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STUDY ON THE EFFECT OF ENZYME AND METHIONINE ON GROWTH PERFORMANCE AND HEMATO-BIOCHEMICAL PARAMETERS IN BROILERS

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ABSTRACT

The experiment was conducted on "Cobb 500" broiler chicks to evaluate the effect of enzymes and methionine supplementation on body weight gain, some hematological parameters (TEC, Hb content, PCV and ESR) and some biochemical parameters (AST and ALT). A total of twenty days old broilers were randomly divided into four equal groups (5×4) as A, B, C and D. Group A was considered as control, fed only with commercial ration, and other (B, C and D) were treated as treatment group, as group B with 1 gm enzymes per liter of drinking water, group C with 1 ml methionine per liter of drinking water and in group D 1 gm enzyme plus 1 ml methionine per liter of drinking water respectively from 0 to 21 days of experiment. It was observed that enzymes and methionine supplementation significantly (P<0.01) increased body. TEC and Hb content increased significantly (p<0.01) in the treated groups as compared to that of control group. ESR, AST and ALT values decreased significantly (p<0.01) in the treated groups from that of the control groups. Therefore, it may be concluded that enzymes and methionine may be used with water to get best result in terms of body weight gain and blood profiles without any detrimental effects on broilers.

Keywords: Broiler, Enzyme, Methionine, Hematological, Biochemical.

Contribution/ Originality

The study is one of very few studies which have investigated the beneficial effects of methionine and enzyme for broiler industry.

1. INTRODUCTION

Poultry meat contributes approximately 37% of total animal protein supplied in the country [1]. There is a great possibility of growth and expansion of the poultry sector both at domestic and commercial level. In the developing country like Bangladesh, unemployment and malnutrition are two major problems. Poultry production can play an important role by providing a large part of increasing demand for animal protein, side by side it is the source of income and can create employment opportunities for the people in the shortest possible time. Broilers are known to live machinery for quick return of edible meat. Broiler production reveals the fact of

maximum return with minimum expense. People from different corner are coming to make the broiler business with a profitable venture.

The nutritive value of available feed stuffs such as wheat, maize rice polish, til oil cake, soybean meal etc. in Bangladesh contain more undigested part [2]. So, the feed utilization and digestibility is poor. Enzyme should have the ability to break down plant cell wall materials and nutrients such protein and starch. Broiler diet is predominantly composed of plant materials, mainly cereals and vegetable proteins plus little amount of animal protein. Many cereals contain some non starch polysaccharides (NSP) such as cellulose, gylose, arabinose and galactonic acid is not easily digested by broiler. The anti nutritional effect of the NSP is manifested by poor growth accomplished by depressed nutrient utilization [3, 4]. The adverse effects can be overcome by dietary supplementation of exogenous enzymes. The use of enzymes in broiler feed has predominantly been related to the hydrolysis of fiber or (NSP) fraction in cereal grains. These NSPs cannot be digested by the endogenous enzymes of poultry and can have anti-nutritive effects. These NSPs can bind to large amount of water and as a result, the viscosity of fluids in the digestive tract is increased. This increased viscosity causes problem in the small intestine because it reduces substrate enzymes interaction, which reduces nutrient availability particularly fat [5] and results increase amount of sticky dropping. Solubilization of insoluble pentosans by the enzymes included led to negative digestibility value of soluble pentosans in the small intestine at the higher levels of supplementation probably was primarily responsible for the improvements in digestibility and production.

Methioinine is essential amino acids of avian species and is thought to enhance growth process. Considering the above facts additional supplementation of enzyme and methionine were supplied to broiler chicks with the following objectives.

To study the effect of enzymes and methionine on growth performance, Hematological parameters and biochemical parameters in boiler chicken.

2. MATERIALS AND METHODS

The experiment was conducted to study the effect of enzymes and methoinine on 'Cobb 500' Broilers. The experiment was conducted from 20 to 40 days of age. Laboratory analysis was performed in the department of Physiology at Bangladesh Agricultural University, Mymensingh. The following produces were followed for conducting the experiment.

2.1. Experimental Design

A total of twenty, 20 days old broiler chicks of "cobb 500 "strain were purchased from" Moshipur, Sirajganj. Birds were housed in proper atmospheric and hygienic condition. The broilers were fed with standard broiler finisher ration throughout the experimental period. These broilers were randomly divided into four equal groups (n=5) namely group A, B, C and D. Initial body weight of each bird was recorded and cased in group wise. Group A was considered as control group and was fed with commercial ration and group B, C and D were treated with supplemented enzyme (Alquerzim, ACI Animal Ltd.) at the dose rate of 1g/L drinking water,

Methionine at the dose rate of 1ml/L drinking water and enzyme 1g/L drinking water plus Methionine 1ml/L drinking water, respectively for next 21 days . Body weight of individual bird was recorded at 7 days interval up to the end of experiment. The birds were sacrificed to collect blood sample for hematological (TEC, Hb, ESR and PCV) and serum for biochemical (AST, ALT) analysis of the birds.

2.2. Experimental Diets

Group A	:	Fed with broiler ration + Fresh drinking water
Group B	:	1gm enzyme (ACI, Pharmaceuticals Ltd.) per liter drinking water+ broiler ration
Group-C	:	1ml Methionine/L drinking water + broiler ration
Group D	:	1gm enzymes/Liter +1ml Methionine drinking water

Table-1. Formulation of commercial ration

Ingredients	Broiler pre starter	Broiler starter	Broiler finisher
Maize	43.00kg	40.32kg	43.64kg
Wheat	10.00kg	10.00kg	10.00kg
Rice polish	4.00kg	8.00kg	10.00kg
Soybean	26.00kg	29.00kg	22.50kg
Meat and bone meal	9.00kg	7.0kg	8.00kg
Oyster shell	1.00kg	1.00kg	1.00kg
Salt	300g	300g	250g
Methionine	200g	200g	180g
Lysine	30g	30g	30g
Vitamin	250g	250g	250g
Premix (broiler) Feed enzyme			50g
Soybean oil	6.5kg	3.5kg	4.00kg
DCP	2.50g	2.50g	-
Choline chloride	100g	100g	100g
Total	100.00kg	100.00kg	100.00kg

Source: Nourish Poultry and Hatchery Ltd. Bangladesh

Table-2. Ingredients and proximate analysis of enzymes (Alquarzim)

Composition	Amount (in gm)
Pepsin	50mg
Pancreatin	100mg
Lipase	10mg
Cellulase	20mg
Excipients to	1mg

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

2.3. Body Weight of Birds

The body weight of each bird was measured with the help of balance on the day 21 of age (0 day experiment) and sequentially 7 days interval upto the end of the experiment.

2.4. Blood Collection

A number of sterile test tubes containing anticoagulant (4% sodium citrate) at ratio of 1:10 were taken. Blood was collected from each group containing five birds through slaughtering. The Hematological studies were performed within two hours of collection.

2.5. Collection and Preparation of Serum Samples

About 3 ml of blood was collected without anticoagulant from the wing vein of chicken and was poured gently in the sterile glass test tubes. The blood containing tubes were placed in a slanting position at room temperature for clotting. The tubes of clotted blood were then placed in the refrigerator (4°C) for overnight and then were centrifuged at 1000 rpm for 15 minutes to have a more clear serum from the blood. The serum was then collected in the screw cap serum vial and stored at -20°C until use.

3. RESULT AND DISCUSSION

Table-3. Body weight (M±SE) in broilers (n=5) on different days after treating with enzymes and methionine.

Group	Pre treatment	Post treatment (%)		
	Body weight (gm) on 21st day of experiment	28th day of experiment	35th day of experiment	42nd day of experiment
A	530.40±9.04b	890.00±10.72d (40.40)	1298.00±14.15d (59.14)	1604.00±21.85d (66.93)
B	535.20±6.79a	914.00±24.69b (41.44)	1410.00±40.59b (62.04)	1772.00±13.43b (69.80)
C	529.00±14.61c	910.00±33.06c (41.87)	1370.00±22.57c (61.39)	1680.00±12.04c (68.51)
D	531.00±10.09b	978.00±7.16a (45.71)	1476.00±25.92a (64.02)	1834.00±14.86a (71.05)

Figures followed by different letter (s) in the same column differ significantly ($p<0.01$)

3.1. Effect of the Body Weight

Body weight of different groups of birds in presented in (Table 3 and Fig. 1). Body weight on 21 days of age (day -0- of experiment) was more or less similar but not statistically significant ($p>0.05$). Highest body weight was recorded in group B and lowest in groups C. The recorded body weights were 530.40±9.04gm in group A, 535.20±6.79gm in group B, 529.00±14.61gm in group C and 531.00±10.09gm in group D.

On 28 days of age (7th day of experiment) it was observed that the body weight in control group A was 890.00±10.72gm and 914.00±24.69 gm., 910.00±33.06 gm 978.00±7.16gm in group B, C and D respectively. All the data were statistically significant ($p<0.01$). The highest and lowest body weight was observed as 978.00±7.16gm and 890.00±10.72gm respectively.

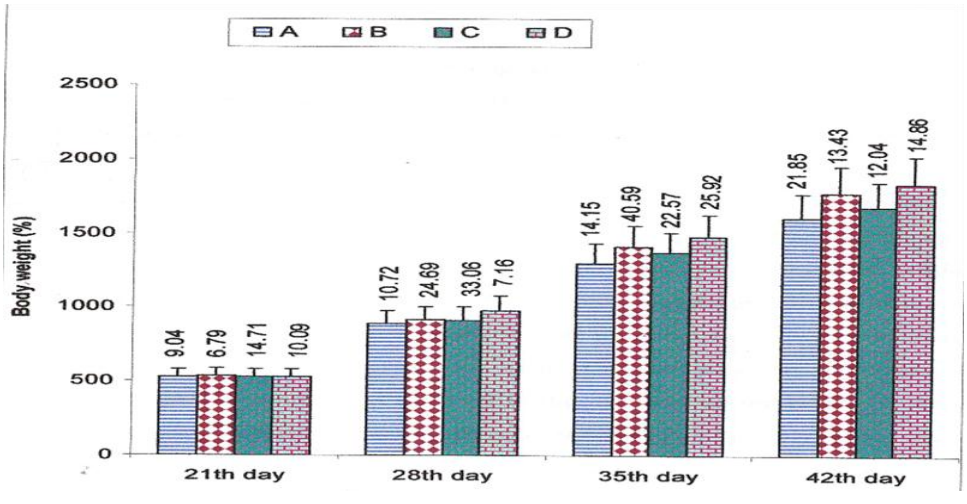
On 35 days of age (14th day of experiment) the body weight in control group A was 1298.00±14.15gm and in the treated groups were 1410.00±40.59gm in group B,

1370.00±22.57gm in group C and 1476.00±25.92gm in group D. The increased rates were statistically significant ($p<0.01$). The highest body weight was recorded in treated group D and lowest in control A. But among the treated groups the lowest body weight was recorded in group C.

On 42 days of age (21th day of experiment) the body weight in control group A was 1604.00±21.85gm and in the treated groups were 1772.00±13.43gm in group B, 1680.00±12.04gm in group C and 1834.00±14.86gm in group D. The body weight gradually increased due to enzyme and methionine supplementation with water and at the terminal day of the experiment the average body weight of all treated groups were statistically significant ($p<0.01$) than the control group. The highest body weight was recorded in treated group D (1834.00±14.86gm).

Data cataloged on 28, 35, and 42 days of age shows that body weight increased significantly ($p<0.01$). The body weight increased slowly in the control group A in respective to the day of experiment but rise of body weight was noticed in the treated groups (B,C and D) in comparison with control group A.

Fig-1. Body weight (mean±SE) in broilers (n=5) on different days after treating with enzyme and methionine. The superscript value above bar indicates standard error



The increased rate of body weight gain in the treated groups might be due to increased digestibility of dry matter, crude protein, non starch polysaccharides and other in digestible food [6, 7]. The increased weight recorded in present findings resembles to the findings of Hosamani, et al. [8]; Jamroz, et al. [9] and Huazhong, et al. [10]. They concluded that body weight increase with enzyme supplementation. Highest weight recorded in the present finding in group D indicates synergistic effect of combine treatment of enzymes and methionine. They better performance might be due to the synergistic action of both of them on the physiology of the birds. Thus methionines are rapidly converted into body protein within a very short period of time of the enzymes with reduced fecal loss of the proteins. This work also deferred earlier report of Han

and Baker [11] who found that weight gain and feed efficacy were not affected by the excess supplementation of methionine or lysine for broiler feed a corn soybean diet but feed intake was reduced at 1% level of inclusions of either amino acid. Christensen, et al. [12] found that efficacy of MHA dependent on its level in the diet and on the levels of methionineand cystine fed with it. It was least effective when fed as the only sulfur amino acid or when fed with cystine. When fed with methionine and cystine. Replacing part of the cystine in a mixture of cystine and MHA with methionine resulted in a marked improvement in performance with L-methionine being slightly more effective than D-methionine the increased weight in present findings also in agreement with the earlier report of Wilson, et al. [13].

3.2. Effects on Hematological Parameters

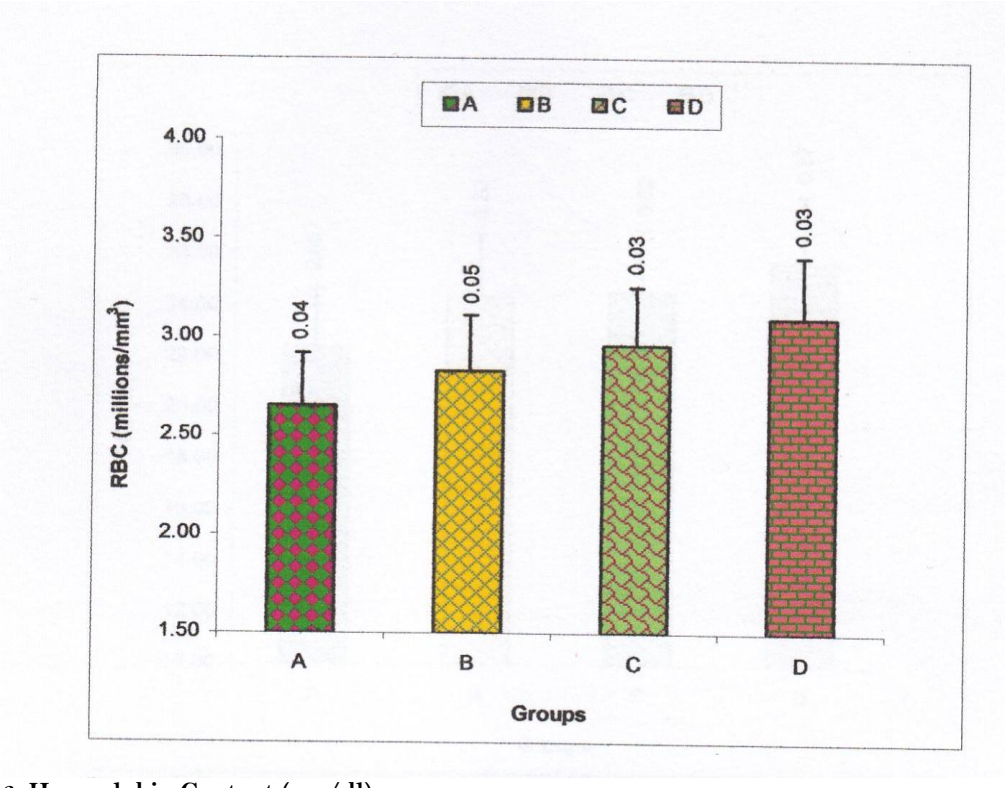
3.2.1. Total Erythrocyte Count (TEC)

Total Erythrocyte count is presented in Table 4 and Fig.2. At final day of experiment (42 days of age) the values of TEC in control group A was 2.65±0.04 million/mm^3 and in the treated group B was 2.83±0.05 million/Imm^3, group C was 2.96±0.03 million/mm^3 and group D was 3.10±0.03 million/mm^3. The highest values of TEC in the group D (3.10±0.03 million/mm^3) and lowest in control group A (2.65±0.04 million/mm ^{3}). All the values of treated groups were significantly ($p>0.01$) higher than the control group A.

Table-4. Hematological parameters (mean±SE) in broilers (n=5) after treating with enzymes and methionine

Group	RBC (millions/mm^3)	Hemoglobin content (gm/dl)	Packed cell volume (%)	ESR mm in 1st hour
A	2.65±0.04d	7.44±0.17d	22.44±0.65c	4.00±0.18a
B	2.83±0.05c	7.70±0.25c	24.40±0.20bc	3.00±0.07b
C	2.96±0.03b	7.60±0.20b	24.60±0.52b	2.02±0.12c
D	3.10±0.03a	7.84±0.31a	25.80±0.57a	1.07±0.04d

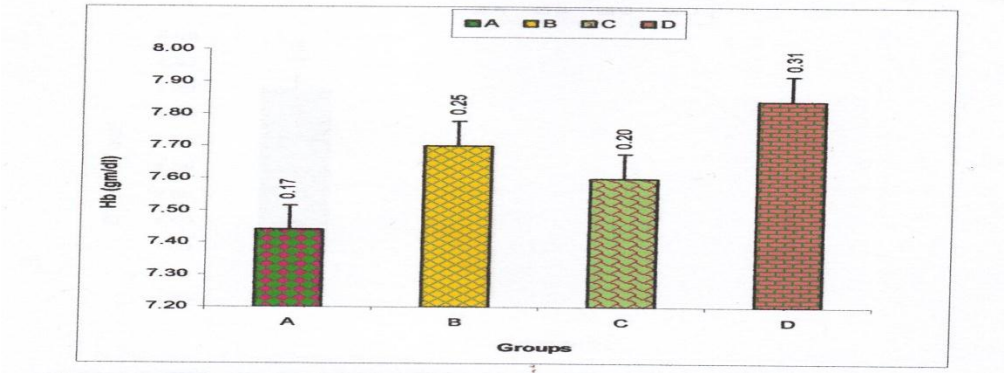
Fig-2. Effect of enzyme and methionine on RBC (millions /mm3)(mean±SE). The superscript value above bar indicates standard error



3.2.2. Hemoglobin Content (gm/dl)

Hemoglobin content in different groups of bird is presented in Table 4 and Fig.3. At final day of experiment (42 days of age) the values of Hb content in control group A was 7.44+0.17 gm/dl and 7.70±0.25 gm/dl, 7.60±0.2 gm/dl and 7.84±0.31 gm/dl in group B,C and D respectively. The highest value of Hb content was recorded in group D (7.84±0.3 1 /dl) and lowest value of Hb was in group A (7.44±0.17 gm/dl). All the values of treated groups were significantly (p>0.0 1) higher than the control group A.

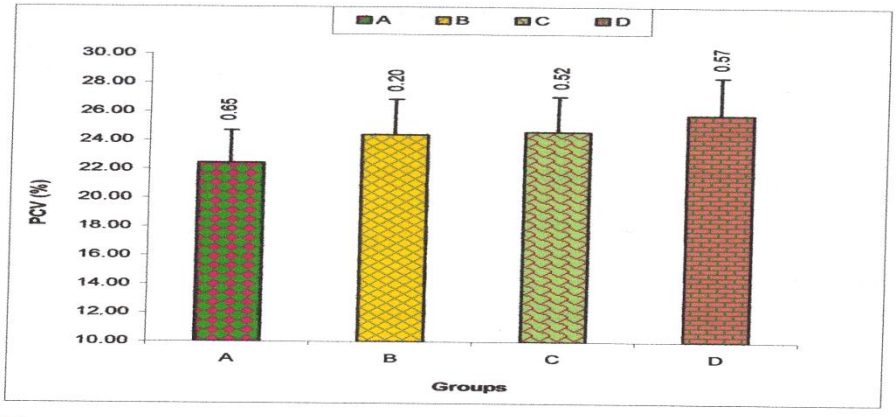
Fig-3. Effect of enzyme and methionine on Hb (gm /dl)(mean±SE). The superscript value above bar indicates standard error



3.2.3. Packed Cell Volume (%)

Packed cell volume (PCV) is presented in Table 4 and Fig.4. At final day of experiment PCV value of groups A. B, C and D were 22.44±0.65%, 24.40±0.2%, 24.60±.0.52% and 25.80±0.57 % respectively. The highest value was found in group D (25.80±0.57 %) and lowest was in control group A (22.44±0.65%). All the values of treated groups were significantly ($p>0.01$) higher than the control group A.

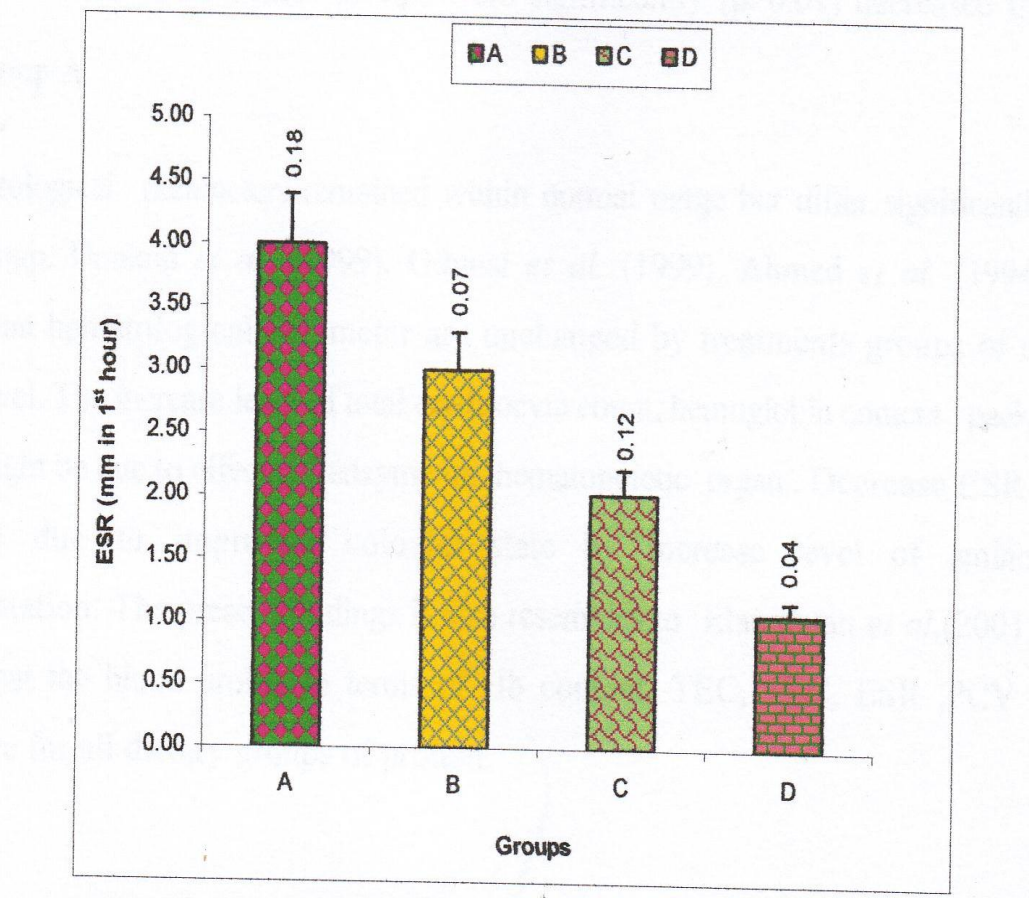
Fig-4. Effect of enzyme and methionine on PCV (%)(mean±SE). The superscript value above bar indicates standard error



3.2.4. Erythrocyte Sedimentation Rate (ESR)

Erythrocyte sedimentation rate (ESR) is presented in Table 4 and Fig.5. At final day of experiment ESR value of groups A. B, C and D were 4.00±0.18, 3.00±0.07, 2.02 ±0.12 and 1.07±0.04 mm in I' hour respectively. The lowest value was found in group D (1.07±0.04 mm in first hour) and highest was in control group A (4.00±0.18 mm in first hour). All the values of treated groups were significantly ($p>0.01$) decreased than the control group A. The hematological parameters remained within normal range but differ significantly with control group. Donkoh, et al. [14]. who reported that hematological parameter are unchanged by treatments groups of dietary protein level. The increase level of total erythrocyte count, hemoglobin content , packed cell volume might be due to effects of enzyme on hernatopoietic organ.. Decrease ESR value might be due to improved coloidal state by increase level of amino acid supplementation. The present findings is also resembles to Elangovan, et al. [15], who reported that the blood profile in terms of Hb content, TEC, TLC, ESR, PCV were comparable for all dietary groups of protein.

Fig-5. Effect of enzyme and methionine on ESR (mm in 1st hour)(mean±SE). The superscript value above bar indicates standard error



4. EFFECTS ON BIOCHEMICAL PARAMETERS

Table-5. Biochemical parameters (meant SE) in broilers (n=5) after treating with enzymes & methionine

Group	AST	ALT (U/L)
A	335.92±14.49a	6.24±0.36a
B	311.74±13.82b	4.76±0.20b
C	305.44±9.83c	4.36±0.09bc
D	283.42±12.33d	4.06±0.12

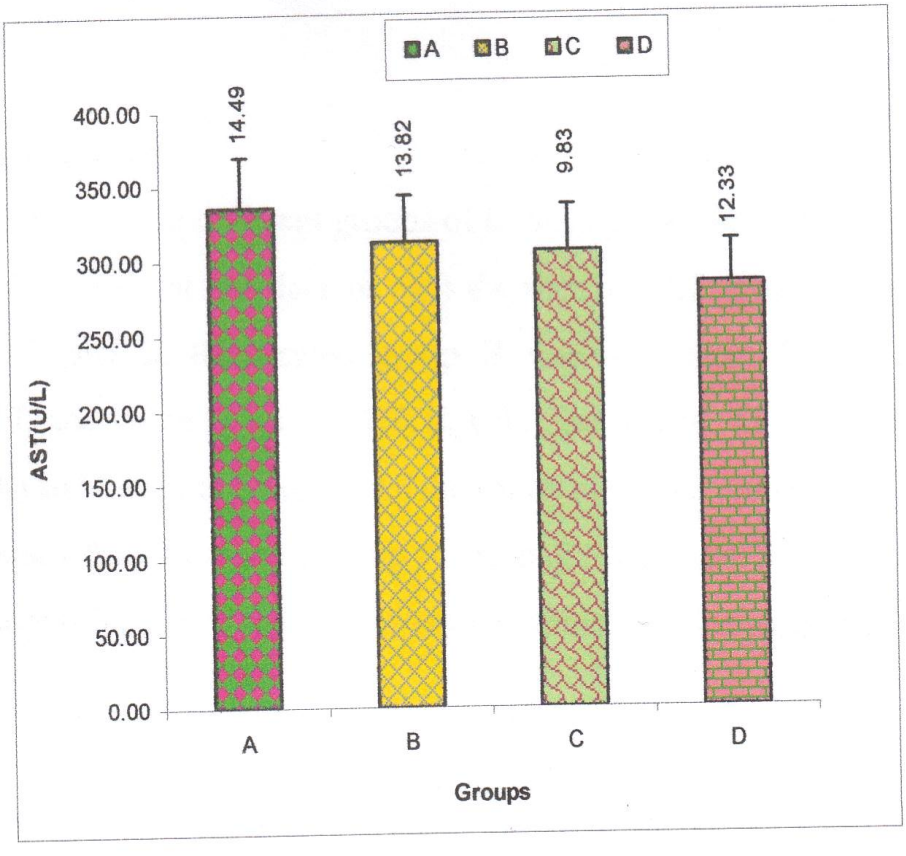
Figures followed by different letter(s) in the same column differ significantly (p<O 01)

4.1. Ast Concentration

The AST concentration in different groups of birds was presented in Table 5 and Fig.6. At final day of experiment (42 days of age) the AST titer was 335.92±14.49 U/L in control group A and in treated groups the values were 311.74±13.82 U/L in group B. 305.44±9.83 U/L in group C and 283.42±12.33 U/L in group D. The highest value was in group A and lowest in

treated group D All the values of treated groups were significantly ($p<0.01$) decreased than the control group A.

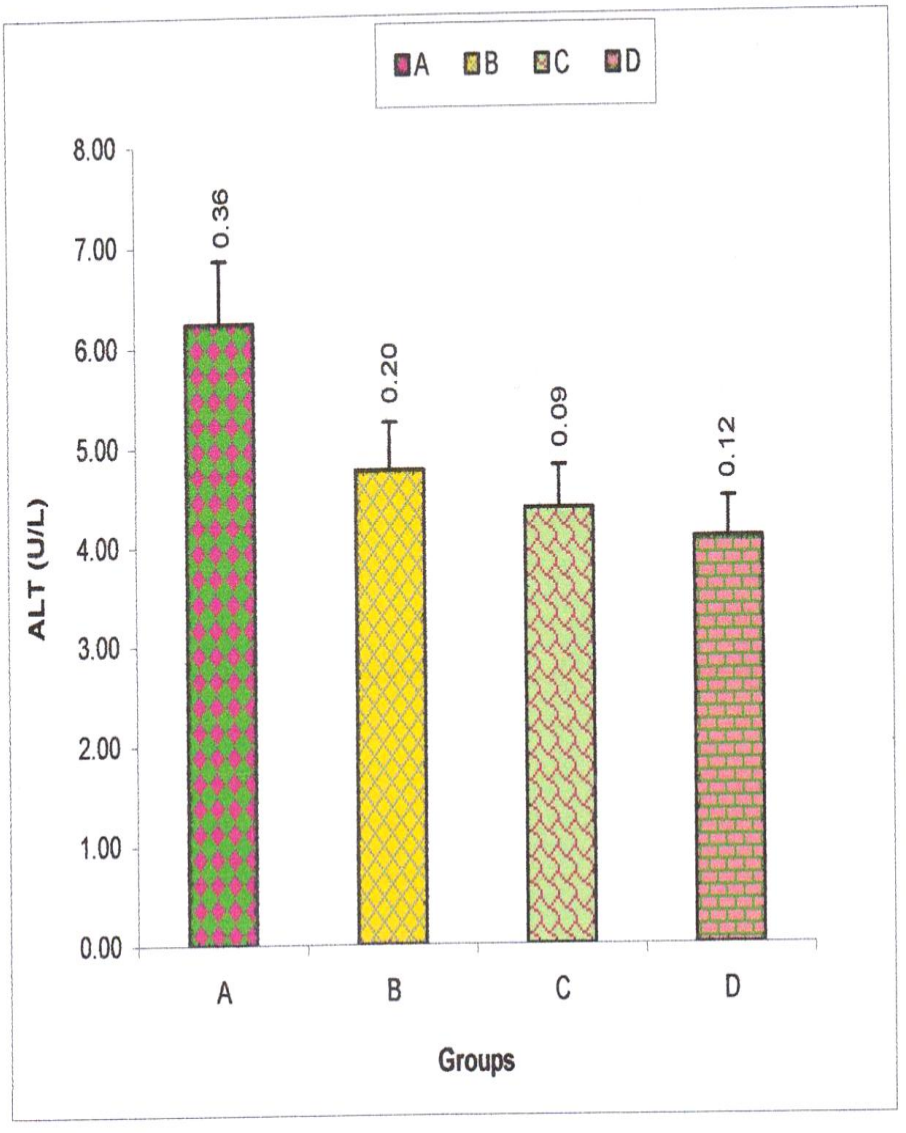
Fig-6. Effect of enzyme and methionine on AST (U/L)(mean±SE). The superscript value above bar indicates standard error



4.2. Alt Concentration

The ALT concentration in different groups of birds is presented in Table 5 and Fig. 7. At final day of experiment (42 days of age) the titer of ALT in control group A was 6.24±0.0.36 U/L and in the treated group B was 4.76±0.0.2 U/L, group C was 4.4.36±0.09 U/L, and group D was 4.06±0.12 U/L. The highest ALT was in the control group (group A) and lowest in group D. All the values of treated groups were significantly ($p<0.01$) decreased than the control group A. Among the groups B, C and D the ALT values were more or less similar and comparison within were insignificant ($P>0.05$).

Fig-7. Effect of enzyme and methionine on ALT (U/L) (mean±SE). The superscript value above bar indicates standard error.



The greatly reduced AST, ALT titre level as observed in the present study implies good health condition with less muscle damage of muscle cell by increase supplementation of protein and enzymes in the agreement with Amubode and Fetuga [16] who reported that high protein diet decrease oxidative damage.

Amubode and Fetuga [16] stated that AST is not affected by higher protein supplementation but our study shows the decrease level of AST this is might be due to less muscle damage due to enzymes supplementation to broiler. Kumar and Rawat [17] shows AST cone in blood serum decrease with age.

5. SUMMARY AND CONCLUSION

It was observed that enzymes and methionine supplementation enhanced growth of broilers. The body weight in the group D was highest which followed in descending order in groups C, B and A, the body weight was significantly ($P<0.01$) increased at the terminal day of experiment in comparison with that of the control group (group A).

Blood parameters like TEC, Hb, concentration and PCV values increased significantly ($p<0.01$) in the treated groups (B, C and D) as compared to that of control group A. But ESR values decreased significantly ($p<0.01$) in the treated groups (B, C and D) as compared to that of control group A. Biochemical parameters like AST and ALT values were decreased significantly ($P<0.01$) in comparison to control group (group A).

It is therefore, suggested that supplementation of enzymes and methionine are essential for rapid muscle development, synthesis of other amino acids, proper digestion of feed stuffs, more use of low quality feed, expected body growth. Due to deficiency, it causes harmful effects in poultry, causes great economic loss. However, effects of enzymes and methionine supplementation remain to be studied. Further studies are necessary to see any adverse effect in relation to histopathology and biochemistry before making any conclusion regarding the beneficial effect in broiler.

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