

Dietary effects of buckwheat (*Fagopyrum esculentum*) and black cumin (*Nigella sativa*) seed on growth performance, serum lipid profile and intestinal microflora of broiler chicks

M.S. Islam¹, M.N. Siddiqui², M.A. Sayed^{3,4#}, M. Tahjib-Ul-Arif¹,
M.A. Islam⁵ & M.A. Hossain¹

¹ Department of Biochemistry and Molecular Biology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

² Department of Biochemistry and Molecular Biology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706, Bangladesh

³ The United Graduate School of Agricultural Sciences, Iwate University, 3-18-8 Ueda, Morioka, Iwate 020-8550 Japan

⁴ Department of Biochemistry and Molecular Biology, Hajee Mohammad Danesh Science & Technology University, Dinajpur-5200, Bangladesh

⁵ Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

(Received 26 November 2015; Accepted 24 January 2016; First published online 30 March 2016)

Copyright resides with the authors in terms of the Creative Commons Attribution 2.5 South African Licence.

See: <http://creativecommons.org/licenses/by/2.5/za>

Condition of use: The user may copy, distribute, transmit and adapt the work, but must recognise the authors and the South African Journal of Animal Science.

Abstract

The study was conducted to investigate the effects of different levels of buckwheat seed (BWS) with black cumin seed (BCS) supplementation on the performance, serum lipid profile and intestinal bacterial flora in broiler chicks. One hundred and twenty day-old Cobb-500 broiler chicks were randomly allotted equally to four experimental groups, designated T₁ (untreated control, no BWS and BCS); T₂ (10% BWS + 1.5% BCS); T₃ (20% BWS + 2.5% BCS); and T₄ (30% BWS + 3.5% BCS), respectively. The study lasted for 30 days. Average bodyweight, weight gain, total feed intake, feed conversion ratio (FCR), and serum lipid profile (serum total cholesterol, HDL-cholesterol, triglycerides) were determined and intestinal bacterial flora (total viable bacteria, *Escherichia coli*, *Salmonella* and *Lactobacillus* sp.) were counted. The results of the study showed that BWS and BCS significantly improved final bodyweight gain of group T₂ compared with the control group. Higher levels of buckwheat and black cumin did not improve growth performance of the chicks. Serum cholesterol and triglyceride concentrations significantly decreased with an elevation of HDL-cholesterol concentration as the level of BWS and BCS increased. In BWS diets supplemented with BCS pathogenic bacteria, *E. coli* and *Salmonella* spp. were suppressed. These findings suggest that 10% BWS with 1.5% BCS supplementation to broiler ration could be considered an alternative to hazardous synthetic antibiotics for safe poultry production.

Keywords: Antibiotic, intestinal bacteria, organic feed, poultry, serum metabolites

Corresponding author: sayed_bmb@yahoo.com

Introduction

Feed additive antibiotics reduce the cost and improve the quantity of food production through more efficient use of natural resources, which is critical to meet the escalating nutritional needs of a growing world human population. Although good results are obtained with these substances, the risk of the development of direct antibiotic resistance of pathogens in the species receiving the feed, as well as indirect resistance to similar antibiotics used in human medicine as the result of food chain residues, led to the ban of all sub-therapeutic levels of growth-promoting antibiotics by the European Union (Council of the European Union, 1970) and many other countries, including Bangladesh. As this may negatively affect the profitability of poultry farming, alternative substances and strategies for growth promotion and disease prevention are being investigated, among which phyto-genic and herbal products have received increased attention, since they have acquired acceptability among consumers as natural additives (Toghyani *et al.*, 2010). In previous studies the authors found that phyto-genic feed additives of plant origin normally proved safe, healthy, eco-friendly, cost effective, not directly associated with problems and less hazardous than synthetic feed additives in poultry production (Islam *et al.*, 2011; Rahman *et al.*, 2013; Islam *et al.*, 2014; Sayed *et al.*, 2015; Siddiqui *et al.*, 2015).

One of the alternatives to synthetic antibiotic feed additives in poultry feed could be the seeds of the much-acclaimed herbal medicinal plant, black cumin (*Nigella sativa* L.). Black cumin is an annual herb of the Mediterranean region and belongs to the family Ranunculaceae. The main active components of black cumin seed include thymoquinone, thymohydroquinone, dithymoquinone, thymol and carvacrol, which are important pharmacologically active substances (Nasir *et al.*, 2005; Al-Saleh *et al.*, 2006). The seeds have been reported to have many biological properties, including antiparasitic, antidiabetic, anticancer (Padhye *et al.*, 2008) and diuretic (Zaoui *et al.*, 2000) effects. Antibacterial and immunostimulant effects of BCS have also been reported (Khan *et al.*, 2012).

Another plant of nutritional and medicinal importance is buckwheat (*Fagopyrum esculentum* Moench), an ancient dicotyledonous crop that belongs to the family Polygonaceae. Its medicinal properties are due the presence of bioactive compounds such as flavones, flavonoids, phytosterols, tocopherols, inositol phosphates, rutin and myo-inositol (Zhang *et al.*, 2012). Additionally, buckwheat is rich in unsaturated fatty acids. Products made from buckwheat are efficient in lowering blood cholesterol concentrations, particularly of low density lipoproteins (LDL) and very low density lipoproteins (VLDL) (Tomotake *et al.*, 2006), which are considered advantageous in preventing human disorders such a cardiovascular diseases, hypercholesterolemia and hypertension (Karamac, 2010).

In previous studies the authors observed that BCS-supplemented diets improved broilers' productive performance such as weight gain, feed intake, feed conversion ratio (FCR) and survivability, and at the same time suppressed harmful intestinal bacteria (Islam *et al.*, 2011; Siddiqui *et al.*, 2015). Similarly, the researchers found that BWS-supplemented diets in broiler feed significantly improved growth performance, increased serum high-density lipoproteins (HDL-cholesterol) and reduced serum total cholesterol, LDL cholesterol and triglyceride concentrations (Sayed *et al.*, 2015). The antimicrobial effect of buckwheat has also been reported by Swiatecka *et al.* (2013). Although several reports have focused on the effects of supplementation of BCS or BWS in various animals, including poultry, none of the studies have evaluated the combined effect of BWS and BCS in broiler diets. Considering the medicinal advantages of BWS and BCS (above), the current study was designed to evaluate the combined effects of BWS and BCS on growth performance, serum biochemical metabolites and intestinal bacterial flora in broiler chickens.

Materials and Methods

One hundred and twenty day-old broiler chicks (Cobb-500) were purchased from a local hatchery. Initially, the chicks were reared in a brooding house for up to 10 days to adjust to environmental conditions. Then the birds were weighed and randomly assigned to five treatment groups of 24 chicks each. Each treatment consisted of three replicates of eight birds (four male and four female) each in a completely randomized design. Birds were provided ad libitum access to feed and water. Care and management of the birds adhered to the accepted guidelines (FASS, 2010). Chicks were vaccinated against Newcastle and Gambaro disease on the fourth and twentieth days, and were examined regularly for abnormal clinical signs (restlessness, lordosis, abnormal gait, vices and depression) as well as feed intake throughout the experiment. Throughout the study, the birds were housed according to standard temperature regimens, gradually decreasing from 32 °C to 24 °C.

A three-phase feeding programme was used, with a starter diet from days 1 to 10, a grower diet from days 11 to 20, and a finisher diet from days 21 to 40. All diets were formulated to meet or exceed the nutrient requirements of broilers, provided by National Research Council (NRC, 1994). Buckwheat seed (BWS) and black cumin seed (BCS) were obtained from a local market. The seeds were coarsely powdered with a mechanical grinder and then mixed directly with manually prepared diets. The birds were allocated to four treatments, and were fed a manually formulated control diet (no BWS and BCS) (T₁) or manually formulated diets supplemented with 10% BWS + 1.5% BCS (T₂), 20% BWS + 2.5% BCS (T₃) and 30% BWS + 3.5% (T₄). The experiment started on the tenth day and lasted a further 30 days. The composition of the grower and finisher diets used in the treatments is presented in Table 1.

Initial bodyweights of the birds were measured before starting the treatments. During the experimental period, growth performance was evaluated. Feed consumption and bodyweight gain were recorded regularly and FCR (feed intake/weight gain) was calculated.

Blood samples were collected from randomly selected birds of each replication at 15 days and at the end of the study (at day 30) by wing vein puncture with sterilized syringes and needles (Islam *et al.* 2011). These samples were centrifuged at 3000 rpm for 10 minutes to obtain serum. Individual serum samples were analysed for total cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides concentrations, using the kit package manufactured by Crescent Diagnostics (K.S.A.).

Faecal samples of chickens were collected from healthy broilers during the last week of the experiment. Fifteen birds (one bird from each replication) were slaughtered, and faecal samples were collected from the large intestine of each bird with sterilized surgical instruments. Microbial populations were

determined by serial dilution of faecal samples. Plate count agar, eosin methylene blue agar, *salmonella*-shigella agar and *Lactobacillus* MRS agar media were used to determine total viable bacteria, *E. coli*, *Salmonella* sp. and *Lactobacillus* sp., respectively. The inoculated media were marked and incubated at 37 °C for 24 - 48 h. The number of colonies (30 - 300) in a particular dilution was multiplied by the dilution factor and expressed as mean $\log_{10} \pm$ SD CFU/g. Colony characteristics such as shape, size, surface texture, edge and elevation and colour developed onto selective media after 24 - 48 h of incubation at 37 °C were recorded as described by Marchant & Packer (1967).

Table 1 Ingredients and chemical composition of the grower diets fed to experimental broilers

Items	Dietary level of buckwheat (BW) and black cumin (BC)							
	11 - 20 days (Grower diet)				21 - 40 days (Finisher diet)			
	T ₁	T ₂	T ₃	T ₄	T ₁	T ₂	T ₃	T ₄
Feed Ingredients (%)								
Maize	51.9	41.9	31.9	21.9	54.9	44.9	31.9	21.9
Soybean meal	22.5	21.57	20.87	20.02	20.9	19.9	20.87	20.02
Rice polish	10.0	10.0	10.0	10.0	7.98	7.98	7.98	7.98
Meat & bone meal	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Protein concentrate	5.8	5.5	5.2	4.9	6.0	5.8	5.8	5.6
Soybean oil	3.27	3.0	3.0	3.0	3.3	3.0	3.0	3.0
Lime stone	0.5	0.5	0.5	0.55	0.8	0.8	0.8	0.80
Di-calcium P	1.0	1.0	1.0	1.1	1.0	1.0	1.0	1.1
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Methionine	0.1	0.1	0.1	0.1	0.08	0.08	0.08	0.08
Broiler premix	0.25	0.25	0.25	0.25	0.32	0.32	0.32	0.32
Toxin binder	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Coccidiostats	0.02	0.02	0.02	0.02	-	-	-	-
Lysine	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01
Enzyme	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Buckwheat seed	0.0	10.0	20.0	30.0	0.0	10.0	20.0	30.0
Black cumin seed	0.0	1.5	2.5	3.5	0.0	1.5	2.5	3.5
Calculated composition								
MJ/kg	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0
Crude protein, g/kg	210	212	213	214	205	207	208	209
Crude fibre, g/kg	45.0	46.3	47.7	49.1	4.40	4.52	4.65	4.72
Calcium, g/kg	11.9	11.8	11.7	11.9	1.30	1.30	1.30	1.31
Phosphorus, g/kg	4.0	3.9	3.8	3.9	0.38	0.38	0.37	0.38
Methionine, g/kg	4.7	4.7	4.7	4.7	0.45	0.45	0.45	0.45
Lysine, g/kg	9.9	10.2	10.5	10.8	0.95	0.98	1.01	1.02

Added broiler premix (Renata Animal Health Ltd.) contained: vitamin A: 4800 IU; vitamin D: 960 IU; vitamin E: 9.2 mg; vitamin K₃: 800 mg; vitamin B₁: 600 mg; vitamin B₂: 2 mg; vitamin B₃: 12 mg; vitamin B₅: 3.2 mg; vitamin B₆: 1.8 mg; vitamin B₉: 2 mg; vitamin B₁₂: 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. Enzyme: phytase (Renata Animal Health Ltd. Bangladesh).

All data were analysed with the SPSS statistical analysis programme (version 17.0). A *P*-value of <0.05 was considered to indicate a significant difference between groups, and a comparison of means was made using Duncan's multiple range test (Steel & Torrie, 1984). The results are expressed as average \pm

standard deviation of three replications ($n = 3$). Data points bearing different letters are significantly different ($P < 0.05$).

Results

The effect of different dietary treatments on bodyweight gain, total feed intake and FCR of broilers are shown in Table 2. Bodyweight gain, total feed intake and FCR in various treatment groups were statistically significant ($P < 0.05$). Bodyweight gain of broilers receiving 10% BWS + 1.5% BCS was statistically higher compared with the other treatments. Total feed intake and FCR of chicks receiving diet with BWS and BCS increased too.

Table 2 Growth performances of broiler chickens fed various experimental diets for 30 days

Parameters	Treatments			
	Untreated control (T ₁)	10% BWS + 1.5% BCS (T ₂)	20% BWS + 2.5% BCS (T ₃)	30% BWS + 3.5% BCS (T ₄)
Body weight gain (g)*	1691.6 ^b	1827.6 ^a	1640.5 ^b	1647.2 ^b
Total feed intake (g)	3034.0 ^b	3276.6 ^a	3237.6 ^a	3231.0 ^a
FCR**	1.79 ^b	1.79 ^b	2.0 ^a	2.0 ^a

BWS: buckwheat seed; BCS: black cumin seed.

*Body weight gain (g): final body weight at 40th day (g) - initial body weight at 10th day (g).

**FCR: feed conversion ratio (total feed intake (g)/body weight gain (g)).

The effects of the experimental diet containing BWS and BCS on serum total cholesterol, HDL-cholesterol and triglycerides concentrations of broilers at the 15th and 30th days of treatment are presented in Figure 1. The results showed that BWS diet supplemented with BCS significantly improved ($P < 0.05$) these biochemical parameters.

Serum total cholesterol concentration of chicks fed BWS and BCS decreased ($P < 0.05$) compared with the control groups at the 15th and 30th days of treatment (Figure 1-A). After 15 days, there was no significant difference between the levels of BWS and BCS supplementation, but at the end (30th day) they differed significantly ($P < 0.05$). The lowest value (60.33 mg/dL) was recorded in birds fed 20% BWS supplemented with 2.5% BCS (T₃). HDL-cholesterol concentrations were significantly higher ($P < 0.05$) in birds receiving diets with BWS and BCS (Figure 1-B). Values were almost non-significant up to 15 days and then increased significantly, irrespective of levels of BWS and BCS, than those of the control. Broilers receiving 10% BWS supplemented with 1.5% BCS (T₂) diet had the highest HDL-cholesterol concentration (42.7 mg/dL).

The reducing tendency ($P < 0.05$) of serum triglycerides levels was also observed in the diets of BWS and BCS compared with the control (Figure 1-B). The lowest concentration of triglycerides was recorded (65.2 mg/dL) in 10% BWS supplemented with 1.5% BCS (T₂) diet. However, the differences observed among the levels of BWS and BCS did not show statistical significance.

Figure 2 shows the effects of varying levels of BWS and BCS-supplemented diets on the population of total viable bacteria, *Escherichia coli*, *Salmonella* sp. and *Lactobacillus* sp. in faeces of broilers. Buckwheat and supplementation of black cumin seed powder in the diets decreased ($P < 0.05$) in the population of harmful bacteria, *Escherichia coli*, *Salmonella* sp. and total viable bacteria than in the control (no BWS and BCS). Total viable bacteria were found to be decreased from Log₁₀ 11.73 CFU/g (T₀) to Log₁₀ 10.90 CFU/g (T₄), *E. coli* from Log₁₀ 9.18 CFU/g (T₁) to Log₁₀ 9.09 CFU/g (T₄) and *Salmonella* sp. from Log₁₀ 9.17 CFU/g (T₁) to Log₁₀ 9.07 CFU/g (T₄). Although the population of beneficial bacterium, *Lactobacillus* sp. was increased in the BWS and BCS groups, these increments were not significantly different from the control.

Discussion

In previous studies, the authors observed that supplementation of BWS and BCS powder in diets significantly decreased serum total cholesterol, triglycerides and LDL-cholesterol concentrations, and the population of *E. coli* in broilers and layers (Islam *et al.*, 2011; Sayed *et al.*, 2015; Siddiqui *et al.*, 2015). The findings of our current research are more or less in accordance to the findings of some earlier researchers (Guler *et al.*, 2006; Ziad *et al.*, 2008; Majeed *et al.*, 2010). The current findings and earlier results (Islam

et al., 2011; Sayed *et al.*, 2015; Siddiqui *et al.*, 2015) suggest that herbal feed additives might be an effective alternative to synthetic antibiotics for the promotion of health and performance of poultry.

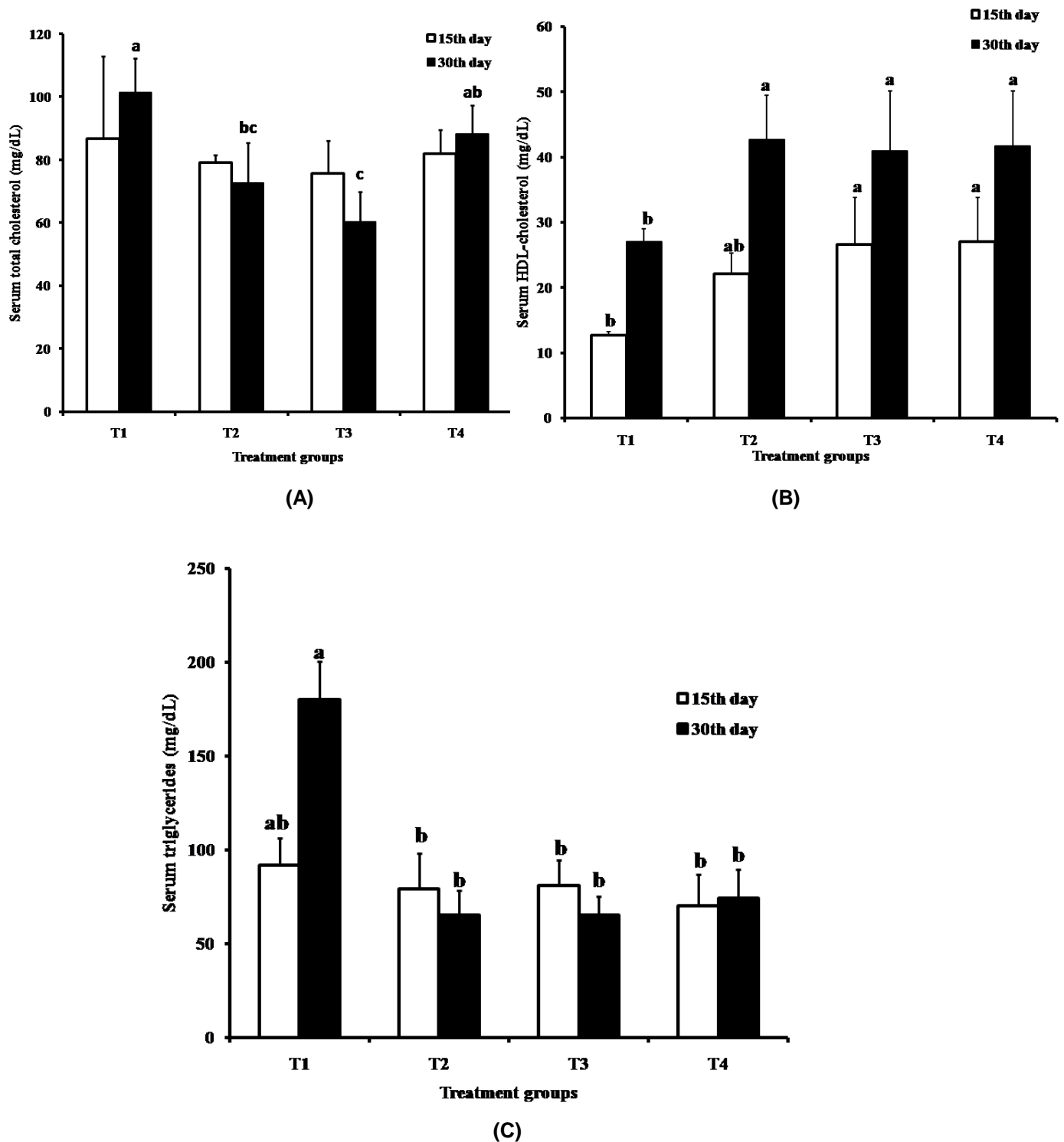


Figure 1 Serum total cholesterol (A), HDL-cholesterol (B), and triglycerides (C) concentrations of broiler chicks fed with an untreated control feed (T₁), 10% BWS + 1.5% BCS (T₂), 20% BWS + 2.5% BCS (T₃), 30% BWS + 3.5% BCS (T₄) at 15th and 30th day of treatment.

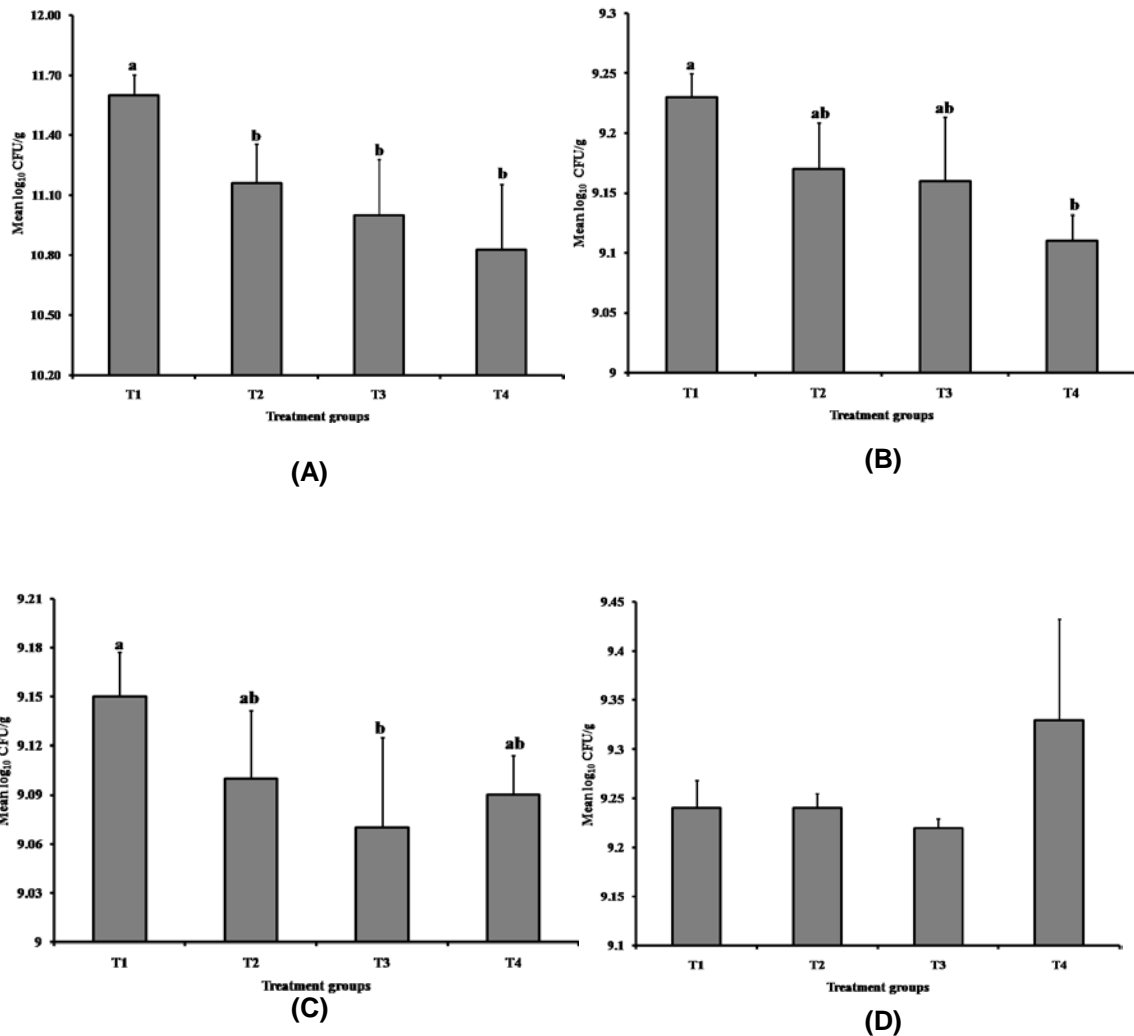


Figure 2 Total viable bacteria (A), *E. coli* (B), *Salmonella* sp. (C) and *Lactobacillus* sp. (D) in the faecal samples of broiler chicks fed an untreated control feed (T₁), 10% BWS + 1.5% BCS (T₂), 20% BWS + 2.5% BCS (T₃), 30% BWS + 3.5% BCS (T₄) at 30 days of feeding. The results are expressed as average \pm standard deviation of three replications (n = 3). Data points bearing different letters are significantly different (P < 0.05).

Several studies have observed that BCS has potential as an alternative to antibiotics and vaccination to improve immunity and to reduce mortality in poultry birds owing to the presence of pharmacologically active constituents such as thymoquinone, dithymoquinone, thymohydroquinone, nigellidine, nigellimine and nigellidine (Abdur-Rehman & Abu-Bakar, 1997; Osman & El-Barody, 1999). BCS supplementation in broiler diets has been reported to strengthen the immune system by preventing lipid peroxidation and liver damage (Sogut *et al.*, 2008), Rutin is the most influential pharmacological compound in buckwheat, and is reported to improve immunity (Sayed *et al.*, 2015). The poor performance of chickens fed higher levels of buckwheat was probably due to the characteristics of hardness and springiness of the buckwheat flour and a high crude fibre content. Birds have limited ability to digest crude fibre owing to an insufficient amount of the cellulase enzyme in their digestive systems (Gupta *et al.*, 2002).

According to the results on the lipid profile of broilers in this study it could be deduced that BWS and BCS have favourable effects on serum metabolites. Both BCS and BWS have been reported to possess hypolipidemic and hypocholesteremic properties in animal studies (El-Bagir *et al.*, 2006; Sayed *et al.*, 2015). In the current study it was observed that BWS and BCS supplementation in broiler diets significantly decreased serum total cholesterol and triglycerides levels, but increased HDL concentrations compared with the control treatment (Figure 1). Similar effects of BCS and BWS on serum lipid profile had been obtained by earlier investigators (Tomotake *et al.*, 2006; Islam *et al.*, 2011; Sayed *et al.*, 2015; Siddiqui *et al.*, 2015; Siddiqui & Sayed, 2015). Al-Beitawi & El-Ghousein (2008) reported that feeding broiler chicks with BCS

supplemented diets reduce plasma cholesterol and triglycerides compared with broiler chicks fed control diet. Reduced serum triglycerides and total cholesterol levels were observed, while the serum HDL cholesterol level increased owing to supplementation of layer rations with black cumin seeds (Akhtar *et al.*, 2003; Siddiqui & Sayed, 2015). Furthermore, Sayed *et al.* (2015) observed similar results with BWS supplementation with chitosan in broiler diets.

Although the mechanism of lipid profile improvement is not clearly understood from the current study, this may be because of possible cholesterol-lowering mechanisms of tocopherols, such as inhibition of cholesterol oxidation (Xu *et al.* 2001) and reduced HMG-CoA-reductase activity (Ha *et al.* 2005). Furthermore, Martin *et al.* (2001) and Torra *et al.* (2001) hypothesized that the cholesterol-lowering mechanism of black cumin fixed oil is dependent on peroxisome proliferator-activated receptor (PPAR α) activation. The volatile oils of black cumin contain quinines, including thymoquinone (TQ) and dithymoquinone, which might be involved in a sharp decrease in serum triglyceride level in hens fed a BCS-supplemented diet (Swamy & Tan, 2000). A serum triglyceride lowering effect of TQ has been reported in rats (Bamosa *et al.*, 2009). In addition, the choleric activity of the seed powder (El-Dhakhny *et al.*, 2000), high level of polyunsaturated fatty acids (Ramadan, 2007) and β -sitosterol in black cumin oil make it effective in lowering blood cholesterol concentration and preventing coronary heart disease (Cheikh-Rouhou *et al.*, 2008). On the other hand, the cholesterol-lowering effect of buckwheat is linked with lower digestibility of buckwheat and the presence of fibre-like substances, which is indicated by an increase in the levels of neutral and acid sterols in rat faeces observed when a diet rich in buckwheat protein products (Tomotake *et al.*, 2001) is administered.

In the present study, a significant reduction ($P < 0.05$) was observed in the population of harmful bacteria, *E. coli* and *Salmonella* spp., as well as total viable bacteria, in the intestine of the chicks fed BCS- and BWS- supplemented diets compared with the control (without BCS and BWS). However, the population of beneficial bacterium, *Lactobacillus* sp., was not significantly affected by BWS and BCS supplementation. These findings are in agreement with those of Alsawaf & Alnaemi (2011) and Islam *et al.* (2011), who reported that black seed and oil inhibited the growth of *E. coli*, *S. aureus*, *Brucella melitensis* and total viable bacteria. Moreover, several studies have referred to the antibacterial effect of BCS and BWS against *E. coli*, *Enterobacteria*, *Enterococcus*, *Streptococcus*, *Lactobacillus* and *Bifidobacteria* (Cabarkapa *et al.*, 2008; Halawani, 2009; Préstamo *et al.*, 2014). Antimicrobial activity of BCS could be attributed to the presence of thymoquinone (TQ) and thymohydroquinone (THQ) in seed oil (Halawani, 2009) while flavonoids and polyphenols influence mainly the inhibitory effect of buckwheat on microorganisms.

Conclusion

In terms of the results obtained from the present investigation it could be stated that BWS- and BCS-supplemented diets improved performance, significantly decreased the levels of serum total cholesterol and triglycerides in broilers with an elevation of HDL-cholesterol and significantly suppressed the growth of pathogenic bacteria (*E. coli* and *Salmonella* spp.). Taken together, these results suggest that poultry diet supplemented with 10% buckwheat seed + 1.5% black cumin seed (T₂) could be regarded as natural feed additives and environment safe diets as alternatives to banned and hazardous synthetic antibiotics. However, further in-depth research is needed to assess the obscure mechanisms of serum cholesterol lowering and antibacterial functions of buckwheat and black cumin seed before they can be used as poultry feed additive.

Acknowledgements

We are grateful to Ministry of Science and Technology, Government of the Peoples' Republic of Bangladesh for their financial support. We are also grateful to Mst. Minara Khatun, Department of Microbiology and Hygiene, Bangladesh Agricultural University for valuable assistance and technical support during the microbiological study.

Author's contribution

MAS designed the study. MAH and MNS supervised the whole study. MSI and MT-U-A conducted the experiment as well as collected different data during study period. MAI provided microbiological data. MNS and MSI wrote the first draft. Finally, MAS revised the manuscript.

Conflict of interest declaration

The authors have declared that there is no conflict of interests.

References

Abdur-Rehman, M.A.Y. & Abu-Bakar, C., 1997. The use of black cumin seed meal (*Nigella sativa* L.) in diets of growing turkeys. Paper presented in 19th MASP Annual Conference, Johar Bahru.

- Akhtar, M.S., Nasir, Z. & Abid, A.R., 2003. Effect of feeding powdered *Nigella sativa* L. seeds on poultry egg production and their suitability for human consumption. *Vet. Arhiv.* 73, 181-190.
- Al-Beitawi, N. & El-Ghousein, S.S., 2008. Effect of feeding different levels of *Nigella sativa* seeds (Black cumin) on performance, blood constituents and carcass characteristics of broiler chicks. *Int. J. Poult. Sci.* 7, 715-721.
- Al-Saleh, I.A., Billedo, G. & Inam, I.E., 2006. Level of selenium, DL- α -tocopherol, DL- γ -tocopherol, all trans retinol, thymoquinone and thymol in different brands of *Nigella sativa* seeds. *J. Food Comp. Anal.* 19, 167-175.
- Alsawaf, S.D. & Alnaemi, H.S., 2011. Effect of *Nigella sativa* (seed and oil) on the bacteriological quality of soft white cheese. *Iraqi J. Vet. Sci.* 25, 21-27.
- Bamosa, A.O., Ali, B.A. & Al-Hawsawi, Z.A., 2002. The effect of thymoquinone on blood lipids in rats. *Ind. J. Physiol. Pharmacol.* 46, 195-201.
- Cabarkapa, I.S., Sedej, I.J., Sakac, M.B., Saric, L.C. & Plavsic, D.V., 2008. Antimicrobial activity of buckwheat (*Fagopyrum esculentum* Moench) hulls extract. *Food Processing Quality Safety* 4, 159-163.
- Cheikh-Rouhou, S., Besbes, S., Hentati, B., Blecker, C., Deroanne, C. & Attia, H., 2008. Sterol composition of black cumin (*Nigella sativa* L.) and Aleppo pine (*Pinus halepensis* Mill.) seed oils. *J. Food Comp. Anal.* 21, 162-168.
- Council of the European Union, 1970. 31970L0524: Council Directive 70/524/EEC of 23 November 1970 concerning additives in feeding-stuffs: Official Journal L 270, 14/12/1970 P. 0001-0017.
- El-Bagir, N.M., Hama, A.Y., Hamed, R.M., Abd El-Rahim, A.G.A. & Beynen, A.C., 2006. Lipid composition of egg yolk and serum in laying hens fed diets containing black cumin (*Nigella sativa*). *Int. J. Poult. Sci.* 5, 574-578.
- El-Dakhkhny, M., Mady, N.I. & Halim, M.A., 2000. *Nigella sativa* L. oil protects against induced hepatotoxicity and improves serum lipid profile in rats. *Arzneimittelforschung* 50, 832-836.
- FASS, 2010. Guide for the Care and Use of Agricultural Animals in Research and Teaching. 3rd ed. Federation of Animal Science Societies Publ., Savoy, IL, USA.
- Guler, T., Dalkilic, B., Ertas, O.N. & Ciftci, M., 2006. The effect of dietary black cumin seeds (*Nigella sativa* L.) on the performance of broilers. *Asian Austral. J. Anim. Sci.* 19, 425-430.
- Gupta, J.J., Yadav, B.P.S. & Hore, D.K., 2002. Production potential of buckwheat grain and its feeding value for poultry in Northeast India. *Fagopyrum* 19, 101-104.
- Ha, T.Y., Han, S., Kim, I.H., Lee, H.Y. & Kim, H.K., 2005. Bioactive components in rice bran oil improve lipid profiles in rats fed a high-cholesterol diet. *Nutr. Res.* 25, 597-606.
- Halawani, E., 2009. Antibacterial activity of thymoquinone and thymohydroquinone of *Nigella sativa* L. and their interaction with some antibiotics. *Adv. Biol. Res.* 3, 148-152.
- Islam, M.R., Siddiqui, M.N., Khatun, A., Siddiky, M.N.A., Rahman, M.Z., Bostami, A.B.M.R. & Selim, A.S.M., 2014. Dietary effect of mulberry leaf (*Morus alba*) meal on growth performance and serum cholesterol level of broiler chickens. *SAARC J. Agric.* 12 (2), 79-89.
- Islam, M.T., Selim, A.S.M., Sayed, M.A., Khatun, A., Siddiqui, M.N., Alam, M.S. & Hossain, M.A., 2011. *Nigella sativa* L. supplemented diet decreases egg cholesterol content and suppresses harmful intestinal bacteria in laying hens. *J. Anim. Feed Sci.* 20, 587-598.
- Karamac, M., 2010. Antioxidant activity of tannin fractions isolated from buckwheat seeds and groats. *J. Am. Oil. Chem. Soc.* 87, 559-566.
- Khan, S.H., Ansari, J., Haq, A.U. & Abbas, G., 2012. Black cumin seeds as phyto-genic product in broiler diets and its effects on performance, blood constituents, immunity and caecal microbial population. *Italian J. Anim. Sci.* 11, 438-444.
- Majeed, L.H.A., Abdelati, K.A., El-Bagir, N.M., Alhaidary, A., Mohamed, H.E. & Beynen, A.B., 2010. Performance of broiler chickens fed diets containing low inclusion levels of black cumin seeds. *J. Anim. Vet. Adv.* 9, 2725-2728.
- Marchant, I.A. & Packer, R.A., 1967. *Veterinary Bacteriology and Virology*. 7th ed. The Iowa State University Press, Iowa, USA. pp. 211-305.
- Martin, G., Duez, H., Blanquart, C., Berezowski, V., Poulain, P., Fruchart, J., Najib-Fruchart, J.C., Glineur, C. & Staels, B., 2001. Statin-induced inhibition of the Rho-signaling pathway activates PPAR α and induces HDL apoA-I. *J. Clin. Invest.* 107, 1423-1432.
- Nasir, Z., Abid, A.R., Hayat, Z. & Shakoor, H.I., 2005. Effect of kalongi (*Nigella sativa*) seeds on egg production and quality in white Leghorn layers. *J. Anim. Plant Sci.* 15, 22-24.
- NRC, 1994. National Research Council: Nutrient Requirements of Poultry. 9th ed. National Academy Press, Washington, DC, USA.
- Osman, A.M.A. & El-Barody, 1999. Growth performance and immune response of broiler chicks as affected by diet density and *Nigella sativa* seeds supplementation. *Egypt Poult. Sci.* 19, 619-634.
- Padhye, S., Banerjee, S., Ahmad, A., Mohammad, R. & Sarkar, F.H., 2008. From here to eternity – the secret of Pharaoh: Therapeutic potential of *N. sativa* seeds and beyond. *Cancer Therapy* 6, 459-510.
- Préstamo, G., Pedrazuela, A., Peñas, E., Lasunción, M.A. & Arroyo, G., 2003. Role of buckwheat diet on rats as prebiotic and healthy food. *Nutr. Res.* 23, 803-814
- Rahman, Z., Siddiqui, M.N., Khatun, M.A. & Kamruzzaman, M., 2013. Effect of guava (*Psidium guajava*) leaf meal on production performances and antimicrobial sensitivity in commercial broiler. *J. Nat. Product.* 6, 177-187.
- Ramadan, M.F., 2007. Nutritional value, functional properties and nutraceuticals applications of black cumin (*Nigella sativa* L.): An overview. *Int. J. Food Sci. Technol.* 42, 1208-1218.
- Sayed, M.A., Islam, M.T., Haque, M.M., Shah, M.J.H., Ahmed, R., Siddiqui, M.N. & Hossain, M.A., 2015. Dietary effects of chitosan and buckwheat (*Fagopyrum esculentum*) on the performance and serum lipid profile of broiler chicks. *S. Afr. J. Anim. Sci.* 45, 429-440.

- Siddiqui, M.N. & Sayed, M.A., 2015. Effect of dietary black seed (*Nigella Sativa* L.) extract supplemented diet on growth performance, serum metabolites and carcass traits of commercial broiler. J. Anim. Sci. Adv. 5 (8), 1380-1385.
- Siddiqui, M.N., Islam, M.T., Sayed, M.A. & Hossain, M.A., 2015. Effect of dietary supplementation of acetone extracts of *Nigella sativa* L. seeds on serum cholesterol and pathogenic intestinal bacterial count in broilers. J. Anim. Plant Sci. 25 (2), 372-379.
- Sogut, B., Celik, I. & Tuluca, Y., 2008. The effects of diet supplemented with black cumin (*Nigella sativa* L.) upon immune potential and antioxidant marker enzymes and lipid peroxidation in broiler chicks. J. Anim. Vet. Adv. 7, 1196-1199.
- Steel, R.G.D. & Torrie, J.H., 1984. Principles and Procedures of Statistics. McGraw-Hill, Tokyo, Japan.
- Swamy, S.M.K. & Tan, B.K.H., 2000. Cytotoxic and immunopotentiating effects of ethanolic extract of *Nigella sativa* L. seeds. J. Ethnopharmacol. 70, 1-7.
- Świątecka, D., Markiewicz, L.H. & Wróblewska, B., 2013. *In vitro* evaluation of the effect of the buckwheat protein hydrolysate on bacterial adhesion, physiology and cytokine secretion of Caco-2 cells. Centr. Eur. J. Immunol. 38, 317-327.
- Toghyani, M., Toghyani, M., Gheisari, A., Ghalamkari, G. & Rezaei, M.M., 2010. Growth performance, serum biochemistry and blood hematology of broiler chicks fed different levels of black seed (*Nigella sativa*) and peppermint (*Mentha piperita*). Livest. Sci. 129,173-178.
- Tomotake, H., Shimaoka, I., Kayashita, J., Yokoyama, F., Nakajoh, M. & Kato, N., 2001. Stronger suppression of plasma cholesterol and enhancement of the fecal excretion of steroids by a buckwheat protein product than by a soy protein isolate in rats on a cholesterol-free diet. Biosci. Biotechnol. Biochem. 65, 1412-1414.
- Tomotake, H., Yamamoto, N., Yanaka, N., Ohinata, H., Yamazaki, R., Kayashita, J. & Kato, N., 2006. High protein flour suppresses hypercholesterolemia in rats and gallstone formation in mice by hypercholesterolemic diet and body fat in rats because of its low protein digestibility. Nutr. 22, 166-173.
- Torra, I.P., Chinetti, G., Duval, C., Fruchart, J.C. & Staels, B., 2001. Peroxisome proliferator-activated receptors: from transcriptional control to clinical practice. Curr. Opin. Lipidol. 12, 245-54.
- Xu, Z., Hua, N. & Godber, J.S., 2001. Antioxidant activity of tocopherols, tocotrienols, and c-oryzanol components from rice bran against cholesterol oxidation accelerated by 2,2 V-azobis (2-methylpropionamide) dihydrochloride. J. Agric. Food Chem. 49, 2077-2081.
- Zaoui, A., Cherrah, Y., Lacaille-Dubois M.A., Settaf A., Amarouch, H. & Hassar, M., 2000. Diuretic and hypotensive effects of *Nigella sativa* in the spontaneously hypertensive rat. Therapie 55, 379-382.
- Zhang, Z.L., Zhou, M.L., Tang, Y., Li, F.L., Tang, Y.X., Shao, J.R., Xue, W.T. & Wu, Y.M., 2012. Bioactive compounds in functional buckwheat food. Food Res. Int. 49, 389-395.
- Ziad, H.M.A. & Mohammad, S.A., 2008. Effect of feeding powdered black cumin seeds (*Nigella sativa* L.) on growth performance of 4-8 week-old broilers. J. Anim. Vet. Adv. 7, 286-290.