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Evaluation of dietary soapnut shell powder supplementation on performance and immune response of broiler breeders

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ABSTRACT

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Present study analyzed the effect of soapnut shell powder supplementation on performance and immune response in broiler breeders. For this purpose, ninety six female and twenty four male broiler breeders of 38 weeks age were distributed randomly into four treatments with three replicates and reared separately for 42 days. The treatments were basal diet as control group (T1) and three groups with basal diet supplemented with graded levels of soapnut shell powder @ 0.0176 (T2), 0.026 (T3) and 0.0528% (T4) an equivalent dose: 0, 50, 75 and 150ppm saponin, respectively. Fortnightly DM intake (g/d) and serum protein profile was comparable ($P>0.05$) among the treatments for both the sexes of the breeder birds. Cell mediated immune response for both female and male birds was significantly ($P<0.001$) higher in T4 and T3 group respectively when compared to control group (T1). T2 group showed higher response than T1 but lower than T4 and T3 in both the sexes. Humoral immune response (log_2) for females was significantly ($P<0.001$) higher in SSP supplemented groups compared to control group (T1), whereas, for males it was significantly ($P<0.05$) higher in T4 and T3 groups compared to control group (T1). T2 group showed intermediate response. It can be concluded that supplementation of soapnut shell powder upto 0.0528% (dose equivalent 150ppm) could be safely included for improved performance and immunity of broiler breeders.
Key words: Soapnut, Cell mediated, Humoral immunity, Broiler breeders.

INTRODUCTION

India's poultry enterprise has undergone a paradigm shift with growth rate of about 8-10% annually. To sustain this high level of production it is mandatory to keep the parent stock of the birds *i.e.* breeders always healthy and productive. Supply of day old chicks is a very vital aspect of poultry production. Development of embryo and hatching of healthy chicks are completely dependent on nutrition of the breeders leading to nutrients deposition in the eggs which will further decide the growth and production of chicks in future. The immune status of the parent stock is also significantly contributes to the proper embryogenesis and subsequently hatching of quality chicks. Antibiotic growth promoters (AGPs) have been used since decades for improving production and immune status of the birds (Lee *et al.,* 2012) but despite of its positive response these AGPs tend to develop of antibiotic resistance by the microorganisms (Bach Knudsen, 2001). Globally, several countries have banned/ restricted the use of these in-feed antibiotics in food animals. Consequently, natural alternative feed additives are being tried in livestock and poultry nutrition for their antibiotic-like effects without causing bacterial resistance. It has been reported that phytogenic feed additives, that are plant derived products could be a substitute of AGPs in improving performance and immunity of the poultry birds (Jacela *et al.,* 2010; Murugesan *et al.,* 2015). These additives also lowers the cholesterol content in both serum and eggs without adversely affecting the feed intake and quality of egg laid (Sharma *et al.,* 2009; Elangovan *et al.,* 2011). North West Himalayan Region of the country has abundance of phytochemical containing herbal plants which may be explored for developing feed additives to both livestock and poultry.

Sapindus mukorossi is a deciduous tree belongs to the family Sapindaceae. It is known by several local names such as soapnut, soapberry, washnut, reetha, aritha, dodan and doadni *etc.* This tree is widely grown in upper reaches of Indo-Gangetic plains, Shivaliks and sub Himalayan tracts at altitudes from 200 to 1500 m. Soapnut tree is largely cultivated for its fruit which is the most valuable part of the plant. Soapnut fruit is a rich source of saponins with several biological and pharmacological properties such as anti-inflammatory, antimicrobial, immunostimulant, hypocholesterolaemic, anticarcinogenic and antioxidant *etc.* (Francis *et al.,* 2002; Guclu-Ustundag and Mazza, 2007). Broiler breeders are experiencing various kind of stresses during their production phase due to heat stress, handling stress etc., which adversely affects their immune status. An immune-compromised breeder stock could hatch out inferior quality chicks and subsequently, broiler production. Thus, there is a need to find an additive which can improve the immunity of

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broiler breeders and maximize their performance. Keeping in view the above facts, the present study was carried out to evaluate the effect of dietary soapnut shell powder supplementation on performance and immune response in broiler breeders.

MATERIALS AND METHODS

Soapnut fruits were purchased from local areas of Palampur, Himachal Pradesh, India. The fruits were dried at room temperature followed by manual separation of seeds. The collected soapnut shells were again dried at room temperature and grinded in the mixer to a uniform particle size and stored in an air tight container for further studies. Saponin was isolated from soapnut shell powder by modified Sharma *et al.* (2012) method and the yield of saponin was 28.4% (DMB).

The experiment was carried out with CARIBRO-VISHAL broiler breeders. For the study, ninety six female and twenty four male broiler breeders of about 38 weeks age were procured. Further, the birds were alienated into four treatments *viz.,* T1 (control), T2, T3 and T4 with three replicates each consisting of 8 females and 2 males. Both the types of birds were reared separately during the entire course of study (42d). Experimental diets contained 0, 0.0176%, 0.026% and 0.0528% SSP (dose equivalent: 0, 50, 75 and 150ppm saponin), respectively. The ingredients and nutrient composition of basal diet was formulated as per ICAR (2013) which is presented in Table 1. All the birds were housed in individual cages with standard management conditions. Data on dry matter intake (DMI) by each replicate was recorded on weekly basis. The body weight of birds were recorded.

Table 1: Ingredients and nutrient composition of basal diet

Ingredients (kg/100 kg feed)	Female	Male
Maize	58.42	57.28
De-oiled rice bran	2.10	23.00
Soybean meal	28.90	15.60
Oyster shell	6.20	0.00
Calcite/ LSP	2.00	1.80
DCP	1.50	1.40
Trace minerals	0.15	0.15
DL-Methionine	0.15	0.12
L-Lysine	0.054	0.13
Common salt	0.259	0.263
Constant*	0.27	0.27
Nutrient composition (as fed basis)		
Crude protein (%)	16.0	15.0
ME (kcal/kg)**	2800	2750
Calcium (%)	3.50	1.00
Total phosphorus (%)	0.70	0.70
Lysine (%)**	0.85	0.80
Methionine (%)**	0.45	0.40

*Constant includes vitamin premixes 0.15, B-complex 0.015, choline chloride 0.05, toxin binder 0.05 and coccidiostat 0.005 %.
**Calculated values as fed basis.

Cell mediated immunity (CMI) measured as foot web index in response to mitogen PHA-P (Corrier and Deloach, 1990) and humoral immune response measured as serum haemagglutination (HA) titre to sheep red blood corpuscles (Siegel and Gross, 1980) on 28th day of feeding trial. Eight female and six male breeder birds from each treatment were used for immunological response criteria. Approximately, 3-5 ml of blood from randomly chosen 8 female and 6 male breeder birds per treatment were collected by jugular vein on II, IV and VI week of feeding trial, in a centrifuge tube under aseptic conditions. Serum sample was harvested from each tube and analyzed for total protein and albumin using diagnostic kit (Coral Clinical System, Goa, India).

Data obtained from the study were subjected to statistical analysis as per Snedecor and Cochran (1994) using Completely Randomized Design (CRD). All the data were subjected to ANOVA using the one way with using SPSS 20.0 statistical software package. The mean differences among different treatments were separated by Duncan's multiple range tests. Therefore, a level of ($P<0.05$) was used as the criterion for statistical significance (Duncan, 1955).

RESULTS AND DISCUSSION

The results of weekly DMI (g/b/d) for both the sex of broiler breeders, for different dietary treatments are presented in Table 2. The average DMI (g/d) was comparable ($P>0.05$) among the treatments for both the sex of broiler breeders. Weekly DMI (g/d) in females were varied from 143.79 to 163.94 in T1, 149.40 to 160.89 in T2, 143.23 to 151.60 in T3 and 142.68 to 152.99g/d in T4, whereas in males, it was varied from 145.69 to 154.27 in T1, 140.57 to 151.08 in T2 135.63 to 149.41 in T3 and 137.21 to 150.43g/d in T4, respectively. Gaurav (2015) reported that the DMI of the birds fed with different diets consisting of Camellia seed and Chlorophytum root saponin were found to be statistically non-significant throughout the experimental period. Gurbuz *et al.* (2011) and Alagawany *et al.* (2016) also reported that daily feed intake was comparable among the treatments following *Yucca schidigera* supplementation in layers.

Overall performance of birds with respect to the body weight (kg), overall gain (kg), body weight (BW) change (g/d) and average DMI (g/d) has been presented in the Table 3. The initial mean body weight of the experimental females birds in T1, T2, T3 and T4 were 3.60, 3.69, 3.68 and 3.50kg, respectively, which get changed to 3.88, 4.02, 4.05 and 3.99kg, respectively. The initial mean body weight of the experimental males birds in T1, T2, T3 and T4 were 4.60, 4.47, 4.61 and 4.41kg, respectively, which get changed to 4.80, 4.72, 4.92 and 4.76kg, respectively. A significant ($P<0.01$) increase in overall gain (kg) and BW change (g/d) was observed in female birds with T4 treatment groups

compare to control (T1) and T2, whereas, T3 treatment group showed intermediate response. Average DMI (g/d) was comparable (P>0.05) among the groups in females. Overall gain (kg), BW change (g/d) and average DMI (g/d) was comparable (P>0.05) among the groups in males. Gaurav (2015) reported that the average daily gain did not differ significantly throughout the trial period in different phases of broilers fed with different sources of saponin. Potter and Shelton (1973) reported that diet with 5% alfalfa meal did not affect the average BWG but diets containing 10% or more alfalfa meal (Yu *et al.,* 1998) affected daily weight gain in geese.

Table 2: Effect of SSP supplementation on weekly DMI (g/d) of broiler breeder

Treatment	I week	II week	III week	IV week	V week	VI week
Females						
T1	161.94	158.70	163.94	160.58	153.96	143.79
T2	159.17	160.89	149.40	151.00	142.28	145.23
T3	143.29	143.23	151.60	148.91	145.94	147.36
T4	147.74	148.58	152.99	142.68	143.54	147.75
SEM	6.35	6.84	6.07	6.84	6.55	4.34
P value	0.139	0.238	0.351	0.337	0.595	0.907
Males						
T1	148.72	148.53	154.27	145.69	149.32	146.66
T2	145.50	150.87	151.08	143.99	140.57	148.59
T3	135.63	149.41	143.57	139.55	137.88	141.21
T4	144.24	139.32	149.36	137.21	144.43	150.43
SEM	7.01	9.36	5.32	3.55	4.42	4.64
P value	0.601	0.816	0.556	0.330	0.313	0.542

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

Table 3: Overall performance of the broiler breeders during the entire feeding trial

Treatment	Initial body weight (kg)	Final body weight (kg)	Overall gain (kg)	BW Change (g/day)	Average DMI (g/d)
Females					
T1	3.60	3.88	0.28^{b}	6.57^{b}	157.15
T2	3.69	4.02	0.33^{b}	7.85^{b}	151.33
T3	3.68	4.05	0.37^{ab}	8.81^{ab}	146.72
T4	3.50	3.99	0.49^{a}	11.67^{a}	147.21
SEM	0.10	0.06	0.05	1.14	5.06
P value	0.398	0.610	<0.01	<0.01	0.449
Males					
T1	4.60	4.80	0.20	4.70	148.86
T2	4.47	4.72	0.24	5.82	146.77
T3	4.61	4.92	0.31	7.29	141.21
T4	4.41	4.76	0.35	8.28	144.16
SEM	0.21	0.16	0.11	2.67	3.07
P value	0.876	0.846	0.757	0.757	0.349

[a,b]Means bearing different superscripts, differ significantly (P<0.05) in a column.

The results of immune response (humoral and cell mediated) is presented in Table 4. Cell mediated immune response (CMIR) *i.e.* foot pad thickness (mm) for both female and male birds was significantly (P<0.001) higher in T4 and T3 group compared to control group (T1). T2 group showed higher response than T1 but lower than T4 and T3 in both the sex of birds. Humoral immune response i.e. HA titre (log_2) for females was significantly (P<0.001) higher in SSP supplemented groups compared to control group (T1), whereas, for males it was significantly (P<0.05) higher in T4 and T3 groups compared to control group (T1). T2 group showed intermediate response. It has been reported that saponins can stimulate secretion of cytokines and trigger innate immunity (Song and Hu, 2009), as well as enhance humoral and cellular immune responses (de Sousa *et al.,* 2004). Our result was in agreement with Zhai *et al.* (2011a,b; 2014) who reported that administration of saponins isolated from ginseng stems and leaves in drinking water of chickens significantly enhanced the immune responses to vaccination against Newcastle disease, avian influenza and infectious bursal disease. Alagawany *et al.* (2016) supplementation of Yucca upto 100 or 150 ppm led to improved immunity functions in layers. Cheeke (2001) reported that saponins are capable to stimulate the immune system and enhance resistance to a disease challenge. Sahoo *et al.* (2015) reported that antibody titre against Newcastle disease virus in broiler chicks on 7 and 14th day of post-vaccination was significantly higher in the yucca treated *Yucca schidigera* extract group than the control group. Thus, present study confirms the role of SSP as immune-modulator and reason for that may be stable gut health maintained by antioxidant activity of SSP (Bharati *et al.,* 2012).

Table 4: Effect of SSP supplementation on immunological parameters of broiler breeders

Treatment	Foot web thickness		Antibody titre (log_2)
	Absolute (mm)	Relative (%)	
Females			
T1	0.21^{c}	100.00^{d}	1.30^{b}
T2	0.44^{b}	109.52^{c}	2.24^{a}
T3	0.75^{a}	257.14^{b}	2.84^{a}
T4	0.94^{a}	347.62^{a}	2.87^{a}
SEM	0.06	0.06	0.20
P value	<0.001	<0.001	<0.001
Males			
T1	0.43^{c}	100.00^{d}	1.77^{b}
T2	0.88^{b}	104.12^{c}	2.35^{ab}
T3	0.95^{a}	120.93^{b}	3.12^{a}
T4	1.10^{a}	155.81^{a}	3.03^{a}
SEM	0.07	0.07	0.27
P value	<0.001	<0.001	<0.05

[a,b,c]Means bearing different superscripts, differ significantly (P<0.05) in a column

Table 5: Effect of SSP supplementation on serum protein profile of female broiler breeders

Attributes	T1	T2	T3	T4	Period mean	P value		
						T	P	T×P
Total protein (g/dl)								
II week	6.44±0.25	6.32±0.18	6.27±0.36	6.33±0.15	6.34±0.11	0.687	0.199	0.920
IV week	5.98±0.16	5.83±0.29	6.07±0.19	6.30±0.28	6.04±0.12			
VI week	6.02±0.03	6.07±0.24	6.28±0.19	6.24±0.26	6.15±0.09			
Treatment mean	6.14±0.11	6.07±0.14	6.20±0.14	6.29±0.12				
Albumin (g/dl)								
II week	2.72±0.14	2.55±0.15	2.60±0.14	2.71±0.15	2.65±0.07	0.622	0.366	0.799
IV week	2.38±0.04	2.51±0.12	2.65±0.09	2.67±0.16	2.55±0.06			
VI week	2.58±0.15	2.43±0.18	2.53±0.06	2.54±0.12	2.52±0.07			
Treatment mean	2.56±0.08	2.50±0.08	2.59±0.06	2.64±0.08				
Globulin (g/dl)								
II week	3.71±0.15	3.77±0.32	3.67±0.39	3.62±0.17	3.69±0.12	0.981	0.520	0.920
IV week	3.60±0.19	3.32±0.37	3.42±0.16	3.63±0.14	3.49±0.11			
VI week	3.44±0.17	3.64±0.21	3.75±0.17	3.70±0.36	3.63±0.11			
Treatment mean	3.58±0.10	3.57±0.17	3.61±0.14	3.65±0.13				
A/G Ratio								
II week	0.74±0.04	0.70±0.09	0.74±0.09	0.76±0.08	0.73±0.03	0.993	0.751	0.804
IV week	0.67±0.05	0.79±0.10	0.78±0.04	0.73±0.03	0.74±0.03			
VI week	0.76±0.08	0.68±0.07	0.68±0.03	0.71±0.09	0.71±0.03			
Treatment mean	0.72±0.03	0.72±0.05	0.73±0.03	0.74±0.04				

T: Treatment; P: Period; T×P: Treatment and Period interaction. Mean bearing different superscripts in a column differ significantly (P<0.05)

Table 6: Effect of SSP supplementation on serum protein profile of male broiler breeders

Attributes	T1	T2	T3	T4	Period mean	P value		
						T	P	T×P
Total protein (g/dl)								
II week	5.40±0.26	5.39±0.43	5.57±0.17	5.34±0.51	5.42±0.17	0.897	0.703	0.922
IV week	5.64±0.59	5.65±0.52	5.84±0.44	5.67±0.43	5.70±0.22			
VI week	5.30±0.45	5.56±0.53	5.77±0.51	5.63±0.51	5.56±0.23			
Treatment mean	5.44±0.24	5.53±0.26	5.73±0.21	5.55±0.26				
Albumin (g/dl)								
II week	1.79±0.17	1.74±0.25	1.81±0.20	1.67±0.07	1.75±0.08	0.998	0.838	0.981
IV week	1.80±0.18	1.89±0.31	1.77±0.12	1.85±0.14	1.83±0.09			
VI week	1.76±0.16	1.70±0.18	1.85±0.09	1.84±0.07	1.78±0.06			
Treatment mean	1.78±0.09	1.78±0.14	1.80±0.08	1.79±0.06				
Globulin (g/dl)								
II week	3.61±0.40	3.65±0.27	3.77±0.30	3.67±0.55	3.67±0.18	0.928	0.841	0.894
IV week	3.84±0.58	3.76±0.56	4.08±0.48	3.82±0.47	3.87±0.24			
VI week	3.54±0.40	3.87±0.54	3.92±0.56	3.80±0.56	3.78±0.23			
Treatment mean	3.66±0.25	3.76±0.25	3.92±0.24	3.76±0.27				
A/G Ratio								
II week	0.53±0.12	0.48±0.07	0.50±0.09	0.50±0.11	0.50±0.04	0.976	0.998	0.995
IV week	0.52±0.10	0.55±0.14	0.46±0.07	0.51±0.08	0.51±0.05			
VI week	0.51±0.06	0.47±0.09	0.51±0.09	0.54±0.13	0.51±0.04			
Treatment mean	0.52±0.05	0.50±0.05	0.49±0.04	0.52±0.06				

T: Treatment; P: Period; T×P: Treatment and Period interaction. Mean bearing different superscripts in a column differ significantly (P<0.05)

Serum protein profile for both female and male broiler breeders are presented in the Table 5 and 6, respectively. Total protein (g/dl), albumin (g/dl), globulin (g/dl) and A:G ratio was found to be comparable (P>0.05) among the treatments. Gaurav (2015) did not observed any significant effect following supplementation of Camellia seed and Chlorophytum root saponin based diet on serum hemato-biochemical profile of chickens. Gupta *et al.* (2005) also reported that there was no effect on serum protein values following supplementation of saponin from *Albizia lebbeck* bark @ 50ppm in male albino rats.

Along with this, it was also found that SSP at various level of incorporation had significantly improved the semen quality, fertility and hatchability performance of broiler breeders without affecting the quality of egg lay (Chaudhary, 2017; Chaudhary *et al.,* 2017).

Thus, from the above findings it can be concluded that supplementation soapnut shell powder upto 0.0528% (dose equivalent 150ppm) can be safely incorporated in the diet of broiler breeders for improved production performance and immune response.

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