Original Research

Efficacy of Supplementing Ashwgandha Extract (*Withania Somnifera*) on Immunity and Serum Biochemistry in Broilers during Summer

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Abstract

The objective of the present study was to determine the effects of different levels of Ashwagandha (Withania somnifera) extract (WSE) on immunity and serum biochemical parameters of broiler chicken reared during hot summer. The average minimum and maximum room temperatures recorded from 0-42 day were 36.8°C±0.25 – 39.7°C±0.25, respectively, with relative humidity ranging from 47 – 74%. Day old broiler chicks (n=160) were randomly allotted to 4 dietary groups with eight replicates of five birds each. The four dietary groups were Positive control (PC) with vit-E (70mg/kg) +Se (0.15mg/kg), Negative control (NC), WSE50 and WSE100 groups supplemented with WSE 50mg/kg and 100mg/kg, respectively. Serum cholesterol levels were significantly (P<0.05) reduced in WSE50 and WSE100 groups, while serum protein levels were unaffected (P>0.05) by supplementation of WSE. Humoral immune response in terms of NDV (Newcastle disease virus) titer and cell mediated immunity in terms of cutaneous basophilic hypersensitivity (CBH) were not significantly influenced by supplementation of WSE in broiler diets.

Key words: Ashwagandha Extract, Cell Mediated Immunity, Humoral Immunity, Serum Cholesterol, Serum Protein

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Introduction

Stress is defined as any biological response developed by an organism when its homeostasis is disrupted (Virden and Kidd 2009). High ambient temperature is considered as a potent climatic stressor causing impaired antioxidant status in poultry (White head and Keller, 2003). Heat stress can result in oxidative stress in birds which has been reported to decrease production performance and immunity hence results in pathogenesis of various diseases (Mujahid *et al.*, 2005). Increase in stress induces sympatho-adrenal activity which further leads to release of corticosteroids, hormones that increase protein and lipid catabolism in turn

elevating plasma cholesterol concentration (Sahin *et al*., 2004). Hypercholesteremia is one of the risk factors for coronary artery disease (CAD) (Gambhir *et al*., 2000). Heat stress decreases the lymphoid organ weights, reduces the levels of total circulating antibodies (Bartlett and Smith, 2003) and causes damage to T-lymhocytes and B-lymphocytes by lipid peroxidation. Dietary modifications are the most practical ways to alleviate heat stress in broilers by addition of anti-oxidants like Vit-E, Vit-C and Selenium (Sahin *et al.*, 2004). Supplementation of medicinal plants as ingredients for broilers is increasing due to its growth promoting effects, immunomodulatory and hypolipidemic effects. *Withania somnifera* also known as ashwagandha has adoptogenic, immunomodulatory, hypoliplidemic and anti-stress activity (Davis and Kuttan, 2000). The main constituents of ashwagandha are *withanolides*, flavonoids and polyphenolic compounds (Uddin *et al.*, 2012). So the present study was aimed to determine the effects of different levels of ashwagandha on immunity and serum biochemical parameters in broilers reared during hot summer.

Materials and Methods

Day old *cobb* broiler chicks (n=160) were randomly assigned to 32 replicates under 4 dietary treatment groups each with 5 chicks and kept in a closed, ventilated, wire-floor battery caged broiler house under uniform conditions of temperature, humidity, and ventilation.

Table 1. Ingredient and nutrient composition of basal diet

Ingredients (%)	**Starter (0-21d)**	**Finisher (22-42d)**
Maize	56.9	61.31
Soya bean meal	35.43	30.6
Vegetable oil	3.355	4.287
Salt	0.423	0.422
Dicalcium phosphate	1.602	1.542
Limestone powder	1.343	1.177
DL-methionine	0.278	0.217
L-Lysine	0.241	0.076
$AB_2D_3K^*$	0.015	0.015
B-complex**	0.015	0.015
Trace mineral mixture***	0.1	0.1
Nutrient Composition		
ME (kcal/kg)	3050	3150
Crude protein (%)	21.5	19.5
Lysine (%)	1.25	0.98
Methionine (%)	0.56	0.48
Calcium (%)	0.9	0.82
Available phosphorous (%)	0.42	0.4

**AB_2D_3K provided per kg diet: Vitamin A 20000 IU, Vitamin B_2 25 mg ,Vitamin D_3 3000IU, Vitamin K 2mg. **Riboflavin 25mg, Vitamin B_1 1mg, Vitamin B_6 2mg, Vitamin B_{12} 40mg, and Niacin 15mg. ***Trace mineral provided per kg diet: Manganese 120mg, Zinc 80mg, Iron 25mg, Copper 10mg, Iodine 1mg.*

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Birds were vaccinated against Newcastle (7th and 28th d) and infectious Bursal disease (15th d) as per the standard vaccination schedule. Maize and soybean meal-based diets were prepared to contain 3050 and 3,150 kcal ME/kg during the starter (0 to 21 d) and finisher (22 to 42 d of age) phases, with respective crude protein contents of 21.5 and 19.5 g/100 g feed (Table 1).

The experimental treatments were as follows: Positive control (PC) (basal diet +Vit-E 70mg/kg + Se 0.15 mg/kg), Negative control (NC)-basal diet, WSE50 (basal diet + 50 mg WS extract/kg diet) and WS100 (Basal diet + 100mg WS extract/kg diet). Water and mash feed were provided *adlibitum* throughout 42 days trial period. The temperature and relative humidity of shed during trial period were 36.8^{o}C±0.25 – 39.7^{o}C±0.25 and 47 – 74% respectively.

Immunity

The humoral immunity was determined in birds by measuring antibody titre to Newcastle disease (ND) vaccine (antibody production against ND virus). At 21st and 42nd days of age blood was collected and serum was separated. Haemagglutination inhibition (HI) activity of serum was estimated and the antibody titers (log_2) were measured following the standard procedure (Wegmann and Smithies., 1966). The cell mediated immune (CMI) response was assessed by measuring cutaneous basophilic hypersensitivity (CBH) to phytohaemagglutinin phosphate (PHA-P). On 40th day of experiment, one bird per replicate was injected with 100 µg of PHA-P suspended in 0.1 ml of phosphate buffer saline (PBS) into the web between third and fourth inter-digital space of right foot, while the left web (control) was injected with 0.1 ml of PBS. The web thickness of both feet was measured by micrometer after 24 h of injection and CBH was calculated by method of Edelman *et al*., 1986.

Serum Bio-Chemistry

On 41st day of experiment, blood collected from one bird per replicate into a clean sterilized tube and kept in a slanted position at room temperature to facilitate separation of serum. Serum protein and cholesterol were determined by using commercially available diagnostic kits (Erba Mannheim Chemicals).

Statistical Analysis

The data was analysed by using SPSS 15th version.

Results and Discussion

The mean (log_2) antibody response to ND vaccine at 3rd and 6th week of age and cell mediated immune response at 41st day were not statistically significant ($P>0.05$). These results are similar to the findings of Mushtaq *et al*. (2012) who supplemented ashwagandha in drinking water (20g/litre). On the contrary Akotkar *et al*. (2007) reported that feeding 1.25% ashwagandha significantly improved HI titer and CMI in

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broilers. Similarly Ansari *et al*. (2013) and Vasanthakumar *et al*. (2015) reported improved ND titer in groups fed WSE at 0.15% and ashwagandha root powder at 2.5% - 5% levels in broiler diets respectively. Improved HI and CMI was also reported by feeding ashwgandha in broiler diets (Davis and Kuttan, 2000; Sujatha *et al*., 2010; and Singh *et al*., 2016). Improved immune parameters may be due to antimicrobial and immunomodulatory effects. Immunomodulatory effects of ashwagandha due to presence of glycowithanolides (Ghosal *et al*., 1989), which stimulates the production, phagocytic activity of circulating macrophages (Malik *et al*., 2007), stimulates bone marrow (Davis and Kuttan, 2000) and increases antibody producing lymphocytes. Decreased immune organ (spleen) weight corroborates the decreased immune response (HI & CMI) in the present study, which might be due to higher temperatures (36.8°C±0.25 – 39.7°C±0.25) during trial period. High temperatures are reported to reduce lymphoid organ weights (Hassan *et al*., 2007) and decreased circulating macrophages and antibodies (Smith, 2003; Mashaly *et al*., 2004; Niu *et al*., 2009). This reduction could be indirectly due to stimulation of hypothalamic pituitary adrenal (HPA) axis which results in release of corticosterone that inhibits antibody production (Gross *et al*., 1992; Star *et al*., 2008).

Table 2: Effect of ashwagandha extract on humoral immunity and cell mediated immunity in broiler chicken (1-42 d)

Treatment	NDV titers (log2)		*PHA-P Response (Thickness Index)
	3rd wk	6th wk	6th week
PC	3.25	3.75	164.1
NC	3.5	2.75	144.3
WSE50	3.25	3.25	150.3
WSE100	3.38	3.75	162.6
SEM	0.133	0.132	2.355
P-Value	0.678	0.103	0.452

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

Table 3: Effect of ashwagandha extract on spleen weights, serum cholesterol and serum total protein levels in broiler chicken (1-42 d)

Treatment	Cholesterol (mg/dl)	Total Protein (g/dl)	Spleen (% live weight)
PC	148.6^{ab}	3.26	0.09
NC	164.4^{b}	2.85	0.08
WSE50	146.2^{a}	3.07	0.09
WSE100	132.8^{a}	3.67	0.07
SEM	3.558	0.127	0.007
P-Value	0.009	0.123	0.774

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

Serum cholesterol concentrations were significantly (P<0.05) decreased in groups supplemented with WSE, while serum protein levels were unaffected (P>0.05). WSE100 group recorded low level of serum cholesterol compared to NC group and comparable to WSE50 and PC group. Increase in stress induces

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sympatho-adrenal activity which further leads to protein and lipid catabolism in turn elevating plasma cholesterol concentration (Sahin *et al*., 2004). Low cholesterol in PC compared to NC may be due to Se, due to inhibitory effect on 3-hydroxy-3-methylglutaryl-coA (HMG-CoA) reductase, a key enzyme in cholesterol biosynthesis (Nassir *et al*., 1997). By quenching free radicals Vit-E reduces the effects of oxidative stress there by reduces corticosteroids secretion (Sahin *et al*., 2002). Similar results were found by Habibian *et al*. (2014) by feeding Vit-E at 250 mg/kg & Se at 0.5-1 mg /kg diet. Ansari *et al*. (2013) reported decreased cholesterol levels by feeding ashwagandha 2.5% & 5.0%. Similarly, Sujatha *et al*. (2010) reported reduced cholesterol levels by supplementation of polyherbal mix at 0.1% level, reduced cholesterol levels due to hypolipidaemic and hypocholesterolaemic effect of *E. officinalis* in polyherbal mixture (Mathur *et al*., 1996). Ahmed *et al*. (2017); Mushtaq *et al*. (2016) and Pedhavi *et al*. (2017) also reported supplementation of ashwgandha led to decreased serum cholesterol. Reduced cholesterol levels in present study could be due to inhibitory effects of ashwagandha exerted at the levels of 3-hydroxyl-3-methyl-glutaryl-coA reductase, a key enzyme in cholesterol biosynthesis (Ansari *et al*., 2013; Ostlund, 2006) and increased excretion of cholesterol and bile acids through faecal sterol excretion (Ebihara and Schneeman, 1989). In the present study serum protein levels in all groups were not significantly influenced. On the contrary Sujatha *et al*. (2010) and Ansari *et al*. (2013) observed increased protein levels by feeding herbal mixture and ashwgandha respectively. Serum protein levels depends on availability of dietary protein, this suggests that proteins of ashwagandha fed diets were more available to the birds (Obikaonu *et al*., 2011.

Conclusion

The results of present study indicated that supplementation of Ashwagandha extract at 100mg/kg diet significantly decreased serum cholesterol levels without any deleterious effects on performance and did not show any significant effect on immunity.

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