lipid lowering activity (Phuwapraisirisan *et al*., 2006). Thus, addition of hogweed leaf powder to broiler diet may improve gut microbiota function and consequently bird growth and performance.

Currently, studies on the productive performance of broilers supplemented with hogweed are limited (Kheiri *et al*., 2014), although the effects of dietary probiotics (Balevi *et al*., 2001; Torres-Rodriguez *et al*., 2005; Jahromi *et al*., 2016; Seidavi *et al*., 2017; Tufarelli *et al*., 2017) and flavophospholipol antibiotics have been extensively studied (Shamsi *et al*., 2015a, b). Therefore, the objectives of this study were to compare the effects of different amounts of hogweed leaf powder, along with probiotics and flavophospholipol antibiotics, on the performance, carcass quality, blood biochemical parameters, immunity, and intestinal flora of broiler chicks.

Materials and Methods

All procedures used in the study were approved by the institution's Ethic Committee, and care was taken to minimize the number of animals used.

Animals and Dietary Treatments

In total, 270-day-old male Ross 308 chicks (Aviagen, Newbridge, Scotland, UK) were randomly assigned to one of six treatment groups while considering that the mean group body weights were similar in each group at the beginning of the experiment. Each group contained 45 birds, and three replicates were made with 15 birds each per group. The study lasted 42 days. Birds were fed isocaloric and isonitrogenous diets, and the ingredients, as well as the calculated nutrient composition, of diets used during starter (1−21 days of age) and finisher (22−42 days of age) periods are shown in Table 1. Nutritional requirements were based on the standard recommendations of the chick's producer (Ross Manual).

The experimental dietary treatments were based on different amounts of hogweed powder, probiotics (PrimaLac), or flavomycin (flavophospholipol antibiotic) as follows: treatment 1: control (basal-diet); treatment 2: basal-diet supplemented with flavophospholipol antibiotics (0.525 g/kg); treatment 3: basal-diet supplemented with probiotics (0.900 g/kg from 1st to 14th days of age, 0.454 g/kg from 15th to 28th days of age, 0.225 g/kg from 29th to 42nd days of age) from 1st to 42nd days of age; treatment 4: basal-diet supple-

Table 1. **Ingredients and nutritional composition of diets**

Ingredients (%)	1−21 days of age	22−42 days of age
Corn	56.9	58.7
Soybean meal (43% CP)	33.1	30.0
Fish meal	3.4	3.5
Soybean oil	2.0	3.5
Dicalcium phosphate	1.55	1.55
Oyster shell	1.03	1.18
DL-methionine	0.01	0.01
Vitamin premix*	0.5	0.5
Mineral premix**	0.5	0.5
Salt	0.26	0.26
Sand	0.75	0.75
Nutritional composition		
ME (kcal/kg)	2,910	3,030
Crude protein (%)	20.1	19.0
Crude fat (%)	4.6	6.14
Calcium (%)	0.95	0.9
Total phosphorus (%)	1.23	1.06
Available phosphorus (%)	0.45	0/36
Methionine (%)	0.5	0.38
Lysine (%)	1.01	1.00
Methionine + Cysteine (%)	0.83	0.71

* Vitamin A, 360,0000 IU; D3, 800,000 IU; vitamin E, 7,200 IU; vitamin B1, 710 mg; vitamin B2, 2,640 mg; vitamin B6, 1,176 mg; vitamin B9, 400 mg; vitamin B12, 6 mg; vitamin K3, 800 mg; pantothenic acid, 3,920 mg; biotin, 40 mg; niacin, 12,000 mg; choline chloride, 200,000 mg. ** Mn, 40,000 mg; Fe, 20,000 mg; Zn, 33,900 mg; Cu, 4,000 mg; I, 400 mg, and Se, 80 mg.

Table 2. **Bacterial content of probiotics used**

Bacteria	CFU/g	pH
Lactobacillus acidophilus	2.5×10^7	5.5-7.0
Lactobacillus casei	2.5×10^7	4.0-9.0
Enterococcus faecium	2.5×10^7	4.0-9.0
Bifidobacterium thermophilum	2.5×10^7	4.0-9.0

mented with 0.25% HP; treatment 5: basal-diet supplemented with 0.50% HP; treatment 6: basal-diet supplemented with 0.75% HP.

Feed Supplements

The probiotic (PrimaLac, Star-Labs/Forage Research Inc. Clarksdale, MS, USA) was a lyophilized mix containing 2.5×10^7 CFU/g *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium thermophilum*, and *Enterococcus faecium* (Table 2). The flavophospholipol antibiotic (Flavomycin-4; HuvePharma Inc., Peachtree City, GA, USA) contained 6.8 g bambermycin per kg of the premix. Hogweed powder was purchased from a local market.

Housing

The birds were housed in 18 pens in a broiler rearing facility. Prior to the experiment, the facility was carefully cleaned, including all drinkers and feeders, and was subsequently disinfected (Aqua GPC® 10). All drinkers and feeders were immersed in a 20% benzalkonium chloride solution and the facility was left to dry for two days. Thereafter, the nonflammable parts were ignited, including the floor and metal walls of the pens. The walls were subsequently sprayed with water and lime. Ventilation was turned on to optimize the climate 24 h before the broilers were brought in. The facility was equipped with eight ventilators and two strong ventilators. A heater was used, and the temperature program was set according to the instructions for Ross 308 broilers (infoworldwide@aviagen.com). Air humidity was maintained at 55-65% in the early growing period by spraying water on the floor. Twenty-Watt lamps were installed at 2.2 m above the floor, and the light was kept on for 23 h daily. The birds were vaccinated against infectious bronchitis (1- and 18-day-old), Newcastle disease (1- and 18-day-old), avian influenza (1-day-old) and Gumboro disease (14- and 24-day-old).

Evaluated Parameters

The feed intake (FI) and body weight (BW) were recorded weekly and feed conversion ratio (FCR) was calculated. Two birds per replicate were selected at 42 days of age after 4 h of fasting. The most representative birds with BWs displaying the mean group weight were carefully selected, sacrificed, and used for measuring carcass yield, and meat and gastrointestinal tract characteristics. The birds were completely pecked using the dry pecking method. The neck, wing tips, gut, and liver were removed, and the empty or edible carcass was weighed. Furthermore, the intestinal segments were measured. Carcass cuts were dissected and weighed.

The birds were fasted for 4 h prior to blood sampling in the morning. At 42 days of age, 5 mL venous blood was collected from the wing vein of the selected bird from each replicate. Blood was transferred from the syringe into a tube coated with 10 mg EDTA and centrifuged at 3,000 rpm×20 min to separate blood cells from the plasma. Plasma was stored at −20℃ until further analyses. The plasma cholesterol and triglyceride levels were determined using enzymatic methods (Teif Azmoon Pars Co., Tehran, Iran), and high density lipoprotein (HDL) and LDL cholesterol levels were measured directly using diagnostic kits (Teif Azmoon Pars Co.). Total cholesterol in plasma samples was colorimetrically determined following the procedure of Barham and Trinder (1972). Plasma triglyceride levels were measured using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol, which is converted to pyruvate and subsequently to lactate. Decrease in absorbance, measured spectrophotometrically, is proportional to the triglyceride concentration in the sample (Schmid and Forstner, 1986). Glucose oxidase and uric acid-uricase kits (both from Teif Azmoon Pars Co.), based on the oxidase-peroxidase method (Trinder, 1969), were used to measure plasma glucose and uric acid levels. Sera for antibodies against Newcastle disease were sampled on days 28 and 42. Non-specific humoral immune response was determined based on the response to sheep red blood cells (SRBC). SRBC was injected on days 21 and 35 and sampling was performed on days 28 and 42.

Microbiota Analysis

On days 21 and 42, two chicks were selected from each replicate and sacrificed. Cecum contents were collected for microbial culture. De Man, Rogosa, and Sharpe agar (MRS, 1.10660.500) was used to culture lactobacilli, nutrient agar (1.05450.0500) was used to determine total aerobic bacteria counts, eosin-methylene blue agar (EMB, 1.01347.0500) was used to culture *E. coli*, and MacConkey agar (105465.0500) was used to culture coliforms. Serial dilutions were prepared for estimating colony forming units (CFU), which were counted after a 72 h incubation period at 37℃ using a colony counter.

Statistical Analysis

Data were analyzed using the general linear model of the SPSS software (1997). The significance level was set at $P<0.05$ and differences among main mean effects were assessed using Duncan's multiple range test.

Results and Discussion

The effects of inclusion of dietary HP, flavophospholipol, and probiotics as feed supplements in broiler diet on bird growth, carcass and blood characteristics, intestinal microflora, and immune response have not been reported. Therefore, the results of this study will be discussed in the context of observations made in other livestock species. Results of the present study are summarized in Tables 3-13. Although the FI of broilers in groups fed 0.50% and 0.75% HP tended to be slightly higher, the difference was not significant. In contrast, birds fed antibiotic, probiotic or 0.50% and 0.75% HP had significantly higher BWG. Conversely, the BWG in

Table 3. **Effect of dietary treatments on cumulative feed intake (g) of broilers**

Treatments	Starter period	Finisher period	Total period
Control	973	2701	3675
Flavophospholipol	978	2690	3668
Probiotics	976	2692	3667
0.25% HP	974	2694	3668
0.50% HP	980	2724	3704
0.75% HP	980	2732	3711
SEM	3.0	14.1	15.0
P-value	0.157	0.235	0.166

SEM: Standard error of means.

Table 4. **Effect of dietary treatments on body weight (g) of broilers at the different growing stages**

Treatments	Starter period	Finisher period	Total period
Control	605^{c}	1342^{c}	1947^{c}
Flavophospholipol	687^{a}	1465^{a}	2152^{a}
Probiotics	671^{b}	1410^{b}	2081^{b}
0.25% HP	609^{c}	1347^{c}	1956^{c}
0.50% HP	681^{ab}	1440^{ab}	2122^{a}
0.75% HP	687^{a}	1452^{a}	2139^{a}
SEM	3.0	11.0	12.0
P-value	0.001	0.001	0.001

Means with different subscripts in the same column differ significantly ($P<0.05$); SEM: Standard error of means.

Table 5. **Effect of dietary treatments on feed conversion ratio (g feed/g gain) of broilers**

Treatments	Starter period	Finisher period	Total period
Control	1.6^{a}	2.0^{a}	1.9^{a}
Flavophospholipol	1.4^{c}	1.8^{c}	1.7^{c}
Probiotics	1.5^{b}	1.9^{b}	1.8^{b}
0.25% HP	1.6^{a}	2.0^{a}	1.9^{a}
0.50% HP	1.4^{bc}	1.9^{bc}	1.70^{c}
0.75% HP	1.4^{c}	1.9^{bc}	1.7^{bc}
SEM	0.01	0.01	0.01
P-value	0.001	0.001	0.001

Means with different subscripts in the same column differ significantly ($P<0.05$); SEM: Standard error of means.

broilers fed 0.25% HP was not significantly different from those of birds fed control diet. This suggests that 0.25% HP is too low to support good growth performance. Compared to other groups, broilers in groups fed antibiotic-, probiotic-, or 0.50% and 0.75% HP-supplemented diets showed significantly improved FCR ($P<0.05$). Thus, supplementation of the basal-diet of broilers with 0.50% or higher levels of HP, as well as with probiotics or antibiotics, resulted in better and efficient growth. In a recent study, Kheiri *et al*. (2014) evaluated the performance traits of broilers fed different levels of hogweed extract via drinking water and observed improved response of the treated birds.

For economic utility, the observed better growth must be accompanied by better carcass yield and quality. However, neither the percentage carcass yield nor the percentage of breast muscle and drumsticks differed significantly among the groups, indicating that the weight gain was evenly distributed over the entire body, with the exception of abdominal fat deposition. A small, but significant decrease in abdominal fat percentage was observed in birds fed 0.5% and

Table 6. **Effect of dietary treatments on relative carcass characteristics and organ yield (%) of 42-day-old broilers**

Treatments	Carcass	Breast	Thigh	Wing	Liver	Gizzard	Abdominal fat
Control	74	28	26	0.8	0.3	0.3	0.18[a]
Flavophospholipol	75	29	27	0.8	0.4	0.3	0.17[b]
Probiotics	75	28	26	0.8	0.3	0.3	0.11[c]
0.25% HP	75	28	26	0.8	0.3	0.3	0.13[c]
0.50% HP	74	29	26	0.8	0.3	0.3	0.11[d]
0.75% HP	75	29	27	0.8	0.3	0.3	0.10[d]
SEM	0.60	0.41	0.22	0.01	0.01	0.01	0.01
P-value	0.449	0.814	0.814	0.407	0.599	0.270	0.0001

Means with different subscripts in the same column differ significantly ($P<0.05$); SEM: Standard error of means.

Table 7. **Effect of dietary treatments on organ weight (g) of 42-day-old broilers**

Treatments	Pancreas	Hearth	Spleen	Thymus	Bursa Fabricius
Control	5	12	2	4	2
Flavophospholipol	6	14	2	5	2
Probiotics	4	16	2	3	2
0.25% HP	4	13	2	5	1
0.50% HP	6	12	2	4	1
0.75% HP	8	11	3	3	1
SEM	1.2	2.2	0.6	0.8	0.4
P-value	0.345	0.774	0.647	0.400	0.409

Table 8. **Effect of treatment on organ weight (g) of 42-day-old broilers**

Treatments	Proventriculus	Small intestine	Large intestine
Control	10	72	22
Flavophospholipol	11	82	23
Probiotics	10	74	20
0.25% HP	9	81	23
0.50% HP	8	93	23
0.75% HP	9	89	27
SEM	1.0	8.0	3.0
P-value	0.685	0.468	0.481

SEM: Standard error of means.

0.75% HP powder. Low fat deposition may be associated with the expression of markers of fat metabolism in blood. Serum HDL levels did not vary significantly among the treatments. However, dietary supplementation with 0.5% and 0.75% HP decreased plasma cholesterol and triglyceride levels. Furthermore, compared to the non-HP treated groups, groups with different amounts of HP supplementation showed significantly lower serum LDL levels. Thus, these markers confirmed that 0.5% and 0.75% HP powder reduced fat deposition in broiler carcass. In agreement with our results, Hajhashemi *et al*. (2014) reported that the essential oil of hogweed increases HDL-cholesterol and decreases LDL-cholesterol levels in rabbits. However, the mechanism via which the hypolipidemic activity of hogweed reduces LDL is still not understood. Possibly, it may reduce LDL by inhibiting intestinal absorption of cholesterol via a mechanism similar to that of ezetimibe (Sweeney and Johnson 2007; Hajhashemi *et al*., 2014). Furthermore, 0.5% and 0.75% HP powder significantly reduced plasma glucose levels, indicating optimal pancreas function in concert with muscle uptake.

Antibody titers against Newcastle disease vaccine were not markedly affected by the treatments, whereas antibody titer against the avian influenza vaccine were significantly higher in the probiotic- and 0.75% HP-supplemented groups than in other groups ($P<0.05$). However, since antibody

Table 9. **Effect of treatment on serum biochemical parameters at 42 days of age**

Treatments	Total protein (g/d*l*)	Albumin (g/d*l*)	Glucose (mg/d*l*)	Cholesterol (mg/d*l*)	Triglyceride (mg/d*l*)
Control	4.4	1.7	207	155^{a}	70^{a}
Flavophospholipol	4.5	2.2	209	157^{a}	72^{a}
Probiotics	4.6	2.3	202	141^{ab}	57^{ab}
0.25% HP	3.6	1.7	208	136^{ab}	51^{ab}
0.50% HP	4.0	1.9	212	128^{b}	39^{a}
0.75% HP	4.7	1.7	214	129^{b}	35^{b}
SEM	0.31	0.32	13.01	7.04	7.07
P-value	0.295	0.619	0.991	0.061	0.009

Means with different subscripts in the same column differ significantly ($P<0.05$); SEM: Standard error of means.

Table 10. **Effect of treatment on serum biochemical parameters at 42 days of age**

Treatments	HDL	LDL	Uric acid (mg/d*l*)	Alkaline phosphatase (mg/d*l*)
Control	80	69^{a}	5.4	1463
Flavophospholipol	82	69^{a}	4.9	1410
Probiotics	79	58^{ab}	3.6	1333
0.25% HP	76	52^{b}	5.0	1096
0.50% HP	81	50^{b}	5.9	1593
0.75% HP	81	50^{b}	4.2	1413
SEM	5.0	4.0	0.8	175.0
P-value	0.963	0.015	0.521	0.513

Means with different subscripts in the same column differ significantly ($P<0.05$); SEM: Standard error of means.

Table 11. **Effect of treatment on antibody titers against Newcastle disease virus and avian influenza virus in broilers**

Treatments	Antibody response to Newcastle vaccine (28 days)	Antibody response to Newcastle vaccine (42 days)	Antibody response to influenza vaccine (28 days)	Antibody response to influenza vaccine (42 days)
Control	4.0	2.0	1.0^{b}	0.3^{b}
Flavophospholipol	4.0	2.0	1.0^{ab}	1.3^{ab}
Probiotics	4.7	2.0	2.0^{a}	1.7^{a}
0.25% HP	3.7	2.0	1.0^{ab}	1.0^{ab}
0.50% HP	3.0	2.0	1.0^{ab}	0.7^{ab}
0.75% HP	3.0	2.0	2.0^{a}	1.7^{a}
SEM	0.72	0.32	0.41	0.45
P-value	0.541	0.656	0.284	0.021

Means with different subscripts in the same column differ significantly ($P<0.05$); SEM: Standard error of means.

production against SRBC, and IgM and IgG levels did not differ significantly among the groups, the increase in anti-influenza antibody titer may have been accidental. In addition, determination of HI antibody titers, which involves a manual dilution step, is not precise. Kheiri *et al*. (2014) reported that the hogweed extract considerably enhanced broiler antibody titer against Newcastle disease virus. This could be because of the presence of flavonoids or furanocoumarins in *H. persicum*, which can increase humoral response by stimulating macrophages and beta-lymphocytes that are involved in antibody synthesis (Sharififar *et al*., 2009). Furthermore, the hogweed extract may also exert a stimulatory effect on lymphocytes and accessory cell types (Vimal and Devaki 2004; Kheiri *et al*., 2014).

The *Lactobacillus* counts in the ileum of birds fed 0.5% or 0.75% HP were significantly higher than those observed after other treatments, whereas *E. coli* counts in all treatment groups were significantly lower than that in the control. This indicates that HP positively affects the gut microbiota and subsequently improves feed digestion and utilization.

In conclusion, addition of 0.50−0.75% HP to broiler diet over the entire rearing period improved weight gain and

Table 12. **Effect of treatment on antibody titers against SRBC, IgG, and IgM at day 28 and 42**

Treatments	SRBC (28 days)	IgG (28 days)	IgM (28 days)	SRBC (42 days)	IgG (42 days)	IgM (42 days)
Control	2.3	1.0[b]	1.3	4.3	2.7	1.7
Flavophospholipol	4.0	1.3[a]	1.7	5.0	3.0	1.7
Probiotics	4.7	2.3[a]	2.7	6.0	3.3	2.7
0.25% HP	2.7	1.7[a]	1.0	4.7	3.3	1.7
0.50% HP	4.0	1.3[a]	1.7	5.0	3.3	1.3
0.75% HP	4.7	2.3[a]	2.7	6.0	3.7	2.3
SEM	1.00	0.71	0.53	0.73	0.72	0.33
P-value	0.586	0.024	0.265	0.531	0.964	0.194

Means with different subscripts in the same column differ significantly ($P<0.05$); SEM: Standard error of means.

Table 13. **Effects of treatment on counts of *E. coli*, coliform, lactobacilli, and total aerobic bacteria in intestinal digesta of 42-day-old broilers (log CFU/g)**

Treatments	*E. coli*	Coliform bacteria	Lactobacilli	Total aerobic bacteria
Control	6.9[a]	6.5	3.0[c]	8.1
Flavophospholipol	5.3[b]	8.0	4.5[b]	8.6
Probiotics	4.3[b]	7.9	5.5[ab]	7.4
0.25% HP	4.1[b]	7.6	4.8[ab]	7.8
0.50% HP	4.8[b]	6.3	5.7[a]	7.4
0.75% HP	4.8[b]	7.7	5.8[a]	8.4
SEM	0.41	0.52	0.34	0.22
P-value	0.002	0.912	0.001	0.781

Means with different subscripts in the same column differ significantly ($P<0.05$); SEM: Standard error of means.

decreased fat deposition due to improved glucose kinetics and the hypolipidemic property of HP, respectively.

Conflict of Interest

The authors declare no conflict of interest regarding the publication and dissemination of knowledge presented in this study.

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