

*Original Article*

Turmeric and Exogenous Enzyme Supplementation Improve Growth Performance and Immune Status of Japanese quail

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ABSTRACT

The aim of the study was to investigate the effect of turmeric and exogenous enzymes on performance, oxidative stress and immunity in Japanese quails (*Coturnix japonica*). Birds (N=10/group) were divided into five groups and three replicate; the first group (T1) was given diet with fish meal and served as the control group. The second group (T2) was given the corn- SBM based diet without any supplements. The third group (T3) was given corn- SBM based diet supplemented with 0.5% turmeric powder. The fourth group (T4) was given corn- SBM based diet supplemented with 0.5% turmeric powder and 0.1% Phytase. The fifth group (T5) was given corn-SBM based diet supplemented with 0.5% turmeric powder and 0.1% Panzyme. The supplementations continued for 4 weeks started from first week of age. Serum samples were collected at the end of the experiment. Results revealed that there was a trend toward increase in final body weight and body weight gain in T5 as compared to all other treatments with a significant reduction in feed consumed compared to T1 (control group) and significant improvement in feed efficiency ratio as compared to T1 and T2. There was a significant increase in carcass weight in T1 compared to T4. Groups treated with turmeric and the exogenous enzymes showed significant increase in total protein and albumin on the other hand, significant reduction in liver enzymes was recorded. We observed a significant suppression of IL-6, IL-10, IL-12 and TNF- α . Oxidative stress parameters showed significant decrease in MDA level but other parameters as CAT, SOD, GSH and GSH-Px revealed significant increase than control group. These results suggested that addition of turmeric together with Panzyme improved the corn-SBM based diet fed to growing quail by improving feed efficiency and anti-inflammatory activity.

Keywords: Cytokines, Growth Performance, Oxidative Stress, Quail, Turmeric

INTRODUCTION

There is a growing interest in developing natural alternatives to antibiotic growth promoters in order to maintain both performance and health. Turmeric (*Curcuma longa*) is a natural herb of the ginger family, Zingiberaceae. Turmeric is a tropical plant native to southern and southeastern tropical Asia. In many Asian countries the use of turmeric as a food spice, colorant and medicine has been a long tradition (HMPC, 2009). It is also strongly alleged that turmeric can improve digestion and nutrient metabolism. The main yellow bioactive substances isolated from the rhizomes of *Curcuma* are curcumin, demethoxycurcumin and bisdemethoxycurcumin which is present to the extent of 2- 5 % of the total spice in turmeric powder. Curcumin is the main important bioactive ingredient responsible for the biological activity of curcuma (Nouzarian et al., 2011). Curcumin has been shown to have several biological effects, exhibiting anti-inflammatory (Holt et al., 2005), antioxidant (Iqbal et al., 2003; Pal et al., 2001) and hypolipidaemic (Ramirez-Tortosa et al., 1999) activities. The anti-inflammatory activity of curcumin was associated with its ability to inhibit the production of proinflammatory cytokines such as TNF- α , IL-1, IL-8, and inducible nitric oxide synthase (Chandramohan and John, 2002) Curcumin has also been studied extensively as a chemopreventive agent in several cancers (Duvoix et al., 2005). Additionally, it has been suggested that curcumin possess hepatoprotective, antitumor, antiviral and anticancer activity (Polasa et al., 1991). It is used in gastrointestinal and respiratory disorders (Anwarul et al., 2006). The significant biological properties of turmeric powder make it a potential substitute for in-feed antibiotics in livestock diets. A number of studies have been conducted to evaluate its effects on the performance of broiler chickens, laying hens and rabbits, however, the results have not been consistent (Nouzarian et al., 2011).

Enzymes are involved in all anabolic and catabolic pathways of digestion and metabolism which helps in improved feed conversion ratio. Corn-SBM diet is mainly used in poultry and was assumed to cause no digestive problem in poultry, so the exogenous enzymes were not required. But researchers have proved that corn-SBM diet contains

numerous anti-nutritional factors, such as β -glucans, β -mannose, protease inhibitors and lectins, and it has been proved that the addition of exogenous enzymes in corn and soybean meal is justified and feasible (Yang et al., 2010). Most of the researchers have concentrated on the destruction of gel-form polysaccharides leached from cereal cell walls in amounts sufficient to depress performance (Annison and Choct, 1991; Bedford, 1993; Chesson, 1993). However, the main beneficial effects of added enzymes have been due to disruption of intact cell walls and release of entrapped nutrients, rather than reduced viscosity. Therefore, by the use of exogenous enzymes added to corn-SBM diet recent studies indicated that there is room for improvement.

Phytate has the potential to bind with proteins at low and neutral pH which reduces protein digestibility (Ready et al., 1982). Phytate, being a strong acid, can also form salts with important minerals thereby reducing their solubility (Eardman, 1979). The addition of the exogenous enzyme phytase has been used to reduce the pollutant residues, through improving the utilization of phytate-bound minerals in pig and poultry diets and decreasing the use of inorganic sources (Zanini and Sazzard, 1999). It improved the digestibility of essential amino acids, crude protein and nitrogen retention (Zhang et al., 2000). Considering the medicinal attributions of *Curcuma longa*, and the use of exogenous enzymes to improve nutrient digestibility in corn/soy-based diets led us to think to evaluate the effects of turmeric powder and commercial exogenous enzymes on growth performance, carcass characteristics, biochemical parameters, oxidative stress status and immunological status in Japanese quails.

MATERIAL AND METHODS

A total of 150 one week old unsexed Japanese quails (*Coturnix coturnix japonica*) purchased from the agricultural technological center, Faculty of Agriculture, Cairo University, Egypt were assigned randomly to five treatment groups having three replicates (A- C, 10 chicks each). Feed was formulated to meet the nutritional requirements as suggested by the NRC, 1994 as shown in table (1). Birds were kept in battery cages, and the temperature was controlled and gradually reduced from 30 °C to 25 °C on day 28. Birds were maintained on a 24-hour consistent lighting schedule. Proper ventilation was achieved by means of the exhaust fans. Birds in the first group (T1) were given diet with fish meal and served as the control group. The other four treatment groups were given corn- Soya bean meal based diet, but each group with a different supplement. The second group (T2) was given the corn- SBM based diet without any supplements. The third group (T3) was given corn- SBM based diet supplemented with 0.5% turmeric powder. The fourth group T4 was given corn- SBM based diet supplemented with 0.5% turmeric powder and 0.1% Phytase-plus broiler 500. The fifth group T5 was given corn- SBM based diet supplemented with 0.5% turmeric powder and 0.1% Panzyme.

Each 3 kg of the diet contains the following vitamins and minerals:

Vit. A 15 mIU, vit. D3 2 mIU, vit. E 1000mg, vit. K3 1000mg, vit. B1 1000mg, vit. B2 5000mg, vit. B6 1500mg, vit. B12 10mg, biotin 50mg, pantothenic acid 10000mg, nicotinic acid 30000mg, folic acid 1000mg, manganese 60000mg, zinc 50000mg, iron 30000mg, copper 4000mg, iodine 300mg, selenium 100mg, cobalt 100mg, carrier(CaCO₃) to 3kg.

(Golden premix- Selim Pharm Elasher, Egypt).

3Phytase-plus broiler 500, each 1 kg contains phytase enzyme 500.000 I.U, vitamin D3 2.000.000 I.U, wheat bran and calcium carbonate up to 1 kg produced by Bytara for Pharmaceuticals Technology under license of VTR Company Sadat Industrial city, Egypt.

Panzyme (multiple enzymes) each 1 kg contains: xylanase enzyme 15.000.000 I.U, acidic proteinase 540.000 I.U, neutral proteinase 450.000 I.U, cellulase 600.000 I.U. produced by Bytara for Pharmaceuticals Technology under license of VTR Company Sadat Industrial city, Egypt.

Performance parameter

Body weight of birds per replicate was recorded on individual basis at weekly intervals. The cumulative feed consumption per replicate was also recorded on weekly basis. Feed efficiency ratio per replicate was worked out at weekly intervals by taking into consideration the weekly body weight gain and the feed consumption of respective replicate. At the end of the feeding trial, six birds per treatment (two birds per replicate) were selected at random and utilized (Esen et al., 2006) for the carcass evaluation study. Each bird was weighed immediately before slaughtering then allowed bleeding. Blood samples were collected, to separate serum samples for measuring the tested parameters. The shanks were cut off at the hock joint. The feathers were removed completely by hand picking leaving the skin intact. Thereafter, the abdominal cavity was opened to expose the visceral organs, and the carcass characteristics were evaluated. The carcass and edible visceral organs were taken; cleaned and weighed (heart, liver, gizzard), and expressed as a percentage of live body weight (Esen et al., 2006).

Serum biochemical parameters

Serum samples were collected at the end of the experiment (4 weeks). Collected serum samples were subjected to measurement of total proteins and albumin according to (Henry 1964) using reagent kits purchased from Spinreact Company (Spain). Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) were measured according to Reitman and Frankel (1957) using reagent kits purchased from Randox Company (United Kingdom). Lipid peroxidation product (malondialdehyde) was measured spectrophotometrically according to the method described by Placer et al., (1966). Glutathione level was assayed colorimetrically according to the method described by Sedlak and Lindsay (1968). Total Superoxide Dismutase (SOD) activity was assayed according to Spitz and Oberley (1989); one unit of SOD is the amount of enzyme required to inhibit the rate of formazan dye formation by 50%. Catalase (CAT) was estimated by following the decomposition of H₂O₂ to H₂O and O₂ at 240 nm for 2 min (Cohen et al., 1970). The enzyme activity was

expressed as units/mg protein; one unit of CAT is the amount of enzyme catalyzing the decomposition of 1 mmol H₂O₂ per min at 25 °C and pH 7.0. Glutathione peroxidase (GPx, GSH-Px) was assayed by the method of Lawrence and Burk (1976) using hydrogen peroxide as substrate. The activity was expressed as U/mg protein; one unit of GSH-Px is the amount of GSH-Px required to oxidise 1 mmole NADPH/min. all oxidative stress parameters were measured using reagent kits purchased from Bio diagnostic company (Egypt) as per manufacture instructions.

Table 1. Ingredient and composition of experimental diets

Ingredients %	T1	T2	T3	T4	T5
Ground yellow corn	57.36	56.81	56.81	56.81	56.81
Soya bean meal (45%) ¹	33.10	33.20	33.20	33.20	33.20
Fish meal (60.05) ¹	2.00	-	-	-	-
Corn gluten (62) ¹	4.95	6.90	6.90	6.90	6.90
Dicalcium phosphate	0.56	0.86	0.86	0.86	0.86
Limestone	1.25	1.35	1.35	1.35	1.35
DL-Methionine	0.09	0.09	0.09	0.09	0.09
Lysine	0.12	0.19	0.19	0.19	0.19
Iodized sodium chloride	0.30	0.30	0.30	0.30	0.30
Minerals and vitamins premix ²	0.30	0.30	0.30	0.30	0.30
Turmeric powder	-	-	0.5	0.5	0.5
Phytase-plus broiler 500 ³	-	-	-	0.1	-
Pan Zyme ⁴	-	-	-	-	0.1
Calculated composition					
Crude protein %	24.01	24.00	24.00	24.00	24.00
ME (kcal/kg)	2900.23	2900.18	2900.18	2900.18	2900.18
Calorie/protein ratio (C/P)	120.82	120.80	120.80	120.80	120.80
Calcium %	0.8	0.8	0.8	0.8	0.8
Phosphorus %	0.3	0.3	0.3	0.3	0.3

¹Determined according to AOAC, 1995.

Immune parameters

Capture and biotinylated antibodies to poultry IL-6, IL-10, IL-12 and Tumour Necrosis Factor (TNF)- α were purchased from BD Pharmingen (San Diego, CA, USA) IL-6, IL-10, IL12 and TNF- α were estimated according to the manufacturer's protocols (Becton Dickinson, San Diego, CA, USA). Briefly, 50 ml/well of capture antibodies (1 mg/ml) were adsorbed overnight on polystyrene Microtiter plates in binding buffer (0.1 MNa₂PO₄, pH 9.0) at 4°C. The supernatants were added followed by biotinylated anti-cytokine-detecting antibodies (0.5 mg/ml). Later, streptavidin-HRP (50 ml/well) followed by orthophenylenediamine was added and the plates were read at 492 nm. The usual steps of blocking, incubation and washing were followed at each step. Titration curves of recombinant IL-6, IL-10, IL12 and TNF- α were used as standards for calculating cytokine concentrations in the samples tested.

Statistical analysis

The obtained data were subjected to one-way ANOVA. Differences between means were tested at the 5% probability level using Duncan's LSD test. All the statistical analyses were done using SPSS program version 16 (SPSS, Richmond, VA, USA) as described by Dytham (2011).

RESULTS

There were no significant differences among treatments in final body weight, body weight gain, and survival percentage (Table 2). There was a trend of higher values in final body weight and body weight gain in T5 compared to all other treatments. There was a significant reduction in feed consumed in T5 compared to T1 (control group) and significant improvement in feed efficiency ratio in T5 compared to T1 and T2.

There was a significant increase in carcass weight in T1 compared to T4 (Table 3). There were no significant differences among treatments in dressing percentage and total edible organs.

A significant increase in total serum protein level was recorded. In contrast, AST and ALT were significantly decreased by addition of turmeric and enzymes as diet supplement (T3, T4 and T5) (Table 4). In the present study, serum concentration of MDA, an indicator of lipid peroxidation, decreased when turmeric was supplemented to diet of quail and the group supplemented with turmeric and Panzyme T5 then group supplemented with turmeric and phytase (Table 5).

In the present study, serum concentration of MDA, decreased when turmeric was supplemented to diet of quail and the group supplemented with turmeric and Panzyme T5 then group supplemented with turmeric and phytase (Table 5). There was a significant increase in the level of SOD and CAT in group T5 which contain turmeric and Panzyme then

followed by significant increase in groups T3 which was supplemented with turmeric 0.5% and T4 which supplemented with turmeric and phytase in compare to control group (Table 5). In our study we reported a significant increase in the levels of both GSH and GPx in groups T3 which was supplemented with turmeric 0.5% and group T5 which was fed turmeric and Panzyme then followed by significant increase in group T4 which fed turmeric and Phytase (Table 5).

In our study, we recorded a significant decrease in the level of TNF in all groups supplemented with turmeric and enzymes T3, T4 and T5 compared with control group T1 (Table 6).

IL-6 showed significant decrease in group T5 which was supplemented with turmeric and Panzyme, followed by significant decrease in groups T3 and T4 which were supplemented with turmeric alone and turmeric with Phytase compared to the control group. IL-10 showed significant decrease in all groups treated with turmeric either alone or with the addition of the exogenous enzymes T3, T4 and T5 compared to the control group.

In case of IL-12, we reported a significant decrease in both groups treated with turmeric and the enzymes T4 and T5 then significant decrease T2 which fed SBM based diet and T3 supplemented with turmeric alone compared to control group (Table 6).

Table 2. Growth performance of Japanese quail fed different experimental diets*

Parameters	T1	T2	T3	T4	T5
Initial weight (g/bird)	21.99±1.05	20.51±0.91	19.99±0.38	21.06±0.21	20.76±0.76
Final weight (g/bird)	197.79±0.99	193.70±4.00	189.76±4.56	193.49±4.45	200.94±8.00
Body weight gain (g/bird/day)	6.28±0.07	6.18±0.16	6.06±0.15	6.16±0.16	6.43±0.29
Feed consumed (g/bird/day)	23.71±0.45 ^a	23.27±1.27 ^{ab}	22.00±0.72 ^{ab}	21.09±0.37 ^{ab}	20.79±0.87 ^b
Feed Efficiency ratio (FER)	0.27±0.01 ^b	0.27±0.01 ^b	0.28±0.01 ^{ab}	0.29±0.00 ^{ab}	0.31±0.01 ^a
Survival rate %	83.33±3.33	90.30±5.78	75.93±3.03	80.00±5.77	87.27±6.38

Values are presented as means ± SE. ; a-b means in the same row with different superscripts are significantly different (p≤ 0.05).

*T1= control with fish meal included, T2= corn –SBM based diet, T3= corn –SBM based diet supplemented with 0.5% turmeric, T4= corn –SBM based diet supplemented with 0.5% turmeric and 0.1% Phytase-plus broiler 500, T5= corn –SBM based diet supplemented with 0.5% turmeric and 0.1% Panzyme.

Table 3. Carcass characteristics of Japanese quail fed different experimental diets after 4 weeks

Parameters	T1	T2	T3	T4	T5
Carcass weight, g	150.72±7.01 ^a	132.15±9.66 ^{ab}	130.48±4.16 ^{ab}	118.94±5.98 ^b	140.84±7.63 ^{ab}
Dressing percentage%	72.99±0.87	70.36±1.39	71.28±0.84	70.51±0.97	71.98±0.73
Total edible organs %	5.06±0.14	5.06±0.10	5.18±0.09	5.35±0.17	5.34±0.11

Values are presented as means ± SE; a-b means in the same row with different superscripts are significantly different (p≤ 0.05).

Table 4. Serum biochemical analysis of Japanese quail fed different experimental diets after 4 weeks

Parameters	T1	T2	T3	T4	T5
ALT (U/L)	4.53±0.48 ^a	4.53±0.21 ^a	3.05±0.05 ^b	3.40±0.24 ^b	2.88±0.95 ^b
AST(U/L)	50.00±1.95 ^a	50.75±2.06 ^a	36.90±1.23 ^b	37.25±1.38 ^b	33.50±2.18 ^b
Total Protein (gm/dl)	3.22±0.27 ^b	3.40±0.32 ^b	5.15±0.35 ^a	5.20±0.31 ^a	5.05±0.52 ^a
Albumin (gm/dl)	1.18±0.36 ^b	1.69±0.30 ^b	2.82±9.25 ^a	2.86±0.42 ^a	2.93±0.39 ^a

Values are presented as means ± SE; a-b means in the same row with different superscripts are significantly different (p≤ 0.05).

Table 5. Oxidative stress parameters of Japanese quail fed different experimental diets after 4 weeks

Parameters	T1	T2	T3	T4	T5
MDA (µg/ml)	0.68±0.07 ^a	0.66±0.10 ^a	0.39±0.44 ^{bc}	0.55±0.02 ^{ab}	0.32±0.02 ^c
SOD (U/ml)	160.75±4.15 ^c	156.92±7.19 ^c	194.25±2.28 ^b	191.25±4.92 ^b	211.75±3.84 ^a
CAT(U/ml)	58.75±4.26 ^c	59.75±2.32 ^c	81.25±4.26 ^b	82.00±3.36 ^{ab}	93.00±3.70 ^a
GSH (U/ml)	2.07±0.33 ^c	2.94±0.49 ^c	5.70±0.26 ^a	4.15±0.51 ^b	6.55±0.22 ^a
GSH-Px(U/ml)	23.75±2.39 ^c	24.75±2.21 ^c	41.75±2.32 ^a	33.50±1.71 ^b	47.00±3.24 ^a

Table 6. Cytokines measured in Japanese quail fed different experimental diets after 4 weeks

Parameters	T1	T2	T3	T4	T5
TNFα (pg/ml)	47.42±4.49 ^a	43.58±9.63 ^a	27.68±2.54 ^b	17.88±1.51 ^b	15.55±1.68 ^b
IL6 (pg/ml)	248.40±17.89 ^a	240.68±15.75 ^a	170.78±10.83 ^b	210.25±13.60 ^{ab}	104.75±5.09 ^c
IL10 (pg/ml)	37.70±3.77 ^a	45.18±3.22 ^a	23.15±4.02 ^b	14.45±1.14 ^b	13.98±2.52 ^b
IL12 (pg/ml)	86.98±2.54 ^a	67.83±7.44 ^b	58.40±9.41 ^b	37.30±2.12 ^c	27.90±2.01 ^c

Values are presented as means ± SE; a-b means in the same row with different superscripts are significantly different (p≤ 0.05).

DISCUSSION

The non-significant difference in performance of birds on corn-SBM based diet T2 indicated that the bioavailability of nutrients of this diet is as compared to the control T1, with fish meal included. This is in accordance with Okan and Ogun (1986) who compared the biological value of soybean meal and fish meal for broiler starter rations. They concluded that soybean meal supplemented with methionine can replace fish meal in starter ration. Haq et al., (1986) concluded that there was no significant differences among groups received a control diet containing 12% fish meal and 3 experimental diets replacing fish meal with 4, 8 and 12% soybean meal in chicken in weight gain, feed intake and feed efficiency. On the other hand Ali et al. (1993) indicated that broilers fed corn-soybean meal based rations gained significantly higher weights, showed better feed conversion ratio, higher dressing percentage with better carcass composition, lower mortality, and higher net profits as compared to those fed on fish meal commercial ration. These differences may be due to variation in diet ingredients used, percentage and quality of fish meal replaced.

Turmeric administration in quail T3 was actually hoped to be able to enhance the metabolism that was found in Al-Sultan and Gameel, 2004 study that found improvement in feed efficiency and growth performance. However, our finding showed that growth performance and feed utilization were not affected by turmeric supplementation in quail (Table 2). This is in accordance with Rahmatnejad et al. (2009) who showed that the diets containing 0.1% turmeric supplementation had no significant effect on broilers performance. These results were consistent with Namagirilakshmi (2005); Emadi and Kermanshahi (2006) and Mehala and Moorthy (2008), who reported that supplementation of turmeric powder in diet had no significant effect on weight gain and feed conversion ratio. On the other hand, Kumar et al., (2005) observed a positive effect of turmeric powder on broiler performance. Isroli et al. (2011) concluded that the insignificant effect of turmeric on feed conversion ratio of broilers was associated with the final body weight and total feed intake that were apparently not affected by turmeric administration, and the hypolipidemic effect of turmeric might hamper its potential to promote the growth of broiler as result that turmeric could not improve the feed efficiency of broilers.

Addition of phytic enzyme to the turmeric supplemented corn-SBM based diet T4 had no effect on growth performance as compared to the control diet T1. Our findings are in agreement with other reports that enzyme supplementation of corn-SBM based diets did not alter average daily gain and feed conversion (West et al., 2007; Aftab, 2009; Madrid et al., 2010; Baurhoo et al., 2011). In contrast in the present study the replacement of fish meal with SBM and turmeric and Panzyme supplementation improved the nutritional value of diet T5, as birds on this diet though non-significantly showed better overall performance, significant decreased feed consumption and significantly improved feed efficiency as compared to the control T1 were observed. This decreased feed consumption in accordance with Baurhoo et al., (2011) finding, the only observed positive effect of enzyme supplementations on broiler performance was reduced consumption of the enzyme supplemented diet compared with the control diet. Interestingly, this reduction was not accompanied by depressed growth. Exogenous enzymes are known to cause enzymatic breakdown of Non starch Polysaccharides (NSP) and release of trapped nutrients (Bedford and Schulze, 1998), leading to improvements in efficiency of feed utilization. It was consistent with several reports that indicated substantial body weight and feed conversion improvements because of enzyme supplementation of corn- SBM based diets (Kocher et al., 2003; Owens et al., 2008). All these findings indicated inconsistent effects of enzyme supplementation of corn- SBM based diets on broiler performance. This could be attributed to differences in the enzyme products studied, diet formulations, animal characteristics, or management practices.

There were no significant differences in carcass characteristics among different groups. This is in agreement with some studies that did not find any significant effect of turmeric supplementation at the rate of 1.0 g/kg (Rahmatnejad et al., 2009) or 2.0 g/kg (El-Hakim et al., 2009) on carcass production. Also Lal et al., (1999) observed no improvement in liver and gizzard by application of Turmeric (*Curcuma longa*). On the other hand, Hussein (2013) found significant higher values of liver and gizzard were obtained from birds on 7 g Turmeric Powder (TP)/ kg diet. However, birds on 5gTP/ kg diet and 9 g TP/ kg diet did not differ significantly.

Our findings are in harmony with studies which reported that carcass characteristics in term of dressing percentage and edible parts were not significantly affected by enzyme supplementation in Japanese quail diet (Arumbackam et al., 2004 and Zahran et al., 2012).

A significant increase in total serum protein level was recorded. In contrast, AST and ALT were significantly decreased by addition of turmeric and enzymes as diet supplement (T3, T4 and T5) (Table 4). These results were in accordance with the study on broiler given a diet mixed with turmeric for 45 days (Kumari et al., 2007).

Enrichment of diets with antioxidant compounds is necessary to improve the oxidative stability, and hence, to increase the quality of product when birds are kept under high ambient temperature (Sahin and Kucuk, 2003). In the present study, serum concentration of MDA, an indicator of lipid peroxidation, decreased when turmeric was supplemented to diet of quail and the group supplemented with turmeric and Panzyme T5 then group supplemented with turmeric and phytase (Table 5). These data are in agreement with previous studies done by various antioxidants supplementation (Ahmadi, 2010; Sahin et al., 2010 and Sahin et al., 2012). Several studies have shown that curcumin has a strong capability for scavenging superoxide radicals, hydrogen peroxide and nitric oxide from activated macrophages, reducing iron complex and inhibiting lipid peroxidation (Sankar et al., 2012; Bayomi et al., 2012; Cai et al., 2012). Similar to our results, Ahmadi (2010) reported that the MDA levels decreased significantly when turmeric was added into the diet at the rate of 0.3 g/kg in broilers. In vitro and in vivo studies have shown that Curcumin activates expression of some intracellular antioxidative defense systems for free radicals (Calabrese et al., 2008; Ho et al., 2011; Mancuso et al., 2012).

Curcumin, one of the most active ingredients in turmeric and responsible for the biological activity, is known to augment antioxidant status especially through SOD which could be due to the increased expression of SOD gene in the chickens fed turmeric (Cheng et al., 2005). Jena et al. (2012) also found that curcumin differentially modulates the expression of SOD in brain cortex and cerebellum of rats with hypothyroidism. Several studies have been reported that broilers fed the combination of Aflatoxin B1 (AFB1) and turmeric had greater activities of SOD, CAT and GPx compared with those fed only AFB1 (Gowda et al., 2009; Yarru et al., 2009). Similarly, Ahmadi (2010) reported that CAT and SOD activities increased when basal diet of broilers was supplemented with 0.3 and 0.6 g/kg turmeric powder.

In our study we reported a significant increase in the levels of both GSH and GPx in group T3 which was supplemented with turmeric 0.5% and group T5 which was fed turmeric and Panzyme then followed by significant increase in group T4 which fed turmeric and Phytase (Table 5). Dietary supplementation of curcumin increased the activities of GPx, glutathione reductase, glucose-6-phosphate dehydrogenase and CAT to 189%, 179%, 189%, and 181% in liver, respectively as compared with corresponding normal diet fed control in mice (Iqbal et al., 2003).

Sharma et al., (2005) reported that expression of the detoxification enzymes glutathione-S-transferase, glutathione reductase, and the antioxidants heme oxygenase-1 (HO-1) and CAT in liver tissues were increased by curcumin supplementation in mice.

Curcumin's antioxidant properties might not be only due to its chemical nature as a free radical scavenger, but also due to its ability to induce GSH linked defense mechanisms against oxidative stress as well as increases in the activity of γ -glutamyl cysteinyl synthase, the rate limiting step in glutathione synthesis (Piper et al., 1998). Moreover, induces de novo synthesis of GSH by stimulating the activity and gene expression of glutamate-cysteine ligase (Zheng et al., 2007).

TNF- α is one of the most versatile pleiotropic cytokine that induces growth stimulation as well as inhibition by self-regulatory mechanisms of its own and plays a crucial role as an immunostimulant and mediator of host resistance to many infectious agents (Ganesh and Bharat, 2007) In our study, we recorded a significant decrease in all groups supplemented with turmeric and enzymes (T3, T4 and T5) compared with control group (T1) (Table 6). This decrease may be attributed to the effect of curcumin. Curcumin can modulate the expression of both TNF and TNF-induced signaling and can also inhibit LPS-induced expression of TNF- α (Bielak et al., 2000; Lantz et al., 2005; Lee et al., 2003). It has also been reported to inhibit lipopolysaccharides (LPS) or phorbol ester, 4 β phorbol 12 β -myristate-13 α acetate PMA-induced TNF- α in dendritic cells, macrophages, monocytes, alveolar macrophages, and endothelial and bone marrow cells (Moon et al., 2006). An almost identical observation has been made in rats, where curcumin treatment attenuated TNF- α in sodium taurocholate-induced acute pancreatitis (Gulcubuk et al., 2006). This effect is apparently mediated by curcumin's ability to prevent TNF, IL-1, and LPS-induced expression of MCP-1 and IP-10 mRNA, and it is completely reversible within 24 h after removing curcumin from the cell culture medium. The inhibition of AP-1 and NF- κ B activation are responsible for this activity of Curcumin (Xu et al., 1998; Ganesh and Bharat, 2007). In another study, curcumin treatment blocked the expression of TNF- α mRNA in the rat model of hemorrhage and resuscitation (Gaddipati et al., 2003).

In another set of experiments, three major active principles namely, 1,7-bis (4-hydroxyphenyl)-1,4,6-heptatriene-3-one, procurcumenol, and epiprocurcumenol isolated from the crude methanol extract of the rhizomes of *Curcuma zedoaria* were reported to suppress the production of TNF- α in LPS-stimulated macrophages (Jang et al., 2001).

In the present study, IL-6 showed significant decrease in group T5 which was supplemented with turmeric and Panzyme, followed by significant decrease in groups T3 and T4 which were supplemented with turmeric alone and turmeric with Phytase compared to the control group. On contrast to our finding Grace et al., 2010 reported that the addition of the polar fractions of *curcuma longa* hot water extracts to human Peripheral Blood Mononuclear Cells (PBMC) resulted in significant increase in the production of the level of TNF- α and IL-6 at 400 and 800 μ g/ml of *curcuma longa* extract. IL-10 showed significant decrease in all groups treated with turmeric either alone or with the addition of the exogenous enzymes (T3, T4 and T5) compared to the control group. Different results may be attributed to route and dose of application (in vivo versus in vitro). In case of IL-12, we reported a significant decrease in both groups treated with turmeric and the enzymes (T4 and T5) then significant decrease in groups T2 which was fed SBM based diet and group T3 which was supplemented with turmeric alone compared to control group (Table 6). Our results are in accordance with studies of Xu et al. (1997) and Lee et al., (2003). Curcumin can also alter the expression and activity of a variety of interleukins, especially IL-1, IL-2, IL-6, IL-8, IL-10, and IL-12 and thus can influence functions of different cells in a variety of ways (Ganesh and Bharat, 2007). For example, treatment of PMBCs with curcumin inhibited LPS-induced IL-1 β , IL-6, and TNF- α . Similarly, in rabbit experiments, curcumin reduced LPS-induced fever by attenuating the expression of IL-1 β , IL-6, and TNF- α in the serum (Lee et al., 2003). This action of curcumin was mediated by suppression of NF- κ B activation and downstream events that blocked these cytokines (Lee et al., 2003). Curcumin reportedly reduced PMA or LPS-stimulated production of IL-1 and IL-8 in human peripheral blood monocytes and alveolar macrophages in a concentration and time dependent manner (Chan, 1995). A similar effect was observed for IL-2 production in PHA-stimulated human PMBCs (Yadav et al., 2005).

Curcumin has the ability to prevent TNF, IL-1, and LPS-induced expression of MCP-1 and IP-10 mRNA, and it is completely reversible within 24 h after removing curcumin from the cell culture medium. The inhibition of AP-1 and NF- κ B activation are responsible for this activity of Curcumin (Xu et al., 1997; Xu et al., 1998). Curcumin exerts anti-inflammatory activity through down regulation of cyclooxygenase-2 (COX-2) and inducible Nitric Oxide Synthetase (iNOS), and reduction of tumor necrosis factor-Alpha (TNF- α -induced expression by suppressing nuclear factor kappa-B (NF- κ B) activation (Mokhtar et al., 2010). On contrary of our results, Chinampudur et al. (2013) recorded significant increase in the levels of IL-2, IL-6, IL-10, IL-12 as result of addition of aqueous based extract of *C. longa* to mouse splenocytes and mouse macrophage.

CONCLUSION

Corn-SBM based diet can replace fish meal based diet to growing quails. Supplementation of turmeric alone although it did not affect the growth performance, it relieved stress and reduced the proinflammatory cytokines. Addition of turmeric together with Panzyme improved the corn-SBM based diet by improving feed efficiency and anti-inflammatory activity.

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