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Research Article

INFLUENCE OF ASHWAGANDHA SUPPLEMENTATION WITH SELENIUM AND VITAMIN E ON HEMATO-BIOCHEMICAL PARAMETERS AND IMMUNE RESPONSE OF BROILER CHICKENS

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ABSTRACT

An experiment was conducted on 300 day-old commercial broiler chicks (Cobb 430 Y) for a period of 42 days. The chicks were distributed randomly into five experimental groups with four replicates having 15 birds each. The group (T_0) was control group and fed as per BIS (2007), whereas treatment groups T_1, T_2, T_3 and T_4 were supplemented with Ashwagandha @ 0.5%, Ashwagandha @ 0.5% plus Vitamin E @ 30 mg/kg, Ashwagandha @ 0.5% plus Selenium @ 0.25 mg/kg and Ashwagandha @ 0.5% plus 30 mg/kg of Vitamin E and 0.25 mg/kg of Selenium, respectively. The blood samples were collected on 0, 28th and 42nd day of feeding trial from two birds per replicate, for analysis of hematological and blood-biochemical parameters. The humoral immunity was estimated by measuring antibody titres by of Newcastle disease (ND) vaccine i.e. antibody production against ND virus. The data collected was statistically analyzed as per Snedecor and Cochran (1994). One Way Anova was applied using SPSS V.16 and means were compared by using Duncan's method. The results of broiler birds of group supplemented with Ashwagandha in combination with Selenium and Vitamin E observed significantly ($p<0.05$) higher values of Hb, PCV, TLC, TEC, ND $\log_2$ titre. The data of hematology, blood-biochemical parameters and humoral immune response of experimental broiler birds supplemented with Ashwagandha in combination with Selenium and Vitamin E recorded improved average values which could be concluded the better health and immunity in broiler birds.

Keywords: Ashwagandha, Selenium, Vitamin E, Blood parameters, Immunity, Broilers

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In India the poultry farming has a potential to bring rapid economic growth, chiefly benefiting the weaker segment of people due its low investment, short maturation period and efficiency of broilers to convert feed into meat. Due to the genetic selection the growth rate of birds is improved and has been profited to the farmers. However, it has augmented sensitivity of these birds to the stressors. The stressors works as internal damage leading to adverse impact on economics traits (White head and Keller, 2003). The heat stress can result in oxidative stress in birds which has been reported to decrease production performance and immunity hence leads to various diseases (Mujahid *et al.*, 2005). The overuse of antibiotics causes high risk of development of resistance, toxicity, high cost of production and environmental hazards and cross resistance as risk factor to human health (Costa *et al.*, 2007). The production of safer poultry meat in an economic way without any chemical and microbial residue is need of the day. Natural growth promoters are identified to be an efficient and effective alternative to antibacterial growth promoters to promote traditional, natural, and alternative health system (Makkar *et al.*, 2007). The Ashwagandha (Withania somnifera) is one such herb which has properties like adaptogenic, immune modulators, immune adjuvant, antidepressant, liver tonic, antioxidant (Ziauddin *et al.*, 1996). It also improves the immunological status and haematological profile, neuroprotective and rejuvenates muscles (Ansari *et al.*, 2008). Selenium works against damage caused by free radical and lipoperoxides with catalase and superoxide dismutase by functioning on the active site of glutathione peroxides (Newberne and suphakarn, 1983; Thompson and scott, 1969). It performs vital role in antioxidant and redox reactions (Bleys *et al.*, 2009), immune function (Mckenzie *et al.*, 1998). Vitamin E plays crucial role in several biochemical and physiological processes, as well as antioxidation (Franchini *et al.*, 1995). Vitamin E supplementation has revealed improvement in growth performance, enhance immunity and restore impaired immunity as well as influence neuroendocrine function (Khan and Thomas, 2004). Vitamin E has been documented to boost immune competence in poultry and enhance their immunity (Erf *et al.*, 1998). Herbal preparation are widely used as feed additives for building immunity in broiler production, enhancing growth, reducing feed cost by improvement in feed conversion ratio (Pandey *et al.*, 2013). Moreover, herbal feed additives has no ill effect on health of birds and by increasing feed conversion ratio, live weight gain it upsurges performance and immunity (Bhardwaj *et al.*, 2011, Kumari *et al.*, 2012). Owing to the overlap between the biological activities of selenium and vitamin E, selenium deficiency contributes to an increase

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in the need for vitamin E in animals. Keeping above facts in view and due to the beneficial properties and common principals of the ashwagandha, selenium and vitamin E it was proposed to undertake a research trial in broiler birds.

MATERIALS AND METHODS

An experiment was conducted on 300, day-old straight run commercial broiler chicks (Cobb 430 Y) for a period 42 days. The experimental birds were randomly distributed into five groups equally. The chicks had similar body weights within the group and between the groups. These treatment groups were further divided into four replicates containing 15 birds each. All the birds were reared on the deep litter system of housing using rice husk as a litter material. The birds were feed as, control group (T_0) standard broiler chicken diet as per BIS (2007). Daily measured amount of feed was offered to experimental birds and calculated the feed intake. The detail of treatment groups of broiler birds is presented in Table 1. The treatment group (T_1) was supplemented with Ashwagandha powder @ 0.5%. The treatment group (T_2) was supplemented with Ashwagandha @ 0.5% plus Vitamin E @ 30 mg / kg of the diet. The treatment group (T_3) was supplemented with Ashwagandha @ 0.5% plus Selenium @ 0.25 mg / kg of the diet and the treatment group (T_4) supplemented with Ashwagandha @ 0.5% plus Vitamin E @ 30 mg/kg plus Selenium @ 0.25 mg / kg of the diet. On 0, 28th and 42nd day of feeding trial, blood samples were collected from two birds per replicate. About 2 ml of blood samples were collected from each bird via brachial wing vein puncture using sterilized needles into vaccutainer containing EDTA. A total of 40 samples were analysed for study of haematological parameters such as blood Hb, PCV, TLC and TEC. Blood haemoglobin (Hb) and packed cell volume (PCV) were estimated by Sahli's haemoglobinometer and Wintrob's methods, respectively. Total erythrocytes count (TEC) and total leukocytes count (TLC) were carried out manually through haemocytometer as per standard method of Natt and Hendricks (1954).

Similarly, blood samples were collected aseptically in clean sterilized glass tubes and kept in slanted position at room temperature for serum collection. The collected serum samples were then centrifuged at 3000 rpm for 5 minutes and transferred to 2 ml Eppendorf tubes which were stored at -20 ºC. Serum samples were analysed for different serum variables like total cholesterol, LDL (low density lipoprotein) and HDL (high density lipoprotein) through spectrophotometer using commercial test kits as per manufacturer's protocol. Low density lipoprotein cholesterol (LDL) was calculated as per formula of Fried Wald *et al.* (1972). Serum total cholesterol was analysed according to Allain *et al.* (1974). The total serum cholesterol was measured through spectrophotometer using the commercial kit supplied by Accurex Biomedical Pvt. Ltd. High density lipotrotein (HDL) and this was was estimated according to Lang and Schettler, (1985). The serum HDL was measured through spectrophotometer using the commercial kit supplied by Accurex Biomedical Pvt. Ltd. The effect of dietary supplementation of Ashwagandha Selenium and vitamin E on humoral immunity in broilers was estimated by measuring antibody titres to Newcastle disease (ND) vaccine i.e. antibody production against ND virus. Broilers were vaccinated against ND by ocular route at 7th and oral route at 28th day of age with Lasota strain (ND Lasota Vac-1000; Ventri Biologicals, Pune, India). On 0, 28th and 42nd days of age, blood was collected from 8 birds per group, two from each replicate and serum was separated. Subsequently antibody specific for ND was detected in sera of chicks by haemagglutination inhibition (HI) test and were expressed as $\log_2$ titers (Allan *et al.*, 1978). One Way ANOVA was applied using SPSS V.16 and the difference between subclass of mean were compared by using Duncan's method.

RESULTS AND DISCUSSION

Hematological Parameters

The blood samples were analysed for haematological parameters such as blood hamoglobin (Hb), packed cell volume (PCV), total erythrocytes count (TEC) and total leukocytes count (TLC) and analysis reports are presented in Table 2.

Hemoglobin (Hb, mg/dl)

The mean value of hemoglobin (Hb) during the 0 day and 28th day of trial were recorded. The statistical analysis of data of Hb values for 0th day and 28th day failed to demonstrate the significant difference ($P<0.05$) between the different groups. Though the numerically higher values can be seen in groups supplemented with *Ashwagandha* in alone and in combination with selenium and Vitamin E compared to non-supplemented group (T_0). At 42nd day of experiment i.e. at end of experiment, the values of Hb were observed as 10.15±0.08, 10.35±0.03, 10.34±0.06, 10.38±0.05 and 10.39±0.05 g/dl, respectively. The data obtained for Hb values at end of trial when analyzed statistically, demonstrated significant difference ($P<0.05$) between the groups. Significantly ($P<0.05$) higher values of Hb were seen in group (T_4), (T_3), (T_1) and (T_2) compared to control group (T_0). The improved Hb values the shows hematinic property of *Ashwagandha*. Our study results are in accordance with Bhardwaj *et al.* (2012), Ansari *et al.* (2013), Kant *et al.* (2014), Singh *et al.* (2016) and Singh *et al.* (2017).

Table 1. Details of Dietary Treatments and Groups of Experimental Broiler Birds

Treatment group	Treatment groups Details	No. of birds/ replications	No. of replications	Total number of birds
	Standard broiler chicken diet as per BIS, 2007	15	4	60
	Standard broiler chicken diet as per BIS, 2007 + *Ashwagandha* @ 0.5%	15	4	60
	Standard broiler chicken diet as per BIS, 2007 + *Ashwagandha* @ 0.5% + Vitamin E @ 30 mg/kg of the diet.	15	4	60
	Standard broiler chicken diet as per BIS, 2007 + *Ashwagandha* @ 0.5% + Selenium @ 0.25 mg/kg of the diet.	15	4	60
	Standard broiler chicken diet as per BIS, 2007 + 0.5% *Ashwagandha* + Vit. E @ 30 mg/kg + Selenium @ 0.25 mg/kg of the diet.	15	4	60
	Total number of experimental broiler birds			300

Table 2. Details of Hematological Parameters Experimental Broiler Birds

Parameters	T_0	T_1	T_2	T_3	T_4	CV
			Hemoglobin (g/dl)			
0^{th} day(g/dl)	9.40±0.04	9.45±0.11	9.45±0.13	9.44±0.11	9.42±0.09	2.863
28^{th} day(g/dl)	9.59±0.05	9.70±0.08	9.66±0.08	9.74±0.08	9.75±0.08	2.170
42^{nd} day(g/dl)	$10.15±0.08^{b}$	$10.35±0.03^{a}$	$10.34±0.06^{a}$	$10.38±0.05^{a}$	$10.39±.0.05^{a}$	1.703
			Packed Cell Volume (PCV %)			
0^{th} day	28.20±0.11	28.34±0.32	28.27±0.36	28.32±0.32	28.25±0.28	2.768
28^{th} day	28.76±0.15	29.10±0.23	28.99±0.24	29.21±0.25	29.25±0.24	2.170
42^{nd} day	$30.45±0.25^{b}$	$31.05±0.10^{a}$	$31.01±0.18^{b}$	$31.13±0.15^{a}$	$31.16±0.14^{a}$	1.703
			Total Leukocyte Count (TLC × 10^3 /µl)			
0^{th} day	21.58±0.05	21.59±0.09	21.55±0.11	21.56±0.08	21.45±0.08	1.072
28^{th} day	22.41±0.05	22.43±0.07	22.53±0.07	22.43±0.06	22.46±0.09	0.843
42^{nd} day	$24.98±0.15^{b}$	$25.45±0.15^{a}$	$25.43±0.14^{a}$	$25.44±0.11^{a}$	$25.46±0.08^{a}$	1.551
			Total Erythrocyte Count (TEC × 10^6 /µl)			
0^{th} day	2.30±0.01	2.31±0.02	2.31±0.01	2.31±0.02	2.31±0.01	1.516
28^{th} day	2.42±0.02	2.47±0.02	2.46±0.01	2.45±0.02	2.47±0.02	2.290
42^{nd} day	$2.49±0.02^{b}$	$2.55±0.01^{a}$	$2.55±0.02^{a}$	$2.57±0.02^{a}$	$2.57±0.02^{a}$	1.982

Note: a, b, c, d Means bearing different superscripts in a row differ significantly (P<0.05).

Packed Cell Volume (PCV, %)

The mean values of packed cell volume (PCV) during the 0 day and 28^{th} day of trial were recorded. The statistical analysis of PCV values data for 0th day and 28^{th} day failed to demonstrate the significant difference (P< 0.05) between the different groups. Though the numerically higher values can be seen in groups supplemented with *Ashwagandha* in alone and in combination with selenium and Vitamin E compared to control group (T_0). The data obtained for PCV values at end of trial (42^{nd} day) when analyzed statically significant difference (P<0.05) was observed between the groups. The significantly (P<0.05) higher values was reported in group (T_4) followed by (T_3), group (T_1) and (T_2) group. Control (T_0) group has significantly (P<0.05) lower values compared to other treatment groups. Higher value of PCV in supplemented group may be due to the hematinic property of *Ashwagandha*. The synergistic effect of *Ashwagandha*, Selenium and Vitamin E can be seen. Bhardwaj *et al.* (2012), Kant *et al.* (2014), Singh *et al.* (2016) and Singh *et al.* (2017) showed significantly higher PCV values in broilers supplemented with *Ashwagandha* and Vitamin E diet.

Total Leukocyte Count (TLC×10^3/µl)

The mean values of total leukocyte count (TLC) during the 0 day and 28^{th} day of trial were recorded. The statistical analysis of TLC values data for 0^{th} day and 28^{th} day did not differ significantly (P<0.05) between the different groups. Though the numerically higher values can be seen in groups supplemented with *Ashwagandha* in alone and in combination with Selenium and Vitamin E compared to non-supplemented group (T_0). At the end of experiment i.e. during 42^{nd} day of trial, the TLC values

Table 3. Details of the blood biochemical parameters and immune response of broiler birds

Parameters	T_0	T_1	T_2	T_3	T_4	CV
Total cholesterol (mg/dl)						
0th day	93.77±0.96	93.78±0.81	93.75±0.79	93.76±0.62	93.75±0.96	2.399
28th day	110.34±0.96	107.41±1.41	106.35±1.11	108.62±0.31	107.22±1.54	3.137
42nd day	141.53±3.05	138.04±0.69	139.05±0.64	137.90±0.78	136.82±1.02	3.192
High density lipoprotein (HDL, mg/dl)						
0th day	56.09±1.11	55.80±0.94	56.01±0.79	56.09±0.99	56.00±0.93	4.588
28th day	64.80±1.17	66.03±0.49	67.25±0.69	67.09±1.03	66.73±0.78	3.753
42nd day	71.95±0.50	72.70±0.41	73.46±0.27	73.71±0.88	73.83±0.52	2.247
Low density lipoprotein (LDL, mg/dl)						
0th day	29.18±1.82	29.35±1.28	29.37±1.30	29.22±1.47	29.35±1.35	13.34
28th day	35.93±1.75	32.10±1.76	30.05±1.11	32.33±1.06	31.35±1.06	13.04
42nd day	59.17±3.36	55.43±0.91	55.63±0.80	54.43±1.51	53.18±0.97	9.37
Humoral response against ND vaccine, ND (log_2) titre						
0th day	1.75±0.31	1.63±0.26	1.63±0.32	1.50±0.33	1.63±0.32	51.56
28th day	6.75±0.25^a	7.88±0.30^b	8.13±0.23^b	7.88±0.40^b	8.13±0.30^b	12.31
42nd day	5.88±0.30^a	7.38±0.32^b	7.25±0.45^b	7.25±0.41^b	7.38±0.38^b	16.59

Note: a, b, c, d Means bearing different superscripts in a row differ significantly (P<0.05).

statistically showed significant difference (P<0.05). The TLC count observed significantly (P<0.05) higher in broilers belonging to supplemented group as compared to control (T_0) group. It may be due to presence of active principle glycowithanolides in roots of *Ashwagandha*. Similar observations were reported by Bhardwaj *et al.* (2012), Singh *et al.* (2016) and Singh *et al.* (2017).

Total Erythrocyte Count (TEC×10^6/µl)

The mean values of total erythrocyte count (TEC) during the 0 day and 28th day of trial were recorded. The statistical analysis of TLC values data for 0 day and 28th day did not differ significantly (P<0.05) between the different groups. At the end of experiment i.e during 42nd day of trial, the TEC showed significant difference (P<0.05) after analyzing the data statistically. The total erythrocyte count was observed significantly (P<0.05) higher in broilers belonging to the supplemented group than compared to control (T_0) group. This may be due to haemo-proliferative and haemo-protective effect of *Ashwagandha* on broiler chicks. It is also attributable to its positive effect on haemopoiesis by stimulating stem cell proliferation and increasing bone marrow cellularity. The haemoprotective effect was attributed due to its antioxidant action that protects RBC from oxidative stress and enhances erythrocyte enzymes. Similarly, Bhardwaj et al. (2012), Singh *et al.* (2016) and Singh *et al.* (2017) reported significantly higher TEC values in the groups supplemented with *Ashwagandha* and Vit. E.

Blood Biochemical Parameters

The blood samples were analysed for blood biochemical parameters such as Serum total cholesterol (mg/dl), High density lipoprotein (HDL, mg/dl), Low density lipoprotein (LDL, mg/dl) and analysis reports are presented in Table 3.

Serum total cholesterol (mg/dl)

The mean values of serum total cholesterol levels of broilers on 42nd day of trial were reported in treatment groups T_1, T_2, T_3 and T_4, respectively. Analyzing the data statistically showed non-significant difference (p<0.05) between the broilers under different groups on 0th, 28th and 42nd day of trial. Though the values of serum total cholesterol was lower in supplemented groups (T_1), (T_2), (T_3) and (T_4) than that of the control group (T_0). The lower value of cholesterol may be owed to decrease in lipid metabolism in supplemented broilers. 3-hydroxy-3-methyl-glutarylcoA reductase is the chief enzyme in biosynthesis of cholesterol. *Ashwagandha* root powder may have indirect inhibitory effects on this enzyme and *Ashwagandha* roots revealed that serum cholesterol value decreases progressively on higher rate of *Aswagandha* supplementation (Ansari *et al.*, 2013). Similarly, present study results are in accordance with the Kale *et al.* (2016).

High density lipoprotein (HDL, mg/dl)

The values of high density lipoprotein (HDL) measured during the 0 day, 28th and 42nd day of the trial statistically

showed the non-significant difference (P<0.05) between the broilers under different groups T_1, T_2, T_3 and T_4, respectively. Though the values of HDL were higher in supplemented group T_1, T_2, T_3 and T_4 than that of the control group T_0. Higher values were observed in group T_4 as 73.83 mg/dl. Our present study results are in accordance with the Kale *et al.* (2016).

Low density lipoprotein (LDL, mg/dl)

The statistical analysis of the data for mean values of low density lipoprotein (LDL) showed the non-significant difference (P<0.05) between the broilers under different groups. Later on 28th day of the experiment, values of LDL for broilers under group on 0, 28th and 42nd day of trial. Though the values of LDL were found lowered in supplemented groups T_1, T_2, T_3 and T_4 than that of the control group T_0. Similarly, present study results are in accordance with the (Ansari *et al.*, 2013) and Kale *et al.* (2016).

Humoral Immune Response

The data pertaining to mean ND titer expressed as $\log_2$ for broiler birds belonging to different group are presented in Table 3. The humoral immune response against ND vaccine measured during the 0 day of the experimental trial. The statistical analysis of the data for $\log_2$ titer showed the non-significant difference between the different groups on 0 day of the experimental. Later on 28th day and 42nd day of the experiment, values of $\log_2$ titer estimated for broilers under group T_0, T_1, T_2, T_3 and T_4, respectively. There was significant difference (P<0.05) observed between the different groups on 28th day and 42nd days of the experiment. At 28th and 42nd day of the sampling and experimental trial the ND $\log_2$ titer was quite higher (P<0.05) in *Ashwagandha* supplemented group either alone or in combination with Selenium and Vitamin E than control group (T_0). This may be due to immune-modulatory effect shown by *Ashwagandha* by increasing the number of lymphocytes due to presence of active principle glycowithanolides in roots of *Ashwagandha* Selenium and vitamin E also increases immunoglobulins by affecting the proliferation of lymphoid cells that may have improved antibody response. Our present study results are in agreement with Vasanthakumar *et al.* (2014) and Tomar *et al.* (2018).

CONCLUSIONS

The study of hematology, blood-biochemical parameters and humoral immune response of experimental broiler birds supplemented with *Ashwagandha* in combination with Selenium and Vitamin E gives improved average values which results in the better health and immunity in broiler birds.

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