

## EFFECT OF PROTEIN AND ENZYME SUPPLEMENTATION ON GROWTH AND HEMATOLOGICAL PARAMETERS IN BROILER CHICKENS

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(Available online at: [www.jsau.com.bd](http://www.jsau.com.bd))

### Abstract

The research work was conducted to evaluate the effects of enzyme and protein supplementation on growth performance and hematological parameters in broiler chickens. A total of 80 (15 days old) broiler chicks were randomly divided into four groups (group A, B, C and D). Group A was considered as control, fed with commercial broiler ration, group B supplemented with protein (60% protein concentrate @ 10% with commercial ration), group C supplemented with an enzyme (Alquerzim (@ 1 g l<sup>-1</sup> drinking water), and group D supplemented with both enzyme and protein with the same dose, respectively. The experiment was continued for the period of 21 days. Results revealed that body weights were increased significantly ( $p < 0.05$ ) in all groups and highest body weights were recorded in Group D. Growth rate was highest at the 1st week (15-21 days of age) of experiment than the 2nd week (22-28 days of age) and no significant increase was observed in the last week (29-35 day of age). No significant differences were observed among the groups for PCV and TEC values. Hemoglobin (g dl<sup>-1</sup>) content increased significantly ( $p < 0.05$ ) and ESR decreased significantly ( $p < 0.05$ ) in group D. Therefore, the study reveals that combined supplementation of protein and enzyme showed better result over control in respect to body weight gain, growth rate and hematological parameters.

**Keywords:** Protein, enzyme, growth performance, hematological parameters, broiler chickens.

### Introduction

In Bangladesh, livestock contributes 1.66% of Gross Domestic Product (GDP) and 3.2% in the growth rate of Gross Domestic Product in the years of 2015-16 (Bangladesh Bureau of Statistics, 2016). Feed is an important factor for broiler production as it constitutes about 65-70% of the total cost of production (Bhuiyan, 1998). Cereal and their by-product contain NSP (non-starch polysaccharides) such as cellulose, xylose, galactonic acid, and arabinose which are not easily digested by poultry (Alam, 2003). Vegetable proteins also contain NSP which trap nutrient inside the cell and digestive enzyme access. Moreover, 70-80% phosphorus exists as phytate in the plant origin feedstuff and birds are unable to hydrolyze due to lack of a necessary enzyme (Kies *et al.*, 2001). So, the anti-nutritive parts of the supplied feed not only depress nutrient utilization accompanied by poor growth but also increase the cost of production. Among total feed-cost, cost of protein ingredient is higher than another ingredient i.e. it involves about 15% of the total feed cost (Banerjee, 1992). For this reason, it is very important to find out possibilities of using a source of an alternative for low-cost protein as a substitute for animal origin protein.

The main goal of enzyme supplementation to poultry diets is to remove or destroy the anti-nutritive factors of different feed ingredients. To reduce the loss of feedstuff along with increasing feed utilization and digestibility to expected level different types of enzymes are used with feedstuff. Enzyme increase feed intake, total tract DM, fat and NSP digestion (Meng *et al.*, 2006). Enzymes increase the digestion of crude protein (Yi *et al.*, 1996), feed conversion ratio 8.87% (Augelovicova and Michalik, 1997) and better protein feed conversion at 17.5-20% level (Min *et al.*, 2007). It not only increase feed intake and conversion but also increases daily weight gain 6.1-7.1% after 20 days (Huazhong *et al.*, 1999) and at the grower period, the rate is higher as 16.4%. Additional use of protein concentrates with commercial feed gives better result in body weight gain of broiler at a reasonable cost within a short period of time (Rajini *et al.*, 1998, Urdaneta-Rincom *et al.*, 2004). Body weight gain with protein concentrates depend on different factors as temperature (Temim *et al.*, 2000, high temperature reduce), seasonal variation (maximum at cold season), sex (Husseini *et al.*, 1987, male grown faster) and age (Nahashon *et al.*, 2005, increase at 5-8 week of age). Protein supplement along with enzyme may also increase the meat production with increasing protein intake, digestion, and conversion rate. Therefore, it is necessary to

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make an economically beneficial feeding practice for broiler by supplying additional protein concentrates with exogenous enzyme for maximum output without deterioration of health. Therefore, the research work was designed to know the potential effect of additional supplementation of protein and enzyme on growth performance and improvement in the physiology of broiler chickens.

## Materials and Methods

### Experimental birds and design

A total of 80 (15 days old, 550 gm weight) broiler chicks were used. The chicks were randomly divided into four equal groups (n=20). Temperature, feeder, waterer, feeder space, floor space, litter, room temperature, lighting, and hygienic measures were provided to the chicks according to the protocol described by Mohiuddin *et al.* (2000). Birds of group A was considered as control fed with commercial ration. Group B supplemented with protein (Jasoprot®, protein concentrate 60%, Jayson Agroviet Ltd. @ 10% with commercial feed), group C supplemented with an enzyme (Alquerzim® @ 1 g l<sup>-1</sup> drinking water) and group D supplemented with both enzyme and protein at the same dose, respectively for 21 days. Body weight of an individual supplemented bird was recorded at 7 days interval up to the end of research. The birds were sacrificed to collect a blood sample for hematological study (TEC, Hb, ESR, and PCV). The research work was conducted in the Department of Physiology, Sylhet Agricultural University, Sylhet.

**Table 1: Formulation of commercial ration**

| Ingredients      | Broiler Starter | Broiler Grower | Broiler Finisher |
|------------------|-----------------|----------------|------------------|
| Maize            | 43.00 kg        | 40.32 kg       | 43.64 kg         |
| Wheat            | 10.00 kg        | 10.00 kg       | 10.00 kg         |
| Rice police      | 4.00 kg         | 8.00 kg        | 10.00 kg         |
| Soyabean         | 26.00 kg        | 29.00 kg       | 22.50 kg         |
| Meat & Bone meal | 9.00 kg         | 7.00 kg        | 8.00 kg          |
| Oyster shell     | 1.00 kg         | 1.00 kg        | 1.00 kg          |
| Salt             | 300 g           | 300 g          | 250 g            |
| Methionine       | 200 g           | 200 g          | 180 g            |
| Lysine           | 30 g            | 30 g           | 30 g             |
| Vitamin          | 250 g           | 250 g          | 250 g            |
| Premix (broiler) | -               | -              | -                |
| Feed zyme        | -               | -              | 50 g             |
| Soyabean oil     | 6.5 kg          | 3.5 kg         | 4.00 kg          |
| DCP              | 2.50 g          | 2.50 g         | -                |
| Choline chloride | 100 g           | 100 g          | 100 g            |
| Total            | 100.00 kg       | 100.00 kg      | 100.00 kg        |

Source: Aleya Feed Ltd., Uttara, Dhaka, Bangladesh

**Table 2. Composition of the supplemented enzyme Alquerzim®**

| Composition  | Amount (in 1 g) |
|--------------|-----------------|
| Pepsin       | 50 mg           |
| Pancreatin   | 100 mg          |
| Lipase       | 10 mg           |
| Cellulase    | 20 mg           |
| Excipient to | 1 g             |

Source: ACI Animal Health, 245 Tejgoan Industrial Area, Dhaka

### Measurement of Body weight

The body weight of each broiler was measured with assessing balance on the 15th day (1st day of the study) and sequentially at 7 days interval up to the end of the study.

### Hematological studies

A blood sample was collected at the end of research. A number of sterile test tubes containing anticoagulant (4% sodium citrate solution) at a ratio of 1:10 were taken. Sequentially chilling was done and blood was collected from each group through slaughtering. The hematological studies were performed within two hours after collection. Total Erythrocyte count (TEC) were measured using hemacytometric method, Hemoglobin (Hb) concentration by

hemoglobinometer and Packed Cell Volume (PCV) by Microhematocrit method according to standard procedures (Das *et al.*, 2014; Bauer *et al.*, 1974; Coles, 1980)

## Results

### Body weight

Body weight of all groups of birds is presented in Table 3. Broiler treated with protein, enzyme and both protein and enzyme supplementation showed a rapid body weight gain than the control group.

The body weights of broilers on day 15th (1st day of research) of age were more or less similar and not statistically significant ( $p>0.05$ ). The recorded body weights were  $555\pm 13.21$  g in group A,  $553\pm 11.68$  g in group B,  $551\pm 10.05$  g in group C and  $548\pm 14.2$  g in group D. On 7th day of research it was observed that the body weight in control group A was  $977\pm 11.76$  g and in the treated group B was  $1057\pm 26.20$  g, in group C was  $1073\pm 37.93$  g and in group D was  $1121\pm 22.33$  g. The results were statistically significant ( $p<0.05$ ) between treated and non-treated control group. The highest body weight was recorded in treated group D and lowest in control group A. Data also showed that the body weight in group B and group C were very close to each other.

**Table 3. Body weight (Mean $\pm$ SE) of birds during treatment**

| Groups                 | No. of birds | Before treatment  |   | After treatment  |  |
|------------------------|--------------|---|---|--|--|
|                        |              | Body weight (g) on 1st day of research (15 days of age) | Body weight (g) on 7th day of research (21 days of age) | Body weight (g) on the 14th day of research (28 days of age) | Body weight (g) on the 21st day of research (35 days of age) |
| A (Control)            | 20           | $555\pm 13.21$  | $977^c \pm 11.76$                                       | $1417^c \pm 22.34$   | $1546^c \pm 25.95$   |
| B (Protein)            | 20           | $553\pm 11.68$  | $1057^{bc} \pm 26.20$                                   | $1572^{bc} \pm 18.55$  | $1810^b \pm 24.14$   |
| C (Enzyme)             | 20           | $551\pm 10.05$  | $1073^b \pm 37.93$                                      | $1595^b \pm 44.00$   | $1768^{bc} \pm 55.38$  |
| D (Enzyme and Protein) | 20           | $548\pm 14.2$   | $1121^a \pm 22.33$                                      | $1642^a \pm 26.91$   | $1882^a \pm 37.87$   |
| Level of Significance  |              |   | **  | **   | **   |

NB: Values followed by the same superscripts in the same column are not statistically significant ( $p>0.05$ ), different superscripts indicate that the difference is significant ( $p>0.05$ ).

On the 14th day of study, the body weight in control group A was  $1417\pm 22.34$  g whereas in the treated groups, body weights were  $1572\pm 18.55$  g in group B,  $1595\pm 44.00$  g in group C and  $1642\pm 26.91$  g in group D, respectively. All the results were statistically significant ( $p<0.01$ ). The highest body weight was recorded in treated group D and lowest in control group A. But among the treated groups the body weight of group B was close to group C. On 21st day of research, the body weight in control group A was  $1546\pm 25.95$  g and in the treated groups were  $1810\pm 24.14$  g in group B,  $1768\pm 55.38$  g in group C and  $1882\pm 37.87$  g in Group D, respectively. The results were statistically significant ( $p<0.01$ ) between treated and non-treated control group. The highest body weight was recorded in treated group D and lowest in control group A. But among the treated groups the body weight of group B was close to group C.

### Total Erythrocyte Count (million $\mu$ l<sup>-1</sup>)

Total erythrocyte count (TEC) is presented in Table 4. At 21st day of research (35 days of age), the values of TEC in all treated groups and control group were more or less similar and the values were within the normal range. The highest TEC was recorded in group D ( $3.01\pm 0.04$ ) and lowest in group A ( $2.83\pm 0.06$ ) million  $\mu$  l<sup>-1</sup>. Although these values show a little fluctuation they were not statistically significant.

### Hemoglobin content (g dl<sup>-1</sup>)

Hemoglobin content in different groups of bird is presented in Table 4. At the 21st day of research the hemoglobin content in control group A was  $7.86\pm 0.23$  g dl<sup>-1</sup> and in the treated groups were  $8.10\pm 0.20$  g dl<sup>-1</sup> in Group B,  $8.56\pm 0.23$  g dl<sup>-1</sup> in group C and  $9.49\pm 0.17$  g dl<sup>-1</sup> in Group D. All the data were statistically significant at ( $p<0.01$ ) level. The highest hemoglobin content was recorded in treated group D.

**Table 4. Hematological parameters (Mean±SE) in the broiler of a different group on the 21<sup>st</sup> day of the experiment after treating with protein and enzyme**

| Group                  | No. of birds | TEC (million $\mu\text{ l}^{-1}$ ) | Hemoglobin content (g $\text{dl}^{-1}$ ) | Packed cell volume (%)   | ESR (mm in 1st hr)       |
|------------------------|--------------|------------------------------------|--|--------------------------|--------------------------|
| A (control)            | 20           | 2.83±0.06                          | 7.86 <sup>c</sup> ±0.23                  | 29.97±0.57               | 4.00 <sup>a</sup> ±0.2   |
| B (protein)            | 20           | 2.97±0.09                          | 8.10 <sup>bc</sup> ±0.20                 | 29.97±0.57               | 2.06 <sup>b</sup> ±0.57  |
| C (enzyme)             | 20           | 2.95±0.10                          | 8.56 <sup>b</sup> ±0.23                  | 29.97±0.57               | 3.01 <sup>ab</sup> ±0.57 |
| D (protein and enzyme) | 20           | 3.01±0.04                          | 9.49 <sup>a</sup> ±0.17                  | 31.32 <sup>a</sup> ±0.88 | 1.00 <sup>b</sup> ±0.28  |
| Level of Significance  |              |                                    | **                                       | **                       | **                       |

NB: Values followed by the same superscripts in the same column are not statistically significant ( $p>0.05$ ), different superscripts indicate that the difference is significant ( $p>0.05$ ).

#### Packed cell volume (PCV%)

Packed cell volume (PCV) is presented in Table 4. At 21st day of the experiment, the value of groups A, B, C and D were 29.97±0.57, 29.97±0.57, 29.97±0.57 and 31.32±0.88%, respectively. The highest value was found in group D but all were more or less similar and not significant ( $P>0.05$ ).

#### Erythrocyte sedimentation rate (ESR)

Erythrocyte sedimentation rate (ESR) is presented in Table 4. At 21st day of research the value of groups A, B, C and D were 4.00±0.2, 2.06±0.57, 3.01±0.57 and 1.00±0.28 mm in 1st hour, respectively. The results were statistically significant at ( $p<0.05$ ). The highest value was observed in control group A and lowest value in group D. Group C was close to group B.

### Discussion

The physical condition of birds of all the treated groups (with protein, enzyme and with protein and enzyme combinedly) was better than the control group. The birds of the treated groups showed good response to attendance, better glossy plumage and they were a good feeder and took feed more readily than the control birds. Data obtained on 7th, 14th and 21st day of the study showed that body weight increased significantly on 7th, at 1% on the 14th and 21st day. The body weight of the control group (Group A) increased very slowly in comparison to the treated group and among treated group (B, C and D). It was highest in group D. Although body weights on the 1st day of the study were more or less similar with distinct fluctuation was observed with the advance of age (7th, 14th and 21st day of research) among different groups and always highest in group D combined protein and enzyme supplemented group. The increased weight recorded in group B (enzyme) and is in agreement with the earlier reports of Jamroz *et al.* (1995), Al Bustany (1996), Hanzhong *et al.* (1999), Hosamani *et al.* (2001) they all reported body weight increase with enzyme supplementation. The increased weight recorded in Group C (protein) is also in agreement with the earlier reports of Elangovan *et al.* (2001), Urdaneta-Rincom *et al.* (2004) and Salauddin *et al.* (2012). They reported that the body weights were increased with protein supplementation in broiler chickens.

The highest weight recorded in the present study in group D indicates a synergistic effect of combined treatments of enzyme and protein. These findings are in agreement with the above-mentioned researchers. As protein is a major source of body protein requirement and enzyme increases feed intake (Abbas *et al.*, 1998, Naber 2002, Meng *et al.*, 2006), digestion of nitrogen, Non Starch Polysaccharides (NSP) and other indigestible part of feed (Yi *et al.*, 1996, Meng *et al.*, 2004, Cowieson *et al.*, 2006) and conversion of feed (Augelovicova and Michalik, 1997, Min *et al.*, 2007). The better performance might be due to the synergistic action of both of them on the physiology of the birds. Thus the major proteins are rapidly digested and converted into body protein within a short period of time by the enzyme with reduced fecal loss of the proteins.

Hemoglobin content significantly ( $p<0.01$ ) increased and ESR significantly ( $p<0.05$ ) decreased with protein and enzyme supplementation and the highest value were in combined protein and enzyme supplemented group (group D). No significant ( $p>0.05$ ) differences were observed among the treatment groups for mean PCV and TEC values in respect to the control after treatment. The unchanged hematological parameters in terms of TEC and PCV observed in the present study is similar to the earlier reports of Ahmed *et al.* (1994), Donkoh *et al.* (1999), Odunsi *et al.* (1999) who reported that hematological parameter is unchanged in protein treatment. Decrease ESR value and increase Hb finding in group D are inconsistent with their findings. Decrease ESR value might be due to the improved colloidal state by an increased level of protein supplementation. Increase Hb might be due to increase level of proteinaceous part of

hemoglobin by an increased level of protein supplementation. This finding is partially supported by Elangovan *et al.* (2001).

## **Conclusion**

It could be concluded that combined supplementation of protein (60% protein@ 10% with commercial ration) and enzyme (1g l<sup>-1</sup> drinking water) is beneficial for broiler growth without making any potential hazards to the physiology of the birds. Further studies are necessary to see any adverse effect in relation to histopathology and more serum biochemistry before making a definite conclusion regarding the economically beneficial field practice.

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