

Effect of Turmeric Rhizome Powder on Immunity Responses of Broiler Chickens

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Abstract: In order to study the effect of Turmeric Rhizome Powder (TRP) on immunity responses in broiler chickens, in a completely randomised design, an experiment with 200 day-old Ross male broiler chickens from 0-49 days with 4 treatments and 5 replicates of 10 birds each was conducted. A corn-soybean meal based diet containing levels of TRP (0.00, 0.25, 0.50 and 0.75%) was used. For measuring immunity responses (white blood cell, immunoglobulins A, G and M), at 14 days of age, one bird from each replicate of treatments was challenged with 0.2 mL Sheep Red Blood Cell (SRBC, 5%) and blood samples were collected at 21 and 42 days of age. Inclusion of TRP significantly ($p < 0.05$) increased immunoglobulins IgA, IgM and IgG (IgY). TRP inclusion into the diets also significantly decreased the ratio of monocytes at 42 days of age ($p < 0.05$). Under the condition of this study, it was concluded that TRP might have some positive effects on immunity response and may be used as immune system fortification in broiler chickens.

Key words: Turmeric, immunity response, immunoglobulin, broiler chickens

INTRODUCTION

Curcumin is the major yellow pigment extracted from turmeric, a commonly used spice, derived from the rhizome of the herb *Curcuma longa*. In the Indian subcontinent and Southeast Asia, turmeric has traditionally been used as a treatment for inflammation, skin wounds and tumors. Clinical activity of curcumin has yet to be confirmed, however, in preclinical animal models, curcumin has shown cancer chemo preventive, antineoplastic and anti-inflammatory properties (Kelloff *et al.*, 1996). Curcumin acts as a scavenger of oxygen species, such as hydroxyl radical, superoxide anion and singlet oxygen (Kunchandy and Roa, 1990; Reddy and Lokesh, 1994; Sharma, 1976; Subramanian *et al.*, 1994; Tonnes and Greenhill, 1992). It interferes with lipid peroxidation (Donatus *et al.*, 1990; Makhopadhyay *et al.*, 1982; Sharma *et al.*, 1972). Certain curcumin metabolites, such as tetrahydrocurcumin, possess anti-inflammatory (Makhopadhyay *et al.*, 1982) and antioxidant activities (Osawa *et al.*, 1995; Sugiyama *et al.*, 1996) similar to those of their metabolic progenitor. Oxidative Stress (OS) may play a role in viral replication, decreased immune cell proliferation and increased sensitivity to drug toxicity (Favier *et al.*, 1991; Jaraga *et al.*, 2002). During infection, free radical damage may be produced, not only by a direct production of oxygen radicals by phagocytes, but also by a Tumor Necrosis Factor (TNF)-mediated generation in target cells. Antioxidants have demonstrated protective capacity for

TNF cytotoxicity (Ferlat and Favier, 1993). A redox imbalance caused by an over production of pro-oxidants or a decrease in antioxidants seems to play an important role in the normal physiological function. Following activation, lymphocytes production increased levels of OS. Lymphocytes from such individuals were more prone to undergo apoptosis or cell death *in vitro* (Buttke and Sandstorm, 1995). Antioxidant supplementation significantly improves some measures of oxidative defence (Batterham *et al.*, 2001). Curcumin enhances the immune response of rats (Chue *et al.*, 2003). Curcumin with its potential properties is a favourite additive. Using curcumin with wide ranges of positive activities might be a suitable additive in poultry that may alternate means of oxidative stresses and improves immunity responses in broiler chickens.

MATERIALS AND METHODS

Diets: Four dietary treatments with levels of 0.00, 0.25, 0.50 and 0.75 % TRP in starter (0-21), grower (21-42) and finisher diets (42-49 days of age) were evaluated in broiler chickens (Table 1). All diets met the National Research Council (NRC, 1994) recommendations.

Birds and housing: This study was conducted at poultry research station of Ferdowsi University of Mashhad, Iran. Two hundred-day-old Ross male broiler chickens were used for this experiment. Birds were housed randomly in

cages with 1m*1.2m floor space. Each experimental diet was tested with 5 replicate cages of ten chickens. Birds were maintained under continuous light and the environmental temperature that initially established on 31 C and then gradually reduced to 20 C by week 7. Feed and water were provided *ad libitum* during starter, grower and finisher periods.

Sample collection: At 14 days of age, one bird from each replicate of treatments was injected with 0.2 mL of SRBC (sheep red blood cell, 5%). At 21 and 42 days of age, one bird from each replicate of treatments was randomly selected and their blood samples were collected. For measuring the concentration of blood immunity parameters (white blood cell and its constituents, eosinophile, lymphocyte, monocyte and neutrophile and immunoglobulins, IgA, IgG and IgM), serum blood samples were separated. The total concentration of immunoglobulins in the sera were determined by immunodiffusion assay and protein electrophoresis.

Statistical analysis: Data were analyzed based on a general linear model procedure of SAS (1993) and treatment means when significant ($p < 0.05$), were compared using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

White blood cell and its constituents (lymphocyte, monocyte, neutrophil, eosinophil) in chickens (Table 2) were not significantly changed at 21 days of age but inclusion of TRP significantly decreased number of monocytes at 42 days of age ($p < 0.05$). The results of Serum immunoglobulins in chickens (Table 3) were also affected by inclusion of TRP into the diets. IgA and IgM at 21 days of age and IgG at 21 and 42 days of age significantly increased ($p < 0.05$) in chickens fed TRP levels. Curcumin, increased the number of T-helper cells in animals that fed it over a period of 3-5 weeks (Yasni *et al.*, 1993). Phagocytosis is the first step of the macrophage response to invading microorganism and

Table 1: Composition of experimental diets of broiler chickens during 0-49 days of age

Ingredients (%)	-----Starter (0-21 days)-----				-----Grower (21-42 days)-----				-----Finisher (42-49 days)-----			
Corn	56.77	56.77	56.77	56.77	63.81	63.81	63.81	63.81	71.52	71.52	71.52	71.52
Soybean meal	32.06	32.06	32.06	32.06	30.13	30.13	30.13	30.13	24.33	24.33	24.33	24.33
Fish meal	4.47	4.47	4.47	4.47	-	-	-	-	-	-	-	-
Turmeric (TRP) ¹	0.00	0.25	0.50	0.75	0.00	0.25	0.50	0.75	0.00	0.25	0.50	0.75
Wheat bran	0.75	0.50	0.25	0.00	0.75	0.50	0.25	0.00	0.75	0.50	0.25	0.00
Dicalcium phosphate	0.93	0.93	0.93	0.93	1.03	1.03	1.03	1.03	0.81	0.81	0.81	0.81
Limestone	1.09	1.09	1.09	1.09	1.24	1.24	1.24	1.24	1.16	1.16	1.16	1.16
Vit. Min. Premix ²	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.34	0.34	0.34	0.34	0.31	0.31	0.31	0.31	0.23	0.23	0.23	0.23
Veg. oil	3.00	3.00	3.00	3.00	2.20	2.20	2.20	2.20	0.69	0.69	0.69	0.69
DL- Methionine	0.09	0.09	0.09	0.09	0.03	0.03	0.03	0.03	-	-	-	-
Calculated analysis												
ME (kcal kg ⁻¹)	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900	2900
CP (%)	20.84	20.84	20.84	20.84	18.12	18.12	18.12	18.12	16.31	16.31	16.31	16.31
Ca (%)	0.91	0.91	0.91	0.91	0.82	0.82	0.82	0.82	0.72	0.72	0.72	0.72
Avail. P (%)	0.41	0.41	0.41	0.41	0.32	0.32	0.32	0.32	0.27	0.27	0.27	0.27
Na. (%)	0.18	0.18	0.18	0.18	0.14	0.14	0.14	0.14	0.11	0.11	0.11	0.11
Arg. (%)	1.40	1.40	1.40	1.40	1.20	1.20	1.20	1.20	1.05	1.05	1.05	1.05
Lys. (%)	1.24	1.24	1.24	1.24	0.98	0.98	0.98	0.98	0.85	0.85	0.85	0.85
Met. + Cys. (%)	0.82	0.82	0.82	0.82	0.65	0.65	0.65	0.65	0.57	0.57	0.57	0.57

¹Turmeric rhizome powder ²Supplied per kilogram of diet: vitamin A, 10000 IU; vitamin D₃, 9790 IU; vitamin E, 121 IU; B₁₂, 20 µg; riboflavin, 4.4 mg; calcium pantothenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin, 30 µg; thiamine, 4 mg; zinc sulphate, 60 mg; manganese oxide, 60 mg

Table 2: Effect of turmeric rhizome powder on blood white blood cells and its constituents in broilers

TRP (%)	Blood tests									
	White blood cell (mL)		Eosinophile (%)		Monocytes (%)		Lymphocytes (%)		Neutrophile (%)	
	21 d	42 d	21 d	42 d	21 d	42 d	21 d	42 d	21 d	42 d
0.00	22480	25520	1.2	1.2	4.2	4.0 ^a	71.4	71.8	23.2	23.0
0.25	23340	27440	1.2	1.4	3.4	3.0 ^a	70.4	70.8	25.0	24.8
0.50	22660	26960	1.0	1.0	3.0	1.4 ^b	71.2	71.6	24.8	26.0
0.75	21500	27180	1.0	0.8	4.0	1.0 ^b	71.0	71.6	24.0	26.6
p values	0.6318	0.1282	0.9523	0.6206	0.75	0.0025	0.9425	0.9035	0.6048	0.0635
± SEM	553.3	324.15	0.19	0.185	0.48	0.28	0.67	0.57	0.57	0.51

^{a,b} Means in each column with different superscripts are significantly different ($p < 0.05$). TRP, Turmeric Rhizome Powder

Table 3: Effect of turmeric rhizome powder on blood immunoglobulins in broilers

TRP (%)	Immunoglobulins					
	IgA (mg dL ⁻¹)		IgG (mg dL ⁻¹)		IgM (mg dL ⁻¹)	
	21 d	42 d	21 d	42 d	21 d	42 d
0.00	40.0 ^b	42.0	423.0 ^b	461.0 ^c	140.0 ^c	140.8
0.25	56.2 ^a	41.0	683.0 ^a	472.0 ^c	190.0 ^a	141.2
0.50	61.2 ^a	41.8	800.0 ^a	614.0 ^b	174.0 ^b	208.0
0.75	42.8 ^b	46.6	507.0 ^b	740.0 ^a	143.6 ^c	187.2
p values	0.0001	0.1209	0.0001	0.001	0.0001	0.3296
± SEM	1.47	0.94	31.97	14.66	2.88	16.98

^{a,b,c} Means in each column with different superscripts are significantly different (p<0.05). TRP, Turmeric Rhizome Powder

curcumin elevated the innate immune response in the body by phagocytosis. It is known that macrophages produce some Reactive Oxygen Species (ROS) during phagocytic process. Accordingly, oxygen radical production indicates the actual killing capacity of the macrophages. It is reported that a defect in production of oxygen radicals leads to an incomplete eradication of bacteria and a higher risk of infection in patients (Cumutte *et al.*, 1974). It is suggested that hydrogen peroxide (H₂O₂) and Nitric Oxide (NO) by macrophages may contribute to inflammation and tissue injury (Loskin and Laskin, 1996; Nathan *et al.*, 1980). NO and Tumor Necrosis Factor (TNF) are key molecules thorough which macrophage effects or interacts with other immune cells. Various cytokines such as TNF are produced by macrophages. TNF plays a major role in the defence against interacellular pathogens. It stimulates T-lymphocyte activation and granuloma formation (Abe, 1999). On the other hand, it was reported that curcumin had a variety of pharmacological effects such as anti-inflammatory activities and an inhibitory effects on the production of TNF by macrophages (Abe, 1999). Therefore, curcumin may kill microorganisms in innate and adaptive immune responses. Oxidative stresses changed cytokine profile and multiplication and inclusion of turmeric alleviate this effect, therefore, immunity responses increased. Turmeric also increased lymphocyte multiplication (Surh, 1999). Knowledge gained in this study found direct application of TRP in the management of poultry health, promising to yield better immune response so better disease resistance and health would be possible. Under the conditions of this study, it was concluded that turmeric rhizome powder might have some positive effects on immunity response and may be used directly in the diet as immune system fortification in broiler chickens.

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REFERENCES

- Abe, Y., H. Hashimotons and T. Horiet, 1999. Curcumin inhibition of inflammatory cytokine production by human peripheral blood monocytes and alveolar macrophages. *Pharmacol. Res.*, 34: 41-47.
- Batterham, M., J. Gold, D. Naidoe, M. Ewing and C.A. Oliver, 2001. Preliminary open lable dose comparison using an antioxidant regimen to determine the effect on viral load antioxidative stress in men with HIV/AIDS. *Eur. J. Clin. Nutr.*, 55: 107-114.
- Buttke, T.M. and P.A. Sandstrom, 1995. Redox regulation of programmed cell death in lymphocytes. *Free Radic. Res.*, 22: 389-397.
- Chue, S.C., M.K. Lai, I.S. Liu, F.C. Teng and J. Chen, 2003. Curcumin enhances the immunosuppressive activity of cyclosporine in rat cardiac allografts and in mixed lymphocytes reactions. *Transplant Proc.*, 35: 1603-1605.
- Cumutte, J.T., D.M. Whitten and B.M. Babior, 1974. Defective superoxide production by granulocytes from patients with chronic granulomatous disease. *N. Engl. J. Med.*, 290: 593-597.
- Donatus, I.A., J. Sardjoko and N.P. Vermeulen, 1990. Cytotoxic and cytoprotective activities of curcumin: Effects on paracetomal-induced cytotoxicity, lipid peroxidation and glutathione depletion in rat hepatocytes. *Biochem. Pharmacol.*, 39: 1869-1875.
- Duncan, D.B., 1955. Multiple range and F-Tests. *Biometrics*, 11: 1-42.
- Favier, A., C. Sappey, P. Leclerc, P. Faure and M. Micoud, 1991. Antioxidant status and lipid peroxidation in patients infected with HIV. *Chem. Biol. Interact.*, 91: 165-180.
- Ferlat, S. and A. Favier, 1993. Tumor necrosis factor and oxygen free radicals: Potential effects for immunity. *C. R. Sean. Soc. Biol. Fil.*, 187: 296-307.
- Jaraga, P., B. Jaruga, D. Gackowski, A. Olczak, A. Halotaw, M. Pawlowska and R. Olinski, 2002. Supplementation with antioxidant vitamins prevents oxidative modification of DNA in lymphocytes of HIV-infected patients. *Free Radic. Biol. Med.*, 32: 414-420.
- Kelloff, G.J., J.A. Crowell, E.T. Hawk, V.E. Stell, R.A. Lubet, C.W. Bone, J.M. Covey, L.A. Doody, G.S. Omenn, P. Grenwald, W.K. Hong, D.R. Parkinson, D. Baghen, G.T. Baxter, M. Blunder, M.K. Doltez, K.M. Eisenhaus, K. Johnson, G.G. Knapp, D.G. Longfellow, W.F. Malon, S.G. Nayfields, H.E. Serrifried, L.M. Swall and C.C. Andisigman, 1996. Strategy and planning for chemopreventive drug development: Clinical developmat plans II. *J. Cell. Biochem.*, 265: 54-71.
- Kunchandy, E. and M.N.A. Roa, 1990. Oxygen radical scavenging activity of curcumin. *Int. J. Pharm.* 87: 79-87.

- Loskin, D.L. and J.D. Laskin, 1996. Macrophages inflammatory mediators and lung injury. *Methods: A companion to methods in Enzymal.*, 10: 61-70.
- Makhopadhyay, A., N. Basu, N. Gllatak and P.K. Gujral, 1982. Anti-inflammatory and irritant activities of curcumin analogues in rats. *Agents Actions* 12: 508-515.
- Nathan, C.F., H.W. Murrey and Z.A. Cohen, 1980. Current concepts: The macrophages as an effector cell. *N. Engel. J. Med.*, 303: 662-665.
- National Research Council, 1994. Nutrient requirement of poultry. National Academy Press, Washington DC.
- Osawa, T., Y. Sagiyama, M. Inayoshi and S. Kawakashi, 1995. Antioxidative activity of tetrahydrocurcuminoids. *Biosci. Biotech. Biochem.*, 59: 1609-1612.
- Reddy, A.C. and B.R. Lokesh, 1994. Studies on the inhibitory effects of curcumin and eagenol on the formation of reactive oxygen species and the oxidation of ferrous iron. *Mol. Cel. Biochem.*, 137: 1-8.
- SAS, 1993. SAS user's guide: version 6.03 Ed., SAS Institute Inc., Cary, North Carolina.
- Sharma, S.C., H. Mukhtar, S.K. Sharma and C.R. Murt, 1972. Lipid peroxide formation in experimental inflammation. *Biochem. Pharmacol.*, 21: 1210-1214.
- Sharma, O.P., 1976. Antioxidant activity of curcumin and related compounds. *Biochem. Pharmacol.*, 25: 1811-1812.
- Subramanian, M., M.N.A. Sreejayan-Rao, T.D.A. Devasagayam and B.B. Singh, 1994. Diminution of singlet oxygen induced DNA damage by curcumin and related antioxidants. *Mutat. Res.*, 311: 249-255.
- Sugiyama, Y., S. Kawakashi and T. Osawa, 1996. Involvement of the di-acetone moiety in the antioxidant mechanism of tetrahydrocurcumin. *Biochem. Pharmacol.*, 52: 519-525.
- Surh, Y., 1999. Molecular mechanisms of chemopreventive effects of selected dietary and medicinal phenolic substances. *Mutation Res.*, 428: 305-327.
- Tonnes, H.H. and J.V. Greenhill, 1992. Studies on curcumin and curcuminoids. XXII: Curcumin as a reducing agent and as a radical scavenger. *Int. J. Pharm.*, 87: 79-87.
- Yasni, K., K. Imaizumi, M. Nakamura, J. Aimoto and M. Sugano, 1993. Effects of curcuma xanthorrhiza roxb. and curcuminoids on the level of serum and liver lipids, serum apolipoprotein A-1 and lipogenic enzymes in rats. *Food Chem. Toxic.*, 31: 213-218.