Original Research

Cottonseed Cake: Effect on Serum and Hematological Parameters of Poultry Broiler Birds

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Abstract

The study was conducted to evaluate the effect of the locally available non-dehulled cottonseed cake on various serum and hematological parameters in the chicken broiler birds under completely randomized block design. For this purpose, day old broiler chicks (n=108) were divided into 4 main treatment groups T_0, T_1, T_2 and T_3. Each treatment group was further divided in 3 replicates with 9 chicks in each. T_0 served as standard control diet and was given standard corn- soy flake- groundnut based ration. Treatment T_1 was given ration containing 20 per cent non-dehulled cottonseed cake and T_2 was given ration containing 20 per cent fermented non-dehulled cottonseed cake whereas treatment T_3 was given ration containing 20 per cent non-dehulled cottonseed cake with additional supplementation of ferrous sulphate (600ppm) and lysine (2%). All the four broiler diets were formulated and prepared conferring to ICAR (2013) standards. Supplementation of non-dehulled cottonseed cake did not show any significant variation in complete blood count except for total leukocyte count (TLC) which was significantly ($P<0.05$) higher in treatment T_1. Plasma phosphorous, triglycerides, high density lipids (HDL), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), total protein (TP) and albumin content were non-significant amongst control and other treatment groups. The average value of cholesterol (mg/dL) and low-density lipids (mg/dL) in blood plasma was significantly ($P<0.05$) higher in treatment T_1 compared to control T_0 and treatment T_2. The average alkaline phosphatase (ALP) value in blood plasma was significantly ($P<0.05$) higher in treatment T_2 compared to control group T_0. The average value of calcium (mg/dL) in blood plasma was significantly ($P<0.05$) higher in T_3 compared to T_2 and numerically higher than control T_0 and T_1. The average value of iron (mg/dL) in blood plasma in control T_0 was significantly ($P<0.05$) higher compared to treatment T_2 and T_3 and numerically higher than treatment T_1. Thus, it was concluded that replacement of 20 per cent non-dehulled cottonseed cake available in the market as a protein replacer in feed of broiler birds has effect on some of the serum and hematological parameters i.e., TLC, cholesterol, low-density lipids (LDL), ALP, calcium and iron.

Key words: Broiler Chickens, Cottonseed Cake, Fermented Cottonseed Cake, Ferrous Sulphate, Lysine, Serum and Hematological Parameters

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Introduction

India ranks first in cottonseed meal production (Indexmundi, 2018a) and third (Indexmundi, 2018b) in its export in the world. Thus, India has tremendous scope for utilization of the cottonseed meal in poultry ration. Feed ingredients prices are rising day by day and cottonseed meal is one of the cheap sources of protein in the ration of animals. It is mainly used in ruminant ration and its use is limited in poultry due to the presence of the polyphenolic compound i.e., gossypol which is an anti-nutritional factor (ANF) and cause adverse effects in the animals. Gossypol form complex with iron thus decrease its absorption which leads to decreased hemopoiesis due to reduced iron availability leading to anemia in animals. Besides this it forms complex with various proteins in the body thus affects various functions of the body dependent on the type of protein which is affected. Various treatment methods have been adopted by various researchers to reduce the concentration of gossypol in the cottonseed meal. Thus, decreasing the ill effects of gossypol in animals. Thus, the present study was designed to evaluate the blood biochemistry of broiler birds fed cottonseed cake-based diet.

Material and Methods

Preparation and Treatment of Substrate

Substrate preparation and treatment was done as per the method of Zhang *et al.* (2007). Substrate (cottonseed cake) was mixed with corn flour in 7:3 ratio. This basal substrate was moistened by taking substrate and water in 1:0.8 ratio and then was autoclaved at 15 psi pressure for 45 minutes.

Fermentation of Cottonseed Cake and Estimation of Gossypol Content

Fermentation of cottonseed cake was done as per the method given by Nie *et al.* (2015a). Probiotic capsule Gitzer was used for fermentation purpose. Capsule was purchased from the local market's drug store. Each gram of Gitzer contained *Lactobacillus acidophilus, L. rhamnosus, Bifidobacterium longum, B. bifidum, Streptococcus thermophilus* (0.48 billion each) and *Saccharomyces boulardii* (0.10 billion). Estimation of total and free gossypol was done by spectrophotometric method suggested by Makkar *et al.* (2007).

The study was conducted at the experimental poultry house and laboratory of the Department of Animal Nutrition, DGCN COVAS, CSKHPKV, Palampur, (Himachal Pradesh, India). The growth studies were conducted through a feeding trial for the period of 42 days which consist of day-old broiler chicks (n=108) divided into 4 main treatment groups T_0, T_1, T_2 and T_3. Each treatment group was further divided into 3 replicates with 9 chicks in each. The total duration of experiment was 42 days.

Four broiler experimental diets for all the three phases (pre-starter, starter and finisher) were formulated and prepared conferring to ICAR (2013) standards. T_0 served as standard control diet and was given standard corn- soy flake- groundnut based ration. Treatment T_1 was given ration containing 20 per cent non-dehulled cottonseed cake and T_2 was given ration containing 20 per cent fermented non-dehulled cottonseed cake whereas treatment T_3 was given ration containing 20 per cent non-dehulled cottonseed cake with additional supplementation of ferrous sulphate (600ppm) and lysine (2%). All the four broiler diets were formulated and prepared conferring to ICAR (2013) standards. The proximate analysis of the samples was done in the laboratory of the Department of Animal Nutrition. The dry matter was determined by oven drying, total ash by muffle burning, fat by Soxhlet extraction with petroleum ether (by use of Plican Socs Plus 08AS DLS instrument), protein by Kjeldahl nitrogen estimation (with the help of instruments: Pelican Kelplus KES 12L R digestion assembly, Pelican p Kelplus Ultimate duo Dist-TS E distillation assembly, SI Analytics Titro Line 7000 titrator), crude fibre by use of Pelican FIBRA Plus FES4 instrument following the procedure as recommended in AOAC (2005). While calcium (Ca) and iron (Fe) were determined with the help of microwave digestion assembly (QLAB PRO Microwave Digestion System) and Atomic Absorption Spectrophotometer (AAS) method (LABINDIA AA8000 Atomic Absorption Spectrophotometer), the digestion for it was done using the method suggested by Palma *et al.* (2015) and phosphorous (P) concentration was determined spectrophotometrically (Spectronic 200 spectrophotometer) using the method proposed by Parks & Dunn (1963). The metabolisable energy (ME) was calculated using the equation as proposed by Lodhi *et al.* (1976).

A total of 108, day old (0 day) broiler chicks (Van cob) were procured from the M/s Uttam Hatchery, Gururkari, Distt. Kangra, Himachal Pradesh. At the start of the experiment, chicks were weighed individually and thereafter were marked and distributed randomly into four dietary groups viz. T_0, T_1, T_2 and T_3 according to the experimental plan in the battery brooder. Each group comprised of three replicates with 9 chicks in each replicate with 27 birds in each treatment. Birds were shifted from battery brooder to deep litter system on 7th day of the experiment. Standard environmental conditions (temperature and relative humidity) were provided to the chicks immediately on the arrival and *ad libitum* feed and lukewarm water were provided. The birds were offered with pre-starter diet for first 14 days, starter diet for next seven days (15- 21 day) and finisher diet for rest of the days (22- 42 days). The day before slaughter pooled blood samples were collected from each replica for the collection of plasma. The birds were kept off feed the day before the collection of blood.

Approximately 3 to 5 mL of blood from randomly chosen birds was collected from wing vein with the help of 24gauge (24G) needle and collected in 10 per cent heparin coated centrifuge tubes. After collection of the whole blood, the blood was centrifuged at 2000 × g for 15 minutes. The resulting supernatant, designated plasma was collected and transferred into a clean polypropylene tube using a Pasteur pipette.

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The samples were then stored at - 20°C and later analysed in the lab by using Erba Mannheim CHEM 5 χ plasma analyzer, with the help of the kits of Agappe diagnostics in the Department of Animal Nutrition, COVAS, Palampur (Himachal Pradesh). While, total erythrocytic count (TEC) and total leukocytic count (TLC) was done by the methods given in book Clinical Avian Medicine- Volume II (Samour, J., 2006).

Statistical Analysis

All the recorded and calculated data were subjected to analysis of variance (ANOVA) by methods of Snedecor and Cochran (1968) using complete randomized block design (CRBD). Results were evaluated at 5 percent level of significance.

Results and Discussion

The average value of calcium (mg/dL) in blood plasma was significantly (P<0.05) higher in T_3 compared to T_2 and numerically higher than control T_0 and T_1. The plasma calcium ranged from 8.05 to 9.70 mg/dL (Table 1). Tang *et al.* (2012) reported no significant difference in plasma calcium level with fermented cottonseed meal-based diet and control diet. The reason might be high fibre diet as fibrous diet increases hydrochloric acid secretion (Sacranie, 2012) leads to enhanced calcium absorption. The average value of phosphorus (mg/dL) in blood plasma exhibited no significant (P>0.05) difference amongst different treatments compared to control (Table 1).

Table 1: Blood plasma mineral content

	Ca (mg/dL)	P (mg/dL)	Fe (mg/dL)
T_0	8.27±*0.54*^{ab}	6.32±*0.17*	3.66±*0.40*^{a}
T_1	9.46±*0.37*^{ab}	6.4±*0.42*	2.91±*0.18*^{ab}
T_2	8.05±*0.14*^{a}	6.58±*0.28*	2.18±*0.17*^{b}
T_3	9.70±*0.24*^{b}	5.4±*0.07*	2.13±*0.16*^{b}
	P<0.05	**NS: P>0.05**	**P<0.05**

Fig. with different superscripts in a column are statistically (p<0.05) significant from each other.

But numerically higher value was observed for treatment T_2. The reason might be increased availability of phosphorous due to the fermentation that was in the form of phytate in the diet. Whereas, Tang *et al.* (2012) reported no significant difference in plasma phosphorous level with fermented cottonseed meal-based diet and control diet. The average value of iron (mg/dL) in blood plasma in control T_0 was significantly (P<0.05) higher compared to treatment T_2 and T_3 and numerically higher than treatment T_1 (Table 1). Gossypol present in cottonseed meal-based diet forms complex with iron thus lowering its availability. Henry *et al.* (2001) also reported a reduced level of plasma iron with 20 per cent cottonseed meal-based diet. Whereas, Ozodogan *et al.* (2010) didn't found cottonseed meal level in the diet to be significant on plasma iron level. However, numerically lowest value of plasma iron was found in 10 per cent cottonseed meal incorporated diet.

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Table 2: Plasma lipid profile

	Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
T_0	125.5±*3.88*^{a}	64.75±*14.05*	95.40±*7.10*^{a}	17.15±*1.22*
T_1	165.53±*7.11*^{b}	50.80±*8.41*	136.77±*4.77*^{b}	18.60±*5.11*
T_2	125.07±*0.43*^{a}	95.57±*11.27*	89.95±*0.45*^{ac}	16.01±*2.36*
T_3	148.47±*10.40*^{ab}	55.36±*7.96*	119.66±*7.17*^{ab}	17.07±*2.61*
	P<0.05	**NS: P>0.05**	**P<0.05**	**NS: P>0.05**

Fig. with different superscripts in a column are statistically (p<0.05) significant from each other.

The perusal of results revealed that the average value of cholesterol (mg/dL) in blood plasma in control T_0 and treatment T_1, T_2 and T_3 was 125.5±3.88, 165.53±7.11, 125.07±0.43 and 148.47±10.40 mg/dL respectively (Table 2) which was significantly higher in treatment T_1 compared to control T_0 and treatment T_2. The reason might be affection of the liver as gossypol was found to affect liver functioning by various researchers (Lee *et al*., 1982; Burgos *et al.*, 1986). However, Mandal *et al.* (2004) and Elangovan *et al.* (2006) reported no significant difference in serum cholesterol level in birds fed 10 per cent cottonseed meal but they had used low inclusion level (10%) of cottonseed meal. Thirumalaismy *et al.* (2016) also reported no significant difference in total cholesterol level among control and cottonseed meal-based diets with or without supplementation of iron. The average value of high-density lipids (HDL) and triglycerides (mg/dL) in blood were found to be non-significant (P>0.05) amongst control and other treatment groups (Table 2). Thirumalaismy *et al.* (2016) also reported no significant difference in HDL and triglycerides level among control and cottonseed meal-based diets with or without supplementation of iron. However, Nie *et al.* (2015b) reported significantly lower hepatic triglycerides in 6 per cent fermented cottonseed meal treatment. The average low-density lipid (LDL) value (mg/dL) was recorded significantly (P<0.05) higher in treatment T_1 compared to control T_0 and T_2 (Table 2). However, Thirumalaismy *et al.* (2016) reported no significant difference in LDL level among control and cottonseed meal-based diets with or without supplementation of iron.

The average value of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) in blood plasma in exhibited no significant (P>0.05) difference amongst different treatments (Table 3). He *et al.* (2015) also reported no significant difference in level of SGOT, SGPT compared to control and different level of inclusion of cottonseed meal. Whereas, Xiong *et al.* (2016) reported an increase in the activity of serum glutamic pyruvic transaminase in birds offered fermented cottonseed meal diet compared to control. The average alkaline phosphatase value (Table 3) in blood plasma was significantly higher in treatment T_2 compared to control group T_0. Affection of biliary tract or bones due to gossypol content could be one of the reasons for increased alkaline phosphate level.

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Table 3: Plasma enzymes

	SGOT (AST)	SGPT (ALT)	ALP
	IU/L	IU/L	IU/L
T_0	259.13±45.63	10.49±1.06	2155.67±73.20^{a}
T_1	248.1±10.59	11.85±1.38	2874.67±172.46^{ab}
T_2	227.73±14.06	8.72±0.68	3178.33±207.49^{b}
T_3	236±17.86	10.79±1.54	3022±212.62^{b}
	NS: P>0.05	**NS: P>0.05**	**P<0.05**

Fig. with different superscripts in a column are statistically (p<0.05) significant from each other.

The average plasma total protein and plasma albumin value (Table 4) in blood plasma was found to be non-significant (P>0.05) amongst control and other treatments. The results were similar to most of the work done in the past i.e., no change in serum total protein and albumin level was observed by most of the researchers at different inclusion levels of cottonseed meal (Mandal *et al.,* 2004; Elangovan *et al.,* 2006; Tang *et al.,* 2012; He *et al.,* 2015 and Thirumalaismy *et al.,* 2016).

Table 4: Plasma proteins

	Total Protein (g/dl)	Albumin (g/dl)
T_0	2.57±*0.16*	1.82±*0.12*
T_1	2.59±*0.09*	1.85±*0.10*
T_2	2.30±*0.34*	1.61±*0.13*
T_3	2.61±*0.04*	1.9±*0.11*
	NS: P>0.05	**NS: P>0.05**

Fig. with different superscripts in a column are statistically (p<0.05) significant from each other.

The average total leukocyte count (TLC) was significantly (P<0.05) higher in treatment T_1 compared to other treatments (Table 5).

Table 5: Complete blood count

	TLC (10^9/L)	TEC (10^{12}/L)	Hb (g/dl)	PCV (%)	MCH (pg)	MCHC (g/dl)
T_0	3.89±*4.33*^{a}	2.35±*0.23*	11.13±*0.24*	41.33±*1.20*	48.52±*5.90*	26.97±*0.72*
T_1	8.20±*1.16*^{b}	2.57±*0.68*	10.8±*0.31*	41±*3.0*	49.00±*13.55*	26.64±*2.18*
T_2	3.92±*1.89*^{a}	2.54±*0.12*	9.87±*0.13*	40.67±*0.33*	39.00±*1.80*	24.27±*0.46*
T_3	4.34±*2.82*^{a}	3.16±*0.34*	9.87±*0.44*	41.67±*0.88*	32.17±*4.55*	23.74±*1.49*
	P<0.05	**NS: P>0.05**	**NS: P>0.05**	**NS: P>0.05**	**NS: P>0.05**	**NS: P>0.05**

Fig. with different superscripts in a column are statistically (p<0.05) significant from each other.

The reason for increased level of TLC might be gossypol content of the diet as gossypol increase the glucocorticoid sensitivity as reported by Hagelstein *et al.* (2014) which leads to increased heterophil count and as differential leukocyte count (DLC) was not done so exact reason couldn't be find out. Besides this reason for increased level of TLC could be stress due to handling or environmental stress and inflammation with or without presence of infection (increased heterophil population) (Jeannine Miesle). However,

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Adeyemo and Longe (2007) observed no significant change in white blood cells (WBCs) when cottonseed cake (CSC) replaced soybean cake (SBC). Thirumalaismy *et al.* (2016) also reported no significant difference in white blood cell count among control and cottonseed meal-based diets with or without supplementation of iron.

The average total erythrocytic count (TEC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was non-significant (P>0.05) amongst the control and different treatment groups (Table 5). Adeyemo and Longe (2007) observed no significant change in red blood cell count, hemoglobin and PCV, when cottonseed cake (CSC) replaced soybean cake (SBC). Similarly, no significant difference in hemoglobin and PCV value was observed on inclusion of cottonseed meal by Henry *et al.*, 2001. Similarly, no significant difference in hemoglobin value was observed on inclusion of cottonseed meal by different researchers (Mandal *et al.* 2004; Elangovan *et al.*, 2006; Karakas *et al*., 2006; Adeyemo 2008 and Ozodogan *et al.*, 2010). Thirumalaismy *et al.* (2016) also reported no significant difference in MCH and MCHC value among control and cottonseed meal-based diets with or without supplementation of iron. However, Karakas *et al*. (2006) reported that MCHC was higher in the 15 per cent cottonseed meal-based diet.

Conclusion

On the basis of the results obtained in the given experiment it could be concluded that replacement up to 20 per cent level non dehulled cottonseed cake in the diet of poultry has effect on some of the serum and hematological parameters (TLC, cholesterol, LDL, ALP, calcium and iron). Whereas, total erythrocytic count (TEC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), plasma phosphorous, triglycerides, high density lipids (HDL), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), total protein (TP) and albumin content were non-significant amongst control and other treatment groups. However, further study is needed to conclude the effect of 20 percent level non dehulled cottonseed cake-based diet on the overall performance of poultry broiler birds.

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