Indian J. Anim. Nutr. 2021. 38 (2): 181-186
doi: 10.5958/2231-6744.2021.00026.8

Indian Journal of
Animal Nutrition

Effect of Dietary Coriander Seed Powder Supplementation on the Serological and Immunological Parameters of Broilers

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ABSTRACT

An investigation on the effect of supplementing coriander seed powder (CSP) in broilers' diet on serum profile and immunological parameters was done. One hundred and twenty day-old commercial broiler chicks were divided into five treatment groups of 24 birds, and each group had three replicates of 8 birds. T_1 served as control and was offered basal diet as per BIS (2007) supplemented with antibiotics but no CSP. Treatment groups T_2, T_3, T_4 and T_5 were offered control diet plus 0.5, 1.0, 1.5 and 2.0% of CSP but no antibiotics as growth promotors. All the diets were isocaloric and iso-nitrogenous in nature. Plasma glucose and serum total cholesterol was decreased significantly ($P<0.05$) in the CSP supplemented groups in comparison to control group. Also, dietary supplementation of CSP resulted in increased ($P<0.05$) HDL and decreased ($P<0.05$) LDL-cholesterol. Serum triglyceride decreased significantly ($P<0.05$) upon CSP supplementation at 1.0% and above. Activity SGOT and SGPT decreased significantly ($P<0.05$) upon CSP supplementation in the diets of broiler. Relative mRNA expression of TLR2 and TLR7 of broilers was found to be enhanced ($P<0.05$) while that of TLR4 was down regulated in all the CSP supplemented groups in comparison to control group. Based on the findings of current study, it was inferred that coriander seed powder supplementation in the broilers diet has beneficial effects on various blood biochemical and immunological indices and can be preferred as feed additives over antibiotics.

Key words: Coriander seed powder, Broiler, Biochemical and Immunological parameters

INTRODUCTION

Poultry including chickens, turkeys, geese and ducks are major groups of food animals which had been domesticated for the production of meat or eggs. The chicken (*Gallus gallus domesticus*) is a type of domesticated fowl that is a subspecies of the red jungle fowl. It is one of the most common and widespread domestic birds. Humans keep chicken primarily for food, consuming both their meat and eggs. In India, the growth in poultry sector has been phenomenal during the last few decades. This has been primarily achieved due to the exploitation of various modern growth-promoting strategies and appropriate measures of disease prevention (Angelakis *et al.,* 2013). But the main constraint for an economic poultry rearing especially in the un-organized poultry sector is threat posed by the pathogens that are present in and around the poultry farms. This has led to reduced profitability of small-scale poultry farming in the country due to high cost of treatment using antibiotics. Moreover, injudicious use of antibiotic may cause antimicrobial resistance along with antibiotic residues in food chain. Considering the ever increase public awareness of ill effects of antibiotics, many countries have banned use of antibiotics as growth promoter in livestock feed. Thus, use of aromatic plants has been an important part of achieving modern intensive poultry production (Ghazanfari *et al.,* 2015).

Coriander (*Coriandrum sativum* L.) is a member of *Apiaceae* family, native to eastern Mediterranean region and southern Europe. It reputedly has health advantages and regarded as both herb and spice. Linalool, an essential oil present in coriander seeds has anthelmintic, antifungal, antioxidant and antimicrobial properties (Silva *et al.,* 2011; Saleh *et al.,* 2014). Additionally, coriander has been advocated as an anti-diabetic remedy (Lewis and Elvin-Lewis, 1977). Anti-diabetic agents can exert beneficial effects in the diabetic patient by improving and or mimicking insulin action and/or by enhancing insulin secretion (Gray

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and Flatt, 1997). Keeping in view the health benefits of coriander, the present study was conducted to investigate the effect of supplementation of coriander seed powder on the serological and immunological parameters in the broiler chickens.

MATERIALS AND METHODS

The present study was conducted at poultry shed of the Department of Animal Nutrition, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar for a period of six weeks. One hundred and twenty day-old broiler chicks were purchased from a local commercial hatchery. The chicks were wing banded, individually weighed and randomly distributed into five treatment groups *viz.* T_1, T_2, T_3, T_4 and T_5. Every treatment group had three replicates of eight birds each. The group T_1 served as control and was fed a basal ration formulated as per BIS (2007) specifications with the supplementation of antibiotics as growth promoting feed additives. In the diets of treatment group T_2, T_3, T_4 and T_5 coriander seed powder (CSP) was supplemented in place of antibiotics at the rate of 0.5, 1.0, 1.5 and 2.0%, respectively. Birds were vaccinated against F_1 strain of Newcastle disease (NCD) on 3rd day and Infectious Bursal Disease (IBD) on 14th day through intraocular/intranasal route. The birds were kept in deep litter system using chopped dry wheat straw. The shed was fumigated with formaldehyde gas (35 ml of commercial formalin and 17.5 g potassium permanganate per hundred cubic feet area) and a thin layer of calcium carbonate was spread on floor prior to placing the bedding material. *Ad-libitum* feeding programme consisted of a pre-starter diet till 7 days, a starter diet fed for succeeding 21 days, and a finisher diet fed at the end until 42 days of age. Fresh drinking water supplemented with hepatoprotective liver tonic was made available round the clock.

Blood samples were collected from the slaughtered birds in non-heparinised tubes.The samples were centrifuged at 3000 rpm for 15 minute and serum obtained was stored at -20°C until analysed. Serum parameters like total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, glucose, SGOT and SGPT were determined using Ebra EM-200 Biochemistry Analyzer (Sr. No. B110318, Ebra Mannheim, Transasia Bio-medicals ltd).

Total RNA was isolated from blood samples by using TRIZOL® kit (Ambion by life technologies) as per the manufacturer's instruction. One ml of TRIZOL® reagent, 200 µl of chloroform was added to 600 µl of blood followed by centrifugation for phase separation and precipitation with isopropanol. Total RNA extracted was dissolved in 20 µl NFW (Nucleus free water) and quantified using Qubit® 2.0 fluorometer (Invitrogen). Reverse transcription was carried out with total reaction volume of 20 µl using Onescript® cDNA synthesis kit (fire script RT-cDNA synthesis kit). The polymerase chain reaction (RT-PCR) cyclic conditions were as initial incubation at 25°C for 10 min, reverse transcription at 42°C for 50 min and deactivation at 85°C for 5 min in thermal cycler (Applied Biosystem, virti). The cDNA was stored at -20°C till further use.

For the analysis of temporal expression profile of different genes, real-time PCR was carried out using Step I plus real-time PCR system. For the real-time PCR reaction, SYBR green dye based universal PCR master mix (Luna) was used. The reaction for various TLRs gene, (TLR 2, TLR 4 and TLR 7) and the endogenous control, β-actin gene was carried out in triplicate along with non-template control as a negative control for each sample. The reaction mixture used to carry out the real-time PCR reaction for TLRs 2, 4 and 7; and β-actin gene contains 1X SYBR green PCR mastermix (Luna, 5 µl), Forward primer (0.3µl), Reverse primer (0.3µl), NFW (1.4µl) and template (3 µl). Amplification was done with initial denaturation for 60 seconds at 95°C, followed by 40 cycles of denaturation for 15 s at 95°C and extension for 30 s at 60°C, and a final melting curve analysis at 60ºC.The average C_T (Threshold cycle) value obtained for the TLRs 2, 4 and 7 gene were normalized to β-actin (endogenous control). The data obtained were subjected to comparative C_T method for the analysis of the

expression levels of targeted TLR gene and an endogenous control. Amplicons were sequenced using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA) on an automatic ABI 3130 xl Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA). The sequence obtained shows 100% nucleotide identity with the TLR sequence of chicken available in the global database.

Statistical analysis

Data was analysed statistically as described by Snedecor and Cochran (1994). Analysis of variance was performed and differences among treatment means were compared by using Duncan's Multiple Range Test (DMRT) as modified by Kramer (1956).

RESULTS AND DISCUSSION

Biochemical parameters of broilers fed different diets are presented in table 1. All the CSP supplemented groups had significantly (P<0.05) lower plasma glucose concentration than the control group. Further, plasma glucose concentration was found to decrease significantly (P<0.05) as the level of CSP was increased from 0.5% in T_2 to 2% in T_5. Decrease in plasma glucose in the CSP supplemented groups might be due to the anti-diabetic property of coriander seeds. In a similar study, Al- Mashhadani *et al.* (2011) reported that inclusion of coriander seed oil at 0.5% and 1% of diet resulted in significant (P<0.05) decrease in serum glucose in broilers. Similarly, serum total cholesterol was also decreased significantly (P<0.05) with the increasing level of CSP supplementation. Control group supplemented with antibiotics had highest while treatment group supplemented with CSP at 2.0% level had lowest serum cholesterol concentration. CSP supplementation at 1.0% or above resulted in significant (P<0.05) increase in serum HDL-cholesterol. On the other hand, serum LDL-cholesterol was decreased significantly (P<0.05) in the CSP supplemented groups as compared to control with the lowest LDL concentration (43.56 mg/dl) being observed in T_5 group supplemented with 2.0% CSP. Hypocholestermic effect of coriander seeds could be due inhibition in the enzyme 3-hydroxy 3-methyl glutaryl CoA reductase (HMG-CoA reductase) that catalyzes the conversion of HMG-CoA to mevalonate. Mevalonate is required for cholesterol biosynthesis (Dhanapakiam *et al.,* 2008). Coriander oil extract interferes with production of mevalonate by acting as a reversible competitive inhibitor of enzyme HMG-CoA reductase (Ciftci *etal.,* 2010), thus results in hypocholesterol-aemia. Chithra and leelamma (1997) have also reported that coriander enhance bile acid synthesis with increased degradation of cholesterol to fecal bile acid and natural sterols which resulted in lowering serum cholesterol level. Additionally, Dhanapakiam *et al.* (2008) also reported decreased LDL and increased HDL cholesterol (P<0.05) in coriander seed-based diet fed animals. A significant (P<0.05) reduction in serum triglyceride was also reported on CSP supplementation at 1.0% or or higher level of inclusion. Similarly, Al-Jaff (2011) illustrated that the inclusion of coriander seed as diet ingredient at levels of 2% and 3% resulted in a significant decreased in serum concentrations of cholesterol and triglyceride. CSP supplemented groups had significantly (P<0.05) lower Serum glutamic

Table 1. Mean values of biochemical parameters of broilers under different dietary treatments

Treatment	Glucose (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Triglycerides (mg/dl)	SGOT (IU/l)	SGPT (IU/l)
T_1	$225.26^{e}\pm1.60$	$142.52^{d}\pm1.26$	$74.44^{a}\pm2.35$	$60.06^{d}\pm1.05$	$86.33^{b}\pm0.88$	$275.10^{e}\pm0.92$	$7.10^{d}\pm0.15$
T_2	$214.46^{d}\pm0.37$	$139.33^{c}\pm1.20$	$78.08^{a\pm}1.27$	$55.01^{c}\pm0.65$	$84.67^{b}\pm1.76$	$260.72^{d}\pm1.19$	$6.53^{c}\pm0.08$
T_3	$208.08^{c}\pm0.98$	$136.07^{b}\pm0.89$	$82.44^{b}\pm0.52$	$52.42^{c}\pm1.09$	$80.00^{a}\pm1.15$	$251.50^{c}\pm1.87$	$6.20^{c}\pm0.17$
T_4	$192.20^{b}\pm1.01$	$134.28^{ab}\pm0.68$	$85.13^{bc}\pm1.10$	$48.51^{b}\pm1.02$	$77.67^{a}\pm1.20$	$243.05^{b}\pm2.28$	$4.93^{b}\pm0.12$
T_5	$183.72^{a}\pm1.68$	$132.55^{a}\pm0.86$	$88.43^{c}\pm0.43$	$43.56^{a}\pm1.24$	$75.33^{a}\pm1.85$	$231.16^{a}\pm0.63$	$4.20^{a}\pm0.06$

Mean values bearing different superscripts in a column differ significantly (P<0.05)

oxaloacetic transaminase (SGOT) concentration as compared to control group. Also, increase of every 0.5% supplementation of CSP from group T_2 to T_5 lowered the serum SGOT concentration significantly (P<0.05). Serum glutamic pyruvic transaminase (SGPT) concentration was also reduced by CSP supplementation as compared to the control group, however, groups supplemented with 0.5 and 1.0% level of CSP had statistically (P>0.05) comparable SGPT concentration. Our findings are in agreement with Al-Mashhadani (2011) and Al-Jaff (2011) suggesting that coriander seeds might have hepato-protective role in birds, and hence it could replace antibiotics as feed additives in their diets.

Relative mRNA expression of TLR2 and TLR7 of broilers was found to be enhanced (P<0.05) in all the CSP supplemented groups, and a down regulation (P<0.05) trend was seen in CSP supplemented groups for TLR4 expression (Tables 2 and 3).Treatment group T_4 (1.5% CSP) had highest relative quantification (RQ) value showing maximum expression of TLR2 while treatment group T_3 (1.0% CSP) had highest RQ value showing maximum expression of TLR7. In case of TLR4 mRNA expression study, a significant (P<0.05) down regulation of RQ value was obtained, being lowest for treatment T_5 (2% CSP) followed by T_4 (1.5% CSP), T_3 (1.0% CSP) and T_2 (0.5% CSP) as compared to control group T_1. TLR2 denotes presence of Gram positive (+) bacteria like *Lactobacillus* that are beneficial for the health of the gut. Up regulation of the TLR2 RQ values for birds fed CSP in comparison to control group suggests an enhanced population of these bacteria in the gut. TLR4 depicts the presence of the Gram negative (-) bacteria like *E. coli* in the gut microflora, therefore, down regulation of TLR4 RQ values for birds fed CSP in comparison to the control group indicates an elevated anti-bacterial activity against these harmful bacteria.TLR7 represents the anti-viral activity of immune system and thus, up regulation of

Table 2. Relative expression of TLRs of the broiler birds under different dietary treatments

Target Gene	TR	CT MEAN	CT SE	$^{\Delta}$CT Mean	$^{\Delta\Delta}$CT Mean	R.Q.
	T_1	21.65	0.07	4.11	0	1.00
	T_2	21.32	0.04	3.97	-0.14	1.36
	T_3	20.78	0.02	3.84	-0.27	1.21
	T_4	20.24	0.10	3.42	-0.69	1.71
TLR2	T_5	20.51	0.11	3.24	-0.48	1.39
	T_1	24.34	0.02	6.8	0	1.00
	T_2	24.20	0.04	6.85	0.05	0.97
	T_3	24.34	0.02	7.4	0.6	0.66
	T_4	24.86	0.00	8.04	1.24	0.52
TLR4	T_5	28.24	0.84	10.7	0	0.46
	T_1	24.45	0.62	9.3	-2.20	1.00
	T_2	27.67	0.68	10.32	-0.38	1.30
	T_3	27.19	0.18	10.25	-0.45	1.37
	T_4	27.02	0.38	10.2	-0.5	1.33
TLR7	T_5	17.54	0.04	9.06	-2.20	1.25
	T_1	20.31	0.65			
	T_2	19.98	0.22			
	T_3	18.84	0.81			
	T_4	17.74	0.20			
β-Actin	T_5	17.85	0.02			

Table 3. Oligonucleotide sequences of sense and antisense primers for real-time PCR products determined

Gene[1]	Primer	Primer sequence	AccessionNo.	ProductSize
β-Actin	Sense	52 -GAGAAATTGTGCGTGACATCA-32	L08165	152
	Antisense	52 -CCTGAACCTCTCATTGCCA-32		
TLR 2	Sense	52 -CATTCACCATGAGGCAGGGATAG-32	AB046533	157
	Antisense	52 -GGTGCAGATCAAGGACACTAGGA-32		
TLR 4	Sense	52 -TTCAGAACGGACTCTTGAGTGG-32	AY064697	131
	Antisense	52 -CAACCGAATAGTGGTGACGTTG-32		
TLR 7	Sense	52 -TTGCTGCTGTTGTCTTGAGTGAG-32	AJ627563	182
	Antisense	52 -AACAACAGTGCATTTGACGTCCT-32		

[1]TLR 2 = Toll-like receptor 2; TLR 4 = Toll-like receptor 4; TLR 7 = Toll-like receptor 7

TLR7 RQ values upon CSP supplementation signifies a boosted immune system response. Modulatory functions of medicinal herbs on immune system have also been reported by earlier study. In a study on medicinal herbs, Jyotsana (2018) had reported that TLR2 mRNA expression was increased ($P<0.05$) and TLR4 mRNA expression was down regulated in broilers fed Ashwagandha root powder supplemented diets. Our results are in harmony with Hosseinzadeh *et al.* (2014) who found that coriander improved performance indices, ileum microflora and immune response in broiler chicks.

CONCLUSION

From the findings of this study, it was concluded that supplementation of coriander seed powder to broiler diets had favourable effects on serum biochemical profile in term of decreased SGOT and SGPT indicating hepato-protective effects and immunological indices of broiler chicken in terms of stimulatory effect on relative mRNA expression of TLR2 and TLR7 and down regulation pattern of TLR4 indicating improved immunity. Therefore, coriander seed powder can be a preferable feed additive in place of antibiotics.

ACKNOWLEDGMENTS

The authors are indebted to the Vice Chancellor, Department of Animal Nutrition and Department of Animal Biotechnology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India for providing the infrastructure facilities that enabled the successful completion of the research.

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Received on 14.09.2021 and accepted on 20.09.2021