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Soapnut shell powder as immunomodulatory and welfare friendly feed additive in broiler chicken

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ABSTRACT

With growing awareness for safe and clean poultry products among consumer, the industry faces a tough challenge of antibiotic free as well as welfare friendly poultry farming. This study was conducted to evaluate the efficacy of soapnut shell powder, rich in saponins, as immune enhancer and stress reliever in broiler chicken. The broiler chicks were randomly distributed into four dietary treatments, viz. T1 (basal diet, control), T2 (basal diet with 100 mg saponin/kg diet), T3 (basal diet with 150 mg saponin/kg diet), and T4 (basal diet with 200 mg saponin/kg diet) and reared up to 42 days of age. The results revealed that supplementing saponin level beyond 100 mg/kg diet significantly reduced body weight gain and feed intake of birds at 4th and 5th week of age with no significant effect on the FCR. The cell mediated and humoral immunity of birds also improved due to saponins @ 100 mg/kg diet or above. However, no significant dietary effects were observed on the weight of immune organs. Similarly, the abdominal fat (%) and H:L ratio of birds significantly decreased at 100 mg saponin level indicating the stress relieving effect of saponins. The study concluded that dietary supplementation of saponins (100 mg/kg diet) in the form of soapnut shell powder not only enhances the immunity but also improves the welfare of birds without affecting feed efficiency. Thus, soapnut shell powder can be used as an alternative option for the antibiotic free broiler chicken production especially in hilly areas where it is cheaply available.

Key words: Broiler, Growth performance, Immunity, Saponins, Stress

Poultry industry in India has witnessed a multifaceted development in areas of nutrition, genetics, engineering, management, and communication to maximize the efficiency of growth performance, meat and egg yield. In modern poultry industry, the use of antibiotics are very much effective in increasing disease resistance as well as overall performance of the poultry birds, and eliminating the use of antibiotics during production may lead to negative effects on the conversion rate of diets (Salois *et al.* 2016). However, because of growing public health concerns of antibiotic use and the increased consumer awareness, the poultry industry is compelled to find alternatives of antibiotics in order to maintain its consumer base.

Investigators have demonstrated that different phytogenic feed additives have the potential to replace the antibiotics as growth promoters and immune enhancers in poultry flocks (Hernandez *et al*. 2004, Cross *et al*. 2007, Windisch *et al*. 2008). Soapnut (*Sapindus mukorossi*) commonly known as ritha, is one such photogenic plant found in diverse geographical areas like Gangetic plains, Western ghats, and Deccan plateau in India. Medicinal value of soapnut due to the presence of saponins, places it among the list of herbs and minerals in Ayurveda. Immuno-stimulatory activities of saponins from different sources have been reported (Cheeke 2001). As per the current knowledge, Yucca saponins (*Yucca schidigera*) are commercially available products that have been used as immunomodulator in chicken (Cheeke *et al.* 2006) and have increased IgG, IgM, T-AOC (total antioxidative capacity), CAT, and SOD levels with positive effects on immune organ maturation (Su *et al*. 2016). Feeding of ginseng stem-and-leaf saponins have shown enhanced immunity in chicken (Zhai *et al.* 2011) and the tea saponins have been reported to enhance immune functions in broilers by stimulating specific cytokines levels (Bhardwaj *et al.* 2014).

However, the literature available pertaining to the use of soapnut shell saponins as poultry feed additive in broiler chicken production is meagre. Thus, the present study was

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undertaken to evaluate the effect of soapnut shell powder saponin on growth performance, immune status and stress level in broiler chicken.

MATERIALS AND METHODS

An experiment was conducted at the Avian Nutrition and Feed Technology Division of ICAR-Central Avian Research Institute, Izatnagar. Day-old broiler chicks (160) (CARIBRO-Vishal) of same hatch were wing banded and weighed individually. The birds were housed in specially designed battery brooder cages with watering and feeding facilities and were reared under standard management conditions. The chicks were randomly distributed into four dietary treatments, viz. T1 (control basal diet), T2 (basal diet with 100 mg saponin/kg diet), T3 (basal diet with 150 mg saponin/kg diet), and T4 (basal diet with 200 mg saponin/kg diet) having 5 replicates per treatment with 8 birds in each (40 birds/treatment) and reared up to 42 day of age. The soapnut shell powder, as a source of saponin, used in this experiment contained 28.4% saponin (Chaudhary 2017) and the amount of the powder added in each dietary treatment was calculated based on this saponin concentration. The iso-nitrogenous and iso-caloric basal diets were prepared as pre-starter, starter, and finisher diets to meet the requirement of all the essential nutrients for growing broiler chicken (ICAR 2013). The ingredients and nutrient composition of basal diets has been given in Table 1.

The data regarding growth performance, viz. body weight gain, feed intake and feed conversion ratio in the control as well as experimental groups were recorded on weekly basis from 0 to 42 days of age. The *in vivo* cell mediated immunity (CMI) as foot web index in response to PHA-P mitogen was evaluated by the method of Cheng and Lamont (1988) on 22nd day post hatch. The humoral immune response was measured as serum haemagglutination (HA) titre to sheep red blood corpuscles (SRBC) after five days of intravenous injection of 1% SRBC at 28th day post hatch in broiler chicken other than used for assessing CMI (Siegel and Gross 1980). The immune response was assessed in 10 birds per treatment. The lymphoid organ weights (spleen, bursa of fabricius, and thymus) were recorded on 42nd day by randomly slaughtering 8 birds per treatment and expressed as percentage of live weight. The abdominal fat percentage and heterophil : lymphocyte (H:L) ratio were studied as stress indicators of birds. The abdominal fat percentage from 8 birds per treatment was recorded at the time of slaughter. The blood smears prepared from fresh blood collected from 8 birds per treatment were stained by Geimsa stain (1:9 dilution for 45 min) to calculate heterophil: lymphocyte (H:L) ratio.

The data obtained from the experiment were analysed by one way ANOVA (Snedecor and Cochran 1994) by using SPSS software package version 20.0. The testing of variables having unequal means were analysed by Duncan's multiple range test (1955).

Table 1. Ingredients and nutrient composition of basal diets

Ingredient (%)	Broiler pre-starter	Broiler starter	Broiler finisher
Maize	57.1	57.9	63.5
Soybean meal	34.2	33.0	28.8
Fish meal	3.0	3.2	2.0
Veg oil	2.4	2.9	2.8
Limestone	0.9	0.9	0.8
Di-calcium phosphate	1.4	1.1	1.2
DL-Methionine	0.2	0.1	0.1
Lysine	0.1	0.0	0.0
Trace mineral premix[1]	0.1	0.1	0.1
Vitamin premix[2]	0.2	0.2	0.2
Vitamin B complex[3]	0.0	0.0	0.0
Choline chloride	0.1	0.1	0.1
Salt	0.5	0.5	0.5
Toxin binder	0.1	0.1	0.1
Total	100	100	100
Nutrient composition of diets			
ME (Kcal/kg)	3003	3051	3104
Crude protein (%)	21.99	21.53	19.55
Lysine (%)	1.20	1.13	0.99
Methionine (%)	0.52	0.49	0.41
Calcium (%)	1.01	0.95	0.85
Available P (%)	0.45	0.41	0.38

ME, Metabolisable energy. [1]Trace mineral mixture each (100 g) contains $FeSO_4$. $7H_2O$, 8 g; $ZnSO_4$. $7H_2O$, 10 g; $MnSO_4$. H_2O, 10 g; $CuSO_4$. $5H_2O$, 1 g; KI, 30 g. [2]Vitamin premix (each g) contains vitamin A, 82.5 IU; vitamin B2, 50 mg; vitamin D3, 12000 unit and vitamin K, 10 mg. [3]Vitamin B complex (each g) contains vitamin B1, 8 mg; vitamin B6, 16 mg; vitamin B12, 80 mg; niacin, 120 mg; calcium pantotheonate, 80 mg; vitamin E, 160 mg.

RESULTS AND DISCUSSION

Growth parameters: The body weight gain of birds did not show any significant dietary effects except the significant ($P<0.05$) decline in 4th week body weight gain due to the saponin level of 200 mg/kg diet compared to the control and other saponin levels which did not differ significantly from each other (Tables 2, 3, 4). There was no significant difference in weekly feed intake of birds except the significantly ($P<0.05$) decreased feed intake at 5th week of age due to the saponin level of 200 mg/kg diet compared to the control. However, the feed intake of birds at saponin level of 100 and 150 mg/kg diet was statistically similar to control as well as 200 mg saponin level. The weekly FCR of birds did not show significant effect of soapnut shell powder feeding.

Though the negative effect on the body weight gain and feed intake in the present study was observed only in the 4th and 5th week respectively, the negative effect of dietary saponins on growth performance are well documented in broiler chicken (Ueda and Tanoue 2000 and Hassan *et al*. 2013). Also, the FCR of birds was not influenced by the saponin supplementation in broiler chicken (Kutlu *et al*. 2001 and Cabuk *et al*. 2004). However, in contrast to our study, several studies have reported significant improvement in growth performance due to saponin supplementation

Table 2. Effect of soapnut shell powder/saponin on weekly body weight gain in broiler chicken

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
T1	98±2.1	165±3.5	317±10.4	372±16.8[b]	392±22.9	392±10.5
T2	102±2.5	163±1.4	317±11.5	360±9.7[b]	344±23.6	382±21.9
T3	95±2.8	160±6.0	300±11.2	376±10.8[b]	329±12.3	345±11.3
T4	101±1.1	161±3.9	396±6.0	320±14.0[a]	343±13.4	360±7.9
P value	0.141	0.782	0.611	0.03	0.135	0.099

Values bearing different superscripts within the column differ significantly (P<0.05).

Table 3. Effect of soapnut shell powder/saponin on weekly feed intake in broiler chicken

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
T1	93±2.76	233±2.21	461±6.80	695±16.40	787±9.74[b]	960.6±4.75
T2	93±2.08	229±3.17	482±17.77	675±15.67	760±23.95[ab]	932±17.53
T3	93±1.14	229±3.04	444±7.30	679±15.76	766±5.21[ab]	917±11.60
T4	92±1.36	227±1.50	447±8.88	636±17.86	718±17.25[a]	943±9.11
P value	0.954	0.406	0.108	0.117	0.046	0.103

Values bearing different superscripts within the column differ significantly (P<0.05).

Table 4. Effect of soapnut shell powder/saponin on weekly FCR in broiler chicken

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
T1	0.96±0.043	1.41±0.040	1.46±0.037	1.88±0.069	2.04±0.133	2.45±0.067
T2	0.91±0.029	1.41±0.023	1.53±0.064	1.93±0.067	2.24±0.112	2.47±0.157
T3	0.98±0.031	1.44±0.069	1.49±0.079	1.81±0.046	2.35±0.105	2.67±0.079
T4	0.91±0.019	1.41±0.042	1.45±0.030	2.01±0.109	2.10±0.107	2.62±0.047
P value	0.319	0.958	0.750	0.347	0.259	0.320

(Nazeer *et al.* 2002, Cabuk *et al.* 2004, Miah *et al.* 2004), whereas Gaurav (2015) and Alagawany *et al.* (2016) did not found significant differences in growth parameters of the birds. These inconsistencies in the results may be due to the difference in the genotype of birds, source of saponin, season, dose level, and duration of supplementation. Since in the present study, no negative dietary effect was observed on the FCR of birds, the decline in body weight gain can be related to corresponding decline of feed intake which may be due to the astringent and irritating taste of saponins (Oleszek *et al*. 1994), reduction in the intestinal motility (Klita *et al*. 1996), and the protein digestibility (Potter *et al*. 1993, Shimoyamada *et al*. 1998). Though, this study asserts that the saponins do not negatively affect the most important economic trait, i.e. FCR of birds, further studies with different doses, season, and duration of supplementation are needed to clearly understand its role in production.

Immunity: There was no significant effect of different saponin levels on the relative weights of spleen, bursa, and thymus of broiler chicken (Table 5). On the other hand, the humoral as well as cell mediated immunity of birds fed different saponin levels improved significantly (P<0.05) with respect to the control diet fed birds. However, the different saponin levels did not significantly from each other in terms of the immunity of the birds. The enhanced immunity in broiler chicken due to the effect of dietary supplementation of saponins found in our study is in agreement with the findings of Cheeke (2001) and Chaudhary (2017) who reported the increased immunity of broiler chicken and broiler breeder, respectively. Similarly, the enhanced immune responses to vaccination against Newcastle disease (Zhai *et al.* 2011a, 2011b, 2014; Sahoo *et al.* 2016), avian influenza and infectious bursal disease (Zhai *et al.* 2011a, 2011b, 2014) were observed due to the saponin supplementation in broiler chicken. The cell mediated immunity (mediated by T cell activity) and humoral immunity (mediated by antibodies) are two aspects of specific immunity. The improved specific immunity of broiler chicken found in the present study as the effect of saponin rich soapnut shell powder feeding can be potential nutritional strategy in disease prevention and ultimately antibiotic free poultry production. Similar to this study, Su *et al.* (2016) did not found any significant effect of dietary yucca extract (containing saponin) on the weight of immune organs (spleen, bursa and thymus) in broiler chicken. However, in contrast, significantly improved immune organ weights have been observed due to the dietary supplementation of polysavone (Alfalfa extract) in broiler chicken (Dong *et al*. 2007). This variation in results may be due to the different sources of saponins and genetic difference of the birds used in different studies.

Table 5. Effect of soapnut shell powder/saponin on immunity and immune organ weight of broiler chicken

Treatment	[1]Foot web index (mm)	[2]Antibody titre (log_2)	Immune organ weight (% live weight)		
			Spleen	Bursa	Thymus
T1	0.596±0.137[a]	6.2±0.663[a]	0.104±0.004	0.194±0.020	0.406±0.040
T2	1.226±0.124[b]	9.2±0.583[b]	0.103±0.003	0.209±0.022	0.449±0.022
T3	1.514±0.317[b]	9.6±0.510[b]	0.105±0.005	0.249±0.027	0.530±0.048
T4	1.366±0.177[b]	9.6±0.678[b]	0.109±0.002	0.244±0.039	0.454±0.047
P value	0.029	0.003	0.634	0.446	0.205

Values bearing different superscripts within the column differ significantly (P<0.05). [1]Cell mediated immunity; [2]Humoral immunity.

Stress indicators: The significant decline of abdominal fat percentage (P<0.05) as well as H:L ratio (P<0.01) in broiler chicken were observed due to the supplementation of dietary saponin compared to control (Table 6). However, though the saponin levels did not differ significantly from each other in terms of abdominal fat and H:L ratio of birds, the abdominal fat (%) of birds fed saponin @ 100 mg/kg diet was statistically similar to that of control diet fed birds.

Table 6. Effect of soapnut shell powder/saponin on stress indicators in broiler chicken

Treatment	Abdominal fat (%)	Heterophil: lymphocyte ratio (H:L)
T1	1.896±0.216[b]	0.468±0.019[b]
T2	1.598±0.208[ab]	0.389±0.018[a]
T3	1.364±0.083[a]	0.350±0.016[a]
T4	1.124±0.103[a]	0.340±0.012[a]
P value	0.017	0.000

Values bearing different superscripts within the column differ significantly (P<0.01).

The birds under stress have greater tendency to deposit abdominal fat and decrease subcutaneous fat. These observations are in agreement with Miah *et al.* (2004), Afrose *et al*. (2010) in broiler chicken, and Jiang *et al*. (2012) in muscovy duck, who reported that saponin from different sources lower the abdominal fat percentage. Similarly, significantly reduced H:L ratio was observed due to dietary supplementation of saponin of soapnut shell powder origin in broiler breeder (Chaudhary 2017).

The decreased H:L ratio indicates better welfare of the birds. Gross and Siegel (1983) first found decreasing numbers of lymphocytes and increasing numbers of heterophils in response to different physiological stressors, the relationship between these leucocytes has become widely accepted as a reliable physiological indicator of stress responses in domestic fowl (Maxwell and Robertson 1998). Gross and Siegel (1983) concluded that the H:L ratio was a more reliable parameter for the measurement of stress in domestic fowl than plasma corticosteroids. Also, the major goals of the poultry industry are to increase the carcass yield and to reduce carcass fatness, mainly the abdominal fat pad which is also an indicator of stress in birds (Rokade *et al.* 2016). Modern broiler strains contain 15 to 20% fat and >85% of this fat is not physiologically required for body function. In general, excessive fat deposition is an unfavourable trait for producers and consumers because it is considered to be the wastage of dietary energy and a waste product with low economic value, which also reduces the carcass yield and affects consumer acceptance. In current study, reduction of abdominal fat percentage and H:L ratio by feeding soapnut shell powder rich in saponins clearly indicate reduced stress and improved welfare of birds and the effect may be due to improved immunity, reduced oxidative stress (Yu *et al*. 2015) and reduced serum cholesterol which is the precursor of serum corticosterone (Rokade *et al.* 2016).

From the above parameters it is clear that supplementation of saponins at the level of 100 mg/kg diet in the form of soapnut shell powder can improve the immunity and reduces stress of birds without hampering the feed conversion ratio (FCR). Thus, it can be concluded that dietary soapnut shell powder can be effectively used as alternative feed additive in place of antibiotics for the improvement of welfare and immunity of broiler chicken.

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