Indian J. Anim. Nutr. 2021. 38 (1): 61-67
doi: 10.5958/2231-6744.2021.00009.8

Indian Journal of
Animal Nutrition

Effect of Herbal Feed Additives on Performance, Immunity, Serum Parameters and *E. coli* Counts of Broilers under Heat Stress

Hanumanth Rao and Srinivas Gurram*
Department of Poultry Science, College of Veterinary Science,
P.V. Narasimha Rao Telangana Veterinary University, Rajendranagar, Hyderabad, 500030, India

ABSTRACT

The present experiment was conducted to investigate the effect of different herbal preparations on the performance of broilers. A total of 250 day-old male broiler chicks were randomly divided into five treatment groups with ten replicates of five birds each. The experimental design consisted of; T_1: control diet, T_2: Herbal powder I (containing *Withania somnifera, Phyllanthus emblica, Glycrrhiza glabra, Tribulus terrestris and Asparagas racemosus*), T_3: Herbal powder II (containing *Withania somnifera, Ocimum sanctum, Mangifera indica and Shilajit*), T_4: Herbal powder III (Ayuce herbal powder), and T_5: Vitamin E (70 mg per kg) and Se (0.15 mg per kg). The results indicated that supplementation of herbal preparations (T_2, T_3 and T_4), and vitamin E and Se significantly ($P<0.05$) increased the body weight gain and feed conversion ratio (FCR) of broilers compared to control group at 42 d of age. However, feed intake was not affected by different herbal supplementation at 42 d of age. The Lipid peroxidation, alkaline phosphatase, blood urea nitrogen, serum cholesterol, blood glucose levels and *E. coli* counts in small intestine were significantly ($P<0.05$) lower in herbal supplemented groups as compared to control and was at par with vitamin E and Se supplemented group. Various carcass parameters, serum total protein, albumin and antibody titer to Newcastle disease (ND) vaccine was similar among the groups. From the results, it could be concluded that poly-herbal preparations can be used as a feed additive for improving performance of broilers during heat stress conditions.

Key Words: Broilers, Body weight, Cholesterol, Herbs, Heat stress, Lipid peroxidation

INTRODUCTION

Heat stress causes huge economic losses to Indian poultry industry leading to heavy mortality and decreased performance. High temperatures, especially when coupled with high humidity, impose severe stress on broiler birds (Sahin *et al.,* 2003; Ajakaiye *et al.,* 2011). In summer, most of the energy produced is shifted to thermoregulatory adaptation, leading to reduced weight gain, reduced immunity, oxidative stress, and high mortality (Maini *et al.,* 2007). The adverse effects of heat stress are manifested in the deterioration of cell function through changes in oxidative metabolism, thereby damaging cell membranes (Mates *et al.,* 1999). Elevated ambient temperature will increase free radicals and other reactive oxygen species (ROS) in tissues that destroy biological macromolecules and disrupt normal cell physiology and metabolism (Spurlock and Savage, 1993). The antioxidants commonly available in the body are vitamin C, vitamin E, folic acid, zinc and chromium. In addition, antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) play vital roles in protecting cells from the damaging effects of ROS (Meister and Anderson, 1983). High ambient temperature will deplete these antioxidants and induce oxidative stress. In addition to oxidative stress, blood glucose and cholesterol concentrations are also significantly increased (Altan *et al.,* 2000) in animal exposed to thermal stress.

Various Ayurvedic herbal preparations have been widely used in poultry to alleviate the negative effects of high environmental temperatures. Herbal products containing different immunomodulatory herbs (*Withania somnifera*), and antistressor (*Phyllanthus emblica, Mangifera indica, Ocimum sanctum*) have been used to improve performance (Reddy *et al.,* 2012). However, these herbs differ widely in their functional properties and a combination of different herbs may complement each other. Keeping this point in mind the present experiment was

*Corresponding author: E-mail: gurramsrinivas4@gmail.com

conducted to study the effect of different herbal preparations on the performance, immunity and antioxidant enzyme activity of broilers.

MATERIALS AND METHODS

In this study, 250 day-old male broiler chicks (Vencobb) were distributed randomly in to five dietary treatments of ten replicates with five chicks in each replicate. At day one, chicks were wing banded and housed under deep litter system with optimum brooding conditions. Standard management practices were followed during the entire experimental period. The birds were fed with maize and soybean meal-based diets containing 3050 and 3150 kcal ME and 21.5 and 19.5 percent crude protein, respectively during starter (1-28 d) and finisher (29-42 d) phases (Table 1). The dietary treatments consisted of; T_1: Control without herbal supplementation, T_2: Herbal powder I (containing *Withania somnifera, Phyllanthus emblica, Glycrrhiza glabra, Tribulus terrestris* and *Asparagas racemosus*) at 250 gm/ton of feed, T_3: Herbal powder II (containing *Withania somnifera, Ocimum sanctum, Mangifera indica* and *Shilajit*) at 1 kg/ton of feed, T_4: Herbal powder III (Ayuce herbal powder supplied by Ayurvet Limited, Baddi, India) at 100 gm/ton of feed, and T_5:

Table 1. Ingredient composition of basal diets (kg/q)

Ingredient	Starter phase (0-28d)	Finisher phase(29-42d)
Maize	56.2	59.1
Oil	4.1	4.1
Soyabean meal	35	32.2
Shell grit	1.83	1.75
Dicalcium phosphate	1.95	1.89
Salt	0.4	0.4
DL-Methionine	0.19	0.15
L-Lysine HCl	0.14	0.15
Trace Mineral Mixture**	0.1	0.1
Vitamin AB_2D_3K	0.02	0.02
Vitamin B-Complex*	0.025	0.025
Coccidiostat	0.05	0.05
Antibiotic	0.05	0.05
Choline chloride(50%)	0.1	0.1
Toxin binder	0.1	0.1
Tylosine	0.05	0.05
Total	100	100
Nutrient composition		
ME (kcal/kg)	3050	3150
Crude protein (%)	21.50	19.51
Lysine (%)	1.21	1.02
Methionine (%)	0.50	0.45
Calcium (%)	1.06	1.01
Available phosphorous (%)	0.46	0.45
Zinc (mg/kg)	22.01	20.26

*Vitamin premix provided per kg diet: vitamin A 200000 IU, vitamin D_3 3000 IU, vitamin E 10 mg, vitamin K 2 mg, riboflavin 25 mg, vitamin B_1 1mg, vitamin B_6 2 mg, vitamin B_{12} 40 mg, and niacin 15 mg; **Trace mineral provided per kg diet: Manganese 120 mg, iron 25 mg, copper 10 mg, iodine 1 mg and selenium 0.1mg.

vitamin E (70 mg per kg) and Se (0.15 mg per kg). Weekly individual body weight and feed consumption of each group were recorded. After the experimental period (42 d), one bird from each replicate of all the treatment groups were sacrificed for recording of carcass parameters. Blood samples were collected on 42 days of age. Serum samples were separated from the blood and were used for the estimation of cholesterol, total protein, albumin, globulin, blood glucose, lipid peroxidation, alkaline phosphatse and blood urea nitrogen using standard diagnostic kits of Erba Pvt. Ltd.

The humoral immunity was estimated in birds by measuring antibody titer to Newcastle disease (ND) vaccine (antibody production against ND virus). Broilers were vaccinated against ND by occular route at 7th and 28th day of age with Lasota strain (ND Lasota Vac-500; Indovax Pvt. Ltd. Hyderabad, India). At 42nd day of age blood was collected and serum was separated for estimation of Haemagglutination inhibition (HI) activity. The antibody titers (log2) were measured following the standard procedure (Wegmann and Smithies, 1966).

On day 42, eight birds from each group were slaughtered and approximately 5 g of ileal digesta was collected in a sterile sampling tube under aseptic conditions and immediately transferred to the laboratory on ice for the *E. coli* count. Eosin methylene blue agar (EMB) was used for *E. coli* growth. Then, 9 sterile test tubes with lids containing 9 ml of phosphate buffer solution (PBS, pH-7.4) as diluent were prepared. Approximately 1g of the intestinal contents was taken by sterile swab, homogenized for 3 min, aseptically mixed, added to the tubes, and diluted up to 10^9. Later, 1 ml of the contents of each test tube was transferred to agar media on petri plates (Gunal *et al.,* 2006). Aerobic bacterial plates (*E. coli*) were placed in an incubator at 37^{o}C for 24 hours. Finally, the intestinal bacterial colony populations formed in each plate was counted by colony counter and the number of colonies was expressed as log10 value.

Data obtained were analyzed for mean, standard errors and analysis of variance as per method of Snedecor and Cochran (1989) and comparison of means were done using methods described by Duncan (1955) using software of Statistical Package for Social Sciences (SPSS) 20.0 version and significance was considered at P<0.05.

RESULTS AND DISCUSSION

Record of temperature was maintained on daily basis, the highest daily average temperature recorded was 41.07 °C and the lowest temperature was 36.8 °C during the experimental period. The temperature-humidity index (temperature 103^{0}F and humidity 58%) was 107±1.10, were above the threshold established for poultry. Thus, the birds were subjected to heat stress.

Supplementation of various herbal preparations (T_2, T_3 and T_4), and vitamin E and Se (T_5) significantly (P<0.05) improved the cumulative body weight gain and feed conversion ratio (FCR) of broilers when compared to control group at 42 d of age (Table 2). Among all the test diets, the highest mean weight gain was recorded in

Table 2. Effect of different herbal feed additives on growth parameters of broilers at 42 days of age

Treatments	Cumulative Weight gain (g) /bird	Feed intake (g)/bird	Feed conversion ratio (FCR)
Control	1960^b	3258	1.67^b
Herbal powder I	1988^{ab}	3299	1.65^a
Herbal powder II	1977^{ab}	3247	1.64^a
Herbal powder III	2013^a	3307	1.64^a
Vitamin E and Se	2017^a	3331	1.65^a
P-value	0.006	0.814	0.003

Value bearing different superscripts within a column are significantly (P<0.05) different

Table 3. Effect of different herbal feed additives on carcass parameters of broilers at 42 days of age

Treatments	Dressed weight (g)	Abdominal Fat (g)	Gizzard (g)	Heart (g)	Liver (g)	Spleen (g)
Control	1664	21.92	41.24	13.04	38.00	3.180
Herbal powder I	1774	20.48	41.50	12.45	42.75	2.450
Herbal powder II	1802	24.94	43.56	10.88	37.42	2.480
Herbal powder III	1784	19.03	41.72	11.55	38.83	3.517
Vitamin E and Se	1797	22.26	44.28	11.58	39.68	2.600
P-Value	0.174	0.322	0.908	0.196	0.764	0.554

Value bearing different superscripts within a column are significantly (P<0.05) different

T_4 and T_5 groups. However, supplementation of herbal preparations did not have any significant (P>0.05) effect on feed intake of broilers at 42 days of age. These findings are in accordance with results of Karangiya *et al.* (2016) and Chaudhari *et al.* (2015) who reported that supplementation of herbs significantly (P<0.05) increased the body weight gain in broilers. This is also in agreement with many studies where supplementation of herbs (Pooja *et al.,* 2017; Mohamed *et al.,* 2012) had a significant (P<0.05) positive effect on the body weight gain and FCR of broilers. Contrary to these findings, Ademola *et al.* (2009) reported that herbs did not show significant (P<0.05) effect on FCR of broilers. The improvement in body weight and FCR may be due to the stimulation of digestive enzymes in the intestines and pancreas, thus improving the digestion of nutrients and the efficiency of feeding, thus increasing the growth rate (Ali, 2011). In addition, herbal preparations have appetizing and stimulatory effects in the digestion process by increasing production of digestive enzymes and juices, which stimulates digestion and peristolic motion, thus improves FCR (Langhout, 2000; Rajeswari and Andallu, 2011).

Highest mortality was noticed in control (4 out of 50 birds) group followed by T_2 (3 out of 50 birds) and T_4 groups (2 out of 50 birds). However, the mortality rate was within the acceptable range and no specific disease outbreak was recorded. Similar results were observed by Shiva Kumar *et al.* (2005). There was no significant (P>0.05) influence of different treatments on carcass parameters like dressing weight and giblet weights (liver, heart and gizzard). Similarly, Kale *et al.* (2014) reported that supplementation of ashwagandha did not show any significant effect on dressing percent, giblet and cooking yield. Similar observations were also made by Dahale *et al.* (2014).

Lipid peroxidation, activity of alkaline phosphatase and serum concentration of urea nitrogen were

Table 4. Effect of different herbal feed additives on lipid peroxidation, alkaline phosphatase and serum urea nitrogen of broilers at 42 days of age

Treatments	Lipid peroxidation (nmol MDA/mg protein)	Alkaline phosphatase (nmol MDA/mg protein)	Blood urea nitrogen (nmol MDA/mg protein)
Control	4.526^{a}	97.49^{a}	65.81^{a}
Herbal powder I	3.747^{b}	83.04^{b}	62.51^{b}
erbal powder II	3.610^{b}	80.80^{b}	62.87^{b}
Herbal powder III	3.371^{b}	76.27^{b}	62.31^{b}
Vitamin E and Se	2.594^{c}	78.92^{b}	61.99^{b}
P- Value	0.025	0.05	0.001

Value bearing different superscripts within a column are significantly (P<0.05) different

Table 5. Effect of different herbal feed additives on carcass parameters of broilers at 42 days of age

Treatments	Cholesterol (mg/dl)	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Blood glucose (g/dl)
Control	194.0[a]	4.327	2.047	2.280	196.92[a]
Herbal powder I	182.8[b]	4.436	1.929	2.506	187.37[b]
Herbal powder II	181.6[b]	3.902	1.935	1.967	185.81[b]
Herbal powder III	180.3[b]	4.386	2.061	2.325	183.70[b]
Vitamin E and Se	186.6[b]	4.327	1.904	2.423	186.40[b]
P-value	0.021	0.116	0.180	0.120	0.003

Value bearing different superscripts within a column are significantly (P<0.05) different

significantly reduced in vitamin E and Se, and herbal supplemented diets as compared to control at 42 d of age (Table 4). Heat stress increased red blood cell susceptibility to lipid peroxidation because of increased free radical generation, as indicated by increased lipid peroxidation concentration in control group. Decreased value of lipid peroxidation in herbal supplemented groups indicate antioxidative effect of these products. Similar results were also reported by Sujatha *et al.* (2010). Herbal preparations are source of phyto-chemicals and functional compounds namely polyphenols, flavonoids and ascorbic acid which ultimately constitute for its high antioxidant activity (Darughe *et al.,* 2012).

Compared with the herbal supplemented groups, the serum concentrations (mg/dl) of glucose and cholesterol of the control group were significantly increased (P<0.05) at 42 d of age, however, no significant (P>0.005) difference was observed among the herbal supplemented groups (Table 5). Similarly, reduced levels of serum LDL, total cholesterol and triglycerides with herbal supplementation in broilers were reported by others (Lanjewar *et al.,* 2008; Khwairakpam *et al.* 2016). However, Dwivedi *et al.* (2015) did not found any difference in blood glucose values due to feeding of herbal preparations. The values of total serum protein, albumin, globulin, and HI antibody titer against Newcastle disease were similar in all the treatments indicating that supplementation of herbal preparations, and vitamin E and Se did not have any significant effect on these parameters (Tables 5 and 6).

Supplementation of various polyherbal preparations, and vitamin E and Se significantly (P<0.05) decreased the *E. coli* counts in small intestine of broilers at 42 days of age (Table 6). In agreement with our results, Allinson *et al.* (2013) reported that herbal extracts enhanced the performance of poultry by significantly decreasing the bacterial count in the intestine. It has been established that herbs in the diets stimulate lactic acid bacteria and decreases pathogenic bacteria such as mesophilic aerobic, coliform and *Escherichia coli,* and thus improves absorption of nutrients leading to better weight gain of the birds.

Table 6. Effect of different herbal feed additives on immunity and *E. coli* counts of broilers

Treatments	HI titre($\log_2$)	*Escherichia coli* ($\log_{10}$ of cfu/ml)
Control	8.012	6.75[a]
Herbal powder I	7.625	6.66[ab]
Herbal powder II	7.875	6.57[b]
Herbal powder III	7.625	6.24[c]
Vitamin E and Se	8.000	6.01[c]
P-value	0.887	0.002

Value bearing different superscripts within a column are significantly (P<0.05) different

Similarly, Taha *et al.* (2019) reported that coriander powder supplementation reduced the ileal total bacteria, *E coli*, and *C. perfringens* counts compared to control group. Similar observations were also reported by Lee *et al.* (2016).

CONCLUSIONS

Dietary supplementation of different herbal supplements, and vitamin E and Se improved the body weight gain, FCR and reduced the *E. coli* colonization in the intestine. Further, these herbal supplements reduced lipid peroxidation in broilers. Therefore, it can be concluded that supplementing broilers with poly-herbal preparations in summer is beneficial to overcome the adverse effect of heat stress.

ACKNOWLEDGEMENTS

The authors are thankful to Department of Poultry Science, CVSc, Rajendranagar, Hyderabad, and Ayurvet Ltd, India for funding the research.

REFERENCES

Ademola, S.G., Farimu, G.O. and Babatunde, G.M. 2009. Serum lipid, growth and haematological parameters of broilers fed garlic, ginger and their mixtures. *World J. Agric. Sci.* 5: 99-104.

Ajakaiye, J.J., Perez-Bello, A. and Mollineda, T.A. 2011. Impact of vitamins C and E dietary supplementation on leukocyte profile of layer hens exposed to high ambient temperature and humidity. *Acta. Vet. Brno.* 79: 377-383.

Ali, A. 2011. Effect of different levels of Chicory, zizaphora, nettle and savoury medicinal plants on the carcass characteristics of male broilers. *J. Med. Plants Res.* 5: 4354-4359.

Allinson, I.B., Ekunseitan, D.A., Ayoola, A.A., Ogunade, I.M. and Njoku, C.P. 2013. Effect of herbal supplement on growth response and faecal egg counts of cockerels. *J. Anim. Feed Res.* 3: 68-73.

Altan, O., Altan, A., Cabuk, M. and Bayraktar, H. 2000. Effects of heat stress on some blood parameters in broilers. *Turkish J. Vet. Anim. Sci.* 24: 140-148.

Chaudhary, R.K., Singh, V.K., Singh, S.P., Gautam, S. and Tewari, D. 2015. Effect of herbal supplements of *curcuma lona, emblica officinalis* and *nigella sativa* on performance of broilers. *Indian J. Anim. Nutr.*31: 90-95.

Dahale, G.S., Wankhade, S.M. and Kale. 2014. Growth performance, serum biochemical profile and carcass quality of broiler chicken fed diets supplemented with shatavari root powder. *Indian J. Anim. Nutr.* 31: 166-171.

Darughe, F., Barzegar, M. and Sahari, M.A. 2012. Antioxidant and antifungal activity of Coriander (*Coriandrum sativum L.*) essential oil in cake. *Int. J. Food Res.* 19: 1253-1260.

Duncan, D.B. 1995. Multiple range and F-tests. *Biometrics.* 11: 1-42.

Dwivedi, V., Singh, V.K., Tewari, D., Gautam, S., Singh, V.B. and Dwivedi, D. 2015. Growth Performance, Blood Constituents and Carcass Traits of Broiler Chicken as Affected by Supplementation of Ashwagandha and Mangrail. *Indian J. Anim. Nutr.* 32: 428-433.

Gunal, M., Yayli, G., Karahan, N. and Sulak, O. 2006. The effect of antibiotic growth promoter, probiotic or organic acid supplementation on performance, intestinal microflora and tissue of broilers. *Int. J. Poult. Sci.* 5: 149-155.

Kale, V.R., Wankhade, S.M. and Kale, S.D. 2014. Effect of dietary supplementation of Ashwagandha on carcass quality of broiler chicken. *Indian J. Anim. Nutr.* 31: 81-85.

Karangiya, V.K., Savsani, H.H., Shrikant, S.P., Garg, D.D., Murthy, K.S., Ribadiya, N.K. and Vekariya, S.J. 2016. Effect of dietary supplementation of garlic, ginger and their combination on feed intake, growth performance and economics in commercial broilers. *Vet. World.* 9: 245-250.

Khwairakpam, R., Tiwari, D.P. and Mondal, B.C. 2016. Effect of dietary incorporation of garlic (allium sativum) and turmeric (curcuma longa) powder and their combination on feed intake, haemato-biochemical parameters and carcass traits in broiler chicken. *Indian J. Anim. Nutr.* 33: 184-190.

Langhout, P. 2000. New additives for broiler chickens. *World's Poult. Sci. J.* 16: 22-27.

Lanjewar, R.D., Zanzad, A.A., Ramteke, B.N. and Deshmukh, G.B. 2008. Effect of dietary supplementation of tulsi (*O. sanctum*) leaf powder on the growth performance and serum lipid profile in broilers. *Indian J. Anim. Nutr.* 25: 395-397.

Lee, J.S., Kim, M.J., Park, S.H., Lee, S.H., Wang, T., Jung, U.S., Im, J., Kim, E.J., Lee, K.W. and Lee, H.G. 2016. Effects of dietary mixture of garlic (*Allium sativum*), coriander (Coriandrum sativum) and probiotics on immune responses and caecal counts in young laying hens. *J Anim. Physiol. Anim. Nutr.* 101: 122-132.

Maini, S., Rastogi, S.K., Korde, J.P., Madan, A.K. and Shukla,

S.K. 2007. Evaluation of oxidative stress and its amelioration through certain antioxidants in broilers during summer. *J. Poult. Sci.* 44: 339-347.

Mates, J.M., Perez-gomez, C. and Nunez D.C.I. 1999. Antioxidant enzyme in human diseases. *Clin. Biochem.* 32: 595-603.

Meister, A. and Anderson, M.E. 1983. Glutathione. *Ann. Rev. Biochem.* 52: 711-760.

Mohamed, A.B., Mohammed, A.M. and Ali, J.Q. 2012. Effect of ginger (*Zingiber officinale*) on performance and blood serum parameters of broiler. *Int. J. Poult. Sci*.11: 143-146.

Pooja, A., Kumar, A. and Singh, P.K. 2017. Garlic and Amla powder addition in diet affects production performance of white leghorn laying hens. *Indian J. Anim. Nutr.* 34: 80-86.

Reddy, E.T., Reddy, P.S., Reddy, P.V.V.S. and Shakila, 2012. Effect of herbal preparations on the performance of broilers. *Tamilnadu J. Vet. Anim. Sci.* 8: 209-214.

Sahin, K., Onderci, M., Sahin, N., Gursu, M.F. and Kucuk, O. 2003. Dietary vitamin C and folic acid supplementation ameliorates the detrimental effects of heat stress in Japanese quail. *J. Nutr.* 133: 1882-1888.

Shiva Kumar, M.C., Javed, M., Pugashetti, B.K. and Sarah, N. 2005. Influence of Supplementation of herbal growth promoter on growth and performance of broilers. *Karnataka J. Agric. Sci.* 18: 481-484.

Snedecor, G.W. and Cochran, W.G. 1989. *Statistical Methods* (8th Ed). The Iowa State University Press. Ames. Iowa, U.S.A.

Spurlock, M.E. and Savage, J.E. 1993. Effects of dietary protein and selected antioxidants on fatty haemorrhagic syndrome induced in Japanese quails. *Poult. Sci.* 72: 2095-2105.

Sujatha, V., Korde, J.P., Rastogi, S.K., Maini, S., Ravikanth, K. and Rekhe, D.S. 2010. Amelioration of heat stress induced disturbances of the antioxidant defense system in broilers. *J. Vet. Med. Anim. Hlth.* 2: 18-28.

Taha, A.E., Saber, S.H., Ramadan, S.S., Ahmed, A.E., Mohamed, E.A., Hussein, E., Islam, M.S., Ayman, A.S. and Mohamed, A.E. 2019. Effects of supplementing broiler diets with coriander seed powder on growth performance, blood haematology, ileum microflora and economic efficiency. *J. Anim. Physiol. Anim. Nutr.* 6: 1-10.

Wegmann, T.G. and Smithies, O. 1966. A simple hema-gglutination system requiring small amounts of red cells and antibodies. *Transfusion.* 6: 67-73.

Received on 30-06-2021 and accepted on 06-07-2021