

Effect of supplementation of Selenium and Ashwagandha (*Withania somnifera*) on some haematological and immunological parameters of broiler chickens

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Abstract: Indian poultry industry is fast growing and poultry meat is not subjected to cultural and religious restrictions. Utilization of medicinal plants as feed ingredient for broilers is increasing due to its growth promoting quality and capacity to reduce antimicrobial resistance among broilers. Selenium is an essential micronutrient required for normal growth and maintenance in poultry. The present experimental study was undertaken to study the effects of selenium and Ashwagandha (*Withania somnifera*) on haematological and immunological parameters of VenCobb-400 broilers. Three hundred and sixty (360) day old chicks were selected and divided in to six groups (T0 to T5) with 3 replicates of each group, 20 chicks in each replicate. Ashwagandha and selenium were supplemented in broiler chicken feed at different levels for different group. The haematological parameters viz. haemoglobin concentration, packed cell volume, total erythrocyte count, total leucocyte count and differential leucocyte count were assessed as per standard procedures. The cell mediated immune response was estimated by skin hypersensitivity test using 1% 2,4-dinitrochlorobenzene reagent in acetone and it was evaluated after 24, 48 and 72 hours post inoculation. The data obtained in the present study were analyzed statistically by using Complete Randomized Design one way Anova procedure of SPSS version 20. The broiler groups treated simultaneously with both the Ashwagandha and selenium showed significantly higher values of haematological parameters when compared to that of other groups. The cell mediated immunity of birds analysed by cutaneous basophil hypersensitivity reaction was found at higher levels in Ashwagandha and selenium treated groups. Supplementation of 2.5g Ashwagandha and 0.15 mg Selenium per Kg of feed was found to be optimum combination for better haematological and immunological attributes for broilers. This may be due to the synergistic effect of Ashwagandha and selenium on the haematology of broilers.

Keywords: Ashwagandha, Broiler, Haematology, Immunity, Selenium

INTRODUCTION

Poultry industry is one of the fast growing sectors of Indian agriculture today, with annual growth rate of 11.44% in broiler production [1]. Poultry meat has significant role in Indian diet and India is the fourth largest producer of poultry meat in world, valued at US \$ 6.6 billion. Favoured by socio-economic conditions like rising purchasing power and changing food habits of the people this sector is driven by increased domestic consumption. Poultry meat is an excellent source of high quality protein, vitamins and minerals and is not subjected to cultural and religious restrictions [2].

Utilization of medicinal plants as feed ingredient for broilers is increasing due to its growth promoting quality and reducing the antimicrobial resistance among broilers. *Withania somnifera* also known as Ashwagandha or Indian Ginseng is a medicinal plant belonging to the family *Solanaceae*.

The main constituents of this plant are alkaloids and steroidal lactone, with withanine as the main alkaloid, which is considered for its biological activity. The extracts of this plant is a potent immune stimulator, antioxidant and anticarcinogenic [3, 4].

Selenium is an essential micronutrient required for normal growth and maintenance in poultry. Selenium has various biological roles like regulation of activity of the enzyme glutathione peroxidase, thyroid hormones, prostaglandins, improvement of immune and reproductive functions, protection against harmful exposure of heavy metals such as mercury and cadmium [5]. Different forms of selenium can be used for supplementation in poultry feed, either as inorganic complex such as sodium selenite or selenate or as part of organic molecules such as seleno methionine, seleno cysteine and seleno cystine. Inorganic selenium retains at lower concentrations in muscle tissue when compared

with organic selenium. The level of selenium in poultry feed varies widely depending on the plant ingredients provided in the ration and the selenium characteristics of the soils in which the ingredients were grown. The levels of selenium in soils globally are decreasing because of the intensity of agricultural cropping [6]. Few studies are reported regarding the effect of Ashwagandha/selenium on broilers, but studies using both selenium and *Withania somnifera* are lacking. So the current study was designed to analyse the combined effect of Ashwagandha and selenium on haematological and immunological profile of broilers.

MATERIALS AND METHODS

Experimental design

The trial was conducted at Poultry Research and Training Centre, Sardar Vallabhbhai Patel University of Agriculture and Technology (SVPUAT), Meerut, India. Three hundred and sixty (360) day old, vaccinated commercial broiler chicks (VenCobb 400 strain) were individually weighed and divided randomly into six treatment groups (T₀- T₅), with three replicates of 20 chicks in each group, on weight basis. All the broiler chicks were fed according to two phase feeding schedule, with a starter diet from 1 to 28 days and finisher diet from 29 to 42 days (Table 1) prepared as per BIS standards. Standard broiler rations with fresh water were provided ad-libitum to experimental birds by adding various concentrations of Selenium and Ashwagandha (*Withania somnifera*) root powder in the rations of treatment groups (Table 2). The chemical composition of Ashwagandha (*Withania somnifera*) root powder on dry matter basis is given in the Table 3. Each group was reared on deep litter upto 6 weeks in an open sided house in pens under standard managerial conditions.

Determination of Haematological parameters

At the end of the experiment 6 birds per treatment group (2 per replicate) were selected randomly and slaughtered humanely. The blood was collected in EDTA containing test tubes (1mg EDTA/ml of blood). These anti-coagulated blood samples were subjected to determine Haemoglobin (Hb) concentration, Packed Cell Volume (PCV), Total Erythrocyte count (TEC), Total Leucocyte count (TLC) and Differential Leucocyte Count (DLC) [7]. Haemoglobin concentration was measured by cyan methaemoglobin method. PCV was measured by a standard manual technique using micro haematocrit capillary tubes centrifuged at 2500 rpm for 5 min. TLC and TEC counts were determined manually by using haemocytometer. DLC counts were made on monolayer blood films, fixed and stained with Giemsa-Wright's stain.

Cell Mediated Immunity

The cell mediated immune response was estimated by skin hypersensitivity test using 1% 2,4-

dinitrochlorobenzene reagent in acetone (DNCB) [8]. Briefly, 4 chicks from each treatment were colour marked and sensitized with 0.25ml of DNCB. The inoculation was made in the interdigital space between the 3rd and 4th toes of right foot by intradermal injection. In the same interdigital space of the left foot (in the same bird), 0.25 ml acetone was injected as control. At 24, 48 and 72 hours post DNCB challenge, the cell reaction caused by DNCB was evaluated as cutaneous basophil hypersensitivity (CBH) response by measuring skin thickness with electronic digital vernier caliper in millimeter (with 0.01mm precision). The cell reaction was calculated as CBH=A-B.

Where, A=Post DNCB injection thickness of the right foot-Pre DNCB injection thickness of the right foot (mm)

B= Post acetone injection thickness of the left foot-Pre DNCB injection thickness of the left foot (mm)

STATISTICAL ANALYSIS

The data for all measured variables were analyzed as a Complete Randomized Design using one way Anova procedure of SPSS version 20 (SPSS for windows, V 20.0; SPSS Inc., Chicago, IL, USA). The significant difference between and within groups for the different variables was determined using Duncan Multi Range Test of Significance.

RESULTS

The mean value of blood parameters of broilers treated with various combinations of Ashwagandha and selenium were given in Table 4. The different blood parameters of broilers differ significantly between treatment ($P<0.05$) as it shows in different superscripts. There was significant difference among selenium and Ashwagandha treated groups when compared to other treatment groups. The highest values of Hb and PCV were obtained in group T₅ (13.42±0.03 and 33.88±0.10). The TEC and TLC were highest in both combination treatment groups (T₄ and T₅), even though there was no significant difference was observed among these groups. The groups treated with both Ashwagandha and selenium showed significantly higher values of haematological parameters when compared to that of other groups. The DLC of T₄ and T₅ groups didn't observe any significant differences except lymphocytes, heterophils and monocytes. The maximum lymphocyte count was observed in the T₄ group (67.99±0.21).

The cell mediated immunity evaluated by cutaneous basophil hypersensitivity reaction at 24, 48 and 72 hours post inoculations with DNCB were expressed in Table 5. The CMI response observed was more prominent at 48 hours of DNCB challenge and was significantly higher in treated groups when compared to control. Present study showed that the highest CMI response was in T₄ group, indicating improved immunity.

Table 1: Ingredients of the starter and finisher diets

Ingredients	Starter (%)	Finisher (%)
Corn	53.55	59.57
Soyabean meal 45% CP	38.93	33.34
Monodibasic phosphate	1.43	1.21
Limestone	1.35	1.38
Vegetable Oil	3.84	3.51
Salt	0.41	0.43
DL-Methionine	0.207	0.214
L-Lysine	0.129	0.197
Choline HCl 60%	0.06	0.05
Mineral-Vitamin premix*	0.1	0.1
Total	100	100

*Composition (per kg): manganese, 75,000 mg; zinc, 70,000 mg; iron, 50,000 mg; copper, 8,000 mg; iodine, 750 mg; vitamin A, 8,000 kIU; vitamin D3, 2,000 kIU; vitamin K3, 1,800 mg; vitamin B1, 1,800 mg; vitamin B2, 6,000 mg; vitamin B6, 2,800 mg; vitamin B12, 12,000 µg; pantothenic acid, 10,000 mg; niacin, 40,000 mg; folic acid, 1,000 mg; biotin, 60,000 µg; selenium, 0.3 mg/kg.

Table 2: Inclusion levels of Ashwagandha and Selenium of different groups of chicks

Group	Basal Ration	Selenium (mg/kg.)	Ashwagandha (mg/kg.)
T ₀	Basal Ration	-	-
T1	Basal Ration	-	250
T2	Basal Ration	0.15	-
T3	Basal Ration	0.20	-
T4	Basal Ration	0.15	250
T5	Basal Ration	0.20	250

Table 3: Chemical composition of Ashwagandha root powder on dry matter basis

Chemical composition	Dry matter basis (%)
Organic matter	97.15
Crud protein	14.15
Crude fiber	11.05
Ether extract	1.90
Total ash	2.85
Nitrogen free extract	70.06
Metabolizable energy (Kcal/kg)	3915

Table 4: Hematological traits of broiler chicks supplemented with Ashwagandha and selenium at the end of the experiment at 6 week.

Attributes/ Group	To	T1	T2	T3	T4	T5	Treatment Mean
Hb	13.13±0.02 ^d	13.24±0.02 ^c	13.27±0.02 ^b	13.31±0.01 ^b	13.41±0.04 ^a	13.42±0.03 ^a	13.29±0.10
PCV	33.05±0.04 ^d	33.14±0.06 ^d	33.31±0.02 ^{cd}	33.47±0.17 ^{bc}	33.71±0.26 ^{ab}	33.88±0.10 ^a	33.42±0.32
RBC	3.11±0.02 ^e	3.21±0.02 ^d	3.30±0.02 ^c	3.36±0.02 ^b	3.49±0.03 ^a	3.49±0.04 ^a	3.32±0.14
TLC	29.88±0.10 ^c	30.17±0.03 ^c	30.22±0.05 ^c	30.33±0.22 ^b	32.05±0.38 ^a	32.06±0.12 ^a	30.88±0.92
Heterophils	28.58±0.07 ^a	27.41±0.04 ^b	26.14±0.07 ^d	25.11±0.05 ^e	25.24±0.08 ^e	26.99±0.09 ^c	26.58±1.26
Lymphocytes	63.02±0.08 ^e	64.31±0.05 ^d	65.95±0.05 ^c	67.31±0.05 ^b	67.99±0.21 ^a	67.43±0.13 ^b	66.00±1.85
Monocytes	3.01±0.11 ^a	2.88±0.06 ^a	2.69±0.04 ^b	2.46±0.03 ^c	2.10±0.11 ^d	2.64±0.03 ^b	2.63±0.03
Eosinophils	3.29±0.02 ^a	3.29±0.02 ^a	3.27±0.01 ^{ab}	3.21±0.04 ^b	3.12±0.03 ^c	3.13±0.04 ^c	3.22±0.07
Basophils	2.12±0.10 ^a	2.12±0.02 ^a	1.90±0.01 ^b	1.84±0.03 ^b	1.70±0.03 ^c	1.70±0.01 ^c	1.90±0.18

Data in table represents Mean ± SD; Where n = 3, values bearing different superscripts in same row differ significantly (P<0.05). Mean ± SED with different superscript in small letter in a row and capital letter in column differ significantly (P<0.05).

Table 5: CMI response of broiler chicks diets supplemented with Ashwagandha and Selenium

Group	T0	T1	T2	T3	T4	T5	Mean ± SD
24 h	0.95±0.02 ^{Ad}	1.02±0.03 ^{Cc}	1.05±0.04 ^{Cc}	1.11±0.03 ^{Cb}	1.18±0.01 ^{Ca}	1.17±0.03 ^{Ca}	1.08±0.09
48 h	0.98±0.02 ^{Ae}	1.50±0.02 ^{Ad}	1.63±0.06 ^{Ac}	1.69±0.04 ^{Abc}	1.96±0.02 ^{Aa}	1.75±0.03 ^{Ab}	1.58±0.31
72 h	0.95±0.01 ^{Ae}	1.30±0.01 ^{Bd}	1.45±0.03 ^{Bc}	1.50±0.02 ^{Bc}	1.81±0.04 ^{Ba}	1.60±0.02 ^{Bb}	1.44±0.27

Mean ± SED with different superscript in small letter in a row and capital letter in column differ significantly (P<0.05).

DISCUSSION

Haematological parameters are good indicators of the physiological status of birds and its changes are of value in assessing the response of birds to various physiological situations. In our study, the groups which were treated with both Ashwagandha and selenium showed significantly higher values of haematological parameters when compared to that of other groups. These findings were augmented by earlier researchers also. The beneficial effects of Ashwagandha as haematinic in broilers were reported by many researchers [4, 9, 10]. Selenium has significant effect on haematology of broilers [1]. This may be due to the synergistic effect of Ashwagandha and selenium in the broiler.

Increased lymphocytes in Ashwagandha treated groups are due to anti-stress activity of *W. somnifera* [9]. Increase in TLC may be also due to stimulating effect of *W. somnifera* on the bone marrow cells [9]. These findings of cell mediated immunity test were in accordance with earlier studies which evidence the immunomodulatory activity of *W. somnifera* due to its glycowithanolides content. The cell mediated immune response is able to recognize a large number of intracellular antigens through of T helper lymphocytes receptors and to eliminate them by the release of cytokines [12]. Higher immune response in the present study may be due to participation of selenium in the mechanism of the major metabolic pathway involving protein synthesis and turnover, carbohydrates, energy, nucleic acids, lipids and haeme syntheses which are involved in immune system in animals. Supplementation with organic selenium imposed better immune response in broilers [13]. The combination treatment with selenium and *W. somnifera* increased disease resistance by enhancing leucocytes and cell mediated immunity. In our studies, combination of 0.15 mg/kg Selenium and 2.5% *Withania somnifera* was found to be optimum for enhancing haematological and immunological traits and we recommend this combination in the basal feed of broilers for the management of immune suppressed diseases.

CONCLUSION

The broiler feed incorporated with herbal ingredients can be used as immunostimulants, as it leaves no antibiotic residues in the meat. The current study was designed with incorporation of only natural immunostimulants and no antibiotics in the feed of Vencobb-400 broilers. The analysis of various combinations of Ashwagandha and selenium revealed

that, the groups treated with both the ingredients shown significant difference in haematological and immunological parameters and among them, the T₄ group had the highest values. From this study, we recommends supplementation of Ashwagandha (*Withania somnifera*) at an inclusion rate of 250 mg/kg along with 0.15 mg/kg Selenium in the feed of broilers as it was having the optimum effect on the immune status of birds.

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