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Research Article
Phytogenic Feed Additive Influences on the Growth Performances, Serum Lipid Profile, Liver Functions and Antibacterial Activity of Broiler Chicks

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Abstract
Objective: This investigation was performed to explore the combined effect of buckwheat (BW), black cumin (BC) and honeyweed (HW) supplemented diet on growth performances, blood metabolites and intestinal microflora of broiler chicks for the production of safe meat. **Materials and Methods:** A total of 60 day-old chicks (Cobb-500) were divided into four groups viz. T1(Commercial control, CC), T2 (Formulated Feed, FF+10% BW), T3 (FF+10% BW+1.5% BC+5% (w/v) HW powder), T4 (FF+10% BW+3% BC+5% (w/v) HW powder) in complete randomized design with three (3) replications, each of which contain 5 birds. **Results:** At the end of the 30 days, T3 diet significantly ($p<0.05$) showed the lowest FCR, mortality rate and the highest body weight gain. Interestingly, T3 diet improved the blood metabolites remarkably ($p<0.05$). Surprisingly, histological study revealed the low deposition of fat in liver in case of BW, BC and HW supplemented feed. Furthermore, T3 supplemented feed also suppressed total viable bacteria in the feces and methanol extract, water extract of combined BW, BC, HW also showed the inhibition zone against the bacteria. **Conclusion:** T3 diet could be used for safe and quality poultry meat production as it showed good FCR and low mortality rate as compared to control treatment.

Key words: Black cumin, buckwheat, honeyweed, lipid profile, histopathology, antibiotic

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Poultry is one of the largest industry in agriculture sector and commercialization of poultry industry has been started in 1980, subsequently declared as an agro based industry in 1994 in Bangladesh. In the decade of eighty, the total investment in poultry sector was only 15 billion BDT (Bangladeshi currency), currently it is now more than 300 billion BDT. The growth rate of poultry industry was 20% from 1980 to 2007. About 65-70 thousand commercial poultry farm, 16 grand parent and 206 parent stock farms are available in Bangladesh. To supply the feed demand in poultry farm, 201 registered feed mills produce feed every day for ensuring the constant poultry meat production. The annual per capita poultry meat consumption is 6.3 kg which is far below from our demand. To achieve this goal, superior poultry meat production must be heightened through poultry rearing. Success of rearing broilers mostly depends on the strain and good quality organic feed[1]. On the other hand, poultry production and profitability depends on various factors like skilled manpower, good poultry shed, vaccination, contagious diseases infected by virus and bacteria, survival rate and mortality[2]. Mortality caused by contamination is another prime obstacle in the poultry sector. These contaminations are accountable for diminished growth rates. For this, antibiotics are added in poultry feed to expedite the production and for sound health of animals. Profitable yield of meat and eggs production depends on utilization of antibiotics that is exhibited tremendously by boosting growth rate, breeding performance, feed utilization and lessening mortality. Regrettably, bacterial resistance are the outcome of over use of antibiotics[3]. Hence, several countries in Europe have restricted the application of hazardous antibiotics in feed as growth promoters in poultry industry[4]. Therefore, the present study was performed to analyze the phytogenic medicinal feed supplements that have the ability to improve the growth performance of broiler chickens. Medicinal plants are one of the major sources of life saving drugs and medicine. According to World Health Organization (WHO), nearly 75-80% of world population still depends on herbal or traditional medicines. Therefore, researchers are investigating the effect of herbal feed additives as alternative to antibiotics to increase immunity and disease prevention in poultry[5].

Buckwheat (*Fagopyrum esculentum*) is a dicotyledonous pseudo cereals belonging to the family of Polygonaceae. Locally it is called *Dhamshi*. This crop is grown on the unproductive land under different natural calamities with minimum agronomical management. Biochemical composition of buckwheat seed is comparable to cereals, whereas amino acid content is more typical of legumes specially lysine, methionine and cysteine. In addition, the high content of unsaturated fatty acids improves the feed value of buckwheat. Therefore, the addition of buckwheat in the diet is useful in heart diseases, hypercholesterolemia or hypertension[6]. Bioactive compound like flavones, flavonoids, phytosterols and myo-inositol intensify its antioxidant capacity[7]. Its best antimicrobial activity against microorganism is due to the presence of hydroxyl group of polyphenol[8]. In a previous study it was observed that the buckwheat seed powder has significant effect on physical and biochemical parameters of broiler[9]. Honeyweed (*Leonurus sibiricus*) is another plant which has curative and remedial value. It belongs to the family of Lamiaceae with a cosmopolitan distribution containing about 236 genera and has been stated to contain 6900-7200 species. Among all the species, *L. siribicus* is very commonly grown on roadsides and crop fields in Bangladesh[10]. *Leonurus sibiricus* have been used as a traditional and medicinal herb for many years in Bangladesh. Biologically active ingredients of honeyweed has high potency to restraint pain, inflammation, atherogenesis, hemostasis, diabetes and cancer[10]. Several studies conducted worldwide have found that *L. sibiricus* species has high antimicrobial activities and has shown a reduction in intracellular reactive oxygen species[11]. Application of different dosages of *L. sibiricus* have changed the body weight, organ relative weight, lipid profile and blood parameters in broilers[12].

Black cumin (*Nigella sativa*) is one of the spice crops under the family of Ranunculaceae, cultivated in Bangladesh. Black cumin is rich in diverse medicinal functioning compounds which are pharmacologically important for animal health. It can alleviate diabetes, cancer, parasitical and bacterial diseases[13]. The effect of dietary black cumin or oils on the performance of broilers have been analyzed[14]. It has positive effect on physical and biological parameters of broilers. It has enormous importance in layer chicken also[2]. *Nigella sativa* also acts as a natural antioxidant and immunostimulant. Due to the double amount of polyunsaturated fatty acid in black seed oil, it is capable to reduce the total cholesterol content[15].

It has been reported that supplementation of either *Nigella* seed or buckwheat has beneficial effect of on different animals encompassing poultry[16]. Combination of buckwheat and black cumin has positive effect on poultry. However, none of the studies have been carried out to evaluate the combined effects of buckwheat, black cumin and honeyweed supplementation on production performance of broiler chicks. Therefore, the objective of this study was to determine the

antibacterial activity and the combined effect of buckwheat, black cumin and honeyweed on growth performances, lipid profile, liver function, histopathology of broiler chicken.

MATERIALS AND METHODS

Collection of broiler chicks: A total of 60 day-old chicks (Cobb-500) were bought from local poultry farm located in Pakerhat, Khansama, Dinajpur, Bangladesh and transferred to Joynal Agro and Feed. The experimental procedures, animal handling and the collection of samples were reviewed and approved by the Animal Welfare and Ethics Committee of Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh.

Accumulation and arrangement of buckwheat, black cumin and honeyweed: Panchagar, a district of northern region of Bangladesh is very popular for cultivation of buckwheat. Buckwheat was purchased from Panchagar and honeyweed was harvested from the fallow high land at Solabaria village of Santhia (24°01'34.5"N, 89°27'28.7"E), Pabna, Bangladesh. Fresh water used to clean the leaves of honeyweed. These wet leaves were sundried and then retained at the temperature of 60°C for three days in the microwave oven. A grinder was used to grinde leaves into powder. Distilled water was used to prepare 5% solution.

Formulation of feed: Experimental diet was formulated for starter (1-15 days) and grower (16-30 days) phases as previously described by Mondal *et al.*[17] (Table 1 and 2). All components required for diet preparation were bought from Dinajpur town. Feed was prepared manually and stored at room temperature.

Design of experiment: A day-old birds were reared in the brooder house upto 10 days for adjusting temperature as described previously[18]. After ten days, 60 broiler chicks were randomly assigned to four dietary groups. Each treatment had three replications with five birds in each group. Brooder house temperature was maintained properly upto 10 days and then gradually decreased from 32-24°C. The birds were weighed prior to the feeding trial. All the birds were examined twicely daily to observe their body fitness. During the experimental period birds were vaccinated as mentioned in a previous study[18]. Different physical, biological and biochemical, parameters were recorded (Table 3). Finally, liver and feces were collected for histopathological study and counting the total viable bacteria, respectively.

Blood collection, lipid profile, SGOT and SGPT determination: At 30th day, blood sample was collected from each replication. Then serum was separated from blood as stated previously[16]. Afterwards, lipid profile and liver function tests were carried out by using the collected sample.

Table 1: Composition (%) of broiler starter diet (100 kg)

Diet composition	FF+10% BW	FF+10%BW+1.5%BC+5%HW	FF+10%BW+3%BC+5%HW
Maize	39.00	39.00	39.00
Soybean meal	25.30	24.36	23.16
Rice polish	10.00	10.00	10.00
Meat and bone meal	4.00	4.00	4.00
Protein concentrate	5.80	5.50	5.20
Soybean oil	3.26	3.00	3.00
Lime stone	0.50	0.50	0.50
DCP	1.00	1.00	1.00
Salt	0.30	0.30	0.30
Methionine	0.12	0.12	0.12
*Broiler premix	0.31	0.31	0.31
Toxin binder	0.30	0.30	0.30
Coccidiostat	0.05	0.05	0.05
Lysine	0.01	0.01	0.01
Enzyme	0.05	0.05	0.05
Buckwheat seed	10.00	10.00	10.00
Black cumin seed	0.00	1.50	3.00
Calculated composition kg feed			
ME (kcal kg^{-1})	3029.00	3034.00	3036.00
Crude protein (%)	22.07	22.17	22.30
Crude fibre (%)	4.57	4.71	4.87
Ca (%)	1.19	1.19	1.18
Available P (%)	0.40	0.40	0.40
Methionine (%)	0.51	0.51	0.51
Lysine (%)	1.04	1.04	1.06

Table 2: Composition (%) of broiler grower diet (100 kg)

Diet composition	FF+10% BW	FF+10% BW+1.5%BC+5%HW	FF+10% BW+3%BC+5%HW
Maize	41.90	41.90	41.90
Soybean meal	22.50	21.57	20.37
Rice polish	10.00	10.00	10.00
Meat and bone meal	4.00	4.00	4.00
Protein concentrate	5.80	5.50	5.20
Soybean oil	3.27	3.00	3.00
Lime stone	0.50	0.50	0.50
DCP	1.00	1.00	1.00
Salt	0.30	0.30	0.30
Methionine	0.10	0.10	0.10
*Broiler premix	0.25	0.25	0.25
Toxin binder	0.30	0.30	0.30
Coccidiostat	0.02	0.02	0.02
Lysine	0.01	0.01	0.01
Enzyme	0.05	0.05	0.05
Buckwheat seed	10.00	10.00	10.00
Black cumin seed	0.00	1.50	3.00
Calculated composition kg^{-1} feed			
ME (kcal kg^{-1})	3145.00	3168.00	3176.00
Crude protein (%)	21.15	21.20	21.39
Crude fibre (%)	4.50	4.63	4.77
Ca (%)	1.19	1.18	1.17
Available P (%)	0.40	0.39	0.38
Methionine (%)	0.49	0.47	0.48
Lysine (%)	0.96	0.95	0.96

*The composition of broiler premix was mentioned previously[18]

Table 3: Growth performances of broilers chicks fed with various diets for a period of 30 days

Parameters	CC (T1)	FF+10% BW (T2)	FF+10%BW+1.5% BC+5% HW (T3)	FF+10%BW+3% BC+5% HW (T4)
Initial body weight at 10th day (g)	266.406±0.41^{a}	265.70±0.35^{ab}	264.98±0.45^{b}	264.98±0.45^{b}
Final body weight at 30th day (g)	1704.00±7.37^{a}	1607.30±7.88^{c}	1729.00±15.94^{a}	1651.00±10.81^{b}
*Body weight gain (g)	1437.50±7.04^{a}	1341.60±8.23^{c}	1464.00±15.79^{a}	1386.00±10.37^{b}
Total feed intake (g)	2419.86±60.2^{b}	2508.20±55.98^{a}	2350.66±106.60^{c}	2371.06±24.33^{c}
**FCR	1.67±.01^{b}	1.86±.00^{a}	1.60±.00^{c}	1.71±0.1^{b}
***Mortality (%)	6.66±3.84^{ab}	8.88±2.22^{ab}	2.22±2..22^{b}	13.33±3..85^{a}

Results are presented as mean±SD o f at least three replications each of which contains five birds. Different letter within a row differ significantly ($p<0.05$) *Body Weight gain (g): Final body weight at 30th day-Initial body weight at 10th day. **FCR (Feed Conversion Ratio): Total feed intake (g)/Body weight gain (g). ***Mortality (%): Number of dead birds/Total number of birds

Preparation of methanol and water extract of BW, BC and HW for antibacterial activity: Exactly 100 g of BW, BC and HW powder was taken in to 500 mL conical flask. Then 400 mL methanol was taken into the conical flask. It was mixed thoroughly and kept this mixture in lab at room temperature for 4 days. Every day, the mixture was vortexed by vortex mixture 4 times to mix properly. Finally, it was filtered by Whatman No.1 filter paper and collected to the supernatant into another new conical flask. The residue of BW, BC and HW powder with filter paper was discarded. The BW, BC and HW extract solution was dried by hot water bath to evaporate the methanol. The final residue was collected and it was found 3.57 g. From this dried sample, 1.5 mg was taken into 50 mL volumetric flask and then methanol was added to make the total volume of 50 mL (up to the mark). It was mixed by vortex mixture to get homogenous solution. Water extract was also prepared following the same procedures. The final concentration of extract was 30 µg mL^{-1} for T2, T3 and T4 whereas T1 was excluded due to commercial feed.

Antibacterial activity test using *E. coli*: The MacConkey agar media was prepared as described previously[16]. The prepared MacConkey agar media plates were taken into the laminar air flow cabinet and *E. coli* bacteria were poured on plates and spread by bacteria spreader to equally distributed bacteria on the plate. The discs containing methanol and water extract were placed in respective portions. Discs were no closer than 15 mm to the edge of the plate and far enough apart to prevent overlapping the zones of inhibition. Tetracycline (30 µg disc^{-1}) discs were used as positive control. Tetracycline

(30 µg disc^{-1}) was placed on the labeled portion of the petridish. Finally, the plates were incubated upside down at 37°C for 16-18 h to show the antibacterial activity. After proper incubation, the antibacterial activity of the test agent was determined by measuring the diameter of inhibition zone in term of millimeter with a calibrated scale.

Statistical analysis: All statistical analyses were performed using the Statistical Package for Social Science (SPSS) version 22.0 for windows (SPSS Inc., Chicago, IL, USA). Differences between treatment means were tested by using Duncan's multiple range test[19]. Differences were considered significant at $p<0.05$.Means and standard deviation were calculated as per standard procedure.

RESULTS

This experiment was carried out to assess the combined effects of three phytogenic feed additives on the growth performance, mortality, serum lipid profile, FCR, liver function test, histological and antimicrobial activity of the broiler chicks.

Growth performances: Table 3 shows the growth performances of broiler chicks. Body weight of each broiler chick was recorded at the 10th day just after completing the brooding period. BW, BC and HW supplemented diets increased the body weight. Throughout the experimental period, satisfactory growth performance was observed in T3 [FF+10% BW+1.5% BC+5% (w/v) HW powder] (Supplementary Fig. 1 and 2).

Serum biochemical parameters: The effect of formulated feed on lipid profile of broiler chicks is presented in Fig. 1-4. The results clearly showed that diet supplemented with BW, BC and HW had significant effects on lipid profile. Lower serum cholesterol level was recorded in T3 group [FF+10% BW+1.5% BC+5% (w/v) HW powder] compared to the control group (Fig. 1). Diet supplemented with BW, BC and HW also decreased serum triglycerides level. There was no significant difference between BW, BC and HW supplemented diets (Fig. 2). HDL-cholesterol level was significantly ($p<0.05$) highest in T3 group [FF+10% BW+1.5% BC+5% (w/v)HW powder] (Fig. 3). Results of the present study showed that the diet supplemented with BW, BC and HW reduced the LDL-cholesterol level compared to control treatment (Fig. 4).

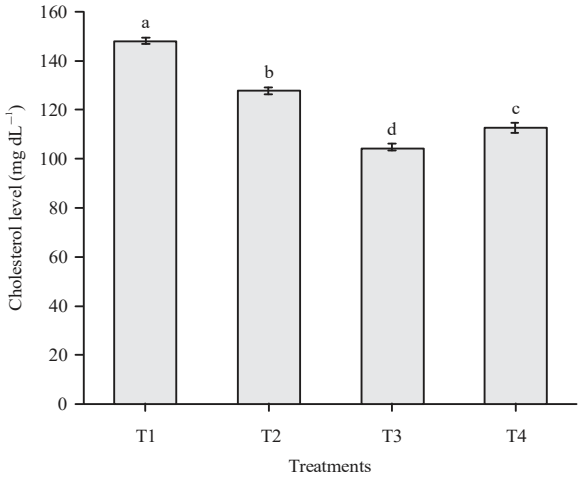
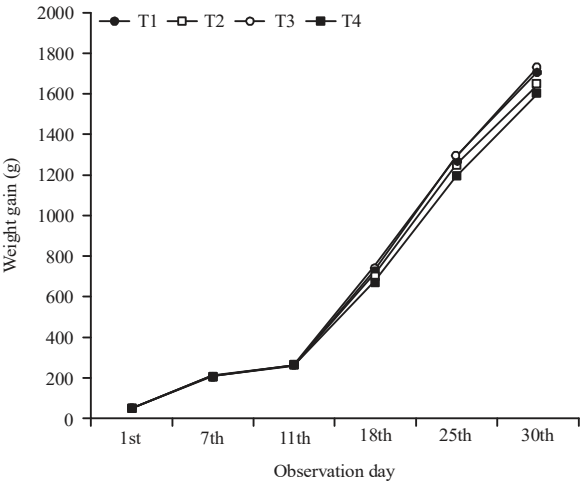


Fig. 1: Impacts of different feed treatments on total cholesterol level of broiler chicks



Suppl. Fig. 1: Impacts of different feed treatments on body weight gain of broiler chicks

Liver function test: The serum SGOT and SGPT are useful biomarkers of liver injury. Figure 5 and 6 shows the liver function test (SGPT and SGOT) of broilers. Lowest SGPT was found inT3 group compared to the other treatment groups. Interestingly, SGOT level was also decreased in T3 treatment.

Histological study: Small, unstained, spherical space was found in cytoplasm of hepatocytes. The space was varying in size but their number was different among the different treated group compared to the untreated control group. The elevated level of fatty change was observed in control group followed by T2, T4 and T3 group (Fig. 7a-d). There was no remarkable Goss morbid lesion related to fatty change in liver on necropsy examination.

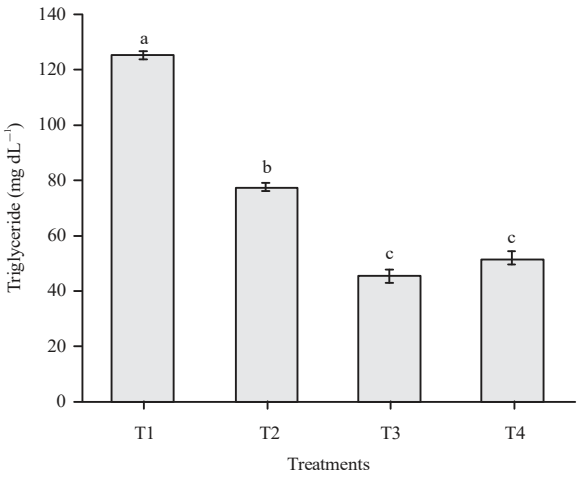


Fig. 2: Impacts of different feed treatments on triglyceride level of broiler chicks

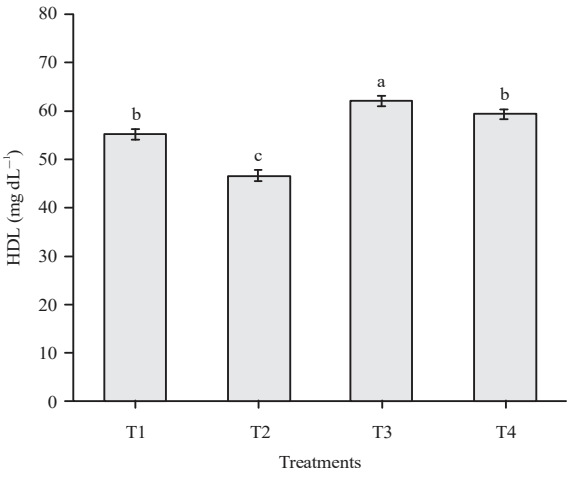
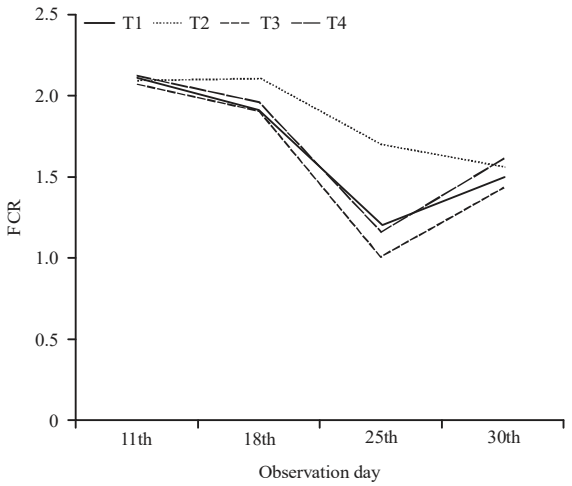
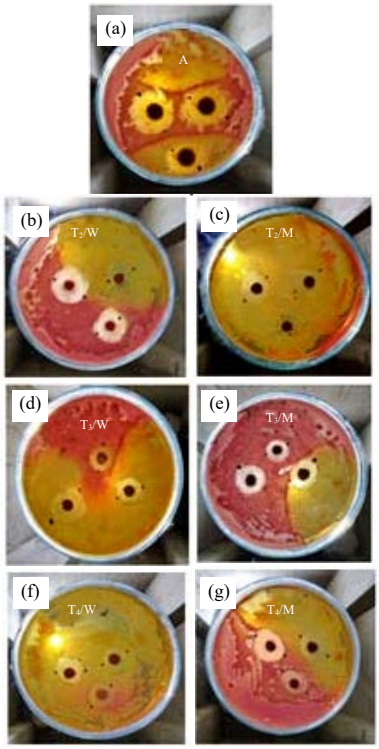


Fig. 3: Impacts of different feed treatments on HDL level of broiler chicks



Suppl. Fig. 2: Impacts of different feed treatments on FCR of broiler chicks

Antimicrobial activity of different diets: Diet supplemented with BW, BC and HW decreased the level of intestinal bacteria At 30th day, the lowest bacteria colony forming unit (CFU) of total viable count was observed in faeces of broilers. Supplementation of BW, BC and HW in the diets decreased ($p<0.05$) the microbial population (Fig. 8). Total viable bacteria were found to be decreased from Log_{10} 11.85 CFU g^{-1} (T1) to Log_{10} 10.36 CFU g^{-1} (T4). The decreased viable bacteria encouraged to test the antibacterial activity of BW, BC and HW. Table 4 shows the antibacterial activity of methanol and water extract of BW, BC and HW. It depicts mild activity against enteric pathogens and tetracycline was used as control to show the antibacterial activity of BW, BC and HW (Supplementary Fig. 3).



Suppl. Fig. 3(a-f): Antibacterial activity of BW, BC and HW: (a) Inhibition zone of antibiotic (T1), (b) Inhibition zone of BW water extract (T2), (c) Inhibition zone of BW methanol extract (T2), (d) Inhibition zone of 10% BW+1.5% BC and 5% HW water extract (T3), (e) Inhibition zone of 10% BW+1.5% BC and 5% HW methanol extract (T3), (f) Inhibition zone of 10% BW+3% BC and 5% HW water extract (T4), (g) Inhibition zone of 10% BW+3% BC and 5% HW methanol extract (T4)

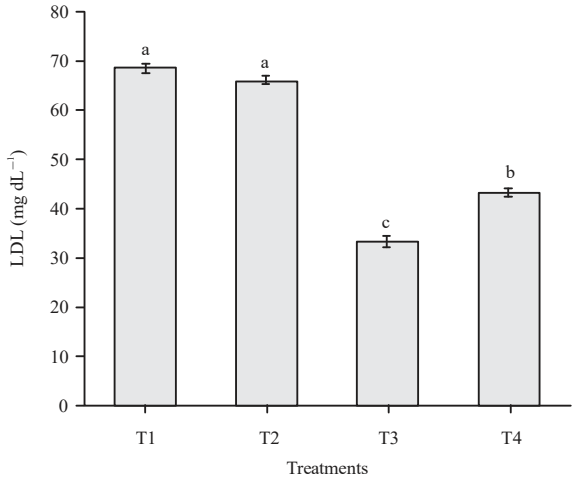


Fig. 4: Impacts of different feed treatments on LDL level of broiler chicks

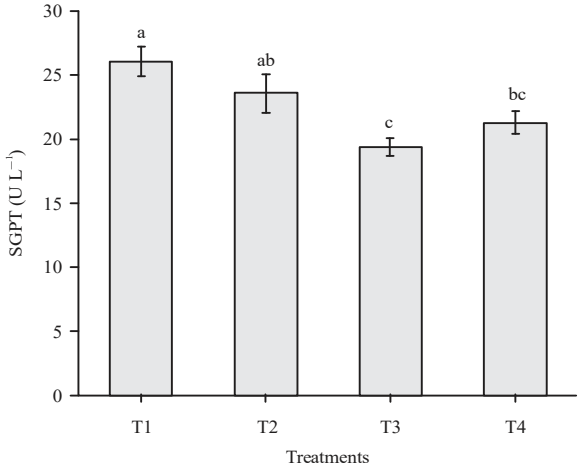


Fig. 5: Impacts of different feed treatments on SGPT level of broiler chicks

DISCUSSION

It is the dire need of the time to find an easy and sustainable solution for poultry industry which makes the quality production as well as maintain the health issues of consumers. Some studies have already been conducted to look for natural and effective alternative to synthetic antibiotics in poultry production, but the combined effect of BW, BC and HW on broiler was assessed the first time.

Effects of formulated diets on growth performances and FCR of experimental birds: The present study showed the better results for body weight gain and FCR compared to the previous study which was conducted to evaluate the

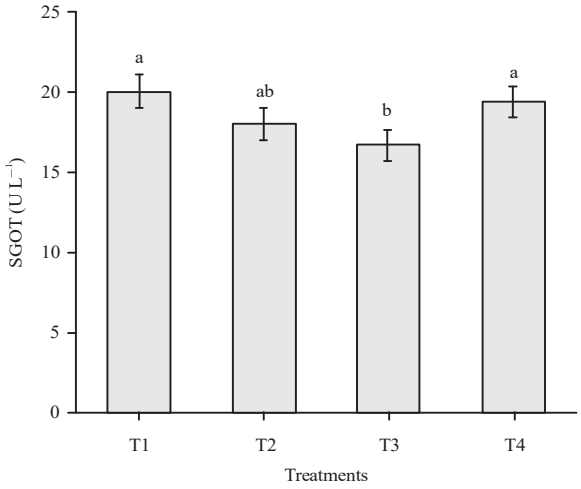


Fig. 6: Impacts of different feed treatments on SGOT level of broiler chicks

Table 4: Antibacterial activity of BW, BC and HW

Water extract treatments (30 µg disc^{-1})	Diameter zone of inhibition (mm)
Tetracycline	11.33±1.52^{a}
BW	6.22±1.52^{b}
10% BW+1.5% BC+5% HW	8.00±1.00^{b}
10% BW+3% BC+5% HW	7.33±0.57^{b}
Methanol extract	
BW	8.33±1.15^{b}
10% BW+1.5% BC+5% HW	9.00±1.00^{ab}
10% BW+3% BC+5% HW	9.33±1.52^{ab}

combined effect of BW and BC supplementation in broiler diet[5]. Black cumin itself is a spice and acts as a digestive stimulator which aids to increase the digestive enzyme[20]. Protein percentage and desirable amount of fiber in buckwheat and antioxidant property of honeyweed maintain the proper digestive ecosystem and immunosystem of broilers. Unfortunately, high dose of BC slightly reduced the body weight gain. High amount of BC may change the flavor of feed and it may be the reason for reducing body weight gain of broiler birds.

Effects of formulated diet on serum lipid profile: The inclusion of BW, BC and HW in the formulated feed had significant effect on total cholesterol. Several previous studies have reported that BW and BC reduced the lipidand cholesterol level in animal[9,21]. Some phyto-chemicals have already been identified in honeyweed[10] which play a significant role to regulate the lipid profile of broiler chicks. In the present study, the diet supplemented with the combination of BW, BC and HW reduced cholesterol and triglycerides levels, but increased HDL concentrations. Same results were obtained when BW and BC was used as supplementary feed ingredients in broiler diets[5,18].

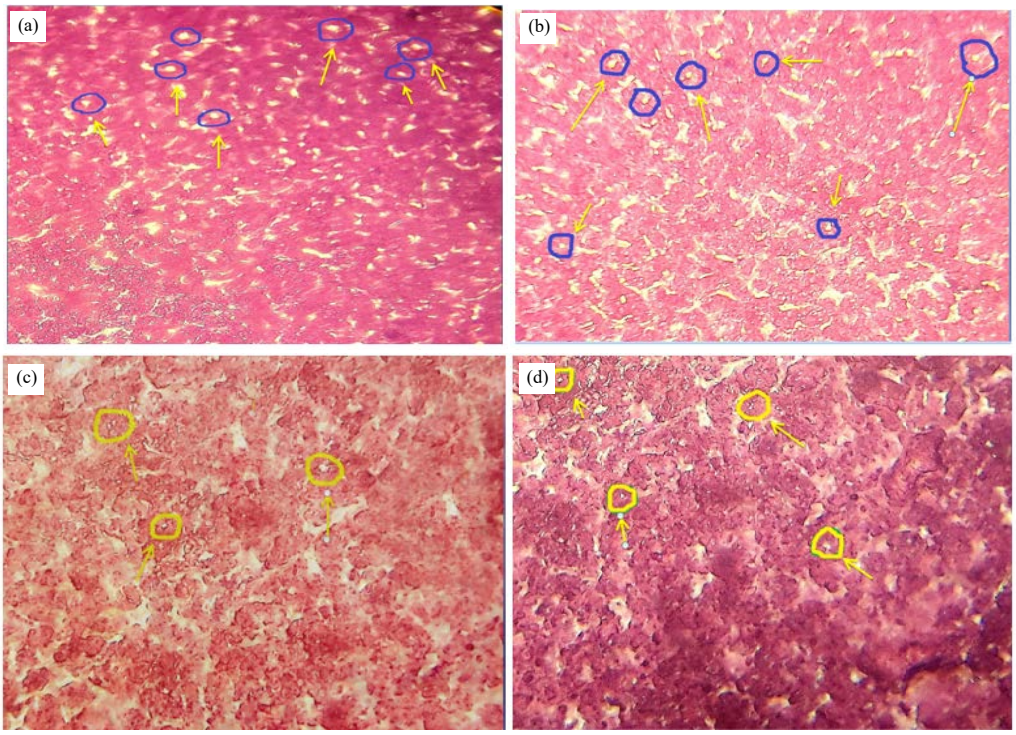


Fig. 7(a-d): Microscopic liver image of different treatment

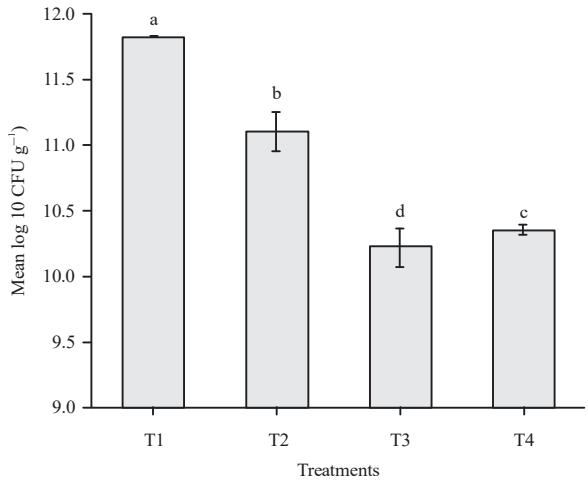


Fig. 8: Total viable bacteria in the faecal samples of broiler chicks feed with different treatments

A previous study conducted by Islam *et al.*[2] also reported the similar results for laying hens. Results of the current study showed that honeyweed has appreciative effects on serum metabolites.

However, the current study demonstrated that BW, BC and HW reduced the total cholesterol and increased the HDL level and triglyceride was lower than those of the birds fed diet supplemented with BW and BC[5].

Effects of formulated diets on liver function test: SGPT and SGOT is mainly concentrated in the liver and kidneys, while trace amounts may be found in the heart and other muscles. When the liver is damaged, high level of SGPT and SGOT concentration is increased in the blood. The SGPT and SGOT concentration was found to be minimum in T3 group compared to the commercial control group. These findings agree with previous findings[22]. In another study, it was also observed that the BC supplementation significantly decreased SGOT in serum of broilers[23]. But the feed supplemented with BW, BC and HW (in combination) decreased SGPT and SGOT compared to the feed supplemented with only black cumin (BC) or the combined effect of other medicinal plants[24]. Combination of BW, BC and HW may maintain cell membrane and protect the liver from inimical agents and free radical. This has been reflected in the reduction of liver enzymes in present study.

Effects of formulated diets on bacteria colony forming unit: In the present study, combination of BW, BC and HW in the diet of broiler significantly ($p<0.05$) reduced the population of harmful bacteria compared to the control group. According to a previous study, the diet supplemented

with BW and BC decreased the number of coliform bacteria in broilers[5]. Ahmed *et al.*[25] reported that both the water and ethanolic extracts of HW reacted differently to inhibit the growth of microorganisms. The present study agrees with findings of Islam *et al.*[2] who reported that BC seed inhibited the growth of *E. coli* and total viable bacteria. Moreover, several studies have referred to the antibacterial effect of BC and BW against *E. coli*[26]. Biochemical compound thymoquinone and thymohydroquinone are found in BC seed oil[26] while flavonoids, polyphenols, caffeic acid, syringic acid, apigenin, 4-Hydroxybenzoic acid, rutin, leosibirinone, ellagic acid, p-coumaric acid are found in HW. These compounds are pharmacologically effective against bacteria[27].

Effects of formulated diets on histological finding: The birds were sacrificed on day 30 and histological examination was performed using a standard method and obscured under microscope using variable objectives (4×, 10×, 40×). Tiny unstained, spherical spaces were found more in control group than that of the treated groups. The commercial boiler feed contain higher concentration of fat and carbohydrate to accelerate the growth of the birds. This excess fat and carbohydrate is stored in muscle and liver as glycogen and fatty acids. However, the population of fat droplets was lower in treated group than that of the untreated group. It clearly indicated the deposition of extra fat in hepatic cytoplasm which was low due to the combined effect of BW, BC and HW and this result agrees with the findings of Sitarek *et al.*[28] who utilized different plant or seeds to observe their effect on the blood cholesterol level or fatty changes in tissue.

The histological findings of liver: Small, unstained, spherical space was found in cytoplasm of hepatocytes. The space was varying in size but their number was varying among the different treated groups compared to the untreated control group (Fig. 7). The increased level of fatty change was observed in T1 followed by T2, T4 and T3. T1and T2 has minimal to moderate degree of intracytoplasmic fatty changes but T3 and T4 has no fatty changes. But necropsy findings showed no gross lesions on liver related to fatty changes in T1 and T2. In addition, combination of BW, BC and HW reduced the fat infiltration of liver. Verbascoside is a bioactive compound found in honeyweed which inhibits the growth of parasite in living system[27], buckwheat have some bioactive compounds, *Nigella sativa* seeds have shown schistosomicidal, curative, prophylactic and chemopreventive activity[29]. That's why, birds fed diet supplemented with BW, BC and HW had no problem of liver parasite.

Effects of formulated diets on antibiotic sensitivity: In Indian sub-continent plants have been used for medicinal purposes for thousands of years, while many studies have proved scientifically their medicinal values. Side effects of natural antibacterial agents are less than that of the synthetic or semi synthetic antibacterial agents. Antimicrobial activity of BW and BC have already been proved by researchers[5]. Now, it is predicting that *L. sibiricus* could be used as natural allelopathic and antimicrobial agent specially in agricultural and food industry[30].The authors found some specific bioactive chemical compound from honeyweed plant, which are very effective against bacteria[25]. Hence, further studies are required to find other pharmacological activities of this plant. However, the results obtained in the present study showed that BW, BC and HW have antibacterial activity against bacteria.

CONCLUSION

Poultry diet supplemented with BW, BC and HW have favourable impact on growth performance, feed intake, serum lipid profile, intestinal bacterial flora and their antibacterial activity. Poultry diet supplemented with T3 [FF+10% BW+1.5% BC+5% (w/v) HW powder] may be used in poultry industry as it showed good FCR, low mortality rate compared to control treatments. Moreover, the T3 reduced the risky serum total cholesterol, LDL and triglyceride as well as increased serum HDL-cholesterol level with highest body weight gain. So, poultry industry can use these alternative feed ingredients in poultry diets for high quality meat production. However, large scale analysis is greatly recommended to check the biochemical mechanisms of combined effect of buckwheat, black cumin and honeyweed in poultry.

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