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ORIGINAL ARTICLE

Effects of *Nigella sativa* and amino acids on growth performance and haematological parameters of broiler

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Abstract

Background: Recently, the use of antibiotic growth promoters has been banned in many countries due to cross-resistance among pathogens and residues in tissues, which led to search for the alternative feed additives in animal production. The aim of this study was to investigate the effects of "Kalo jeera" (*Nigella sativa*) powder and amino acids supplementation on the growth of broiler chicks and their blood parameters.

Methods: A total of 100 day-old (Cobb® 500) chicks were randomly divided into four groups such as T_0, T_1, T_2, T_3, and each group contained 25 chicks. The group T_0 was used as control and groups T_1, T_2 and T_3 were treated group. Chicks of control group were fed with only basal diet and no supplements were supplied, and chicks of treatment group including T_1, T_2 and T_3 were supplemented with amino acids, *Nigella sativa* powder and combination of *Nigella sativa* and amino acids, respectively. Body weight was recorded on 7th, 14th, 21th, 28th and 35th day of age. Haematological tests were performed at 15th, 25th and 35th day of age.

Results: The results showed that net weight gain and feed conversion ratio were improved in the treated groups than that of the control group. Among the treated groups, the significant level of improvement was achieved in the T_3 group followed by T_1 and then T_2. Total erythrocyte count, packed cell volume and haemoglobin concentration were significant ($p<0.01$) in the treated groups as compared to control.

Conclusions: The results suggest that better growth performance could be achieved in broilers supplemented with *Nigella sativa* and amino acids in combination and those can be used as growth promoters.

Key words: *Nigella sativa*, Amino acid, Broiler, Growth performance, Blood parameters.

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Introduction

Poultry farming is one of the most successful and rapidly growing industries in Bangladesh which provides not only high quality protein in the form of meat and eggs but also generates employments. The use of antibiotics as a principal growth promoter in poultry feed often resulted in the incidence of cross resistance among pathogens and also a source of residues in animal body tissues (Schwarz *et al.*, 2001). Consequently, the European Union including many other countries have banned the use of antibiotics as a growth promoter in animal feeds (Toghyani *et al.*, 2010). Ultimately, the use of antibiotics in the poultry feed was reduced. These led to a search for alternative natural growth promoters in animal production. Herbal medicinal plants and their extracts have been used as feed supplements since ancient times. *Nigella sativa* (Kalanji or black cumin) under the family of Ranunculaceae is utilized as a medicinal plant, herb and spice worldwide (Akhtar *et al.*, 2003). The *Nigella sativa* contains about 20.7 to 26.7% crude protein, 35.6 to 42.1% fixed oil and 0.5–1.6% volatile oil (AL-Gaby, 1998). *Nigella sativa* has been extensively studied for its active constituents in which oils are the principle components (Tawfeek *et al.*, 2006; AL-Douri *et al.*, 2010; Nasir and Grashorn, 2009, 2010; Khan *et al.*, 2012). The active compositions of Nigella seed are the volatile oils thymoquinoline and dithymoquinoline, both of which have antitumor properties (Abu-AL-Basal, 2011). In addition, *Nigella sativa* have been reported to have many biological properties including an antibacterial activity (Hanafy and Hatem, 1991; Mouhajir *et al.*, 1999; Nair *et al.*, 2005), antioxidant (Tawfeek *et al.*, 2006), antiparasitic (Mahmoud *et al.*, 2002), antidiarrheal (Gilani *et al.*, 2004), antidiabetic (Meral *et al.*, 2001), diuretic (Zaoui *et al.*, 2000), and protective effect against liver damage (Mahmoud *et al.*, 2002). It has been considered one of the greatest forms of healing medicine available, especially in Middle East and Asia, as it was mentioned that black seed is the remedy for all diseases except death in one of the prophetic hadith. It is also recommended for use on regular basis in "Tibb-e-Nabwi" (prophetic medicine) (Al-Bukhari, 1976). The commercial farming is becoming challenging for obtaining the desired weight without the use of antibiotics as growth promoters; therefore natural products capable of meeting the challenge are desired. Different studies on the effect of *Nigella sativa* seed on broiler performance have been carried out. Improved average daily weight gain and better feed conversion ratio (FCR) in broilers was achieved with fed 1% *Nigella sativa* seed in broiler diet (Guler *et al.*, 2006; Durrani *et al.*, 2007; AL-Beitawi *et al.*, 2009) in different countries such as Turkey, Pakistan, Jordan. El-Ghammry *et al.* (2002) and Hassan *et al.* (2004) reported an increased body weight by incorporating grounded *Nigella sativa* seed in broiler feed. Heat stressed broilers fed black cumin oil (0.5, 1%), seeds (1, 2%) or meal (10, 20%) in feed showed better results than the control group. Significant improvement was observed regarding FCR, crude protein conversion (CPC), calorie conversion ratio (CCR) and feed consumption of the birds (Hermes *et al.*, 2009). Some authors report that Nigella seed has immuno-stimulant effects, thus maintaining good animal health (Afifi, 2001; Mahmoud *et al.*, 2002; AL-Beitawi and El-Ghousein, 2008). Hermes *et al.* (2009) also reported that *Nigella sativa* oil at 0.5 and 1% significantly increased white blood cells (WBCs) and packed cell volume (PCV) compared to the control, while decreasing triglycerides, and alanine aminotransferase (ALT). To the best our knowledge, the report on the effects of *Nigella sativa* along with amino acids on growth performance, and haematological values of broiler chickens in Bangladesh, particularly for coastal area of southern districts is still absent. Therefore, the present study was designed to investigate the effects of dietary supplementation of *Nigella sativa* (with and without amino acid supplementation) as an alternative growth promoter on growth performance and blood parameters of broilers raised under coastal climate condition of Bangladesh.

Material and Methods

This study was conducted at a poultry farm located at Uzirpur, Barishal Sadar, Barishal. A total of 100 one-day-old chicks (Cobb-500),

"Kalo jeera" (*Nigella sativa*) seeds (from local market) and amino acids (Availa Z/M, Renata Ltd.) were purchased. After washing and drying "kalo jeera" seeds were crushed into powder. Broiler chicks (n=100) were randomly divided into four groups, namely T_0 (control group), T_1, T_2, and T_3 (treatment groups), and each group contained 25 chicks. Chicks of group T_0 were fed with basal diet only and no "kalo jeera" and amino acids supplements were supplied. Besides basal diet chicks of treatment groups T_1, T_2, and T_3 were supplemented with amino acid (1% in drinking water), kalo jeera (1% in drinking water), and both amino acid and "kalo jeera" (1% in drinking water), respectively. Chicks of both control and treatment groups were monitored closely for 35 days and following parameters were studied.

Body weight and FCR

The body weight of each bird was measured using a balance on a weekly intervals until 35 days and feed conversion ratio (FCR) was measured at the end.

Blood collection

Whole blood samples were collected with anticoagulant (4% sodium citrate) from wing vein of chicken of both control and treated groups at 15th, 25th and 35th day. Total erythrocyte count (TEC), hemoglobin (Hb) estimation and determination of packed cell volume (PCV) were conducted.

Determination of total erythrocyte count (TEC)

For erythrocyte count, dry clean red pipette was dipped into the blood and 0.5 mark blood was drawn into the pipette. Then the tip of the pipette was cleaned by cotton and immediately placed into Hayem's solution and was filled upto 101 marks. The pipette was agitated vigorously by an electric shaker for proper mixing. The unit was expressed in millions per mm^3 of blood.

Estimation of haemoglobin (Hb)

0.1N Hydrochloric acid (HCL) was taken in the graduated diluting tube upto 2 mark with the help of a dropper. A 0.02 ml of blood was added directly into the diluting fluid by Sahli pipette. Distilled water was added drop by drop and stirred unit the color of the content matches to that of the standard color of the comparator. The haemoglobin (Hb) was recorded within 10 minutes and was expressed in gm%.

Determination of packed cell volume (PCV)

After completion of ESR recording the Wintrobe's tubes were placed in the centrifuge machine and centrifuged at 3000 rpm for 30 minutes. Then the hematocrit of PCV was recorded. The percent volume occupied by the hematocrit was calculated by using the following formula.

$$\text{PCV\%} = \frac{\text{Height of the red cell volume in cm}}{\text{Height of total blood in cm}} \times 100$$

Statistical analysis

The data were recorded in excel sheet and were analysed statistically among the treatment and groups of chicken by the analysis of variance (ANOVA) technique in completely randomized design by using SAS statistics data editor. Significantly different means among treatments were separated as per the standard method of Duncan at 5% level of probability ($P < 0.05$).

Results and Discussion

For commercial purpose, broilers are usually reared for 4 to 5 weeks and during this time body weight is increased with the age of birds. In our study, body weights were similar at the day 7 within both control and treated groups (Fig. 1). At day 14, body weights were increased in the group T^1 and T^3 but in group T^2 was similar to that of control. However, significant differences were observed at 21, 28, and 35 days of age where body weights were increased within all the treated groups. Similar findings were demonstrated by Jahan *et al.*, (2015) showed that body weights were increased within linear pattern at the increase level of dietary *Nigella sativa* seed meal. Ziad *et al.*, (2008) also supported our findings that broilers feed on 1.5% crushed *Nigella sativa* had higher live body weight and net body weight gain in broiler diet. Our results showed that net weight gain (Fig. 2) and FCR (Fig. 2) were improved in the treated groups than that of the control group. Among the treated

groups, the significant level of improvement was achieved in the T_3 group followed by T_1 and then T_2. In terms of FCR, significant improvement was observed in the group treated with both *Nigella sativa* and amino acids, particularly in the day 28 and 35 (Fig. 2). The improvement of growth performance suggested that *Nigella sativa* may be associated with highly active components particularly thymoquinone and thymohydroquinone which have robust antioxidant activities. Similar studies were performed by Boka *et al.* (2014) and found a great enhancement in FCR in broilers supplemented with 0, 1, 2, and 3% *Nigella sativa* seeds, and the best FCR was recorded in broilers fed on 2% *Nigella sativa*. Jahan *et al.*, (2015) showed that FCR was improved using 1.5% *Nigella sativa* seeds at the early age of 14 days old of chick. Total erythrocyte counts were significantly greater in quail chicks receiving 1% and 1.5 % *Nigella sativa*. Hemoglobin and packed cell volume also were non-significantly higher in the same chicks (Shokrollahi and Sharifi, 2018). In harmony with our current results, Toghyani *et al.* (2010) suggested that black kumin seeds enhanced total erythrocyte counts, hemoglobin and packed cell volume levels in broilers. In our study, total erythrocyte count (Fig. 3) was not markedly different within both control and treated groups. However, at the age of day 35, significant improvements were noticed. Moreover, packed cell volume (Fig. 4) were increased within treated groups where significantly improved at day 35. By contrast, haemoglobin concentration (Fig. 5) were also significantly ($p<0.01$) increased in the treated groups as compared to that of control but markedly improved at the day 15. These results suggest that *Nigella sativa* may have hematopoietic effect. We observed that the group supplemented with only *Nigella sativa* has improvement but not at a significant level. However, the group supplemented with amino acid only showed a significant level of improvement. At the same time, the group supplemented with both *Nigella sativa* and amino acid has improvement which is above the total of both supplementations that may be due to their synergistic effect.

It may be concluded that supplementation of *Nigella sativa* (Kalo jeera) and amino acid (Availa Z/M®) with drinking water may be used for economical and efficient production of broilers.

Figure 1: (A) Amino acid (Availa Z/M®) and (B*) Nigella sativa* (kalo jeera).

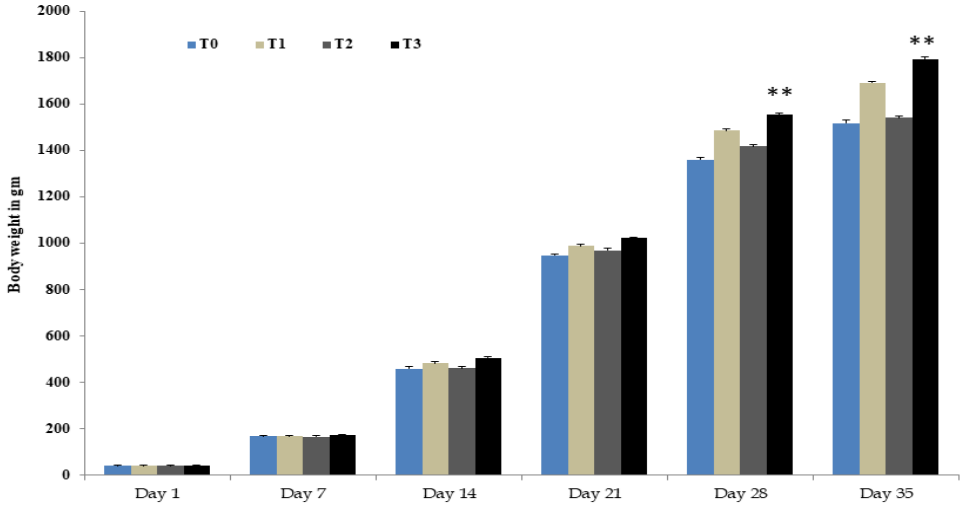

Figure 2: Body weight changes 1st, 7th, 14th , 21th, 28th and 35th days of treatment with *Nigella sativa* (kalo jeera) and amino acid (Availa Z/M) in broiler (n =25 in each group). Data are expressed as mean ± SD, * p < 0.01 significantly different for broiler control. Feed with kalo jeera and amino acid, significant at 1% level of probability (0.00-0.01) of drinking water.

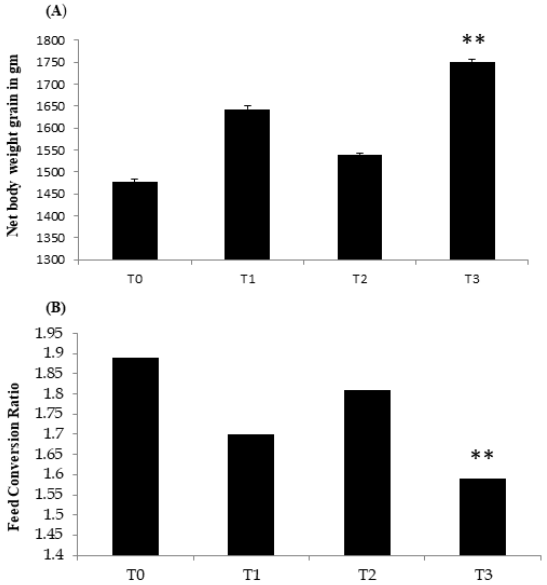

Figure 3: Effects of *Nigella sativa* (Kalo jeera) and amino acid (Availa Z/M®) on (A) net body weight grain and (B) feed conversion ratio (FCR) of Broilers at 35th Day.

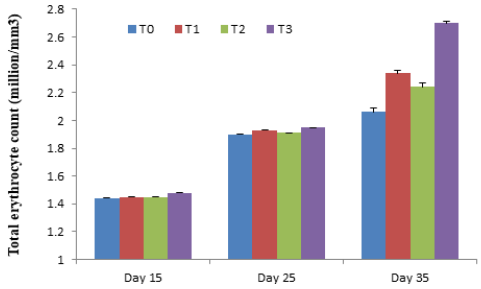

Figure 4: Effects of *Nigella sativa* (Kalo jeera) and amino acid (Availa Z/M®) on 15th, 25th and 35th day of broiler. Total erythrocyte count (million/mm3) in different group of broiler. Data are expressed as mean ± SD, * $p < 0.01$ significantly different for broiler control. Feed with kalo jeera and amino acid, significant at 1% level of probability (0.00-0.01) of drinking water.

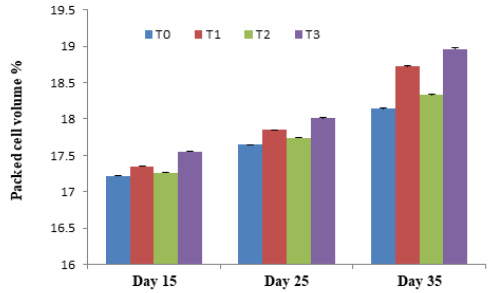

Figure 5: Effects of *Nigella sativa* (Kalo jeera) and amino acid (Availa Z/M®) on 15th, 25th and 35th day of broiler. The pack cell volume (PCV) % in different group of broiler. Data are expressed as mean ± SD, * $p < 0.01$ significantly different for broiler control. Feed with kalo jeera and amino acid, significant at 1% level of probability (0.00-0.01) of drinking water.

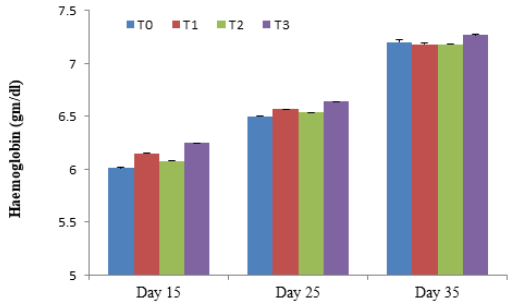

Figure 6: Effects of *Nigella sativa* (Kalo jeera) and amino acid (Availa Z/M®) on 15th, 25th and 35th day of broiler. The haemoglobin (Hb) (gm/dl) in different group of broiler. Data are expressed as mean ± SD, * $p < 0.01$ significantly different for broiler control. Feed with kalo jeera and amino acid, significant at 1% level of probability (0.00-0.01) of drinking water.

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