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Full Length Research Paper

Effects of supplementing *Aloe vera* gel and garlic powder on blood biochemical parameters and immune response of broiler

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The present study was designed to evaluate the effects of supplementing different levels of *Aloe vera* gel and garlic powder on blood biochemical parameters and immune response of broiler. For this purpose, one hundred and sixty, one-day-old, Ross chicks were used, in completely randomized design, in four treatments and each with four replicates. The treatment groups consisted of: control group fed only with non-supplemented basal diet, group 2 supplemented with 3% *aloe vera* in drinking water, group 3 supplemented with 3% garlic powder in diet and group 4 supplemented with 1.5% *aloe vera* in drinking water + 1.5% garlic powder in diet, which were reared for 42 days. The result of this experiment showed no significant difference in heterophil to lymphocyte ratio at 42 days of age between control and treated groups. The other results of this investigation showed that broilers receiving *Aloe vera* gel and garlic powder had lower blood glucose, uric acid, total cholesterol, High-density lipoprotein (HDL), Low-density lipoprotein (LDL) and Triglycerides concentrations compared to control group. However, higher total protein concentrations were observed with supplementation of *Aloe vera* gel and garlic powder than control group. Although, there was no significant difference between the control and treated groups in antibody titres against Newcastle and Influenza, the highest amount of anti body titres for Newcastle and Influenza were observed in group - 4 on 18 and 28 days.

Key words: *Aloe vera* , garlic powder, blood parameters, immune parameters , broiler.

INTRODUCTION

A number of additives are often used in poultry diets and most of these do not contribute any nutrients. Most additives are used to improve physical characteristics of diet, feed acceptability or bird health (Leeson and Summers, 2008). There is no doubt that the antibiotics play an important and essential role in production and animal health but at the same time may affect negatively the health of consumers. The use of antibiotics as growth promoter has led to the development of bacterial resistance. On other hand, it may also result in residue problems in the tissues of birds and animals. Many studies have been carried out on using additives, including herbs as alternatives to antibiotics with direct or indirect effects in poultry products (Taylor, 2001). Medicinal herbs, as a

new class of additives to animal and poultry feeds have beneficial properties such as antioxidant, anti-microbial, and anti-fungal effects (Hardy, 2002) as well as immune modulation effects, which has lead to increased use of herbs. Furthermore, many countries around the world with abundant medicinal herbs resources can use these herbs as natural feed additives for animals and poultry. *Aloe vera* is among the most well-known herbs. A member of *Liliaceae,* it is similar to cactus in appearance and mostly grows in arid regions of Asia and Africa (Boudreau and Beland, 2006). *A. vera* has been widely used for the treatment of various ailments for millenia. The whole leaf of *A. vera* contains over 200 compounds including aloesin, anthraquinones (aloin and aloe-emodin), acemannan, saponins, sterols, aminoacids and vitamins (Grindlay and Reynolds, 1986).

Most of the compounds have various biological activities with potential health benefits such as antibacterial, antiviral, wound healing, antioxidant, immune modulatory, antineoplastic and antidiabetic activities (Pandey et al., 2010). The biological and toxicological effects of *A. vera* gel have been extensively studied. An important property of *A. vera* that has been the subject of many *in-vivo* and *in-vitro* experiments is improvement in immune response, probably due to its acemannan content (Harlev et al., 2012; Djeraba and Quere, 2000; Zhang and Tizard, 1996; Karaca et al., 1995). Another study reported an improvement in antibody titre in broilers against Newcastle Disease Virus (NDV) as a result of adding acemannan (0.1 and 0.05%), polysaccharide (0.1%), and *A. vera* gel (0.1%) to broiler feed (Jiang et al., 2005).

Garlic is one medicinal plant that has several biological functions making it suitable for preventing and controlling many disorders from metabolic and cardiovascular diseases to some infections (Corzo-Martinez et al., 2007). Allicin the main bioactive component of garlic may account for some effects of garlic (Amagase et al., 2001). *In-vitro* studies have shown that garlic possesses antibacterial, antifungal, antiparasitic, antiviral (Ankri and Mirelman, 1999), antioxidant (Prasad et al., 1995), as well as antithrombotic, vasodilatory and anticancer (Agarwal, 1996) activities. It has benefits in lowering total plasma cholesterol, reducing blood pressure and decreasing platelet aggregation (Sterling and Eagling, 2001). Kyo et al. (2001) examined the effects of garlic extract on immune functions using various kinds of mouse models, and suggested that it could be a promising candidate as an immune modifier, which main-tains the homeostasis of immune functions. The aim of this study was to investigate the effect of supplementing different levels of *A. vera* gel and garlic powder on blood biochemical parameters and immune response of broiler.

MATERIALS AND METHODS

Birds and diets

In this investigation, 161-day old Ross 308 broiler chickens were reared in 16 pens with 4 treatments and 4 replications in a poultry farm in Amol city for 42 days. Treatments in this experiment consist of control (no additives), treatment supplemented with 3% *A. vera* to drinking water, treatment supplemented with 3% garlic powder in diet, treatment supplemented with 1.5% *A. vera* to drinking water + 1.5% garlic powder in diet. Garlic powder and *A. vera* were purchased from local markets and M/s. Barij Essence Pharmaceutical Co. The birds were exposed to 23 h light: 1 h darkness during each 24h period throughout the 42 days of trial. Starter and grower diets were offered from 1 to 21 and 22 to 42 days of age, respectively. Feed and water were provided *ad libitum* throughout the experiment. The composition and nutrients content of the basal diets is shown in Table 1. The diets were formulated to meet or exceed the National Research Council (NRC, 1994) requirements.

Immune parameters

Two 18 and 28 day-old birds per replicate were randomly chosen and blood samples were collected from the brachial vein and centrifuged for 20 min at 1500 rpm to obtain serum. Antibody titers against Newcastle and influenza viruses were measured using Hemagglutination inhibition test. Two 42-day old birds per replicate were selected and blood samples were collected with syringes containing heparin to avoid blood clot formation. Blood smears were prepared using May–Greenwald–Giemsa stain. 100 leukocytes per samples were counted by heterophil to lymphocyte separation under an optical microscope. Heterophil to lymphocyte (H/L) ratio was calculated and recorded according to Gross and Sigel (1983).

Blood biochemical parameters

Two 42 day-old birds per replicate were randomly chosen, then slaughtered and blood samples were collected into vials containing The role of ethylenediamine tetraacetic acid (EDTA) (to avoid blood clot formation) and centrifuged for 20min at 1500rpm to separate the serum. The serum samples were stored at -20^{o}C for the analysis of serum glucose (Coles, 1986), total protein (Wotton, 1964), uric acid (Trinder, 1969), total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides (Franey and Elias, 1986).

Statistical analyses

All data were analyzed using the CRD (completely randomized design) by 1-way ANOVA procedure of SAS software (SAS Institute, 2003). Duncan's multiple range tests was used to compare differences among treatment means (Duncan, 1955).

RESULTS AND DISCUSSION

The results in Table 2 showed significant differences in blood glucose, total cholesterol, HDL and LDL ($p<0.05$) among different treatments. Broilers receiving *A. vera* gel and garlic powder had lower blood glucose, uric acid, total cholesterol, HDL, LDL and triglyceride concentra-tions compared to the control group. The total protein, uric acid and triglycerides did not differ significantly among the treatment groups ($p>0.05$). However, higher total protein concentration was observed with addition of *A. vera* gel and garlic powder in comparison to control group. Further, it is observed that the lowest uric acid, total cholesterol, HDL, LDL and triglyceride concentrations

Table 1. Composition of experimental chicken diets and calculated major components (% as fed).

Ingredient (%)	1 - 21 days	22 - 42 days
Corn grain	54.17	63.49
Soybean meal	39.84	30.72
Soybean oil	2.12	1.84
$CaCO_3$	1.18	1.07
Dicalcium phosphate	1.56	1.73
Common salt	0.34	0.33
Vitamin premix[1]	0.25	0.25
Mineral premix [2]	0.25	0.25
DL- methionine	0.20	0.27
L- lysine HCL	0.10	0.06
Nutrients composition	-	-
Metabolizable energy (mcal/ kg)	2900	3005
Crude protein	22.50	20.70
Crude fiber	4.10	2.59
Calcium	0.92	0.90
Available phosphorous	0.45	0.40
Lysine	1.38	1.12
Methionine + Cystine	0.92	0.92

[1]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, [2]Each kilogram of mineral supplement contains: Mn, 40000 mg, Zn, 33880 mg, Fe, 20000 mg, Cu, 4000 mg, I, 400 mg, Se, 80 mg, choline chloride, 100000 mg.

Table 2. Effect of adding *A. vera* gel and garlic powder in blood biochemical parameters of broiler.

Parameter	Control	Group 2	Group 3	Group 4	SEM
Glucose (mg/dl)	326.16^{a}	285.63^{b}	253.38^{c}	255.12^{c}	12.43
Total protein (g/dl)	7.06	7.31	7.48	7.15	0.16
Uric acid (mg/dl)	58.18	51.03	53.12	51.18	2.13
Total cholesterol (mg/dl)	148.65^{a}	135.81^{b}	126.59^{c}	124.78^{c}	8.78
HDL (mg/dl)	68.16^{a}	63.26^{b}	61.18^{b}	60.36^{b}	5.71
LDL (mg/dl)	31.75^{a}	26.34^{b}	24.52^{b}	23.68^{b}	4.83
Triglycerides (mg/dl)	105.38	103.65	102.36	101.58	15.35

Group 2: addition of *A. vera* 3% in drinking water; Group 3: addition of garlic powder 3% in diet; Group 4 addition of *A. vera* 1.5% in drinking water + addition of garlic powder 1.5% in diet; a, b, c: means within the same raw with no common superscript differ significantly ($p<0.05$); SEM=Standard Error Mean.

were observed with broilers that were receiving 1.5% *A. vera* gel + 1.5% garlic powder (Group 4). The results reported by Taimorizade et al. (2008) indicated that extracts derived from oregano and garlic could decrease triglycerides (TG) in broilers. The reduction of serum LDL and raising HDL cholesterol (check with table?) by adding garlic powder observed in the present study might be due to the reduction of synthetic enzyme activities. Garlic has a dose-dependent inhibition effect on hepatic β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA) reductase, cholesterol 7α-hydroxylase, and fatty acid synthetase (Qureshi et al., 1983). Carrijo et al. (2005) did not report any alternation in serum levels of cholesterol and triacylglycerols after feeding broiler chicks with a diet containing up to 1% of garlic powder. The effects of adding *A. vera* gel and garlic powder on immune response of broiler were shown in Table 3.

The results of this experiment showed that there was

Table 3. Effect of adding *A. vera* gel and garlic powder in immunity response of broiler.

Parameter	Control	Group 2	Group 3	Group 4	SEM
Anti body titres - Newcastle (log_2)					
18 days	4.1	4.5	4.3	4.8	0.53
28 days	5.3	5.6	5.4	5.8	0.23
Anti body titres - Influenza (log_2)					
18 days	4.3	5.1	5.3	5.7	0.53
28 days	5.4	5.6	5.8	6.1	0.37
H/L ratio	0.58	0.61	0.63	0.65	0.28

Group 2: addition of 3% *A. vera* in drinking water; Group 3: addition of 3% garlic powder in diet; Group 4 addition of 1.5% *A. vera* in drinking water + addition of 1.5% garlic powder in diet; SEM=standard error mean.

no significant difference in antibody titres against Newcastle and Influenza and H/L ratio between the control and treated groups. The highest heterophil to lymphocyte (H/L) ratio was observed in Group 4 while the lowest was observed with control group. The highest amount of antibody titres to Newcastle and influenza were observed in Group 4 in 18 and 28 days. As seen in the table, the lowest amount of antibody titres to Newcastle and Influenza were observed with control group. Experiments on chickens suggest promoted macrophage activities in broilers caused by the acemannan contained in *A. vera* (Djeraba and Quere, 2000; Karaca et al., 1995). In a study on *A. vera* effects on humoral immunity of broilers, Darabighane et al. (2012) reported an increase in antibody titre against Newcastle disease virus (NDV) on days 24 and 38 by adding *A. vera* gel to broiler feeds (at 1.5, 2 and 2.5%).

The findings of the present study are consistent with those of Valle-Paraso et al. (2005) who reported that broilers treated with 2% *A. vera* gel (mixed with their drinking water) showed significant increase in antibody titre against NDV on days 37 and 52, compared to the control group. In another study by Alemi et al. (2012) *A. vera* gel powder (0.5, 0.75 and 1%) added to broilers feeds resulted in an increase in antibody titre against NDV. On the other hand, assessment of blood parameters showed an increase in total white blood cell and lymphocyte counts on days 37 and 52 for broilers that received 2% *A. vera* gel (mixed with drinking water) compared to the control group (Valle-Paraso et al., 2005). Another study on the effects of *A. vera* gel powder on antibody titre against SRBC found a significant increase in antibody titre for the groups treated with 0.75 and 1% *A. vera* gel powder compared to the control group and the group receiving 0.5% (Mahdavi et al., 2012). Jafari et al. (2008) reported that the inclusion of 1 and 3% of garlic powder did not enhance the serological response of broilers to Newcastle vaccine. The finding of this investigation is in agreement with results reported by Valle-Paraso et al., 2005, Mahdavi et al., 2012, Darabighane et al., 2012 and Alemi et al.2012.

CONCLUSION

Based on this study, it can be concluded that adding *A. vera* with garlic powder improved the immune system functions and reduced the concentrations of blood glucose, uric acid, total cholesterol, HDL, LDL and triglycerides in broiler.

Conflict of Interest

The authors declare no conflict of competing interest.

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