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Evaluation of hematological and serum biochemical response of broiler chicken to Ashwagandha root powder supplementation

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Abstract
An experiment which lasted for 42 days was carried out to investigate the hematological and serum biochemical response of broiler chicken to Ashwagandha root powder. A total of 300, day-old commercial broiler chicks were used for the experiment. The birds were randomly allotted into six dietary treatments with five replicates per treatment and each replicate has ten birds. Feeding trial was conducted in two different growth phases i.e. starter (0-28d) and finisher (29-42d). The first group was kept as a control (T_1) and given the basal diet without antibiotic while second (T_2) basal diet with antibiotic, third (T_3), fourth (T_4), fifth (T_5) and sixth (T_6) groups were supplemented with Ashwagandha root powder @ 0.25, 0.5, 0.75 and 1%, respectively in the diet. At end of feeding trial (42 days), one bird from each replicate was selected and blood samples were collected. Thus a total of 30 samples were analyzed using automatic hemoanalyzer and kits for hematological and serum biochemical parameters. Significant ($P< 0.05$) increase was observed in hemoglobin concentration, red blood cell, lymphocyte count of the Ashwagandha supplemented groups as compared to control. The serum biochemical parameters measured were significantly ($P< 0.05$) different in Ashwagandha supplemented groups showing a better response. Cholesterol, serum triglyceride and LDL (Low density Lipoprotein) decreased significantly ($P< 0.05$), while there was a significant increase in HDL (High density lipoprotein) levels in 0.75% and 1.0% Ashwagandha supplemented groups. Supplementation of Ashwagandha root powder to broiler chickens boosted their immunity as well as improved their general well-being, thus, recommended in broiler chicken production.

Keywords: Ashwagandha, broilers, hematology, cholesterol, LDL, HDL

Introduction
Use of synthetic growth promoters like antibiotics has led to success in limiting most of the prevalent bacterial diseases which affected man and animals in epidemic proportions. But, inadvertent and overuse of antibiotics resulted in disadvantages like high cost of production, toxicity and development of resistance and environmental and health hazards. To eliminate antibiotics, numerous substances, commonly known as natural growth promoters (NGPs) have been identified as effective alternatives to antibiotics. Commonly used growth promoters are prebiotics, probiotics, synbiotics, enzymes, acidifiers and phytobiotics. Phytobiotics are NGPs, which have been growing in popularity as feed additives, due to their beneficial effect on gut health and immunity and growth performance. *Withania somnifera* (Ashwagandha) holds a celebrated position in the Indian *materia medica*. Ashwagandha *(Withania somnifera)* is a plant of Solanaceae family. Ashwagandha plant constitutes alkaloids and steroidal lactones, but the withanine, the main alkaloid found in its roots and leaves is thought to be responsible for its biological activity. Other constituents include saponins containing an additional acyl group (sitoindoside VII and VIII). It improve feed intake, body weight gain, FCR, hematological profile and immunological status, neuro-protective and rejuvenate muscles (Ansari *et al.*, 2008) [2]. The extract of this plant is a potent immune stimulator, antioxidant, anticarcinogenic, antimetastatic (Davis and Kuttan, 2000; Mishra *et al*., 2000 and Sharma *et al*., 2010) [6, 15, 21] and antibacterial (Owais *et al.*, 2005) [18]. The use of Ashwagandha has been mainly associated to its modulatory effects on the immune system (Gautam *et al*., 2004) [10]. It has been reported that Ashwagandha significantly increases the white blood cell and erythrocyte counts (Manish *et al*., 2004 and Senthilnathan *et al*., 2006) [14, 20]. Preparations obtained from this plant have been shown to enhance circulating antibody titer, increase the activity of lysosomal enzymes and increase phagocytosis (Agarwal *et al*., 1999) [3].

Ashwagandha is reported to be general tonic, anti-stress, hepato protective, haematinic, growth promoter and antioxidant in human practice and anticoccidial agent in poultry practice (Das *et al*., 2001) [5].

Materials and Methods

The experiment was conducted at poultry unit of College of Veterinary and Animal Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana. A total of three hundred, day-old commercial broiler chicks were used for the experiment. The birds were randomly allotted into six dietary treatments with five replicates per treatment and each replicate has ten birds. The birds were from the same hatch and were reared under uniform management condition upto six weeks of age. All the birds irrespective of their treatments were fed maize crumble for first three days of their age, followed by experimental ration prepared as per BIS (2007) [4]. Level of crude protein in starter (0-4weeks) and finisher (4-6weeks) ratio was 23 and 20%, respectively. The respective ME content was 3000 and 3200 Kcal/kg are presented in Table 1. The first group was kept as a control (T_1) and given the basal diet without antibiotic while second (T_2) basal diet with antibiotic, third (T_3), fourth (T_4), fifth (T_5) and sixth (T_6) groups were supplemented with Ashwagandha root powder @ 0.25, 0.5, 0.75 and 1%, respectively in the diet. The experimental birds were reared under deep litter system. The litter was regularly racked to avoid any lump formation. Birds were vaccinated against F_1 strain of Ranikhet disease on 3rd day and IBD on 14th day. At the end of the experiment, one bird per replication (5 birds per treatment) were selected randomly to collect blood sample into well labelled and sterilized vials containing Ethylene Diamine Tetra Acetic Acid (EDTA) as anticoagulant for hematological examination. The samples were investigated for Hemoglobin content, Red blood cells, white blood cells, Heterophils and Lymphocyte using auto analyzer. For serum collection, samples were centrifuged at 3000 rpm for 15 minutes and serum obtained was stored at -20 °C until analysis. Serum parameters were determined by auto analyzer using commercial kits for different serum variables like Total cholesterol, Triglycerides, HDL and LDL.

Ethical approval

The animal experiment was conducted in accordance with guidelines approved by the Institutional Animal Ethics Committee (IAEC), 235/CPCSEA dated 1-8-2000 in the Department of Animal Nutrition, LUVAS.

Statistical analysis

The data were analyzed using general linear model procedure of statistical package for social sciences 20th version (SPSS) [25] and comparison of means tested using Duncan's multiple range test (DMRT) [7] and significance was considered at $P< 0.05$.

Table 1: Chemical composition of feed ingredients used in ration formulation

Ingredient	CP (%)	CF (%)	EE (%)	TA (%)	Lysine* (%)	Methionine* (%)	ME* (kcal/kg)
Maize	9.31	2.48	3.49	2.25	0.18	0.15	3300
Soyabean meal	45.40	3.96	3.16	8.47	2.57	0.76	2230
Fish meal	47.40	1.82	5.16	26.62	1.42	1.42	2210
Ashwagandha root powder	2.91	6.34	2.30	4.41	-	-	245

*Calculated values (Singh and Panda, 1992) [23], CP- Crude Protein, CF- Crude Fibre, EE- Ether Extract, TA- Total Ash, ME- Metabolizable Energy

Results

Hematology

Data pertaining to hematological parameters of the experimental birds under different dietary treatments are presented in Table 2. Mean values of Hb (g/dl) ranged from 10.21 g/dl (T_1) to 10.90 g/dl (T_6) and significantly ($P< 0.05$) higher values were observed in T_4, T_5 and T_6. The values were significantly ($P< 0.05$) higher from control group. Mean values of RBC (red blood cell) and WBC (white blood cell) were found significantly ($P< 0.05$) higher in 0.75% (T_5) and 1.0% (T_6) Ashwagandha supplemented group as compared to other treatments, antibiotic supplemented group and control group.

Mean values of heterophil % ranged from 27.56% (T_6) to 28.24% (T_2) and lowest heterophil count was observed in T_6 group followed by T_4 and T_5 group. Lymphocytes mean value ranged from 58.62% (T_2) to 59.61% (T_5 and T_6) and highest lymphocyte % was observed in T_5 and T_6. Mean values for H: L ratio ranges between 0.463 (T_6) to 0.482 (T_2) and significantly ($P< 0.05$) lowest ratio was found in T_4, T_5 and T_6 groups as compared to control group (T_1) and antibiotic supplemented group (T_2).

Table 2: Mean values of hematological parameters of birds under different dietary treatments

Treatments	Hemoglobin g/dl	RBC×10^6/µl	WBC×10^3/µl	Heterophil %	Lymphocyte %	H: L
T_1	10.21^{a}±0.06	1.95^{a}±0.01	27.50^{a}±0.05	28.17^{bc}±0.03	58.72^{a}±0.01	0.480^{b}±0.00
T_2	10.23^{a}±0.03	1.94^{a}±0.01	27.49^{a}±0.05	28.24^{c}±0.04	58.62^{a}±0.02	0.482^{c}±0.00
T_3	10.25^{a}±0.07	2.00^{a}±0.01	27.48^{a}±0.05	28.08^{b}±0.05	58.68^{a}±0.04	0.479^{b}±0.00
T_4	10.45^{b}±0.03	2.23^{b}±0.03	27.41^{a}±0.81	27.61^{a}±0.04	59.44^{b}±0.07	0.464^{a}±0.00
T_5	10.80^{c}±0.07	2.39^{c}±0.05	28.62^{b}±0.05	27.65^{a}±0.03	59.61^{c}±0.06	0.464^{a}±0.00
T_6	10.90^{c}±0.04	2.38^{c}±0.04	28.66^{b}±0.05	27.56^{a}±0.03	59.61^{c}±0.06	0.463^{a}±0.00

Means bearing different superscripts in a column differ significantly ($P< 0.05$)

Serum parameters

Data pertaining to serum parameters of the experimental birds under different dietary treatments are presented in Table 3 and Figure 1.

Serum cholesterol

Values of serum cholesterol content under different dietary treatments ranged between 130.60 mg/dl (T_6) to 154.20 mg/dl (T_2). Lowest serum cholesterol value was reported in 1.0% Ashwagandha root powder (T_6) supplemented group. This was significantly ($P< 0.05$) differing from the control group and other dietary treatments. Dietary treatments T_4 and T_5 also showed significantly ($P< 0.05$) lower serum cholesterol content than the control group (T_1).

Serum triglyceride

Mean values of serum triglycerides (mg/dl) under different dietary treatments ranged from 60.60 mg/dl (T_6) to 93.20 mg/dl (T_1). This differed significantly ($P< 0.05$) with the control group. Groups supplemented with higher levels of Ashwagandha root powder showed significant reduction in serum triglycerides levels.

Table 3: Mean values of serum cholesterol, triglycerides, HDL and LDL under different dietary treatments

Treatments	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
T_1	$151.60^{c}\pm2.77$	$93.20^{c}\pm2.96$	$43.40^{ab}\pm2.38$	$73.40^{c}\pm6.17$
T_2	$154.20^{c}\pm1.88$	$91.60^{c}\pm2.25$	$39.82^{a}\pm3.12$	$72.60^{c}\pm5.89$
T_3	$151.00^{c}\pm1.64$	$84.80^{c}\pm2.03$	$44.28^{ab}\pm3.10$	$74.60^{c}\pm1.57$
T_4	$140.60^{b}\pm1.86$	$72.60^{b}\pm3.12$	$51.60^{b}\pm3.37$	$67.20^{bc}\pm3.34$
T_5	$135.60^{ab}\pm1.81$	$69.60^{ab}\pm1.81$	$66.88^{c}\pm3.21$	$57.40^{ab}\pm4.28$
T_6	$130.60^{a}\pm1.29$	$60.60^{a}\pm6.96$	$65.22^{c}\pm3.85$	$52.80^{a}\pm3.12$

Means bearing different superscripts in a column differ significantly ($P< 0.05$)

Serum HDL

Dietary supplementation of Ashwagandha root powder significantly ($P< 0.05$) improved the HDL value than the control group. Mean value of HDL was highest in T_5 (66.88 mg/dl) and followed by T_6 (65.22 mg/dl), T_4 (51.60 mg/dl) and T_3 (44.28 mg/dl) treatment groups.

Serum LDL

Mean values of LDL in different dietary treatments ranged from 52.80 mg/dl (T_6) to 74.60 mg/dl (T_3). Lowest value of LDL was observed in 1.0% Ashwagandha supplemented group (T_6) followed by 0.75% (T_5) and 0.50% (T_4) and was significantly ($P< 0.05$) lower from antibiotic and control group.

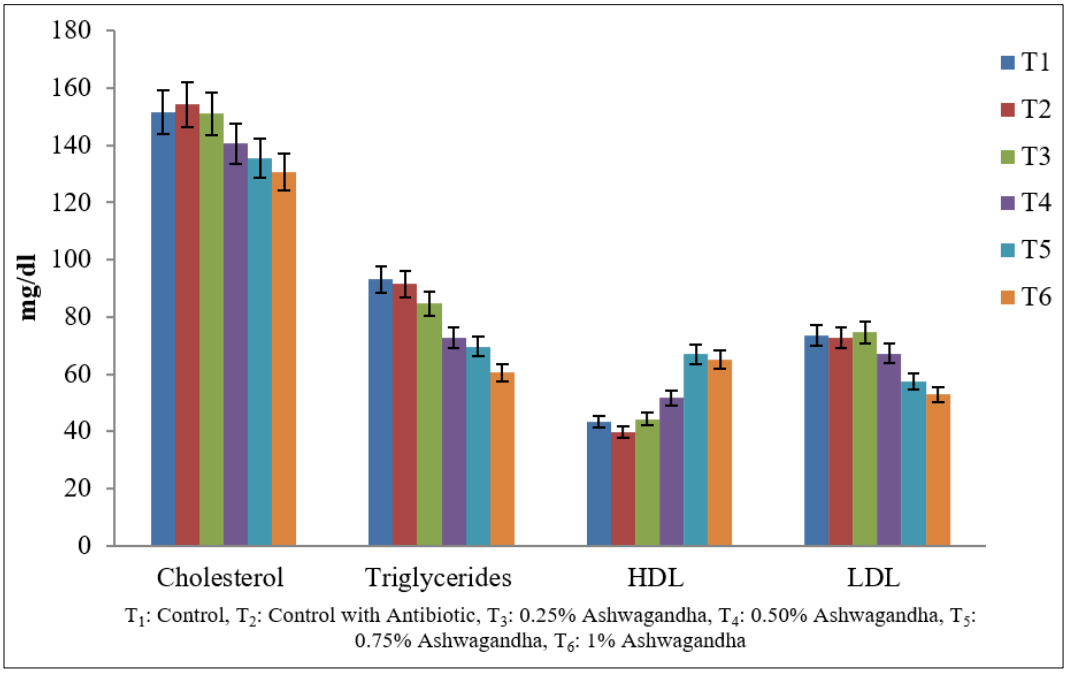

Fig 1: Serum cholesterol, triglycerides, HDL and LDL under different dietary treatments

Discussion

Mean values of Hb (g/dl) ranged from 10.21 g/dl (T_1) to 10.90 g/dl (T_6) and significantly ($P< 0.05$) higher values were observed in T_4, T_5 and T_6. The values were significantly ($P< 0.05$) higher from control group. Mean values of RBC (red blood cell) and WBC (white blood cell) were found significantly ($P< 0.05$) higher in 0.75% (T_5) and 1.0% (T_6) Ashwagandha supplemented group as compared to other treatments, antibiotic supplemented group and control group. Mean values of heterophil% ranged from 27.56% (T_6) to 28.24% (T_2) and lowest heterophil count was observed in T_6 group followed by T_4 and T_5 group. Lymphocytes mean value ranged from 58.62% (T_2) to 59.61% (T_5 and T_6) and highest lymphocyte% was observed in T_5 and T_6. Mean values for H : L ratio ranged between 0.463 (T_6) to 0.482 (T_2) and significantly ($P< 0.05$) lowest ratio was found in T_4, T_5 and T_6 groups as compared to control group (T_1) and antibiotic supplemented group (T_2). Hematological parameters are good indicators of the physiological status of birds, and the changes are helpful in assessing the response of birds to various physiological situations. Our results are in agreement with findings of Shefali *et al.* (2008) [22], who showed increased hemoglobin and hematocrit, in broilers fed *W. somnifera* aqueous roots (WSR). Micro-mineral composition of WSR in their study showed that WSR was a rich source of iron, which might have increased the level of hemoglobin in broilers. This hemoprotective effect of WSR is consistent with the findings of Kumar *et al.* (2006) [13], who observed ameliorative effects of simultaneous feeding of powdered *W. somnifera* roots in chlorpyrifos intoxication in white leghorn cockerels. Similarly, improvement of erythrocyte count and increasing the hemoglobin concentration after administration of *Nigella sativa* were explained by an increased number of cells in bone marrow that reached advanced developmental stages and the acceleration effect of medicinal plants on the cellular respiratory mechanism (Ebaid *et al.*, 2011) [8]. Thus, protein formation needed cellular events such as mitosis generated in mitochondria, which have enzymes involved in the

biosynthesis of haem, an important component in erythropoiesis. Our results are supported by Singh *et al.* (2016) [24] who observed that the groups treated with both Ashwagandha and selenium showed significantly higher values of hematological parameters when compared to that of other groups. Mushtaq *et al.* (2012) [16] evaluated that the TLC values were significantly higher in the group receiving 20g plant extract water compared with control. Increase in the TLC values may be due to stimulating effect of *W. somnifera* on the bone marrow cells as has been reported by Davis and Kuttan (2000) [6] in the mice injected (intraperitoneally) with powdered roots of *W. somnifera* at 20 mg/dose/animal for 10 days. The increase in the presence of α-esterase positive cells in the bone marrow showing the enhancement of stem cells differentiation due to *W. somnifera* was another indicator of its hematopoietic stimulatory effect.

Values of serum cholesterol content under different dietary treatments ranged between 130.60 mg/dl (T_6) to 154.20 mg/dl (T_2). Lowest serum cholesterol value was reported in 1.0% Ashwagandha root powder (T_6) supplemented group. This was significantly ($P< 0.05$) differing from the control group and other dietary treatments. Dietary treatments T_4 and T_5 also showed significantly ($P< 0.05$) lower serum cholesterol content than the control group (T_1). Mean values of serum triglycerides (mg/dl) under different dietary treatments ranged from 60.60 mg/dl (T_6) to 93.20 mg/dl (T_1). This differed significantly ($P< 0.05$) with the control group. Groups supplemented with higher levels of Ashwagandha root powder showed significant reduction in serum triglycerides levels. Dietary supplementation of Ashwagandha root powder significantly ($P< 0.05$) improved the HDL value than the control group. Mean value of HDL was highest in T_5 (66.88 mg/dl) and followed by T_6 (65.22 mg/dl), T_4 (51.60 mg/dl) and T_3 (44.28 mg/dl) treatment groups. Mean values of LDL in different dietary treatments ranged from 52.80 mg/dl (T_6) to 74.60 mg/dl (T_3). Lowest value of LDL was observed in 1.0% Ashwagandha supplemented group (T_6) followed by 0.75% (T_5) and 0.50% (T_4) and was significantly ($P< 0.05$) lower from antibiotic and control group. Our results are in accordance with Rehman *et al.* (2013) [19] who reported that water based infusion of *A. sativum* and *W. somnifera* in 1:6 at the rate of 10 ml/l in broiler chicks reduced total cholesterol, triglycerides, low density lipoproteins while increased high density lipoproteins. The cholesterol lowering effect of *W. somnifera* could be due to elevated excretion of cholesterol and bile acids through fecal sterol excretion. It could also be attributed to higher phytosterol contents, which might lead to decrease in intestinal transit time for cholesterol and carbohydrate absorption from gut (Ebihara and Schneeeman, 1989) [9]. *Withania somnifera* root powder might have indirect inhibitory effects exerted at levels of 3 hydroxy-3- methyl-glutaryl-coA reductase, a key enzyme in cholesterol biosynthesis (Ansari *et al.*, 2013) [1]. *W. somnifera* root powder has higher fiber and phytosterol contents which are known to have a greater affinity for micelles than cholesterol because of their greater hydrophobicity. Therefore, they can easily displace intestinal cholesterol from the micelles, reducing intestinal cholesterol absorption, and consequently reduce hepatic and plasma cholesterol concentrations (Ostlund, 2007) [17]. The findings of study conducted by Hemalatha *et al.* (2004) [11], that administration of aqueous extract of *Withania somnifera* significantly lowered the serum cholesterol, serum lipid peroxidase, and hepatic lipid peroxidase level were in relevance with our study. Visavadiya and Narasimhacharya (2006) [26], who added root powder of *W. somnifera* to the diet of Hypercholesteraemic male albino rats at rate of 0.75 and 1.5 g/rat/day and registered significant increase in plasma HDL-cholesterol levels. Kale *et al.* (2016) [12] concluded that lowered serum lipid profile i.e. triglyceride, cholesterol and HDL are suggestive of profound hypolipidemic and hypocholesteremic properties of *Withania somnifera* root powder.

Conclusion

It was concluded that the inclusion of Ashwagandha root powder at the level of 0.75 and 1.0 percent in broiler ration as herbal feed additive could be beneficial in improving hematological and blood biochemical profile of broilers.

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