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EFFECT OF DIETARY SUPPLEMENTATION OF ACETONE EXTRACTS OF *NIGELLA SATIVA* L. SEEDS ON SERUM CHOLESTEROL AND PATHOGENIC INTESTINAL BACTERIAL COUNT IN BROILERS

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ABSTRACT

This study aimed to investigate effects of varying doses of *Nigella sativa* seed powder or acetone extracts in diet on feed intake, mortality, serum lipid profiles and population of intestinal microflora of broilers. A total 168, day-old broiler chicks (Cobb 500) were *ad-libitium* manually prepared feeds supplemented with 0, 1.5, 2.5, 3.0% seed powder or 0, 0.2, 0.4% acetone extracts of *N. sativa* seed for 4 weeks. The experiment was conducted in a complete randomized design (CRD) with seven treatments and three replications. *N. sativa* supplemented feed had no significant effects on feed intake, body weight and mortality rate of broiler. However, supplementation of either 3.0% seed powder or 0.4% extracts of *N. sativa* seeds significantly ($p<0.05$) decreased serum cholesterol and triglycerides contents in broiler. Furthermore, both *N. sativa* seed powder and extract supplemented feed also suppressed harmful bacterial (*Escherichia coli*) population in the feces. These results suggest that *N. sativa* seed might have potential as an alternative to hazardous synthetic feed additives (antibiotics) to formulate low cost and environment-friendly diet for the broiler.

Key words: Broiler, *Nigella sativa* seeds, Serum cholesterol, *Escherichia coli.*

INTRODUCTION

Feed additives have been widely used in poultry industry since long time as a tool to increase animals' performance in regard to growth, egg production, and feed efficiency (Collington *et al*. 1990). A number of feed additives including antibiotics have been widely used in the poultry industry for several decades. The use of antibiotics as feed additives is hazardous due to cross-resistance amongst pathogens and residues in tissues (Schwarz *et al*. 2001).

Considering the multifaceted detrimental effects, the use of antibiotic growth promoters has been banned in many countries including Bangladesh. Consequently, the use of antibiotics in poultry diets has been reduced. Natural feed additives of plant origin are generally believed to be safe, biodegradable, healthier, less hazardous than the synthetic chemicals. Therefore, research for alternative natural growth promoters from plants or their extracts is becoming more important due to their antimicrobial effects (Ramakrishna *et al*. 2003; Jang *et al*. 2004).

One of the alternatives to hazardous synthetic chemicals (antibiotics) is addition of aromatic plants and their extracts. *Nigella sativa* L. is an annual herbaceous aromatic plant belonging to the Ranunculaceae family, growing in countries bordering the Mediterranean Sea (Denli *et al*. 2004; Cheikh- Rouhou *et al*. 2007). The black seeds of *N. sativa* has been reported to have many biological properties including anti-parasitic (Mahmoud *et al*., 2002), anti-diabetic (Al-Hader *et al*. 1993), anticancer (Padhya *et al*. 2008) and diuretic effects (Zaoui *et al*. 2000). Antibacterial activity of *N. sativa* seed extracts has also been reported (Nair *et al*. 2005 and Islam *et al*. 2011). Positive impacts of supplementation of *N. sativa* seed powder to poultry diets have been reported (Akhtar *et al*., 2003). Supplementation of *N. sativa* seeds in diets of rats significantly decreases serum triglyceride and increase HDL level (Tayyab *et al*. 1995; Chaudary *et al*. 1996). However, scant information is available on the effect of *N. sativa* seed supplementation in diets on population of intestinal bacteria and performance of broilers. The mode of action of the beneficial effects of *N. sativa* seed supplemented poultry feed is also poorly understood. Therefore, to assess the beneficial effects and commercial potentials of *N. sativa* seeds as an alternative to antibiotic additives in poultry feed, a thorough investigation is needed in both layers and broilers.

In our previous study, we demonstrated that supplementation of seed powder of *N. sativa* significantly decreased egg cholesterol and suppressed population of *Escherichia coli* in the feces of laying hens (Islam *et al*. 2011). The objective of this study was to evaluate the effect of supplementation of varying doses of *N. sativa*

seed powder or its acetone extracts on feed intake, body weight, mortality, fat content, serum lipid profile and population of intestinal bacteria in the broilers.

MATERIALS AND METHODS

A. Preparation of *N. sativa* seed powder: *N. sativa* L. seeds were obtained from a local market of Dinajpur (Bangladesh). The seeds were coarsely powdered by a mechanical grinder and then directly mixed with manually prepared diets in appropriate doses (Tables 1, 2 and 3).

B. Extraction of *N. sativa* seeds: To obtain extracts, 3 kg coarsely powdered seeds of *N. sativa were* dissolved in 6.0 L acetone and were kept for 72 hours with occasional shaking for extraction of secondary metabolites. The acetone extract was filtered with cheese cloth followed by filter paper. The extract was dried by a vacuum rotary evaporator (HAHN SHIN HS-2005V-N) connected with HAHN water bath B-480 at 40º C. Stock solution of crude acetone extract was prepared by diluting with 10% aqueous ethanol and mixed with the manually prepared diet at 0.2 and 0.4% extracts in the feed.

C. Experimental birds and design: A total of 168, day-old broiler chicks (Cobb 500) were purchased from a local hatchery (CP Bangladesh Ltd., Konabari, Gazipur, Bangladesh). Initially the chicks were reared at brooding house up to 10 days to adjust with the environmental conditions. Then, the chicks were randomly assigned to different seven dietary treatment groups of 24 chicks each. Each treatment comprised of three replicates with eight birds in each in a completely randomized design. The composition of manually prepared experimental diet according to NRC, (1994) used in different treatments for the broiler is presented in Table 1, 2 and 3.

D. Parameters studied: The experimental birds were randomly assigned to diets and fed *ad-libitium*. During the experimental period (4-weeks), growth performance of the birds was evaluated. Body weight was measured for all birds at the beginning of the experiment, and it was repeated daily at the beginning of the week at the same time. Live weight gain was calculated by subtraction the live weight at the beginning of the week from the live body weight of the next week. Feed consumption is the amount of feed consumed every day; it was calculated for each treatment at daily basis. Then feed conversion ratio (FCR) was calculated every week at the amount of feed consumption per unit of body gain. Mortality was recorded throughout the study. Excreta at final week were collected and analyzed for culturable bacterial counts using some selective media. All the samples were cultured primarily in nutrient agar at 37°C for 24 h, and then subcultured onto the MacConkey agar, EMB agar and S-S agar by streak plate method to observe the morphology. Morphological characteristics (shape. size, surface texture, edge, elevation, color, opacity etc.) developed after 24 h of incubation were carefully studied as described by Marchant and Packer (1967).

E. Blood collection and serum lipid profile analysis: The blood samples from wing vein of each bird were collected at 2-weeks interval beginning at 1-week of feeding using sterilized syringes and needles (Islam *et al.* 2011). Each syringe with blood sample was kept at normal temperature in an inclined position. After 20 minutes, the blood serum was collected and centrifuged for 15 min at 2500 rpm. After centrifugation, the supernatant was carefully separated by a micropipette and preserved in an eppendorf vial. The collected serum was stored at -15ºC until determination of total cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides using lipid profile kit (Crescent Diagnostics).

F. Statistical analyses: The data thus collected were analyzed using analysis of variance technique through the MSTATC program according Kuehl (1994). The means were compared using Duncan's Multiple Range Test. Statements of statistical significance were based on $P < 0.05$.

RESULTS

A. Growth performances: The effect of varying levels of *N. sativa* seed powder on body weight, body weight gain, total feed intake, feed conversion ratio and mortality of broilers is shown in Table 4. Average body weight and body weight gain were improved by 3.0% dose of *N. sativa* seed supplementation in the diets, whereas, feed intake and feed conversion ratio decreased by 3.0% seed or 0.4% acetone extract of *N. sativa* supplemented diets compared with the control treatments. The mortality rate slightly decreased by supplementation of broiler-ration with *N. sativa* supplemented seed powder and their acetone extract.

B. Serum lipid profile: Effects of supplementation of *N. sativa* seed powder on the contents of serum cholesterol, HDL and triglycerides concentration in broilers at 2, 3, and 4-weeks are presented in Fig. 1. The results revealed serum cholesterol concentration significantly ($p<0.05$) differed among different treatments of seed powder and seed extracts (Fig. 1-A). Cholesterol contents in blood serum remained statistically unchanged by dietary supplementation of seed powder up to 2-weeks and then significantly decreased irrespective of doses of seed powder or extracts than those of control. The lowest serum cholesterol content was recorded in broilers fed 0.4% acetone extract supplemented diet until 4-weeks.

Serum triglycerides content did not affect by feeding either *N. sativa* seed powder or acetone extracts supplemented until 3-weeks (Fig. 1-B). However, feeding seed powder or seed extracts significantly ($p<0.05$) decreased serum triglycerides irrespective of supplemental levels.

Almost no significant differences in blood serum HDL content were recorded by the treatments of *N. sativa* seed powder or acetone extracts (Fig. 1-C). Although acetone extract at 0.4% significantly decreased after 2-weeks treatment, however, statistically no differences were observed at 4-weeks treatment irrespective of the inclusion levels of the extract.

C. Bacterial counts in use feces: Figure 2 shows the effect of different dietary levels of *N. sativa* seed powder on *Escherichia coli* and total viable bacterial count in excreta of broilers. Both *E. coli* and total bacterial counts were significantly ($p<0.05$) decreased by *N. sativa* seed powder supplemented diets irrespective of inclusion levels. Interestingly, broilers those fed control diets without addition of any seed powder also had significantly lower number of *E. coli* and total viable bacteria than those of control. Supplementation of seed powder at 1.5% seed powder significantly suppressed bacterial population in feces than control and then remained statistically unchanged with increasing doses of seed powder (Fig. 2- A)

Acetone extract of *N. sativa* seed supplementation in diets also showed almost similar effects on both *E. coli* and total culturable bacterial counts in the feces of broilers (Fig. 2-B). Seed extracts as low as 0.2% significantly suppressed the population of *E. coli* as well as total cultural bacterial counts in the feces.

Table 1. Ingredients and chemical composition of the experimental broiler starter diets

Items	Dietary level of *N. sativa, %*					
	T_0	T_2	T_3	T_4	T_5	T_6
***Feed Ingredients* (%)**						
Maize	49.60	49.60	49.60	49.60	49.60	49.60
Soybean meal	26.35	25.64	25.16	24.92	25.64	24.92
Rice polish	10.00	10.00	10.00	10.00	10.00	10.00
Meat and bone meal	8.00	8.00	8.00	8.00	8.00	8.00
Soybean Oil	3.50	3.00	2.73	2.57	3.00	2.57
Lime stone	0.85	0.85	0.85	0.85	0.85	0.85
DCP	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	0.12	0.12	0.12	0.12	0.12	0.12
Broiler premix	0.25	0.25	0.25	0.25	0.25	0.25
Toxin binder	0.30	0.30	0.30	0.30	0.30	0.30
Choline chloride	0.07	0.07	0.07	0.07	0.07	0.07
Coccidiostate	0.05	0.05	0.05	0.05	0.05	0.05
Lysine	0.01	0.01	0.01	0.01	0.01	0.01
Enzyme	0.05	0.05	0.05	0.05	0.05	0.05
Growth promoter	0.06	0.06	0.06	0.06	0.06	0.06
N. sativa seed	0.00	1.50	2.50	3.00	1.50	3.00
Calculated composition/ Kg feed						
ME, kcal/kg	3084	3106.5	3124.5	3133	3106.5	3133
Crude protein, %	21.40	21.35	21.30	21.28	21.35	21.28
Crude fibre, %	3.77	3.71	3.78	3.78	3.71	3.78
Ca, %	1.11	1.12	1.12	1.13	1.12	1.13
P, %	0.54	0.54	0.55	0.55	0.54	0.55
Methionine, %	0.48	0.48	0.48	0.48	0.48	0.48
Lysine, %	1.19	1.19	1.18	1.18	1.19	1.18

Table 2. Ingredients and chemical composition of the experimental broiler starter diets

Items	Dietary level of *N. sativa, %*					
	T_0	T_2	T_3	T_4	T_5	T_6
***Feed Ingredients* (%)**						
Maize	52.00	52.00	52.00	52.00	52.00	52.00
Soybean meal	22.70	21.98	21.50	21.26	21.98	21.26
Rice polish	12.00	12.00	12.00	12.00	12.00	12.00

Meat and bone meal	7.00	7.00	7.00	7.00	7.00	7.00
Soybean Oil	3.50	3.00	2.73	2.57	3.00	2.57
Lime stone	1.00	1.00	1.00	1.00	1.00	1.00
DCP	0.80	0.80	0.80	0.80	0.80	0.80
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.10	0.10	0.10	0.12
Broiler premix	0.25	0.25	0.25	0.25	0.25	0.25
Toxin binder	0.30	0.30	0.30	0.30	0.30	0.30
Choline chloride	0.06	0.06	0.06	0.06	0.06	0.06
Coccidiostate	0.02	0.02	0.02	0.02	0.02	0.02
Lysine	0.01	0.01	0.01	0.01	0.01	0.01
Enzyme	0.05	0.05	0.05	0.05	0.05	0.05
Growth promoter	0.05	0.05	0.05	0.05	0.05	0.05
N. sativa seed	0.00	1.50	2.50	3.00	1.50	3.00
Calculated composition/ Kg feed						
ME, kcal/kg	3096	3118.5	3137.5	3143	3118.5	3143
Crude protein, %	19.66	19.58	19.52	19.57	19.58	19.57
Crude fibre, %	3.77	3.78	3.79	3.80	3.78	3.80
Ca, %	1.15	1.16	1.17	1.17	1.16	1.17
P, %	0.56	0.57	0.57	0.57	0.57	0.57
Methionine %	0.43	0.43	0.43	0.43	0.43	0.43
Lysine %	1.06	1.06	1.05	1.05	1.06	1.05

Table 3. Ingredients and chemical composition of the experimental broiler starter diets

Items	**Dietary level of *N. sativa, %***					
	T_0	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**
***Feed Ingredients* (%)**						
Maize	55.00	55.00	55.00	55.00	55.00	55.00
Soybean meal	21.50	20.78	20.30	20.06	20.78	20.06
Rice polish	10.70	10.70	10.70	10.70	10.70	10.70
Meat and bone meal	6.00	6.00	6.00	6.00	6.00	6.00
Soybean Oil	3.50	3.00	2.73	2.57	3.00	2.57
Lime stone	1.00	1.00	1.00	1.00	1.00	1.00
DCP	0.70	0.70	0.70	0.70	0.70	0.70
Salt	0.27	0.27	0.27	0.27	0.27	0.27
Methionine	0.08	0.08	0.08	0.08	0.08	0.08
Broiler premix	0.25	0.25	0.25	0.25	0.25	0.25
Toxin binder	0.30	0.30	0.30	0.30	0.30	0.30
Choline chloride	0.05	0.05	0.05	0.05	0.05	0.05
Coccidiostate	0.05	0.05	0.05	0.05	0.05	0.05
Lysine	0.02	0.02	0.02	0.02	0.02	0.02
Enzyme	0.05	0.05	0.05	0.05	0.05	0.05
Growth promoter	0.065	0.065	0.065	0.065	0.065	0.065
N. sativa seed	0.00	1.50	2.50	3.00	1.50	3.00
Calculated composition/ Kg feed						
ME, kcal/kg	3120	3142.5	3130.5	3169	3142.5	3169
Crude protein, %	18.85	18.76	18.69	18.66	18.76	18.66
Crude fiber, %	3.69	3.70	3.71	3.71	3.70	3.71
Ca, %	1.06	1.07	1.07	1.08	1.07	1.08
P, %	0.51	0.50	0.52	0.52	0.50	0.52
Methionine %	0.40	0.40	0.40	0.40	0.40	0.40
Lysine %	1.01	1.00	1.00	0.99	1.00	0.99

Added broiler premix (Renata Animal Health Ltd.) @ 250 g per 100 kg which contained: vitamin A: 4800 IU; vitamin D: 960 IU; vitamin E: 9.2 mg; vitamin k_3: 800 mg; vitamin B_1: 600 mg; vitamin B_2: 2 mg; vitamin B_3: 12 mg; vitamin B_5: 3.2 mg; vitamin B_6: 1.8 mg; vitamin B_9: 2 mg; vitamin B_{12}: 0.004 mg; Co: 0.3 mg; Cu: 2.6 mg; Fe: 9.6 mg; I: 0.6 mg; Mn: 19.2 mg; Zn: 16 mg; Se: 0.48 mg; DL – Methionine: 20 mg; L- lysine:12 mg.

Table 4. Effect of dietary supplementation of *Nigella sativa* L. on growth performances of broilers

Parameters	*Nigella sativa* supplemented diets[1], %						
	CD*	0	1.5	2.5	3.0	0.2 Extract	0.4 Extract
Average body weight(g)	1996.57	2032.27	1892.53	1851.62	2045.24	1983.84	2016.29
Average weight gain(g)	1718.47	1760.27	1614.83	1576.82	1784.74	1716.14	1765.19
Total feed intake(g)	3054.58	3135.83	3120.41	3125.00	3038.75	3118.75	3047.08
Feed conversion ratio	1.77	1.78	1.93	1.98	1.70	1.81	1.72
Mortality (%)	0.00	8.00	0.00	4.00	4.00	0.00	0.00

* Commercial diet

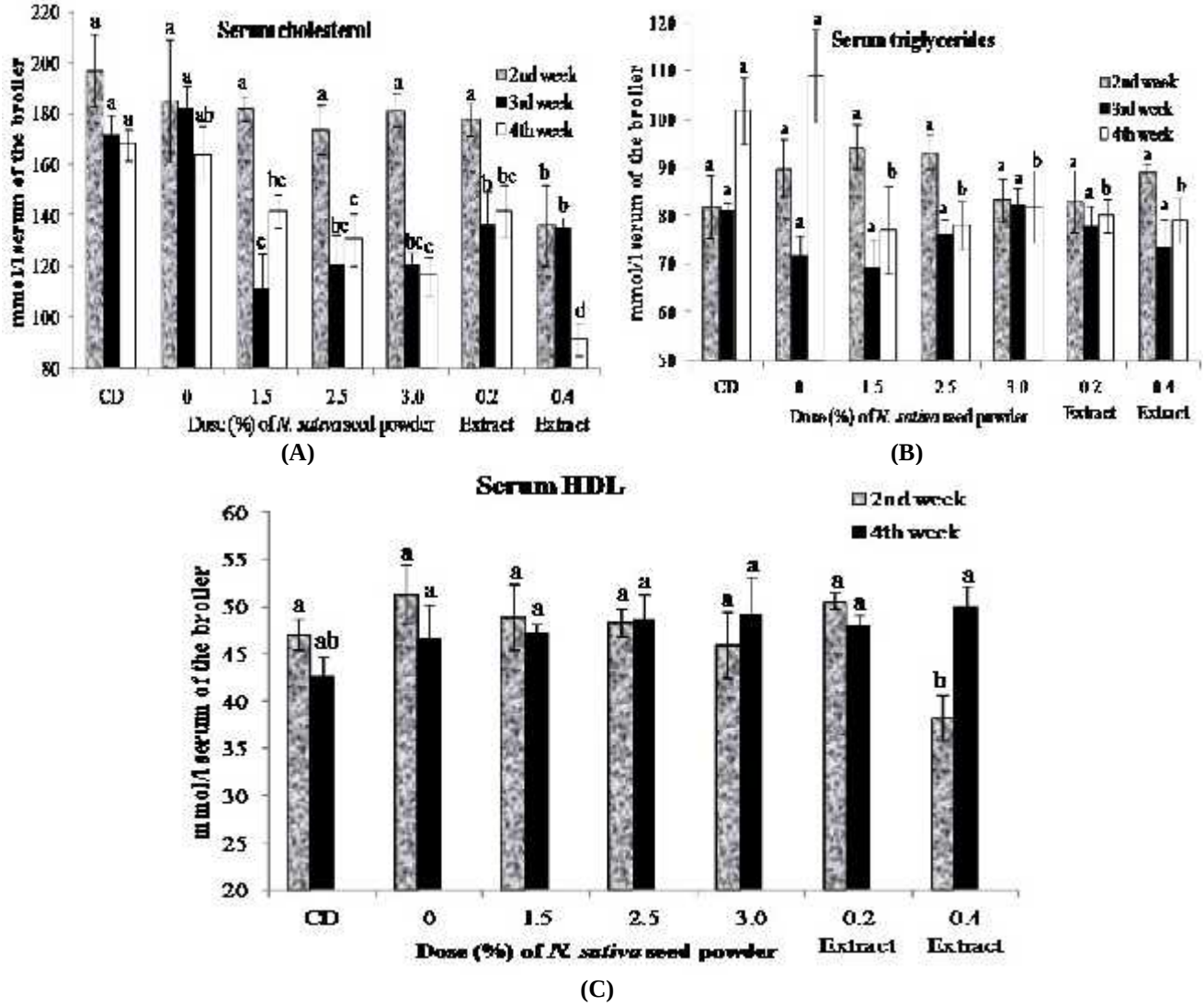

Fig. 1: Effects of varying levels of *Nigella sativa* L. seed powder or seed extracts supplementation in diets on (A): serum cholesterol; (B): serum triglyceride and (C): serum HDL (mmol/l) of broiler chicks at 2, 3, and 4-weeks of feeding. The data represent the average ± standard error of at least three replications each of which has 8 birds. Data points bearing different letters are significantly different at p<0.05. CD, commercial diet.

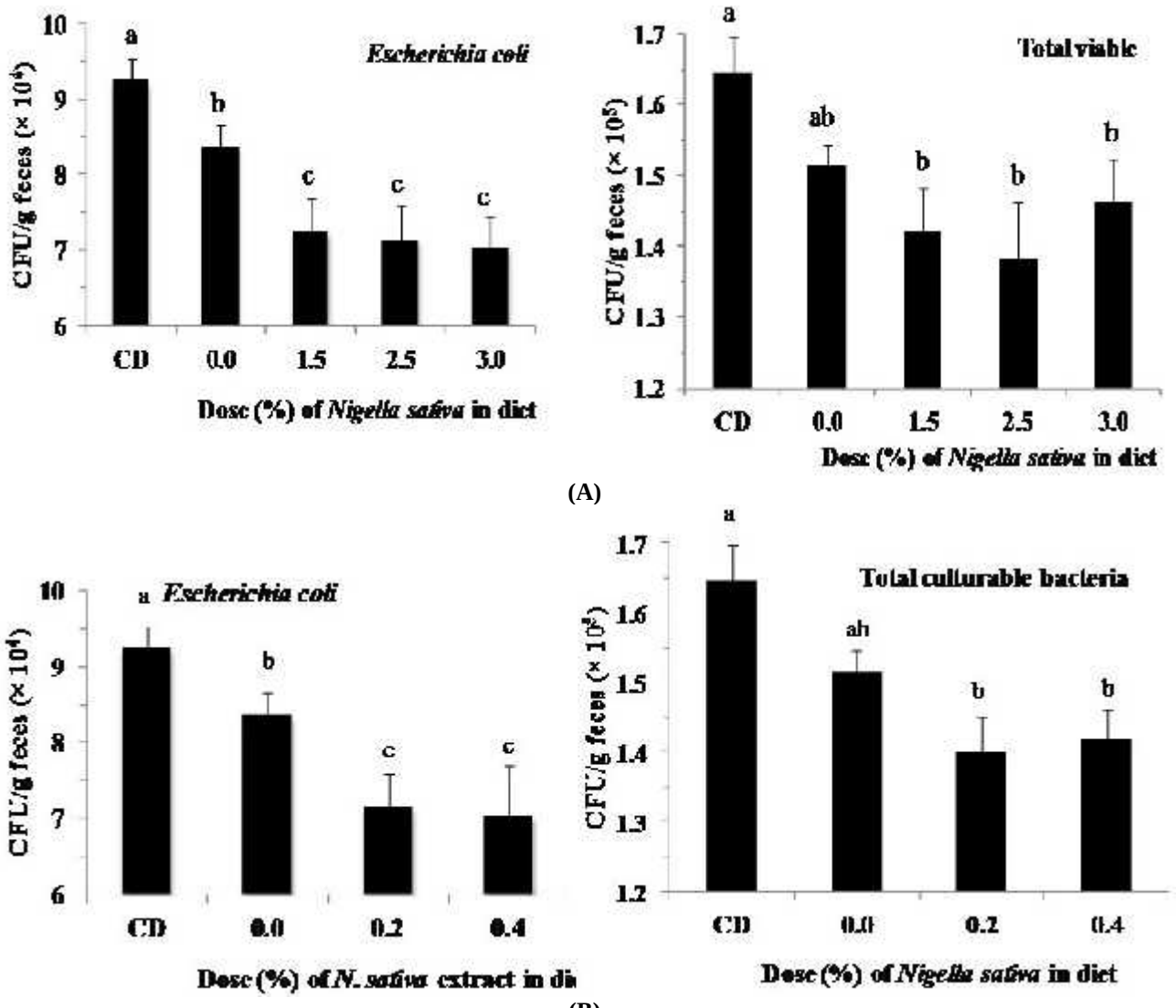

Fig. 2: Effects of varying levels of *Nigella sativa* seed extracts supplemented diets on *Escherichia coli* and culturable bacteria (total viable) in the excreta of broiler chicks at 4 weeks of feeding. (A): *Nigella sativa* seed powder supplemented diets; (B) *Nigella sativa* seed extract supplemented diets. The data are the average ± standard error of at least three replications each of which 8 birds. Data points bearing different letters are significantly different at p< 0.05. CFU, colony forming unit.

DISCUSSION

In this study, the effects of *N. sativa* seed and acetone extract supplemented diets on average body weight, body weight gain, total feed intake, feed conversion ratio, mortality, fat content, lipid profile in blood serum and population of intestinal bacteria of broilers (Cobb 500) were investigated. Both *N. sativa* seed and acetone extract supplemented diets significantly decreased cholesterol and triglyceride contents in blood and suppressed the number of harmful bacteria such as *E. coli* in the feces of the broilers without affecting growth and other parameters studied (Table 4; Fig. 1,2). Taken together, these results suggest that supplementation of *N. sativa* seeds or seed extracts to diets could be considered as an alternative natural growth promoter to hazardous synthetic antibiotics for safe poultry meat production. In our previous study, we observed that supplementation of *N. sativa* seed powder in diets significantly decreased population of *E. coli* in the feces of layers (Islam *et al*. 2011). The findings of our research on the effect of *N. sativa* seeds in poultry diets were more or less in agreement with the findings of some earlier researchers (Ramakrishna *et al*. 2003; Jang *et al*. 2004; Ziad *et al*. 2008). As both seed powder and acetone extracts showed almost identical results on decrease of serum lipid profile and number of *E. coli* in feces of the broilers, it can be

suggested that secondary metabolites present in the seeds might be responsible for these bioactivities of the popular medicinal herb. A further bioassay-guided should be useful to elucidate the active principle of acetone extract of *N. sativa* seed.

It has been reported that substitution of soybean meal by *N. sativa* meal at 3.0% in broiler diets significantly increased body weight and body weight gain (Abdel-Mageed, 2002). Mehmet *et al*. (2008) found that feed consumption reduced linearly by increasing doses of black seed extract in 0 to 12 weeks of age. However, in the present study, *N. sativa* supplemented diets had no significant effect on average body weight, average body weight gain and total feed intake up to 4.0%. Our findings are in full agreement with those of Durrani *et al.* (2007), where they observed improved broiler performance by supplementation of 2 and 4 % *N. sativa* seeds. Contrarily, Ismail (2011) reported that both *N. sativa* seeds and their extracts supplemented diet increased ($P<0.05$) feed intake compared to the control diet. But feed intake was not significantly different between the *N. sativa* seeds and their extracts.

One of the interesting findings of our study is that *N. sativa* seed supplemented diets significantly decreased blood serum cholesterol and triglycerides but increased HDL contents compared with control (Fig. 1). Akhtar *et al.* (2003) also observed that serum triglycerides and total cholesterol contents were reduced, while serum high density lipoprotein cholesterol level was increased. This could probably be attributed to the possible cholesterol lowering mechanisms of tocopherols explored in number of research investigations i.e. like inhibition of cholesterol oxidation (Xu *et al*. 2001) and reduced HMG-CoA-reductase activity (Qureshi *et al*. 2002; Ha *et al*. 2005). Although mechanism of blood cholesterol lowering effect of *N. sativa* seed powder is not clearly understood from our study, however, Martin *et al*. (2001) and Torra *et al.*(2001) hypothesized that cholesterol lowering mechanism of *N. sativa* seed oil is dependent on Peroxisome Proliferator-Activited Receptor (PPARα) activation. The mode of action of cholesterol reduction associated with consumption of fixed and essential oils of *N. sativa* seed is multidimensional. The fixed oil of *N. sativa* seed is rich in polyunsaturated fatty acids which mainly accounts for cholesterol lowering potential (Cheikh-Rouhou *et al*. 2007; Atta 2003; Ramadan 2007).

Another important finding of our research is the suppression of number of *E. coli* in the feces of broilers (Fig. 2). Intestinal bacteria play an important role in the health status of host animals including poultry. Therefore, a common approach to maintain host health is to increase the number of desirable bacteria (e.g. probiotics) in order to inhibit colonization of invading pathogens (Guo *et al*. 2004). In the present study, supplementation of both seed powder and acetone extracts of *N. sativa* in the diet of broilers significantly ($P<0.05$) decreased the harmful bacterial count of *E. coli*, as well as total culturable bacteria (Fig. 2). Our results are in agreement with those observed by Alsawaf and Alnaemi, (2011) and Islam *et al*. (2011). Our current findings and previously described results suggest that herbal feed additives might be an effective alternative to synthetic antibiotics for the promotion of health and performance of poultry (Cross *et al*. 2007; Islam *et al*. 2011).

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