Pakistan J. Zool., vol. 48(6), pp. 1715-1719, 2016.

Effect of Herbal Extracts on Serum Minerals, Lipid Profile and Anti-NDV-HI Antibody Levels of Vaccinated Broiler Chicks

Kamran Safdar,[1] Khushi Muhammad[2] and Rubina Naz[3,*]
[1]*Gomal College of Veterinary Sciences, Gomal University, D.I. Khan,. KPK, Pakistan*
[2]*Department of Microbiology, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan*
[3]*Institute of Chemical Sciences, Gomal University, D.I. Khan, KPK, Pakistan*

Article Information
Received 10 September 2015
Revised 26 December 2015
Accepted 20 March 2016
Available online 25 September 2016

Authors' Contribution
KM conceived and designed the study. KS bred the broiler chicks and collected blood samples. KS and RN analyzed the sera samples. KS analyzed the data and wrote the article. KM and RN assisted in manuscript preparation.

Key words
Herbal extracts,
Serum minerals,
Garlic,
Lipid profile

ABSTRACT

The present study was designed to evaluate the effects of different herbal extracts on the skeletal strength, nervous system, lipid profile and anti-Newcastle Disease Virus-Hem-Agglutination Inhibition (Anti-NDV-HI) antibody levels of the Newcastle disease virus (NDV) immunized broiler chicks. The study was conducted on seven groups of one day old broiler chicks labeled as A,B,C,D,E,F and G (each containing 15 birds) in three replicates of five birds. Each bird of the group A, B, C, D, E and F was fed with 0.2% aqueous extract of *Aloe vera*, Zingiber officinale , Safi, Allium sativum, Probiotic (Dahi) and Curcuma longa in addition to the basal diet, respectively. Group G was kept as control, fed on basal diet only. Serum levels of cholesterol, triglycerides, sodium, calcium and anti-NDV-HI antibody titer were measured**.** During this study, it was observed that the Garlic extract increased the serum level of Calcium while it decreased the cholesterol level in broiler chicks. So these two results with respect to garlic were significantly different from other herbs which show that garlic probably increased the calcium absorption in intestine and resultantly enhanced the skeletal strength. On the other hand garlic also decreased the total cholesterol level in blood making the broiler meat presentable for heart patients. There was no significant effect of any of the herbal extracts on the anti-NDV-HI antibody level of the broiler chicks.

INTRODUCTION

Antibiotics have been extensively used in animal feed as feed additives and growth promoters. Indiscriminate use of antibiotics in animal feed is hazardous due to cross resistance and multiple resistances of intestinal pathogens (Schwarz *et al*., 2001). Keeping in view this ill effects, European Union has banned the use of most of the antibiotics in poultry feed. For the last few years, efforts are being made to investigate new and promising feed-additives including probiotics, prebiotics, enzymes, and plant extracts in animal feed (Sarica *et al*., 2005; Hernandez *et al*., 2004; Demir *et al*., 2003).

Herbs are used as chemotherapeutics in poultry. In past recent years, aromatic plants and their extracts are used in animal feed (Mikulski *et al*., 2008). Some herbs or herbal extracts improve feed intake, secretion of digestive tract juices and immune system of animals (Mikulski *et al*., 2008). Essential oils (derived from herbs) and spices or their purified compounds have antimicrobial properties (Faleiro *et al*., 2003). Herbal essential oils in poultry feed or water improves their feed conversion ratio and weight gain (Bassett, 2000) and also have antibacterial potential (Azaz *et al*., 2002; Dorman and Deans, 2000) and antioxidant activity (Botsoglou *et al*., 2002, 2004). The FCR of the birds is enhanced by stimulating endogenous enzyme activity and facilitating nitrogen absorption (Gill, 2001). Addition of alfalfa, thyme or garlic in broiler's feed reduce cholesterol and lipoprotein in their meat (Ponte *et al*., 2004; Bolukbasi *et al*., 2006; Konjufca *et al*., 1997; Habibian Dehkordi *et al*., 2010). Some of the commercial herbal products such as mentofin, safi, and Suduri have antibacterial activity against urease producing intestinal poultry bacteria and may exert immune enhancing effect due to reducing ammonia production on the farms (Yasmeen *et al*., 2012). Keeping in view promising results of the herbs, this project was planned to investigate the effects of probiotics and aqueous extracts of medicinal herbs on serum biochemistry of broiler chicks.

MATERIALS AND METHODS

Preparation of aqueous extracts

Fresh leaves of *Aloe vera*, rhizome of ginger, bulb of garlic, and dry powder of turmeric (100 gm each) were separately homogenized in 50 mls of distilled water, soaked overnight and centrifuged. The supernatant was used as aqueous extract (Babayi *et al.*, 2004). Ten % stock solution of each aqueous extract was prepared and stored.

The study was conducted on seven groups of one day old broiler (Hubbard classic breed) chicks labeled as

* Corresponding author: nazrubina25@gmail.com
0030-9923/2016/0006-1715 $ 8.00/0

Table I.- Effect of aqueous herbal extracts and probiotic on the serum level of calcium (mg/100 ml), cholesterol (mg/100ml), triglyceride (mg/100ml), sodium (U/L) in broilers.

Aqueous extract	18th day	25th day	32nd day	39th day
Calcium (mg/100 ml)				
Aloe vera	5.9 ± 1.0^{c}	5.2 ± 0.2^{b}	5.5 ± 0.0^{b}	4.9 ± 1.0^{a}
Ginger	4.6 ± 0.4^{a,b}	4.4 ± 0.2^{a}	5.0 ± 0.2^{a,b}	4.6 ± 0.4^{a}
Safi	6.6 ± 0.4^{a,b}	4.4 ± 0.2^{a}	5.0 ± 0.2^{a,b}	4.6 ± 0.4^{a}
Garlic	8.9 ± 0.8^{d}	13.8 ± 0.3^{c}	18.8 ± 0.7^{c}	23.6 ± 1.1^{b}
Probiotic	5.2 ± 0.2^{a,b}	4.3 ± 0.5^{a}	5.1 ± 0.0^{a,b}	5.0 ± 0.2^{a}
Turmeric	4.8 ± 0.6a	4.7 ± 0.2^{a,b}	4.8 ± 0.2^{a}	5.6 ± 0.6^{a}
Control	5.2 ± 0.2^{a,b}	4.5 ± 0.5^{a}	4.6 ± 0.2^{a}	4.8 ± 0.2^{a}
Cholesterol (mg/100ml)				
Aloe vera	167.0 ± 20.4^{a}	225.6 ± 11.7^{b}	222.1 ± 30.4^{a,b}	169.9 ± 33.7^{b}
Ginger	183.4 ± 7.8^{a}	233.9 ± 25.6^{b}	246.4 ± 63.3^{b}	179.9 ± 8.5^{b,c}
Safi	188.1 ± 9.0^{a}	214.6 ± 13.0^{b}	176.0 ± 55.9^{a,b}	190.8 ± 6.6^{b,c,d}
Garlic	170.0 ± 8.8^{a}	165.7 ± 5.1^{a}	155.6 ± 5.4^{a}	142.8 ± 2.5^{a}
Probiotic	183.7 ± 5.6^{a}	239.1 ± 29.0^{b}	174.8 ± 50.6^{a,b}	202.2 ± 9.2^{c,d}
Turmeric	170.8 ± 7.2^{a}	240.0 ± 20.4^{b}	202.8 ± 30.1^{a,b}	208.6 ± 4.8^{d}
Control	187.0 ± 11.5^{a}	240.0 ± 20.4^{b}	209.4 ± 30.1^{a,b}	194.1 ± 7.1^{b,c,d}
Triglyceride (mg/100ml)				
Aloe vera	115.5 ± 16.0^{a}	85.8 ± 10.0^{b}	162.7 ± 33.2^{a}	105.2 ± 10.2^{a}
Ginger	128.2 ± 14.6^{a}	82.1 ± 13.0^{b}	110.0 ± 58.5^{a}	95.2 ± 2.6^{a}
Safi	135.7 ± 22.9^{a}	111.3 ± 11.8^{b,c}	103.2 ± 12.5^{a}	93.5 ± 35.5^{a}
Garlic	150.2 ± 49.9^{a}	160.2 ± 43.5^{a}	128.9 ± 79.2^{a}	67.7 ± 38.7^{a}
Probiotic	133.5 ± 14.5^{a}	153.3 ± 48.0^{a,c}	120.5 ± 78.2^{a}	100.4 ± 18.0^{a}
Turmeric	136.6 ± 6.1)^{a}	76.4 ± 10.7^{b}	107.7 ± 13.5^{a}	98.8 ± 10.4^{a}
Control	126.0 ± 9.3^{a}	106.5 ± 10.8^{b,c}	149.3 ± 48.8^{a}	93.9 ± 24.7^{a}
Sodium (U/L)				
Aloe vera	44.4 ± 1.7^{a}	93.2 ± 77.0^{a}	64.8 ± 7.2	47.5 ± 1.6^{a,b}
Ginger	51.4 ± 4.2^{a}	63.6 ± 10.2^{a}	61.4 ± 15.2^{a,b}	46.6 ± 4.9^{a,b}
Safi	52.8 ± 12.3^{a}	67.9 ± 6.9^{a}	51.5 ± 3.2^{a,b}	41.4 ± 2.1^{a}
Garlic	47.5 ± 2.9^{a}	85.1 ± 34.8^{a}	57.9 ± 6.5^{a,b}	52.1 ± 9.0^{b}
Probiotic	54.2 ± 7.4^{a}	68.8 ± 3.8^{a}	50.3 ± 1.9^{a}	43.6 ± 4.6^{a,b}
Turmeric	52.9 ± 8.9^{a}	62.8 ± 5.2^{a}	48.2 ± 4.5^{a}	41.6 ± 7.1^{a}
Control	55.7 ± 12.4^{a}	75.5 ± 9.3^{a}	53.1 ± 3.1^{a,b}	41.2 ± 3.0^{a}

Mean ± SD

Figures in the same columns having different superscripts are significantly different ($p<0.05$)

A, B, C, D, E, F and G (each containing 15 birds) in three replicates of five birds. Each bird of the group A, B, C, D, E and F was fed with 0.2% aqueous extract of *Aloe vera*, *Zingiber officinale* , Safi, *Allium sativum*, Probiotic (Dahi) and *Curcuma longa* in addition to the basal diet, twice a day from day 11 till the end of experiment, respectively. Group G was kept as control, fed on basal diet only. Ten percent Stock solution of Probiotic (Dahi) was prepared twice a week while that of SAFI was prepared weekly in clean filtered water.

Blood samples were collected by puncturing the ulnar vein of broilers, using 5-ml plastic syringes, and 25 x 7 mm needles. Twenty one blood serum samples were collected from NDV vaccinated broilers weekly *i.e*., on 18th day (G1), 25th day (G2), 32nd day (G3) and 39th day(G4). Broilers were fed a corn and soybean meal-based diet, which supplied the nutritional requirements (Rostagno *et al.*, 1995).

Serum levels of cholesterol, triglycerides, sodium, calcium were determined using commercial kits of Cromatest purchased from Linear Chemicals S.L. Spain, using standardized method described by German Society for Clinical Chemistry (1972). Sample readings were recorded using spectrophotometry (Chemistry Analyzer, Metro Lab, Model: 1600 plus) with wave length adequate for each test. Antibody titer against ND Virus was

determined by performing Haemagglutination inhibition test (Hirst, 1942; Allan and Gough, 1974) to check the immune response of broilers to ND virus.

The data thus obtained was analyzed using ANOVA and Duncan's multiple range tests by SPSS statistical program version 15.0. Serum components were expressed as means and standard deviation. Means were compared by Duncan multiple range test. Significance was considered at 5% probability level.

RESULTS AND DISCUSSION

Table I shows the effect of aqueous herbal extracts and probiotic on the serum level of calcium, cholesterol, triglyceride and sodium in broilers. Garlic has increased the level of calcium in the sera of broilers whereas level of cholesterol is decreased. None of the other herbal extracts had any significant effect on sera level of triglyceride and sodium.

Table II shows that there is no effect of herbal extract on antibody titre. This showed that in this experimental design the aqueous extract of garlic probably enhanced the intestinal absorption of calcium by modulating the activity of Ca-ATPase enzyme present in the plasma membrane. While other aqueous herbal extracts did not significantly affected the calcium level of broilers. The present study is to some extent in agreement with the past study conducted by Mukherjee *et al.* (2006), which suggested the significant effect of oil extract of garlic by promoting intestinal transference of calcium in rats.

Effect of other herbal extracts on cholesterol level was not significant. This study is to some extent in accordance with the study conducted by Durak *et al.* (2004), who concluded that garlic extract supplementation improves blood lipid profile by lowering the serum levels of **s**erum total cholesterol, low-density lipoprotein (LDL) and very–low-density lipoprotein (VLDL) cholesterols in humans.

This result is to some extent against the study conducted by Durak *et al.* (2004), who suggested that garlic extract supplement lowers the serum level of triglycerides in humans. But according to Dong *et al.* (2015) in humans, obesity is associated with increased or decreased levels of serum biochemical indicators. Due to long-term intense selection for fast growth rate, modern broilers have the problem of excessive fat deposition, exhibiting biochemical or metabolic changes. So the results shown in Table I indicate that as broilers have the tendency of excessive triglyceride synthesis that was why any of the herbal extract had not significantly lowered the serum level of triglycerides.

The herbal extracts had no significant effect on the serum level of sodium in broiler chicks as shown in Table I. This result is to some extent against the study conducted by Oluwole (2001), who indicated that higher doses of garlic increase the serum level of Sodium in rats. Our results were different from Oluwole (2001) because the chicks were bred in an open shed in the extreme hot and dry climate of DIKhan district, so it is possible that probably due to the heat stress endured by the broiler chicks the sera level of sodium were not significantly different for any of the herbal extract.

Furthermore all the herbal extracts and probiotic did not significantly altered the immune response of broiler chicks against the Newcastle Disease Virus vaccine as tabulated in Table II. Although the antibody titer of broilers against ND Virus was sufficient to ward off any ND outbreak.

Table II.- Effect of aqueous herbal extracts and probiotic on antibody response of broilers to NDV vaccine to anti-NDV-HI antibody Titre.

Name of extract	25th day	32nd day	39th day	CGMT
Aloe vera	2	2.2	1.5	1.9 ± 0.4^{a}
Garlic	1.3	2	1.5	1.6 ± 0.4^{a}
Ginger	1.7	2	1.2	1.6 ± 0.4^{a}
Turmeric	1.7	2.1	2	1.9 ± 0.2^{a}
Probiotic	1.5	2.1	2	1.9 ± 0.3^{a}
Safi	1.5	2.2	2.1	1.9 ± 0.4^{a}
Control	1.5	2.3	1.5	1.8 ± 0.5^{a}

CGMT, Cumulative Geometric Mean Titre

CONCLUSION

The results presented in this study suggest that herbal extracts should be added in the feed of broilers in minute quantities. This will improve the quality of their feed and will have benefits for the consumer of the broiler meat. The commercial feed of broilers is prepared to make it cost effective and thus the quality of the feed is low which is not good for the consumers. So by adding these and other herbal extracts with proven medicinal benefits will definitely enhance the quality and medicinal value of the broiler feed with negligible impact on the cost.

ACKNOWLEDGEMENTS

The financial support for the present research was provided by the late Professor Dr. Amanullah Akhtar,

Principal, Gomal College of Veterinary Sciences, Gomal University, D.I. Khan, KPK. He also allowed to use the Poultry Farm of the college for this research. The staff of the college also provided technical and non-technical support throughout the research.

Professor Dr. Masood Rabbani, Director of University Diagnostic Laboratory, University of Veterinary and Animal Sciences, Lahore, kindly granted permission to analyze the sera samples in his laboratory.

Conflict of interest

The authors disclose no conflicts of interest for the present research.

REFERENCES

Allan, W.H. and Gough, R.E., 1974. A standard haemagglutination-inhibition test for Newcastle disease: A comparison of macro-and micromethods. *Vet. Rec.*, **95:** 120-123.

Azaz, D., Demirci, F., Satil, F., Kürkcüog, L.U. and Baser, K.H.C., 2002. Antimicobial activity of some Satureja oils. *Z. Naturforsch.*, **57:** 817-821.

Babayi, H., Kolo, L., Okogun, H. and Ijah, U.J., 2004. The antimicrobial activities of methanolic extracts of *Eucalyptus camaldulensis* and *Terminalia catappa* against some pathogenic microorganisms. *Biokemistri*, **16**: 106-111.

Bassett, R., 2000. Oreganos positive impact on poultry production. *World Poult.* Elsevier **16:** 31-34.

Bolukbasi, Ş.C., Erhan, M.K. and Özkan, A., 2006. Effect of dietary thyme oil and vitamin E on growth, lipid oxidation, meat fatty acid composition and serum lipoproteins of broilers. *S. Afr. J. Anim. Sci.*, **36:** 189-196.

Botsoglou, N.A., Christaki, E., Florou-Paner, P., Giannenas, I., Papageorgiou, G. and Spais, A. B., 2004. The effect of a mixture of herbal essential oils or a-tocopheryl acetate on performance parameters and oxidation of body lipid in broiler. *S. Afr. J. Anim. Sci.*, **34:** 52-61.

Botsoglou, N.A., Florou-Paner, P., Christaki, E., Fletouris, D.G. and Spais, A.B., 2002. Effect of dietary oregano essential oil on performance of chickens and on iron induced lipid oxidation of breast, thigh and abdominal fat tissues. *Br. Poult Sci*., **43:** 223-230.

Demir, E., Sarica, S., Ozcan, M.A. and Suicmez, M., 2003. The use of natural feed additives as alternatives for an antibiotic growth promoter in broiler diets. *Br. Poult Sci*., **44:** S44-S45.

Dong, J.Q., Zhang, H., Jiang, X.F., Wang, S.Z., Du, Z.Q., Wang, Z.P., Leng, L., Cao, Z.P., Li, Y.M., Luan, P. and Li, H., 2015. Comparison of serum biochemical parameters between two broiler chicken lines divergently selected for abdominal fat content. *J. Anim. Sci.*, **93:** 3278-3286.

Dorman, H.J.D. and Deans, S.G., 2000. Antimicrobial agent from plants: antimicrobial activity of plant volatile oils. *J. appl. Microbiol*., **88:** 308-331.

Durak, I., Kavutcu, M., Aytaç, B., Avci, A., Devrim, E., Özbek, H. and Öztürk, H.S., 2004. Effects of garlic extract consumption on blood lipid and oxidant/antioxidant parameters in humans with high blood cholesterol. *J. Nutr. Biochem*., **15:** 373–377.

Faleiro, M.L., Miguel, M.G., Ladeiro, F., Venanclo, F., Taveres, R., Brito, J.C., Figueiredo, A.C., Barroso, J.G. and Pedro, L.G., 2003. Antimicrobial activity of essential oils isolated from Portuguese endemic species of thymus. *Lett. appl. Microbial*., **36:** 35-40.

German Society for Clinical Chemistry, 1972. Recommendations of the German Society for Clinical Chemistry. Standardisation of methods for the estimation of enzyme activities in biological fluids. Experimental basis for the optimized standard conditions. . *Z. Klin. Chem. Klin. Biochem*., **10:** 281-291.

Gill, C., 2001. Safe and sustainable feed ingredients. *Feed Int*., **22:** 40-45.

Habibian Dehkordi, S., Zamani Moghadam, A., Maghsoudi, N., Aali, E., Gerami, R. and Dehsadeghi, E., 2010. The effects of fresh garlic on the serum concentration of total cholesterol, total triglyceride and adipose tissues of broilers. *Biochim. biophys. Acta*, **19:** 363-365.

Hernandez, F., Madrid, J., Garcia, V., Orengo, J. and Megias, M.D., 2004. Influence of two plants extracts on broiler performance, digestibility and digestive organ size. *Poult Sci*., **83:** 169-174.

Hirst G.K., 1942. The quantitative determination of influenza virus and antibodies by means of red cell agglutination. *J. exp. Med*., **75:** 49–64.

Jinag, L, Zhang, F.Y., Xu, Y., Xinting, Z. and Yang, D.P., 2005. Effect of gel polysaccharide and acemannan from *Aloe vera* on broiler gut flora, microvilli density, immune function and growth performance. *Chinese J. Vet. Sci*., **25:** 668-671.

Konjufca, V.H., Pesti, G.M. and Bakalli, R.I., 1997. Modulation of cholesterol levels in broiler meat by dietary garlic and copper. *Poult. Sci*., **76:** 1264-1271.

Mikulski, D., Zdunczyk, Z., Jankowski, J. and Juskiewicz, J., 2008. Effects of organic acids or natural plant extracts added to diets for turkeys on growth performance, gastrointestinal tract metabolism and carcass characteristics. *J. Anim. Feed Sci*., **17:** 233-246.

Mukherjee, M., Das, A.S., Das, D., Mukherjee, S., Mitra, S. and Mitra, C., 2006. Role of oil extract of garlic (Allium sativum Linn.) on intestinal transference of calcium and its possible correlation with preservation of skeletal health in an ovariectomized rat model of osteoporosis. *Phytother. Res.*, **20:** 408-15.

Oluwole, F.S., 2001. Effects of Garlic on hematological and biochemical parameters. *Afr. J. biomed. Res*., 4: 139-141.

Ponte, P.I.P., Mendes, I., Quaresma, M., Aguiar, M.N.M.,

Lemos, J.P.C., Ferreira, L.M.A., Soares, M.A.C., Alfaia, C.M., Prates, J.A.M. and Fontes, C.M.G.A., 2004. Cholesterol levels and sensory characteristics of meat from broiler consuming moderate to high levels of alfalfa. *Poult. Sci*., **83:** 810-814.

Rostagno, H.S. and Pupa, J.M.R., 1995. Diet formulation for broilers based on total versus digestible amino acids. *J. appl. Poult. Res.*, **4:** 293–299.

Sarica, S., Ciftci, A., Demir, E., Kilinc, K. and Yildirim, Y., 2005. Use of antibiotic growth promoter and two herbal natural feed additives with and without exogenous enzymes in wheat based broiler diets. *S. Afr. Anim. Sci*., **35:** 61-72.

Schwarz, S., Kehrenberg, C. and Walsh, T. R., 2001. Use of antimicrobial agents in veterinary medicine and food animal production. *Int. J. Antimicrob. Agents*, **17:** 431-437.

Yasmeen, R., Hashmi, A.S., Anjum, A.A., Saeed, S. and Muhammad, K., 2012. Antibacterial activity of indigenous herbal extracts against urease producing bacteria. *J. Anim. Pl. Sci.*, **22**: 416-419.