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Biological Effects of Commercial Enzymes, Probiotics and Liver Tonic on Live Weight and Hematobiochemical Parameters in Broiler

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Abstract **– The study was conducted on broiler chicks to investigate the effects of probiotics, enzymes and liver tonic supplementation on live weight and hemato-biochemical parameters. A total number of 250 day-old broiler chicks were allowed to take rest for 13 days for the adaptation and supplied with normal diet and water. After day 13 they were randomly divided into four equal groups (N=50) and kept the groups as T0 (control), T1 (probiotics), T2 (enzyme) and T3 (probiotics plus enzymes) for 21 days. Live weight was measured on 1st day of experiment (day 14) and sequentially at 7 days interval up to 21st day of experiment (day 35). Blood samples were collected from the randomly selected birds (N=10) from each group at the end of experiment on day 35. Live weight were increased significantly ($p<0.01$) in the treatment groups as compared to control group. Total Erythrocyte Count (TEC), Hb concentration and Packed Cell Volume (PCV) were increased significantly ($p<0.01$) but the Erythrocyte Sedimentation Rate (ESR) and Total Leukocyte Count (TLC) decreased significantly ($p<0.01$) in all the treatment groups. Aspartate Transaminase (AST) and Alanine Transaminase (ALT) values were also decreased significantly ($p<0.05$) in the treatment groups. Probiotics, enzymes and liver tonic would be a better supplementation on live weight gain without any detrimental effects in broilers.**

Keywords **– Probiotics, Enzymes, Liver Tonic, Live Weight, Hematobiochemical Parameter, Broiler.**

I. INTRODUCTION

Diet of broiler is mainly processed with plant materials chiefly cereals and vegetable proteins and small amount of animal protein. Many cereals and their by-products contain non-starch polysaccharides (NPS) such as cellulose, hemicellulose, xylose, arabinose, galactonic acid which are not easily digested by poultry. The nutritive value of available feed stuffs such as wheat, maize, rice polish, til oil cake, soyabean meal etc. in Bangladesh contain more indigestible part [1]. Furthermore, most of the feed ingredients contain some anti-nutritional factors and non-digested part which inhibit feed utilization. The anti-nutritive effect is manifested by depressed nutrient utilization accompanied by poor growth. This adverse effect can be overcome by supplementation of exogenous carbohydrase (xylanase) enzymes that is observed by the viscosity of intestinal contents and to improve digestibility of starch, protein, fat and apparent metabolisable energy inbroiler feed [2].

Probiotics are feed additives that contain live microorganisms and promote beneficial effects to the host by favoring the balance of the intestinal microbes [3] including live bacteria, yeast, their metabolites and pH adjusters, which contribute to maintain balance in intestinal microflora [4]. Probiotics are living microorganisms which is beneficial to the individual and antagonistic to harmful microbes [5] assists in preventing colonization of pathogen in the intestinal tract and in producing certain enzyme like substances [6] claimed to exert beneficial effects on live weight gain, feed consumption, feed conversion ratio and livability [7].

Rapid and maximum growth in a minimum period with efficient feed utilization is of utmost importance for the profitable broiler production, as the feed itself contributes about 70% of the total expenditure on poultry farming [8]. Liver function is very essential for huge amount of metabolism for the rapid and maximum growth in broiler. It is also reported that liver tonic have been indicated to exert immunomodulatory action, which confer birds with greater general immunity from various diseases, disorders and tolerance against toxins leading to lower mortality, morbidity, adaptability and metabolism and enhanced productivity.

Considering the above facts the study was done to determine the effects of enzymes, probiotics and liver tonic supplementation on live weight, hematological parameters like Total Erythrocyte Count (TEC), Hemoglobin concentration (Hb), Packed Cell Volume (PCV), Erythrocyte Sedimentation Rate (ESR) and Total Leukocyte Count (TLC) along with serum biochemical parameters like Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT).

II. MATERIALS AND METHODS

Description of the Study Area

The present study was conducted during the period from March to May 2015 in Sabbir Broiler Farm, Jamalpur sadar, Jamalpur, Bangladesh under the supervision of the department of Physiology, Sylhet Agricultural University, Sylhet, Bangladesh. Laboratory tests were conducted at the District Veterinary Hospital, Jamalpur and Fair Diagnostic and Clinic (Pvt.) Limited, Jamalpur, Bangladesh.

B. Experimental Design

A total number of two hundred and fifty (250) day-old broiler chicks of Cobb 500 strain were obtained from a reputed hatchery (Nourish poultry and Hatchery Ltd.). All the birds were reared under standard husbandry. All the day old broiler chicks were allowed to take rest for 13 days for the adaptation and supplied with normal diet and water. After 13th day two hundred (200) birds were randomly selected and divided into four equal groups (N=50) and marked them as group T_0, T_1, T_2 and T_3. Broiler chicks of group T_0 were kept as control and fed with only the commercial broiler ration and fresh drinking water for 21 days but the broiler chicks of group T_1, T_2 and T_3 were treated with probiotics, enzymes, probiotics plus enzymes plus liver tonic respectively along with commercial broiler ration for 21 days.

C. Experimental Diets

The commercial broiler ration (broiler starter, grower and finisher marketed by Nourish poultry and Hatchery Ltd., Bangladesh), commercial probiotics (Protexin®, Novartis Bangladesh Ltd.), commercial enzymes (Alquerzim®, ACI Animal Health, Bangladesh) and commercial liver tonic (Livavit®, Square Pharmaceuticals Ltd, Bangladesh) were purchase from the local market of Jamalpur. From 14 days of age of birds the probiotics, enzymes and liver tonic were supplemented along with normal diet. T_0 were fed with commercial broiler ration and fresh drinking water, T_1 were treated with probiotics (Protexin®) @ 1gm/L drinking water and commercial broiler ration, T_2 were treated with enzymes (Alquerzim®) @ 1gm/L drinking water and commercial broiler ration, T_3 were treated with probiotics (Protexin®) @ 1gm/L plus enzymes (Alquerzim®) @ 1 gm/L plus liver tonic (Livavit®) @ 1ml/L drinking water drinking water and commercial broiler ration for 21 days.

D. Measurement of Live Weight of Broiler Chicks

Live weight of broiler chicks was measured with the help of electric balance on the day 14 of age (1st day of experiment) and sequentially at 7 days interval up to the end of the experiment up to day 35 of age (21st day of experiment).

E. Collection of Blood Samples

For the hematobiochemical examinations, blood samples were collected aseptically from the randomly selected 10 (i.e. n = 10) birds from each and every group at the end of experiment on day 35 of age (21st day of experiment). At slaughter, from each bird 5ml of blood sample was collected and was transferred immediately to a clean, dried, sterile test tube containing anticoagulant (EDTA) @ 1:10 for the hematological studies and were performed within two hours after collection of blood samples. Another 5ml of blood sample was collected from the same and each bird and was transferred immediately to a clean, dried, sterile test tube without anticoagulant which was used to collect the serum for biochemical studies.

F. Separation and Preservation of Serum

The blood samples (without anticoagulant) containing test tubes were placed at normal temperature at 45^0 angle. After 24 hours the upper most light yellowish liquid (serum) were collected from the test tubes in another tubes by the help of individual syringe and needle. Then the serum was centrifuged at 3000rpm for 15 minutes to discard the unwanted erythrocytes and this process was repeated for 3 times. After centrifuged these erythrocytes were accumulated at the bottom of the tubes. Then finally the clean and pure serum samples were collected by the help of individual syringe and needle in the bijou bottles and were stored at -20^0C for the further use as biochemical studies.

G. Hematological and Biochemical Examination

Estimation of Total Erythrocyte Count (TEC), Hemoglobin (Hb) concentration, Packed Cell Volume (PCV), Erythrocyte Sedimentation Rate (ESR) and Total Leukocyte Count (TLC) were performed on day 35 of age (21st day of experiment) following standard methods [9]. Blood sera biochemical parameters AST and ALT were measured from the bird's serum on day 35 of age (21st day of experiment) by the use of specific test kit (liquiUV Test for AST and ALT, Trade Worth Lab. Ltd., Germany) and auto analyzer (Humalyzer 3500).

H. Statistical Analysis

Experimental data were calculated and expressed as Mean ± SE on live weight, hematological parameters (e.g. TEC, Hbconcetration, PCV, ESRand TLC) and serum biochemical parameters (e.g. AST and ALT) were analyzed statistically using MS-STAT statistical software [10] with the help of a one way ANOVA method was made by F variance test.

III. Results

Effects of probiotics, enzymes and liver tonic supplementation with water on live weight, hematological parameters and biochemical parameters of different groups of broiler chickens are shown at Table 1, 2 and 3 respectively

Table 1: Effect of probiotics, enzymes and liver tonic on live weight in gm (Mean ± SE) of different groups of broiler

Groups	Live weight in gm (Mean ± SE)			
	Pre-treatment	Post treatment		
	14 day of age (1st day of experiment)	21 day of age (7th day of experiment)	28 day of age (14th day of experiment)	35 day of age (21st day of experiment)
T_0 (control)	368±0.95	680±0.77	1167±0.80	1447±0.75
T_1 (probiotics treated)	362±1.25	698±0.95**	1217±1.05**	1610±1.00**
T_2 (enzymes treated)	367±0.82	702±0.65**	1226±1.02**	1613±1.03**
T_3 (probiotics + enzymes + liver tonic treated)	364±0.98	740±0.96**	1266±0.62**	1697±0.52**

****= Significant at 1% (p<0.01) level of probability. N = 50 birds in each group.**

Table 2: Effect of probiotics, enzymes and liver tonic on hematological parameters (Mean±SE) of different groups of broiler on 21st day of experiment (35 day of age)

Groups	Hematological parameters (Mean±SE)				
	TEC (million/mm^3 of blood)	Hb (gm/dl)	PCV (%)	ESR (mm in 1st hour)	TLC (thousand/ mm^3 of blood)
T_0 (control)	2.36±0.024	7.67±0.015	25.60±0.024	3.25±0.025	24.86±0.42
T_1 (probiotics treated)	2.71±0.022**	8.62±0.021**	32.35±0.027**	2.35±0.030**	22.06±0.56**
T_2 (enzymes treated)	2.68±0.017**	8.56±0.049**	30.96±0.056**	2.42±0.015**	21.12±0.86**
T_3 (probiotics + enzymes + liver tonic treated)	2.98±0.027**	8.88±0.042**	33.87±0.070**	1.12±0.020**	19.76±0.43**

****= Significant at 1% (p<0.01) level of probability. n = 10 birds (randomly from 50 birds) in each group.**

Table 3: Effect of probiotics, enzymes and liver tonic on serum biochemical parameters (Mean±SE) of different groups of broiler on 21st day of experiment (35 day of age)

Groups	Biochemical parameters (Mean±SE)	
	AST (U/L)	ALT (U/L)
T_0 (control)	43.50±1.32	7.05±0.19
T_1 (probiotics treated)	38.67±1.76*	5.67±0.22*
T_2 (enzymes treated)	37.56±0.98*	5.12±0.33*
T_3 (probiotics + enzymes + liver tonic treated)	29.45±1.41*	3.98±0.24*

***= Significant at 5% (p<0.05) level of probability. n = 10 birds (randomly from 50 birds) in each group.**

IV. Discussion

Data cataloged on 7th, 14th and 21st day of experiment shows that the live weight were increased significantly (p<0.01). The body weight increased slowly in the control group T_0 in respective days of experiment but rise in body weight was noticed in the treated groups (T_1, T_2 and T_3) and it is highest in group T_3 in comparison with control group T_0. Although body weight on 1st day of experiment was more or less similar a distinct fluctuation was observed with the advanced of age (7th, 14th and 21st day of experiment) among different groups and always highest in group T_3. The highest live weight recorded in the present findings in group T_3 at 21st day of experiment (35 day of age) indicates synergistic effect of combined treatments of probiotics, enzymes and liver tonic.

These finding are supported by many studies [7, 11, 12, 13, 14, 15, 16]. The effect of dietary supplementation of probiotic lactobacillus significantly enhanced body weight gain in broilers [17]. Increasing the growth performance of broiler chickens by supplementing their diets with exogenous enzymes can also contribute to positive changes in gut health [18]. Diet supplementation with probiotic and prebiotic increased body weight of broilers at 28 and 42 days of age (P<0.05) compared to control birds [19]. Studies also reported [20] that inclusion of probiotics in the diets of broilers will bring about improved live weight and feed conversion ratio. Multi-enzymes supplementation showed significant (P<0.05) positive effect on weight gain (last three weeks), feed intake (last two weeks), FCR (1st, 2nd, 4th and 5th week) and nutrient retention in T3 when compared with control [21]. Live weight of broiler significantly increased by probiotics, enzymes [21] and liver tonic [8, 22] those support the present study.

Result showed that TEC, Hb concentration and PCV were increased but ESR and TLC were decreased in the birds of probiotics, enzymes and liver tonic treated groups than control group. TEC, Hb concentration and PCV showed significantly (p<0.01) higher values in broiler of T_1, T_2 and T_3 groups than control group T_0 but the ESR and TLC were decreased significantly (p<0.01)in broiler of T_1, T_2 and T_3 groups than control group T_0. The different values of hematological parameters were remain within normal range but statistically significant within the comparison of treatment and control group. The increased level of TEC, Hb content, PCV and ESR might be due to the initiative effects on hematopoietic organs and the better hematological profile recorded in the present findings in group T_3 at 21st day of experiment (35 day of age) indicates synergistic effect of combined treatments of probiotics, enzymes and liver tonic.

The hematological parameters of present findings resembles to other study [23] where it is reported that the number of erythrocyte and other components of blood varied due to the influence of sex, environment, exercise, nutritional status and climate. Probiotic improves hematology of broiler chicken. Hb, RBC, PCV were increased at 1.5% with probiotic *Saccharomycescerevisiae* [24]. This investigation supported by the present study. TEC, PCV and Hb content increased significantly (p<0.01) in the probiotics and enzymes treatment groups as compared to that of control group [15] that strongly supported by present study. Probiotic and enzyme positively affect hematological parameters reported by many investigators such as [4, 21, 25, 26]. However, it is also reported that probiotics and enzymes significantly increased the TEC, Hb and PCV but significantly decreased the ESR and TLC of broiler which resembles the present findings [21].

On day 35 of age (21st day of experiment) result observed that AST and ALT were decreased in the birds of probiotics, enzymes and liver tonic treated groups than control group. AST and ALT showed significantly (p<0.05) decreased in broiler of T_1, T_2 and T_3 groups than control group T_0. The lowest AST and ALT recorded in the present findings in group T_3 at 21st day of experiment (35 day of age) indicates synergistic effect of combined treatments of probiotics, enzymes and liver tonic.

This present finding is in agreement with other study [21] that Serum Alanine Aminotransaminase (ALT) and Serum Aspertate Aminotransaminase (AST) levels were decreased significantly ($p<0.01$) in probiotics and enzymes treated groups than the control group in broiler. AST and ALT values decreased significantly ($p<0.01$) in the enzyme and multivitamin treated groups than control [27] but decreased non-significantly in herbal liver tonic Xlivpro premix supplemented group than untreated group [28].

V. Conclusion

The study demonstrated that enzymes, probiotics and liver tonic have specific effect on live weight gain of broiler without any detrimental effects in broiler. So, they would be a better supplementation on live weight gain of broiler. Further study should be needed including histopathology and molecular effects of the agents.

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