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HYPOLIPIDEMIC EFFECT OF BLACK CUMIN (*Nigella sativa*) OIL ON ADULT LAYER BIRDS

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ABSTRACT

The present study was aimed to assess the effect of black cumin (*Nigella sativa*) oil on serum lipid profile and β-hydroxy, β-methylglutaryl coenzyme-A reductase (HMG CoA reductase) gene expression in adult layer birds. Thirty six weeks old White Leg Horn (WLH) layer birds were randomly divided into five groups consisting of 6 birds each. Their diets were supplemented with 0 (control), 1.0, 2.0, 3.0 and 4.0 g black cumin oil kg^{-1} feed, respectively. Blood samples were collected on day 0, 14, 28, 42 and 56 and study further continued for one more month without supplementing the diet with herbal preparations in order to observe the withdrawal effect. Gene expression studies of HMG CoA reductase were carried out in the liver tissue of the birds by real time quantitative PCR analysis on day 56. Serum total lipids, cholesterol, tri-glycerides, HDL, LDL and VLDL-cholesterol varied highly significantly ($p<0.01$) between treatments and intervals. Maximum reduction in HMG CoA reductase gene expressions was noticed in group fed diet supplemented with 4 g cumin oil kg^{-1}. The reduction pattern in gene expression and serum lipid profile in treatment groups fed diets supplemented with black cumin oil were in the order of 4> 3> 2> 1> 0 g cumin oil kg^{-1} feed. The study revealed that the supplementation of black cumin oil @ 4 g kg^{-1} feed is most effective in reducing serum lipid profile and HMG CoA reductase gene expression after 56 days. These effects gradually reduced with time after the withdrawal of oil.

Key words: Black cumin oil, gene expressions, HMG CoA reductase, lipid profile, White Leg Horn layer birds

INTRODUCTION

Hyperlipidemia is characterized by derangements in carbohydrate, protein and lipid metabolism. These complications are caused by complex interaction of genetics, environmental stressors and life-style choices (Gupta *et al*., 2009). Individuals with insulin resistance commonly have increased triglyceride, low density lipoprotein (LDL), total cholesterol and decreased high density lipoprotein (HDL) level (Kontosh and Chapman, 2006). *Nigella sativa* commonly known as *kalonji*, black seed or black cumin, belongs to the class of phytogenic immuno-stimulants that help in establishment

and strengthening of para-immunity (Akhtar *et al.,* 2003; Nasir and Grashorn, 2009). The seeds of *N. sativa* have been reported to have many biological properties including hypolipidemic and hypotensive actions (Zaoui and Cherrah, 2000). Solvent extracted fixed oil of black cumin has 42.76% linoleic acid, 16.59% oleic acid, 8.51% palmitic acid, 4.71% eicosatrienoic acid, 5.98% eicosapentaenoic acid (EPA) and 2.97% decosahexaenoic acid (DHA) (Kaskoos, 2011). High unsaturated fatty acid content signified its health benefits. In view of above, the present study was undertaken to elucidate the hypolipidemic activity of black cumin oil in adult layer birds as well as estimate lipid profile and gene expression of β-hydroxy β-methyl glutaryl CoA (HMG CoA) reductase regulatory enzyme associated with lipid metabolism.

MATERIALS AND METHODS

The study was conducted on 30 healthy White Leg Horn layer birds of 36 weeks age. The birds were randomly divided into 5 groups having 6 birds each. These groups were fed diets supplemented with black cumin oil @ 0, 1, 2, 3 and 4 g kg^{-1} feed. The basal diet consisted of 2700 ME kcal kg^{-1} and 17% protein. One millilitre blood was collected from each group on 0^{th}, 14^{th}, 28^{th}, 42^{th} and 56^{th} day and the study further continued for one month without supplementing the diet with herbal preparations in order to observe the withdrawal effect. The serum total lipids were estimated by the method of Frings *et al.* (1972), whereas serum cholesterol, triglycerides, HDL, LDL and VLDL-cholesterol were estimated with the help of diagnostic kits (Erba). The gene expression study of HMG CoA reductase was carried out in liver tissue of layer birds by real time quantitative PCR analysis on day 56. Total RNA was extracted from liver by using trizol reagent as per the manufacturer's instructions (Zhou *et al.*, 2002). The comparative quantification of different gene mRNAs was done by the ΔΔCT method (Livak and Schmittgen, 2001). The primers used in this study were GAPDH-F and GAPDH-R with primer sequence (5′-3′) as CCACATGGCATCCAA GGAGT and GAACTGAGCGGTGGTGAAGA (amplicon size 101 bp), respectively. Also, HMG CoA reductase-F and HMG CoA reductase-R with primer sequence (5′-3′) as TAGGCGTAGCAG GACCACTA and TGCTCCTCCACCGAGACATA (amplicon size 125 bp), respectively, were used. The annealing temperature was 55°C.

The data was analyzed using hierarchical analysis of variance (Steel and Torrie, 1992). The comparisons among treatment means was made by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The serum total lipids, cholesterol, triglycerides, HDL, LDL and VLDL-cholesterol varied highly significantly ($P<0.01$) between treatments and intervals. The reduction in serum lipid profile was observed in White Leg Horn layer birds fed with medicinal oils in a dose-dependent manner. The percent reduction in serum total lipids were 7.11, 10.44, 13.97 and 17.0 in 1, 2, 3 and 4 g kg^{-1} black cumin oil treated groups, respectively, on day 56 (Table 1). Maximum reduction was observed in 4 g kg^{-1} treated group. In a similar experiment, Shrivastava (2012) reported higher reduction (43.75%) in Jabalpur colour birds. Bagir *et al.* (2006) found 18 and 19% reduction on serum total lipids on dietary supplementation of 1 and 3% black cumin seeds for three months. The hypocholestremic effect was in treatment groups fed diets supplemented with black cumin oil was in the order of 4 > 3> 2> 1> 0 g cumin oil kg^{-1} feed. The maximum reduction (19.27%) was observed in birds fed with black cumin oil @ 4g kg^{-1} feed (Table 2). Similar effects were reported by Labhal *et al.* (1997) who

Table 1: Effect of black cumin oil on serum total lipids (mg dL^{-1}) in White Leg Horn layer birds

Black cumin oil (g kg^{-1})	Days						
	0	14	28	42	56	70	84
Control	722.41 ±10.09	724.19^{B} ±6.28	725.41^{D} ±7.80	723.34^{C} ±11.86	718.97^{C} ±4.69	731.23^{C} ±7.58	726.40^{C} ±8.19
1.0	712.69 ±5.16	709.15^{AB} ±8.88	$689.87^{B;C}$ ±5.59	672.41^{B} ±13.32	658.74^{B} ±12.07	665.60^{B} ±8.21	672.26^{B} ±7.21
2.0	725.77^{b} ±5.91	$722.86^{b;AB}$ ±7.16	$697.02^{b;C}$ ±8.13	$667.35^{ab;B}$ ±11.14	$649.95^{a;B}$ ±7.69	$657.53^{a;B}$ ±8.56	$658.20a^{B}$ ±9.69
3.0	713.65^{c} ±8.60	$694.19^{b;A}$ ±11.70	$660.17^{b;A}$ ±10.72	$631.50^{ab;A}$ ±9.69	$613.94^{a;A}$ ±8.08	$619.90^{a;A}$ ±7.86	$626.57^{a;AB}$ ±9.59
4.0	723.05^{d} ±7.63	$703.13^{cd;AB}$ ±7.94	$676.78^{c;B}$ ±11.72	$632.51^{b;A}$ ±9.45	$600.08^{a;A}$ ±11.70	$608.83^{ab;A}$ ±8.76	$617.60^{ab;A}$ ±8.95

Mean values with different superscripts in a row (lower case) and column (upper case) vary significantly ($P_{0.05}$)

observed that feeding *N. sativa* oil was as efficient as the cholesterol-reducing drug simvastatin. They reported that two capsules of 500 mg black seed oil twice a day for two weeks to nine patients reduced blood cholesterol levels significantly. Black cumin seed oil significantly reduces serum cholesterol (Akhtar *et al.,* 2003) by more than 20% (Tahir *et al.*, 1993). Bagir *et al.* (2006) reported that serum cholesterol concentrations were lowered by 15 and 23% on supplementation of black cumin seeds at 1 and 3% inclusion levels for 3 months. Tasawer *et al.* (2011) reported the effects of *N. saitva* seed oil on lipid profile in cardiac patients of 26-69 years. Cholesterol was reduced by 14.58% whereas the reduction obtained on treatment with statin daily for 6 months was negligible.

The serum triglycerides exhibited similar effect by black cumin oil as those of lipids. The maximum reduction in triglycerides was observed in 4 g kg^{-1} black cumin oil treated group (12.84%) on day 56 (Table 3). Mathur *et al.* (2011) reported that administration of *N. sativa* orally to rats significantly decreased serum triglycerides. Similar reduction in serum triglyceride levels was reported by Akhtar *et al.* (2003) and Ali *et al.* (2014). Bagir *et al.* (2006) observed 18 and 12% reduction in serum triglyceride levels on supplementation of black cumin in White Leg Horn birds at 1 and 2% level. Significant reduction in serum triglyceride levels were also reported by Tahir *et al.* (1993) and Tasawer *et al.* (2011).

There was maximum increase in HDL- cholesterol level in 4 g kg^{-1} black cumin oil treated group of (12.87%) [Table 4]. Akhtar *et al.* (2003) reported a significant increase in HDL level in layers on administration of 0.0, 0.5, 1.0 and 1.5% cumin seeds in powder form. Increase in HDL-

Table 2: Effect of black cumin oil on serum cholesterol (mg dL^{-1}) in White Leg Horn layer birds

Black cumin oil (g kg^{-1})	Days						
	0	14	28	42	56	70	84
Control	233.58 ±4.13	232.82 ±2.26	233.97^{C} ±3.40	232.09^{C} ±3.79	231.98C ±2.79	235.78C ±2.20	234.87C ±2.40
1.0	229.95^{b} ±3.37	228.13^{b} ±1.96	$220.98^{b;AB}$ ±2.94	$214.40^{ab;B}$ ±8.55	$209.30^{a;B}$ ±5.73	$210.65^{ab;B}$ ±4.05	$213.68^{ab;B}$ ±4.39
2.0	234.52^{b} ±3.33	232.70^{b} ±3.00	$226.57^{b;B}$ ±2.24	$212.63^{ab;AB}$ ±4.65	$206.18^{a;B}$ ±3.49	$209.85^{a;B}$ ±4.09	$209.77^{a;B}$ ±3.02
3.0	229.83^{b} ±1.85	223.87^{b} ±5.03	$212.65^{b;A}$ ±3.56	$202.43^{ab;AB}$ ±2.42	$193.73^{a;A}$ ±3.24	$196.37^{a;A}$ ±2.74	$199.12^{ab;A}$ ±2.92
4.0	234.20^{d} ±3.04	227.03^{cd} ±2.44	$217.25^{c;AB}$ ±3.61	$202.38^{b;A}$ ±3.74	$189.07^{a;A}$ ±4.04	$193.17^{ab;A}$ ±3.24	$196.97^{ab;A}$ ±2.38

Mean values with different superscripts in a row (lower case) and column (upper case) vary significantly ($P_{0.05}$)

Table 3: Effect of black cumin oil on serum triglycerides (mg dL^{-1}) in White Leg Horn layer birds

Black cumin oil (g kg^{-1})	Days						
	0	14	28	42	56	70	84
Control	255.24 ±3.14	258.55 ±6.21	257.47^{B} ±4.52	259.14^{B} ±8.66	255.00^{B} ±3.73	259.66^{B} ±5.46	256.66^{B} ±5.81
1.0	251.66 ±7.18	249.07 ±6.87	244.14^{AB} ±3.97	242.41^{AB} ±7.30	242.33^{B} ±4.10	243.33^{B} ±3.40	243.66^{B} ±2.80
2.0	256.74^{b} ±3.89	257.45^{b} ±8.37	243.88$^{b;AB}$ ±7.33	242.08$^{ab;AB}$ ±3.13	237.57$^{a;AB}$ ±7.36	237.83$^{ab;AB}$ ±7.63	238.66$^{ab;AB}$ ±7.83
3.0	253.98^{b} ±8.39	246.45^{b} ±5.65	234.86$^{ab;A}$ ±5.93	226.63$^{ab;A}$ ±5.80	226.47$^{a;AB}$ ±4.81	227.17$^{ab;AB}$ ±4.88	228.33$^{ab;A}$ ±4.82
4.0	254.65^{b} ±5.79	249.06^{b} ±8.02	242.28$^{b;AB}$ ±8.16	227.74$^{ab;A}$ ±4.89	221.94$^{a;A}$ ±5.00	222.50$^{ab;A}$ ±5.14	223.66$^{ab;A}$ ±5.18

Values with different superscripts in a row (lower case) and column (upper case) vary significantly ($P_{0.05}$)

cholesterol was also reported by Tahir *et al.* (1993) after oral administration of cumin oil to rats and Ati *et al.* (2009) on administration of cumin seeds in the feed of broilers. Tasawer *et al.* (2011) reported the significant increase (3.18%) in good cholesterol level in cardiac patients of 26-69 years fed with of *N. saitva* seed oil after 6 months.

There was maximum reduction in LDL- cholesterol and VLDL-cholesterol levels in 4 g kg^{-1} black cumin oil treated group (30.81%) on 56 days of treatment (Table 5). Jain *et al.* (1993) and Steiner *et al.* (1996) showed that black cumin and its preparations significantly reduced plasma

Table 4: Effect of black cumin oil on serum HDL cholesterol (mg dL^{-1}) in layer birds

Black cumin oil (g kg^{-1})	Days						
	0	14	28	42	56	70	84
Control	41.52 ±1.26	40.43 ±0.77	40.61 ±1.07	41.04^{A} ±0.95	41.62^{A} ±1.18	42.29^{A} ±1.60	41.99^{A} ±0.91
1.0	42.13 ±0.58	42.60 ±0.99	42.93 ±1.06	43.43^{AB} ±0.64	44.76^{AB} ±1.03	44.24^{AB} ±0.98	43.71^{AB} ±0.86
2.0	41.04^{a} ±0.75	41.67^{ab} ±0.79	43.04^{ab} ±0.80	44.30$^{b;B}$ ±0.97	45.68$^{b;B}$ ±0.36	45.51$^{b;B}$ ±0.83	44.55$^{b;B}$ ±0.90
3.0	39.57^{a} ±0.90	41.77^{ab} ±0.49	42.16^{ab} ±0.63	43.05$^{b;AB}$ ±0.79	44.45$^{b;AB}$ ±1.11	44.24$^{b;AB}$ ±1.01	43.60$^{b;AB}$ ±0.66
4.0	40.92^{a} ±0.41	41.97^{ab} ±0.90	42.69^{ab} ±0.83	44.39$^{b;B}$ ±0.82	46.19$^{b;B}$ ±1.66	46.06$^{b;B}$ ±1.06	45.36$^{b;B}$ ±1.53

Values with different superscripts in a row (lower case) and column (upper case) vary significantly ($P_{0.05}$)

Table 5: Effect of black cumin oil on serum LDL cholesterol (mg dL^{-1}) in layer birds

Black cumin oil (g kg^{-1})	Days						
	0	14	28	42	56	70	84
Control	141.01 ±3.82	140.67^{C} ±3.53	141.86^{C} ±3.73	139.23^{B} ±4.23	139.37^{C} ±2.64	141.56^{C} ±2.89	141.54^{C} ±2.84
1.0	137.49^{b} ±4.60	135.72$^{b;BC}$ ±1.86	129.23$^{b;AB}$ ±2.93	122.49$^{ab;A}$ ±9.71	116.08$^{a;B}$ ±5.26	117.75$^{ab;B}$ ±4.42	121.24$^{ab;B}$ ±4.32
2.0	142.13^{b} ±4.09	139.54$^{b;B}$ ±4.49	134.75$^{b;AB}$ ±3.40	119.92$^{ab;A}$ ±4.27	112.98$^{a;B}$ ±4.35	116.77$^{a;B}$ ±4.37	117.49$^{a;AB}$ ±4.15
3.0	139.47^{b} ±3.11	132.80$^{b;A}$ ±5.51	123.51$^{b;A}$ ±3.47	114.06$^{ab;A}$ ±2.48	103.99$^{a;AB}$ ±3.94	106.69$^{a;AB}$ ±3.48	109.85$^{a;AB}$ ±2.55
4.0	142.35^{c} ±3.62	135.26$^{c;AB}$ ±3.16	126.10$^{b;A}$ ±4.27	112.45$^{b;A}$ ±3.92	98.49$^{a;A}$ ±4.08	102.61$^{ab;A}$ ±3.02	106.87$^{ab;A}$ ±2.40

Values with different superscripts in a row (lower case) and column (upper case) vary significantly ($P_{0.05}$)

Table 6: Effect of black cumin oil on serum VLDL cholesterol (mg dL $^{-1}$) in layer birds

Black cumin oil (g kg^{-1})	Days						
	0	14	28	42	56	70	84
Control	51.05 ±0.63	51.71 ±1.24	51.49^{B} ±0.91	51.83^{B} ±1.73	51.00^{B} ±0.75	51.93^{B} ±1.09	51.33^{B} ±1.16
1.0	50.33 ±1.44	49.81 ±1.37	48.83^{AB} ±0.79	48.48^{AB} ±1.46	48.47^{B} ±0.82	48.67^{AB} ±0.68	48.73^{AB} ±0.56
2.0	51.35 ±0.78	51.49 ±1.68	48.78^{AB} ±1.47	48.42^{AB} ±0.63	47.52^{AB} ±1.47	47.57^{AB} ±1.53	47.73^{AB} ±1.57
3.0	50.80^{b} ±1.68	49.29^{b} ±1.13	46.97$^{ab;A}$ ±1.19	45.33$^{ab;A}$ ±1.16	45.29$^{a;AB}$ ±0.96	45.43$^{ab;AB}$ ±0.98	45.67$^{ab;AB}$ ±0.96
4.0	50.93^{b} ±1.16	49.81^{b} ±1.61	48.46$^{ab;AB}$ ±1.63	45.55$^{ab;AB}$ ±0.98	44.39$^{a;A}$ ±1.00	44.50$^{ab;A}$ ±1.03	44.73$^{ab;A}$ ±1.04

Values with different superscripts in a row (lower case) and column (upper case) vary significantly ($P_{0.05}$)

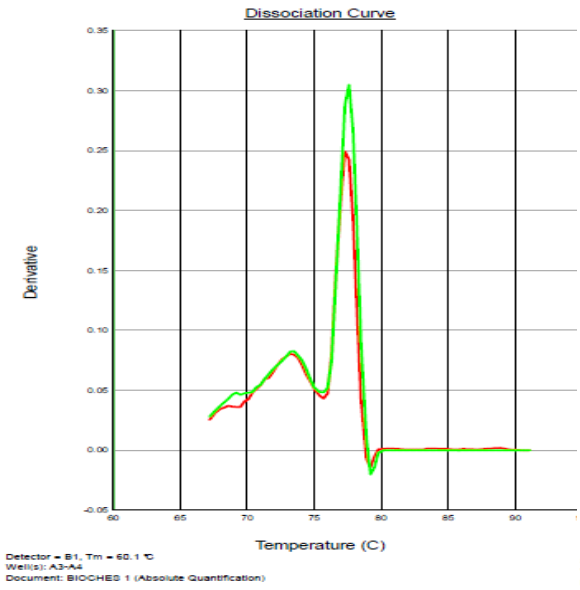

Fig. 1. Dissociation curve of HMG CoA Reductase

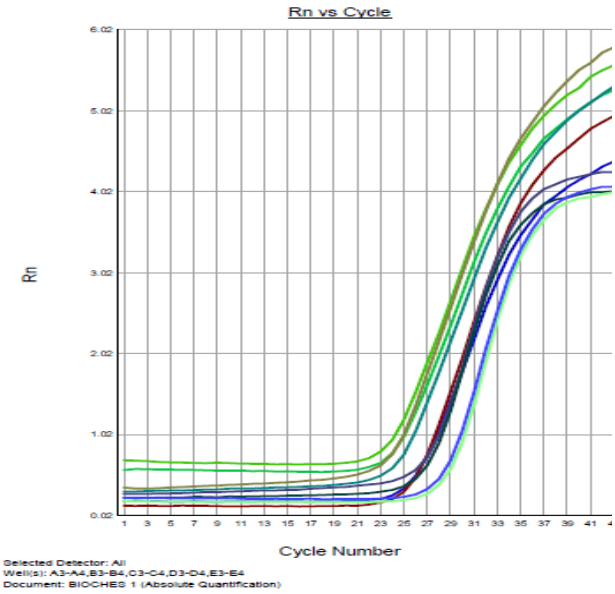

Fig. 2: Amplication curve of HMG CoA reductase

LDL-cholesterol in humans. Najmi *et al.* (2008) reported that after oral administration of *N. sativa* oil @ 2.5 ml twice daily for a period of 6 weeks there was significantly reduction upto 23% LDL-cholesterol levels and upto 15.2% VLDL-cholesterol levels in cardiac patients (Tasawer *et al.*, 2011). Kokdil *et al.* (2004) reported that administration of *N. unguicularis* fixed oil orally to Wistar Kyoto rats significantly decreased serum VLDL- cholesterol levels.

Gene expression of β-hydroxy, β-methylglutaryl coenzyme-A reductase (HMG CoA reductase) was less in all treatment groups in comparison to that of control group. The reduction pattern in gene expression was in the order of 4> 3> 2> 1 >0 g kg^{-1} of black cumin oil treated groups (Fig. 1 and 2). Ahmad and Beg (2013) found that the oral feeding of 100 mg methanolic or 20 mg volatile oil extract from cumin per rat/day significantly decreased the hepatic HMG-CoA reductase activity. Mitchell *et al.* (2013) reported diurnal variations of HMG-CoA reductase activities in turkey liver. Parallel diurnal variations for HMG-CoA reductase with maxima near 1000 hr, were demonstrated. Turkeys fed a diet containing 1% cholesterol for two weeks had reduced HMG-CoA reductase (97%).

Conclusion: The present study revealed that black cumin oil exerts the hypocholestremic effects in birds. The hypocholestremic effect is due to the reduction in the expression of HMG CoA reductase activity. However, black cumin oil @ 4g kg^{-1} diet is the most effective supplementation in reducing the serum lipid profile on 56 day of experiment, but after withdrawal of the oils these effects gradually decrease.

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