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The Effects of a Combination of Garlic, Oyster Mushroom and Propolis Extract in Comparison to Antibiotic on Growth Performance, Some Blood Parameters and Nutrients Digestibility of Male Broilers

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ABSTRACT

A study was conducted to evaluate the combined effects of garlic, oyster mushroom and propolis extract on the growth performance, organ weights, antibody response to Newcastle Disease Virus (NDV), serum lipid concentrations and nutrient digestibility of male broilers. A total of 192 day old chicks (Ross 308) were randomly assigned to 3 treatments with 4 replications (16 birds per replication). Experimental treatments were a maize- soybean based diet as control, control diet supplemented with a combination of garlic (30 g/kg), oyster mushroom (2 g/kg) and propolis extract (0.2 g/kg) known as GMP, and control diet supplemented with Virginamycin (0.25 g/kg) as antibiotic. The inclusion of GMP decreased ($p < 0.05$) live body weight, average daily gain and feed intake but had no effect on feed conversion ratio when compared to control diet. GMP and antibiotic had no effects on relative weight of organs. Antibiotic decreased ($p < 0.05$) the relative weight of small intestine segments. GMP decreased total cholesterol but did not affect other serum lipids when compared to control group. Antibiotic increased ($p < 0.05$) cholesterol concentration in serum. The inclusion of GMP to the diet improved antibody response to NDV when compared to control and antibiotic diets. In conclusion, the results showed that GMP decreased growth performance but improved immunity of broiler chickens. More studies should be performed to confirm the action modes of such combinations.

INTRODUCTION

Antibiotics are widely used as growth promoters in poultry production. In recent years, usage of antibiotics as growth promoter in poultry diet has been banned due to concerns about their residues in animal tissues and subsequent induction of emerging antibiotic resistant strains of microorganisms (Roe & Pillai, 2003 and Saleha *et al*. 2009) and the inclusion of antibiotics to animal diet was banned in some parts of the world (Simon, 2006). Therefore, researchers are looking for safe alternatives candidates such as natural products and phytobiotics. Recently, natural materials such as garlic, mushrooms and propolis have been investigated. Garlic has antioxidant, antimicrobial and antiviral properties (Corzo-Martinez *et al*. 2007). Oyester mushroom (*Pleurotus ostreatus*) are known to have antioxidant and immunomodulatory effects (Shamtsyan *et al*. 2007 and Elmastas *et al*. 2007) and has been shown to improve growth, immunity and intestinal health (Guo *et al*. 2003; Machado *et al*. 2007 and Giannenas *et al*. 2010). Propolis is a resinous substance which worker bees collect from various sources to seal hive grooves and mummify dead invaders (Burdock, 1998). It has antimicrobial, anti-inflammatory and immunomodulatory properties (Dobrowolski *et al*. 1991and Bankova *et al*. 2000). The beneficial effect of propolis on growth performance and immune response in both broiler (Khojasteh Shalmani & Shivazad, 2006 and Ziaran *et al*. 2005) and layer (Galal *et al*. 2008) have been reported.

Based on these findings, it can be proposed that the combination of these materials could be of particular benefit and be useful as a substitute for antibiotics. There are no reported studies about the combined effects of these natural substances on poultry performance. Therefore the present study was carried out to study the effects of a combination of garlic, oyster mushroom and propolis extract on the performance and health attributes of broiler chickens.

MATERIALS AND METHODS

Animals and feeds

A total of 192 one day old male broilers (Ross 308) were randomly assigned to three experimental treatments. Each treatment consisted of four replicate pens with 16 birds each. Each pen was 150×110 cm with one hanging tube feeder and one suspended drinker. The experiment was a completely randomized design and dietary treatments were as follows: 1) a maize-soybean meal based diet as control, 2) control diet contained a combination of garlic (30 g/kg), oyster mushroom (2 g/kg) and propolis extract (0.2 g/kg) as GMP diet, and 3) control diet contained Virginamycin (0.25 g/kg) as antibiotic diet. Control diet was formulated to meet or exceed nutrient requirements according to the NRC (1994) nutrient requirements for broiler chickens and the other 2 diets were prepared separately using the same ingredients as in the control diet and supplements incorporated to basal diet on a weight: weight ratio basis. Preliminarily a diet with determined amount of garlic powder prepared and then oyster mushroom powder added and mixed thoroughly. Finally, propolis extract sprayed on this combination and mixed again to make GMP diet. Garlic and oyster mushroom (*Pleurotus ostreatus*) powder were purchased from local industry (Grandis®[1] and Isatis®[2], respectively) and propolis extract was prepared using the method of Yaghoubi *et al*. (2007) by some modification. Feed and water were offered *ad libitum* and the light program was 23L/1D.

Performance and carcass traits

At 21 and 42 days of age, the feed consumption and total weight of each pen were used to calculate live body weight (LBW), average daily gain (ADG), feed intake (FI) and feed conversion ratio (FCR).

[1] Laleh-Bahar Hamadan Food Industry Co. (Private Joint Stock), Hamadan, Iran.

[2] Isatis mushroom producing Institute, Yazd, Iran.

At both ages, two birds from each pen were picked out randomly, bled and eviscerated. Organs (spleen, bursa and liver), abdominal fat and the segments of small intestine (duodenum, jejunum and ileum) were removed, emptied, weighed and recorded as percentage of LBW. The relative lengths of small intestine segments were measured as percent of total length.

Table 1 - Composition of chicken diet and calculated major components (g per 100g as fed).

Ingredients	1 – 21days	22 – 42days
Maize	54.17	63.49
Soybean Meal(48% CP)	39.84	30.72
Soybean Oil	2.12	1.84
Dicalcium phosphate	1.56	1.73
Calcium CO3	1.18	1.07
Common Salt	0.34	0.33
Vitamin Premix*	0.25	0.25
Mineral Premix**	0.25	0.25
DL- Methionine	0.20	0.27
L- Lysine HCl	0.10	0.06
Calculated nutrients content		
Metabolizable energy (MJ/kg)	12.1	12.7
Crude protein (%)	22.5	21.0
Calcium (%)	0.92	0.90
Available phosphate (%)	0.45	0.40
Lysine (%)	1.38	1.12
Methionine + Cysteine (%)	0.92	0.92

* Each kilogram of Vitamin supplement contains: Vitamin A, 3600000 IU; vitamin D3, 800000 IU; vitamin E, 7200 IU; vitamin K3, 800 mg; vitamin B1, 720 mg; vitamin B2, 2640 mg; vitamin B3, 4000 mg; vitamin B5, 12000 mg; vitamin B6, 1200 mg; vitamin B9, 400 mg; vitamin B12 , 6 mg; biotin, 40 mg; choline chloride, 100000 mg; antioxidant, 40000 mg.

** Each kilogram of Mineral supplement contains: Mn, 40000 mg; Zn, 33880 mg; Fe, 20000 mg; Cu, 4000 mg; I, 400 mg; Se, 80 mg.

Blood parameters

At days 21 and 42 of age, two bird two birds from each pen were bled. Blood samples were centrifuged at 2000× g for 15 min to obtain serum, and frozen at -24^{oC} until utilized for measuring the concentrations of total cholesterol (CHL), triglycerides (TG), high density lipoprotein (HDL) and very low density lipoprotein (VLDL). Serum lipids were assessed by spectrophotometer (Jasco, V570, Japan) equipped with one centimeter quartz cells employing CHL, TG

and HDL diagnostic kits (Pars Azmoon, Iran) and VLDL was calculated by dividing TG by 5.

Each bird was vaccinated by commercial Newcastle Disease Virus (NDV) vaccine via eye-drop at day 6 of age, and via drinking water at days 16 and 26 of ages. Additionally, at 21, 33 and 42 days of age two birds from each pen were bled to obtain serum for measuring antibody to NDV by means of ELISA using the Newcastle Disease Antibody Test Kit from IDEXX Laboratories Inc. (Westbrook, ME, USA).

Nutrient Digestibility

Diets contained Cr_2O_3 as an indigestible marker at a level of 2 g/kg during 31 to 35 days of experiment. At day 33, feces samples were collected from each pen and immediately frozen at -24°C. Fecal samples were defrosted and dried at 80°C for 48 h. Crude protein (CP) and organic matter (OM) were determined according to AOAC (1995). The Cr_2O_3 content was determined by the method described by Fenton & Fenton (1979). The digestibility (Dig) of nutrients for each diet was calculated according to the following equation:

Dig (%) = 100 – 100 × [(Cr_2O_3 Diet × Nutrient Feces) / Cr_2O_3 Feces × Nutrient Diet)]

Where Cr_2O_3 Diet and Cr_2O_3 Feces = respective concentrations of Cr_2O_3 in the diet and feces samples (g/kg); and Nutrient Diet and Nutrient Feces = respective concentrations of the nutrients in the diet and feces samples (g/kg)

Analytical measurements

Data were analyzed as a completely randomized design by ANOVA using the General Linear Model (GLM) procedure of SAS (2002). The significance of differences between means was assessed using Duncan's Multiple Range Test. Differences were considered significant when $p < 0.05$.

RESULTS

The inclusion of a combination of garlic, mushroom and propolis extract (GMP) lowered ($p < 0.05$) LBW when compared to the control group at both 21 and 42 days of age (Table 2). There was no significant ($p > 0.05$) difference between the antibiotic and control groups. As compared to the control diet, GMP decreased ($p < 0.05$) ADG at the end of the experimental period while

Table 2 - Effects of antibiotic and GMP on growth performance of broiler chickens at 21 and 42 days of age.

	Age	Control	Antibiotic	GMP[2]	SEM[3]	P-value
LBW[1] (g)	21	774[a]	783[a]	628[b]	23.5	0.001
	42	2507[a]	2571[a]	2115[b]	68.9	0.002
ADG (g/bird)	1-21	34.2[a]	35.0[a]	27.9[b]	1.08	0.002
	21-42	81.7[ab]	85.0[a]	72.1[b]	2.16	0.028
	1-42	58.4[a]	59.9[a]	48.3[b]	1.44	0.003
FI (g/bird/d)	1-21	52.7[a]	52.1[a]	46.2[b]	1.01	0.001
	21-42	164.9[a]	163.7[a]	142.4[b]	4.05	0.017
	1-42	103.9[a]	103.5[a]	90.6[b]	2.36	0.012
FCR (g/g)	1-21	1.57	1.48	1.67	0.039	0.165
	21-42	2.07	1.93	1.97	0.030	0.126
	1-42	1.89[a]	1.73[b]	1.88[a]	0.018	0.010

1. live body weight (LBW), average daily gain (ADG), feed intake (FI) and feed conversion ratio (FCR)
2. GMP: a combination of garlic, mushroom and propolis.
3. SEM: standard error of means.
([a-b]) Means values with different superscripts within a row differ significantly ($p<0.05$).

Table 3 - Relative weight of organs (% of live weight) in the experimental male broilers at 21 and 42 days of age.

Age	21					42				
Organs	Control	Antibiotic	GMP[1]	SEM[2]	P-value	Control	Antibiotic	GMP	SEM	P-value
Carcass Yield	55.93[b]	60.27[a]	56.02[b]	0.601	0.0020	62.98	65.00	62.32	0.977	0.5267
Liver	3.13	3.14	3.08	0.074	0.9567	2.47	2.40	2.59	0.062	0.4669
Spleen	0.11	0.10	0.10	0.007	0.7772	0.13	0.11	0.11	0.006	0.2593
Bursa	0.22	0.21	0.21	0.014	0.9496	0.15	0.15	0.14	0.010	0.7549
Abdominal Fat	1.09[a]	1.08[a]	0.68[b]	0.059	0.0078	1.08	1.45	1.08	0.086	0.1224

1. GMP: a combination of garlic, mushroom and propolis.
2. SEM: standard error of means.
([a-b]) Means values with different superscripts within a row differ significantly (p<0.05).

antibiotic had no effect (p > 0.05). Feeding the GMP supplemented diet resulted in lower (p < 0.05) FI when compared to control birds, while no difference was seen between birds fed antibiotic and those received the control diet (p > 0.05). Addition of antibiotic to the diet improved (p < 0.05) FCR in comparison to the control group, while GMP did not affect this ratio at the end of experimental period (p > 0.05).

Although antibiotic increased (p < 0.05) carcass yield at 21d, there was no effect (p > 0.05) at the end of experimental period (Table 3). Overall, none of the additives affected relative weight of organs over the whole period of experiment as compared to control group (p > 0.05).

GMP and antibiotic had no (p > 0.05) effect on the relative length of small intestine segments compared to control diet at either 21 and 42 days of age (Table 4). Antibiotic-fed birds had lower (p < 0.05) duodenum, jejunum and ileum relative weights as compared to control birds at both 21 and 42 days of age, while GMP had no (p > 0.05) effect.

Table 4 - Relative lengths (% of small intestine length) and weights (% of live body weight) of small intestine segments in the experimental male broilers at 21 and 42 days of age.

Age	21					42				
Treatments	Control	Antibiotic	GMP[1]	SEM[2]	P-value	Control	Antibiotic	GMP	SEM	P-value
Length										
Duodenum	18.05	17.88	18.25	0.305	0.8606	17.09	17.16	17.79	0.279	0.5508
Jejunum	40.19	40.82	39.15	0.443	0.2452	39.09	40.95	39.56	0.363	0.1673
Ileum	41.73	41.30	42.60	0.533	0.4727	43.01	41.10	42.64	0.441	0.1720
Weight										
Duodenum	1.57[a]	0.97[b]	1.61[a]	0.066	<0.0001	0.60[b]	0.53[c]	0.72[a]	0.020	0.0001
Jejunum	2.31[a]	1.68[b]	2.22[a]	0.082	<0.0001	1.18[a]	0.98[b]	1.25[a]	0.033	0.0002
Ileum	1.69[a]	1.31[b]	1.78[a]	0.063	0.0002	1.02[a]	0.88[b]	1.00[ab]	0.028	0.0433

1. GMP: a combination of garlic, mushroom and propolis.
2. SEM: standard error of means.
([a-c]) Means values with different superscripts within a row differ significantly (p<0.05).

Table 5 - Serum lipid concentrations (mg/dL) in the experimental male broilers at 21 and 42 days of age.

	Age	Control	Antibiotic	GMP[1]	SEM[2]	P-value
TG[3]	21	112.03[a]	47.73[b]	86.49[ab]	12.254	0.0441
	42	83.17	94.89	93.21	3.909	0.4582
CHL	21	132.28	152.78	113.46	8.050	0.1326
	42	94.92[b]	119.91[a]	70.35[c]	7.102	0.0024
HDL	21	75.66	52.94	53.82	5.840	0.2083
	42	44.75	58.11	46.06	4.206	0.4462
VLDL	21	22.41[a]	9.54[b]	17.30[ab]	2.451	0.0441
	42	16.63	18.97	18.64	0.782	0.4578

1. GMP: a combination of garlic, mushroom and propolis.
2. SEM: standard error of means.
3. TG: triglyceride, CHL: total cholesterol, HDL: high density lipoprotein, VLDL: very low density lipoprotein.
[(a-c)] Means values with different superscripts within a row differ significantly ($p<0.05$).

DISCUSSION

Since it has been demonstrated that antibiotic residues can induce resistant strains of bacteria, research has focused on alternatives to these additives, particularly on natural substances. The main purpose of the present work was to evaluate the effect of a combination of the natural materials including garlic, mushroom and propolis (GMP) in comparison to antibiotic. The inclusion of a combination of these natural products decreased growth performance of broiler chickens when compared to birds fed with control diet or those received antibiotic in the diet. The main component of GMP was garlic which comprised 30 g/kg of the diet. The most active and important component of garlic is sulfur. Garlic has a sulfur content of about 100 mg/kg of dry weight (Fenwick *et al*. 1985). It has been shown that higher doses of sulfur (up to 330 mg/kg) caused a reduction in feed intake and consequently body weight of broilers (Meinhart & Fenske, 1977). The sulfur content of the garlic used in the present experiment was about 300 mg /kg that probably lowered palatability, depressed FI and consequently decreased LBW and ADG.

Antibiotic increased ($p < 0.05$) serum total cholesterol when compared to control (Table 5), while GMP decreased ($p < 0.05$) the serum cholesterol at the end of experimental period. Experimental additives had no effects on TG, HDL and VLDL when compared to the control group at the end of the experiment.

The GMP induced a lower antibody titer to NDV at day 21 of age, but there were significantly ($p < 0.05$) increased antibody titers at 33 and 42 days of age as compared to the control group (Figure 1). The GMP treatment increased antibody response to NDV when compared to the antibiotic group at all sampling intervals. Antibiotic treated chicks showed similar antibody response to NDV at day 21 of age when compared to the control birds, but inclusion of antibiotic increased ($p < 0.05$) response at 33 and 42 days of age.

Inclusion of GMP or antibiotic had no effect ($p > 0.05$) on crude protein or organic matter retention when compared to control (Table 6).

Table 6 - Digestibility coefficient (%) of crude protein (CP) and organic matter (OM) in the experimental male broilers at 33 days of age.

	Control	Antibiotic	GMP[1]	SEM[2]	P-value
CP	54.6	58.5	57.1	2.00	0.0125
OM	71.9	75.3	73.5	0.85	0.2870

1. GMP: a combination of garlic, mushroom and propolis.
2. SEM: standard error of means.
[(a-b)] Means values with different superscripts within a row differ significantly ($p<0.05$).

The antibiotic used in the present study could not improve the LBW, ADG, but improved FCR when compared to control group. In agreement with this finding, Guo *et al*. (2004) reported that the inclusion of Virginamycin as an antibiotic in the diet of broilers had no effects on growth performance.

None of GMP or antibiotic could affect the relative weight of organs. This finding for antibiotic is in agreement with observation by Denli *et al*. (2003).

GMP did not affect relative length and weight of small intestine segments while antibiotic decreased relative weights of duodenum, jejunum and ileum. It has been shown that the microbial population in broilers' gut can increase the production of polyamines and volatile fatty acids (VFAs) which both adhere to absorption sites in small intestine (Adibmoradi *et al*. 2006). VFAs increase the thickness of gastrointestinal walls thereby increasing the weight of small intestine. Preventing the colonization of microbial populations in the gut allows antibiotics to decrease the microbial

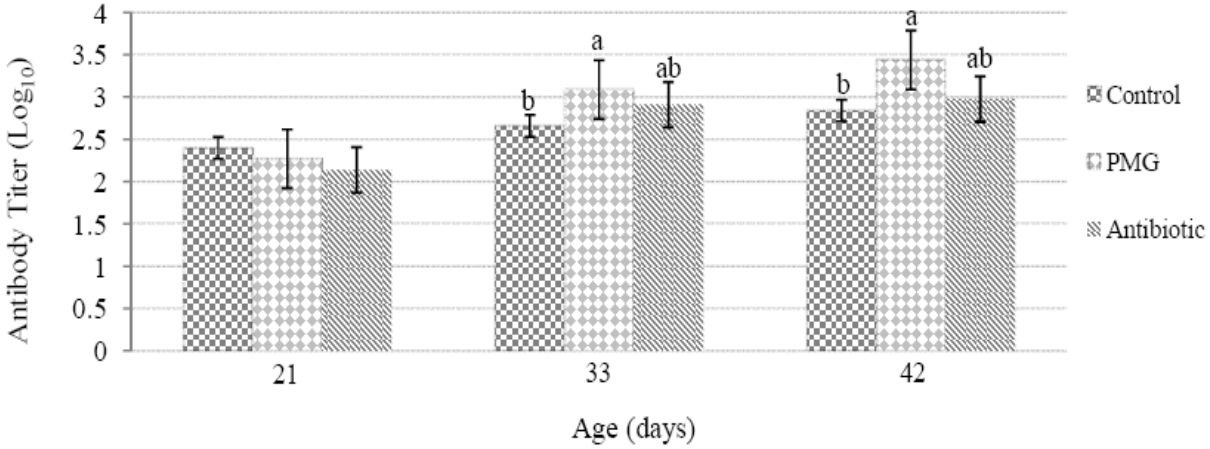

Figure 1 - Antibody titer (Log10) to Newcastle disease virus in the experimental male broilers at 21, 33 and 42 days of age.

production of volatile fatty acids and polyamines that lead to lower intestine weight, as seen in present study.

GMP lowered cholesterol concentration in serum while had no effects on other lipids in serum. Horton *et al*. (1991) reported that inclusion of 10 g/kg garlic in broiler diet could decrease cholesterol concentration, without any effect on HDL and TG. Cheung (1998) found that mushroom inclusion in the diet reduced serum cholesterol concentration and Fuliang *et al*. (2005) observed that propolis depressed serum total cholesterol in rats. Based on these findings, it is reasonable to observe that a combination of garlic, mushroom and propolis extract could decrease total cholesterol. The garlic component of GMP contains some organosulfur compounds such as allicin and ajeon (Matthew *et al*. 2003) and it is likely that this compounds can construct disulfide internal proteins which inactivate thiol (-SH) group in enzymes such as HMG-CoA reductase. This inactivation leads to inhibition and excretion of this enzyme; hence the production of cholesterol is lowered.

The GMP and antibiotic supplementation increased the antibody response to NDV in the present study. There is no report on the direct effects of this combination on antibody titer in literature. However, the beneficial effects of mushrooms or propolis extracts on immune system have been reported. Ziaran *et al*. (2005) demonstrated that low doses of propolis extract (40 and 70 mg/kg) improved cellular and humoral immunity of broilers. Guo *et al*. (2003) supplemented broiler diets with mushroom and concluded that it can improve immunity against pathogens. The beneficial effect of antibiotics on immune response also has been reported. Huang *et al*. (2007) showed that dietary inclusion of Flavomycin induced a higher antibody response to NDV but had no effect on immunoglobulin production, as compared to control birds.

GMP and antibiotic treatments could not affect protein and organic matter retention. In agreement with this finding, Mountzouris *et al*. (2010) showed no beneficial effect of antibiotic on nutrient digestibility in broilers.

CONCLUSION

The combination of the garlic, oyster mushroom and propolis extract in amounts that used in present study decreased bird's body weight and weight gain, which shows there were no significant synergetic and complementary effects between the three in broiler chickens. Inclusion of GMP and antibiotic showed immunomodulatory effect on broiler chickens, as the birds fed with these additives had higher antibody titer concentration to NDV. More investigations with fractioned parts of GMP seem interesting to explore the mode of action of fractions on immune system in birds.

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