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Effect of dietary supplementation of turmeric (*Curcuma longa*) powder on haematological and biochemical profile of commercial broiler chicken

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Abstract

This experiment was conducted to study the effect of inclusion of different levels of turmeric (*Curcuma longa*) powder on hematological and biochemical profile of commercial broiler. A total of 144 day-old (Cobb 400) broiler chicks were allocated randomly to four dietary treatments for 42 days of age with three replicates (12 birds/ replicate). The experimental diets were as follow: control (T_0), 0.25 % turmeric powder (T_1), 0.5 % turmeric powder (T_2), 0.75 % turmeric powder (T_3). It was found that total RBC count in the T_2 group showed significantly (P \leq 0.05) higher value as compared to control group. Moreover, the total lymphocyte count was significantly (P \leq 0.05) higher in T_3 , T_2 and T_1 group as compared to T_0 group. The biochemical parameters (total serum cholesterol, HDL, LDL and ALT) except serum glucose, triglycerides and glutathione peroxidase differed significantly (P \leq 0.05) among the experimental groups in this study.

Keywords: turmeric, Curcuma longa, broiler, haematological, biochemical

Introduction

Phytogenic feed additives are plant-derived products used in animal feeding in order to improve performance of agricultural livestock. This class of feed additives has recently gained increasing interest in recent years, especially for use in poultry. This appears to be strongly driven by the ban on most of the antibiotic feed additives within the European Union in 1999, a complete ban enforced in 2006, and ongoing discussions to restrict their use outside the European Union due to speculated risk for generating antibiotic-resistance in pathogenic micro biota.

Natural phytobiotic like turmeric played an important role as feed additive from long time ago. The active ingredients found in Turmeric (*Curcuma longa*) are curcumin, demethoxy-curcumin, bisdemethoxycurcumin, (Wuthi-Udomler *et al.*, 2000) ^[40] and tetrahydrofuran-cuminoids (Osawa *et al.*, 1995) ^[29]. Curcumin is the main important bioactive ingredient responsible for the biological activity. These active components have unique properties like such as anti-inflammatory (Holt, 2005) ^[18] and antioxidant (Jayaprakasha *et al.*, 2005; Karami *et al.*, 2011) ^[20, 21]. Some pharmacological activities of Turmeric (*Curcuma longa*) as nematocide (Kiuchi *et al.*, 1993) ^[22], hypolipidemic (Ramirez-Tortosa *et al.*, 1999) ^[32] and anti-inflammatory (Ammon *et al.*, 1993; Holt *et al.*, 2005) ^[5, 18] were demonstrated. Additionally, it has been suggested that curcumin possess hepatoprotective, antitumor, antiviral and anticancer activity (Polasa *et al.*, 1991) ^[30]. Keeping this view in mind, the research was conducted to investigate the effect of feeding turmeric (*Curcuma longa*) powder on the growth performances and carcass characteristics of commercial broilers.

Materials and Method

This study was conducted at the Instructional Poultry Farm (August,2016 to October,2016), College of Veterinary Science, Khanapara, Guwahati to study effect of supplementing different levels of turmeric (*Curcuma longa*) powder on broiler haematological and biochemical profile. One hundred and forty four day-old (Cobb 400) broiler chicks were allocated randomly utilizing a complete randomize design (CRD) with four dietary treatment for a period of 42 days with three replicate each of 12 birds per replicate. The experimental diets were as follow: control (T₀), 0.25 % turmeric powder (T₁), 0.50 % turmeric powder (T₂), 0.75% turmeric powder (T₃). The ingredient and chemical composition of the diets is presented in Table (01) & (02).

 Table 1: Ingredients and nutrient composition of basal diet (broiler starter and broiler finisher) as per bis (2007)

Ingredients (Kg)	Starter (0-28 days)	Finisher (29-42 days)				
Maize	50.0	52.5				
Rice polish	7.0	7.0				
Ground nut cake	17.0	12.0				
Soya bean meal	22.5	24.0				
Vegetable oil	1.0	2.0				
Mineral mixture	2.0	2.0				
Common salt	0.5	0.5				
Nutrient composition						
Dry matter (%)	88.4	86.6				
Crude protein (%)	22.04	20.99				
Ether extract (%)	3.5	5.0				
Crude fibre (%)	4.0	3.5				
Nitrogen free extract (%)	63.16	64.51				
Total ash (%)	7.3	6.0				
Metabolizable energy (Kcal/Kg)*	2803.11	2901.53				

* calculated values

(N.B. Vitamin premix Provita M was added @ 20g per quintal in both starter and finisher diet.)

Table 2: Nutrient composition of turmeric powder

Nutrient composition	Turmeric powder
Dry matter (%)	88.2
Crude protein (%)	9.40
Ether extract (%)	11
Crude fibre (%)	2.5
Nitrogen free extract (%)	68.8
Total ash (%)	8.3
Curcumin (%)	3.00

At the end of the feeding trial, for estimation of hematological parameter like hemoglobin (Hb), Packed Cell Volume (PCV), total Red Blood Corpuscles (RBC), total White Blood Corpuscles (WBC) and WBC differential count, about 2ml of blood was collected aseptically with anticoagulant from 5 birds of each group. Then the blood was estimated for the above parameter with the instrument "Automatic Hemolyzer" in Teaching Veterinary Clinical Complex, College of Veterinary Science, AAU, Khanapara.

Serum cholesterol

For estimation of total serum cholesterol, five birds were selected randomly from each group and about 5 ml blood was collected aseptically from each bird. Then the blood samples were centrifuged for separation of serum. Then the total serum cholesterol was estimated using spectrophotometer (Systronics model No. 106) with Lyphochek Cholesterol Kit (Cholesterol Oxidase Peroxidase methodology) supplied by AGAPPE Diagnostics Ltd.

Calculation

Absorbance of test sample

Total cholesterol (mg/dl) =

Absorbance of standard

Serum triglycerides

For estimation of serum triglycerides, five birds were selected randomly from each group and about 5 ml blood was collected aseptically from each bird. Then the blood samples were centrifuged for separation of serum. Then the total serum cholesterol was estimated using spectrophotometer (Systronics model No. 106) with Liquichek Triglycerides Kit (GPO-TOPS methodology) supplied by AGAPPE Diagnostics Ltd.

Calculation

Absorbance of test sample Triglycerides (mg/dl) = _____ × 200 Absorbance of standard

High density lipoprotein (HDL)

For estimation of serum direct HDL, five birds were selected randomly from each group and about 5 ml blood was collected aseptically from each bird. Then the blood samples were centrifuged for separation of serum. Then the total serum direct HDL was estimated using spectrophotometer (Systronics model No. 106) with CliniQuant-FSR HDL-Direct Reagent Kit (Accelerator Selective Detergent Methodology) supplied by Meril Diagnostics Pvt. Ltd.

Calculation

Direct HDL (mg/dl)=

Absorbance of test sample x concentration of calibrato

Absorbance of calibrator

Low density lipoprotein (LDL)

The total serum LDL was estimated using the following Friedewald formula (Knopfholz *et al.*, 2014)^[23].

Calculation

Direct LDL (mg/dl) = (Total Cholesterol) - (Direct HDL) - (<u>Triglycerides</u>)

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Serum Glucose

For estimation of serum Glucose, five birds were selected randomly from each group and about 5 ml blood was collected aseptically from each bird. Then the blood samples were centrifuged for separation of serum. Then the total serum Glucose was estimated using spectrophotometer (Systronics model No. 106) using the Lyphochek Glucose Kit (GOD-PAP methodology) supplied by AGAPPE Diagnostics Ltd.

Calculation

Serum glucose (mg/dl) =
$$2000 \times 100$$

Absorbance of standard $\times 100$

Serum glutamate pyruvate transferase (SGPT)/ alanine transaminase (ALT)

For estimation of serum ALT, five birds were selected randomly from each group and about 5 ml blood was collected aseptically from each bird. Then the blood samples were centrifuged for separation of serum. Then the total serum ALT was estimated using spectrophotometer

 $\times 200$

(Systronics model No. 106) using the Lyphochek SGPT/ALT Kit (IFCC recommended methodology) supplied by AGAPPE Diagnostics Ltd.

Calculation

Serum ALT (mg/dl) = (OD/min) $\triangle \times 1768$

Glutathione peroxidase

Glutathione peroxidase (GPx) was estimated as per Hafeman et al. (1974)^[17]. The haemolysate was diluted 1:200 times with distilled water 0.2 ml of reduced glutathione (2Mm), 0.2 ml of sodium phosphate buffer and 0.1 ml of 0.01M sodium azide in test, control and blank tubes were added. Again 0.1 ml haemolysate and 0.2 ml of distilled water were added to test and control tubes. In the blank only 0.3ml of distilled water was added. After 5 minutes of incubation, 0.2ml of prewarmed 1.2Nm H₂O₂ (at 37 ⁰C) was added to test and blank except control where 0.2ml of distilled water was added. After 3 minutes interval, 4ml of metaphosphoric acid precipitation solution was added to all the tubes and centrifuged. To 2ml of filtrate pipette from all the tubes, 2ml of 0.4M sodium hydrogen phosphate solution and 0.1ml of DNTB reagent were added. Absorbance was read using spectrophotometer (Systronics model No. 106) at 420nm and value was expressed as U/ml.

Statistical analysis: One way Analysis of Variance was performed by the software SAS system (Local, X64_7PRO).

and lymphocyte count recorded in the present study did not differed significantly (P>0.05) among the different treatment groups (Table 03 and 04). The findings of Al-Jaleel (2012)^[2] and Sugiharto et al. (2011) [35] also indicated that there were no significant (P>0.05) differences in haemoglobin, PCV and total WBC count due to supplementation of turmeric powder in the feed of broiler chicken. On the other hand, few workers (Kumari et al., 2007; Noori et al., 2011; Ukoha and Ununkwo, 2016 and Attia et al., 2017) [24, 27, 37, 9] found significantly (P≤0.05) higher values in Hb and PCV in broiler chicken due to supplementation of turmeric powder at different levels. The total RBC count recorded in T₃ group showed significantly (P≤0.05) higher values as compared to T_0 group. However, there were no significant differences in value of total RBC count between T_0 , T_1 and T_2 groups. The present findings were in agreement with the reports of Al-Sultan (2003) and Ukoha and Ununkwo (2016) [37] who observed significantly (P≤0.05) higher values in total RBC count in broiler chicken supplemented with 0.50, 1.00, 2.00 and 3.00% turmeric powder. The significant increase in the RBC count might be due to the presence of iron which was an essential co-factor for cytochrome oxidase enzymes at cellular level metabolisms and required for red blood cell production (Rudrappa, 2009) ^[34]. The total lymphocyte count showed significant (P<0.05) increase in all the three turmeric treated groups as compared to the control group. The increase in lymphocyte count might be due to the immunomodulatory (Antony *et al.*, 1999)^[6] effect of turmeric powder and thereby helps in activation of immune responses and increasing the lymphocyte count (Surh, 1999)^[36].

Result and Discussion

All the haematological parameters except total RBC count

	_	-		
Group Parameters	T ₀₀ (Control)	T ₁ (TP-0.25%)	T ₂ (TP-0.50%)	T ₃ (TP-0.75%)
Haemoglobin (g/dl)	$9.50^{a}\pm0.20$	$9.96^{a}\pm0.12$	$9.90^{a} \pm 0.12$	$9.66^a\pm0.25$
PCV (%)	$32.66^a\pm0.49$	$35.44^{a} \pm 1.02$	$33.70^{a} \pm 1.47$	$34.76^{a} \pm 1.16$
Total RBC (million/mm ³)	$2.46^a\pm0.02$	$2.55^{ab}\pm0.03$	$2.52^{ab} \pm 0.04$	$2.59^{\mathrm{b}} \pm 0.03$
Total WBC (thousand/mm ³)	$14.13^{a}\pm0.8$	$14.61^{a} \pm 1.30$	$17.41^{a} \pm 1.49$	$17.45^{a} \pm 0.58$
Neutrophil (thousand /mm ³)	$1.95^{a}\pm0.33$	$2.42^{a}\pm0.45$	$3.19^{a} \pm 0.50$	$3.28^{a}\pm0.95$
Eosinophil (thousand /mm ³)	$0.24^{a}\pm0.04$	$0.25^{a} \pm 0.03$	$0.19^{a}\pm0.03$	$0.19^{a} \pm 0.04$
Monocyte (thousand /mm ³)	$0.88^{a} \pm 0.27$	$1.50^{a} \pm 0.74$	$1.82^{a}\pm0.67$	$0.61^{a}\pm0.75$
Lymphocyte (thousand /mm ³)	$82.98^a\pm3.67$	$91.58^b\pm3.38$	$93.84^{b} \pm 1.97$	93.86 ^b ±0.75

Table 3: Mean (± se) values of haematological parameters of broilers under different treatment groups

Means bearing same superscripts in a row did not differ significantly

The mean (±SE) values of all the biochemical parameters (total serum cholesterol, HDL, LDL, ALT) except triglycerides, serum glucose and glutathione peroxidase recorded in the present study differed significantly (P≤0.01) among different experimental groups (Table 03 and 04). The total serum cholesterol recorded in the present study was significantly (P \leq 0.01) lower in T₃ and T₂ (140.97 and 148.24 mg/dl) as compared to T_0 and T_1 group (158.87 and 160.83 mg/dl). All the blood lipid metabolites (cholesterol, HDL and LDL) except triglycerides tested in the present study were significantly improved by inclusion of turmeric powder in broiler chicken diet. These findings were in agreement with the reports of earlier workers (Daneshyar et al., 2011; Vashan et al., 2011; Faghani et al., 2014; Maaty et al., 2014; Fallah and Mirzaei, 2016 and Arslan et al., 2017) [11, 38, 13, 26, 14, 7] who reported that dietary supplementation of turmeric powder at different levels caused a significant decreased in the values of total cholesterol, LDL while HDL concentration in serum in broiler chickens as compared to control group without any

treatment. The depression in cholesterol level in the turmeric treated groups might be due to the inhibition of the active enzyme hepatic 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) which was responsible for cholesterol synthesis in the liver (Crowell, 1999) ^[10]. This suggested the hypocholesterolemic and hypolipidemic action of turmeric powder on broiler chicken. Furthermore, the reduction in blood cholesterol could be attributed to reduction in the levels of some hormones secreted by the cortex of the adrenal glands, which decreases the secretion of fatty acids from the adipose tissue or as a result of fat oxidation process, leading to depression of levels of fatty acids including blood cholesterol (Ganong, 2005) ^[15]. Contrary to the present findings, few workers namely Ashayerizadeh et al. (2009) [8], Nouzarian et al. (2011) [28], Reddy et al. (2012b) [33] and Abou-Elkhair et al. (2014) ^[1] reported non-significant (P>0.05) differences in the concentration of serum cholesterol in the turmeric treated group when compared with control group.

Group Parameters	T ₀ (Control)	T1 (TP-0.25%)	T ₂ (TP-0.50%)	T ₃ (TP-0.75%)
Total serum Cholesterol (mg/dl)	$158.87^a\pm2.31$	160.83 ^a ± 2.29	$148.24^{b} \pm 3.62$	$140.97^{b} \pm 3.06$
Triglycerides (mg/dl)	$111.79^{a} \pm 2.27$	$107.68^{a} \pm 3.22$	$107.89^{a} \pm 2.32$	$103.58^{a} \pm 4.97$
HDL (mg/dl)	82.13 ^c ±5.13	$97.68^{ac} \pm 3.66$	105.68 ^{ab} ±8.06	$119.22^{b} \pm 8.17$
LDL (mg/dl)	$54.39^{a} \pm 5.21$	$21.74^{b} \pm 6.01$	$20.98^{\text{b}}\pm8.90$	$20.89^{b} \pm 8.44$
Serum Glucose (mg/dl)	$217.91^{a} \pm 2.74$	$217.60^{a} \pm 2.86$	$214.66^{a} \pm 2.83$	$215.06^{a} \pm 4.33$
ALT (U/ml)	$29.00^{a}\pm1.94$	$19.86^{b} \pm 3.01$	$19.76^{b} \pm 1.17$	$19.51^{b} \pm 0.60$
Glutathione Peroxidase (U/ml)	$105.96^{a} \pm 7.18$	$115.05^a\pm4.12$	$115.34^a\pm3.05$	$118.82^{a} \pm 1.99$

Means bearing same superscripts in a row did not differ significantly

The mean $(\pm SE)$ values of triglycerides did not differ significantly among the different treatment groups (Table 03). The results indicated that the turmeric powder supplementation had no influence on the levels of triglycerides in serum of broiler chicken. These findings were in agreement with the reports of Ashayerizadeh et al. (2009) ^[8], Vashan et al. (2011) ^[38], Fallah and Mirzaei (2016) ^[14] and Arslan et al. (2017)^[7] who found no significant differences in the concentration of triglycerides in broiler chicken supplemented with turmeric powder. Contrary to the present findings, Daneshyar *et al.* (2011)^[11], Nouzarian *et al.* (2011) ^[28], Hussein (2013) ^[19], Faghani et al. (2014) ^[13] and Maaty et al. (2014) [26] suggested that supplementation of turmeric powder in the broiler diet decreased the levels of triglycerides in the blood serum due to the hypolipidaemic action of turmeric powder.

The mean (±SE) values of HDL of the different treatment groups were 82.13, 97.68, 105.68 and 119.22 mg/dl for T₀, T₁, T_2 and T_3 groups, respectively. The HDL values were found to be significantly (P \leq 0.05) higher in T₃ and T₂ group as compared to control T₀ groups. The present results corroborated with the findings of few workers (Daneshyar et al. (2011)^[11], Vashan et al. (2011)^[38], Faghani et al. (2014) ^[13], Maaty et al. (2014) ^[26] and Arslan et al. (2017) ^[7] who reported significant increase in HDL concentration in blood serum of broiler chicken as compared to control. However, Ashayerizadeh et al. (2009)^[8] and Nouzarian et al. (2011)^[28] found no significant differences in HDL levels among the different treatment groups fed with turmeric powder in the ration. The significant effect of turmeric powder in increased HDL level could be explained by the hypocholesterolemic and hypolipidemic effect of curcumin when added to diet (Alwi et al., 2008)^[4]. The possible mechanism of modulating anti-lipid effect due to the bioactive components which might be responsible for the selective inhibition of 11 beta-HSD1 (a key metabolic enzyme) which decreased absorption of cholesterol and increased in the activity of cholesterol-7alpha-hydroxylase, an enzyme that catalyzed the formation of bile acid from cholesterol (Daniells, 2015)^[12].

All the three levels of turmeric powder (0.25, 0.50 and 0.75%) showed decreased LDL levels compared to the control group. The LDL values of the different groups were 54.39, 21.74, 20.98 and 20.89 mg/dl for T_0 , T_1 , T_2 and T_3 groups, respectively. The present findings were in agreement with the reports of few previous workers (Faghani *et al.*, 2014; Maaty *et al.*, 2014 and Fallah and Mirzaei, 2016) ^[13, 26, 14] who found that there was significant effect in LDL concentration between the control and the turmeric powder treated groups. The significant (P<0.05) effect of turmeric increased the population of receptors for LDL in the liver and this effect directly increased the breakdown of LDL-cholesterol (Godkar *et al.*, 1996) ^[16]. Moreover, they also found that the

antioxidant effect of turmeric inhibited lipid peroxidation and prevented the oxidation of LDL. Contrary to the present findings, Ashayerizadeh *et al.*(2009) ^[8], Daneshyar *et al.* (2011) ^[11], Vashan *et al.* (2011) ^[38], Nouzarian *et al.* (2011) ^[28] and Arslan *et al.* (2017) ^[7] reported non-significant (P>0.05) differences in serum LDL concentration due to supplementation of turmeric powder in broiler diet.

The mean values of serum glucose did not differ significantly among different treatment groups (Table 03). The results of present study indicated that turmeric powder the supplementation had no effect on the levels of serum glucose in broiler chicken. Similar findings were also reported by Fallah and Mirzaei (2016) [14], who found no significant differences in glucose levels in broiler chicken supplemented with turmeric powder. Contrary to the present findings, Abou-Elkhair et al. (2014)^[1] and Qasem et al. (2016)^[31] suggested that inclusion of turmeric powder in the broiler diet reduced serum glucose level as compared to non-supplemented group. The mean (±SE) values of ALT recorded in the present experiment differed significantly (P<0.05) among the different treatment groups (Table 03). The ALT/SGPT values were recorded as 26.29, 26.02, 24.27 and 25.32 U/ml for T₀, T_1 , T_2 and T_3 groups, respectively. The results clearly indicated that supplementation of turmeric powder significantly (P<0.01) decreased serum ALT concentration in T_1 , T_2 and T_3 groups compared to the control group. The results thus indicated that inclusion of turmeric powder in the broiler diet had no toxic effects on the liver. This finding was in agreement with the reports of Maaty et al. (2014) [26] and Qasem et al. (2016) [31] who observed significant reduction in the activity of ALT in serum of broiler chickens in the turmeric treated group compared to control group. The hepatoprotective activity of turmeric powder might be attributed to the fact that curcumin has been reported to be excellent biological chain breaking antioxidants that protects cells and tissues from lipid peroxidative damage induced by free radicals (Osawa et al., 1995; Lee et al., 2004) [29, 25]. However, in similar studies, Vashan et al. (2011) [38], Reddy et al. (2012b) [33], Hussein (2013) [19] and Abou-Elkhair et al. (2014) ^[1] found non-significant differences in serum ALT level among the turmeric supplemented groups.

The mean (\pm SE) values of glutathione peroxidase enzyme in the present study did not showed any significant (P>0.05) differences among the different treatment groups (Table 03). The results of the present experiment indicated that inclusion of turmeric powder in the broiler diet had non-significant effect on the levels of glutathione peroxidase enzyme in the blood. However, the levels of GPx were numerically higher in turmeric treated groups. This indicated that turmeric might increase the antioxidant activity in the body of the broiler chicken. Contrary to the reports of present study, Vashan *et al.* (2011) ^[38] and Wang *et al.* (2016) ^[39] found significant increase in the enzymatic activity of GPx in turmeric supplemented broiler chicken.

In respect of haematological parameters, it was found that inclusion of turmeric powder at the rate of 0.75% showed improvement in the total RBC count in broiler chicken compared to other groups. Moreover, the total lymphocyte count in blood increased in all the three levels (0.25, 0.50 and 0.75%) of turmeric powder as compared to control group.

All the blood lipid metabolites (Cholesterol, HDL and LDL) except triglycerides were significantly ($P \le 0.05$) improved due to dietary supplementation of turmeric powder in broiler chicken. There was significant ($P \le 0.05$) decrease in the total serum cholesterol and LDL concentration while HDL concentration was significantly increased in broiler chicken at 6 weeks of age. In respect of total serum cholesterol and HDL, it was found that supplementation of turmeric at the rate of 0.50 and 0.75% showed better results as compared to other levels (0.00 and 0.25%). Moreover, dietary supplementation of turmeric powder elicited significant ($P \le 0.05$) decrease in the level of serum ALT in all the three turmeric treated groups.

Thus, from this study, it can be recommended that turmeric powder can be used economically as a natural feed additive in broiler chicken diet at the level of 0.75% due to its beneficial effects on commercial broiler chicken. In addition turmeric powder exhibited hypocholesterolemic and hypolipidemic effect on broiler chicken. The effective level of supplementation of turmeric powder of 0.75% might be due to the hormesis effect. Further in depth studies may be required using different levels of turmeric powder as feed additive in broiler chicken to validate the present results.

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