Effect Of Enzyme and Vinegar (Acetic Acid) on Growth Performance and Hematological Parameters in Broiler

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Abstract

The experiment was conducted on "Cobb 500" broiler chicks to investigate the effects of enzymes and vinegar on live weight and hematological parameters (TEC, Hb concentration, PCV, ESR, TLC, AST and ALT). A total 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 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 group T_1, T_2 and T_3 were treated with enzymes (Alquerzim®) @ 1gm/L, vinegar @ 1gm/L, enzymes (Alquerzim®) plus vinegar @ 1ml/L drinking water with normal diet for 21 days respectively. Live weight as gm of broiler chicks were measured on the day 14 of age (1st day of experiment) and sequentially at 7 days interval up to the end of the experiment (day 35 of age i.e 21st day of experiment). For the hematological examinations, blood samples were collected 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). It was observed that live weight was increased significantly ($p<0.01$) in the treatment groups (T_1, T_2 and T_3) as compared to control group T_0. TEC, Hb concentration and PCV were increased significantly ($p<0.01$) but the ESR and TLC significantly ($p<0.01$) decreased in all the treatment groups as compared to control group T_0. Serum biochemical parameters like AST and ALT values were also decreased significantly ($p<0.05$) in the treatment groups as compared to control group T_0. Therefore, it may be concluded that additional enzymes and vinegar had better performance in terms of live weight gain and hematological parameters without any detrimental effects in broilers.

Keywords: Broiler; Growth performance; enzymes; vinegar; hematological parameters; Galactosidases; Xylanases; pectinases; raffinose; hemicelluloses.

Introduction

Poultry production can play a significant role by providing a large part of escalating 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. Broiler birds are known to live machinery for quick return of edible meat. Poultry meet contributes approximately 37% of total animal protein supplied in the country (Kabir *et al*., 2005). 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, malnutrition and unemployment are two major problems. Broiler production reveals the fact of maximum return of minimum expense. Broiler farming is developed all over the country and recognized as a beneficial endeavor. It is a means of quick return (Hamid *et al*., 2001). Its success depends on how hurriedly a bird attains a maximum marketable age in a minimum period.The feed accounts 70% of the total cost of broiler production. Thus, it is essential to utilize most efficiently to have minimum production cost. A number of feed additives like antibiotics, steroids, vitamin, minerals and other growth promoters have been used to improve the performance of broiler growth. The excessive dependency on medications threatens the mankind in antibiotic resistance. However, it is also discouraged to use growth promoters because of their residual effect in broiler meat. People from different corner are coming to make the broiler business with profitable venture.Enzyme and vinegar protects deficiency diseases and stimulate growth rate. Beside this, enzyme supplement along with vinegar reduces mortality, keep birds healthy, increase feed intake, improve digestion and feed conversion rate. Most of the feed ingredients contain some anti-nutritional factors and indigestible part, which hinders feed utilization and bird's performance.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 (Jin *et al*., 2000). So, the feed digestion and utilization 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 and little amount of animal protein. Many cereals and their by-products contain non-starch polysaccharides (NPS) such as cellulose, xylose, arabinose, galactonic acid which are not easily digested by poultry.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 in broiler feed (Choct *et al*., 1995). Vinegar is a diluted solution of acetic acid in water and it is one of the organic acids legally allowed to use all over the world as food additives and preserves (Ricke, 2003). It is quite often use to improve the productive performance of meat breeds and barley feed conversion in order to improve the absorption of minerals, vitamins and speed recovery from stress (Fernandes, 2008). The short chain fatty acids (SCFA) considered as the potential alternative to antibiotics (Van Immerseel et al., 2004). Vinegar is one such SCFA, which has higher bactericidal activity when the acid is undissociated. The bacterial cell takes up undissociated fatty acid, which by ionizing fatty acid inside the bacterial cell, there is a change in the intracellular pH leading to the death of bacterial cells (Khan and Iqbal 2016). Organic acids added to feed for their various beneficial effects on gut function and microflora, feed preservation from microbial invasion, inhibition of pathogenic bacteria, enhancing mineral absorption and improvement of nutrient digestibility (Dehghani-Tafti and Jahanian, 2015).Since the levels of SCFA are quite low in the intestine of young chicks, so they may be the best candidates for use of acidifiers (Panda et al., 2009; Abudabos et al., 2017). The addition of organic acids in diet can have a beneficial effect on the performance of poultry

by decreasing pathogenic bacteria. Currently, drinking water (DW) acidification is another achievement in the broiler industry used for improving performance. Following studies indicated that addition of organic acid to the drinking water helps to reduce the level of pathogens in the water and the crop or proventriculus, to regulate gut microflora, to increase the digestion of feed and to improve growth performance (Khan and Iqbal, 2016).

MATERIALS AND METHODS

The experiment was carried out to investigate the effects of enzymes and vinegar on broiler chicken to assess the growth performance as live weight and some hematobiochemical parameters.
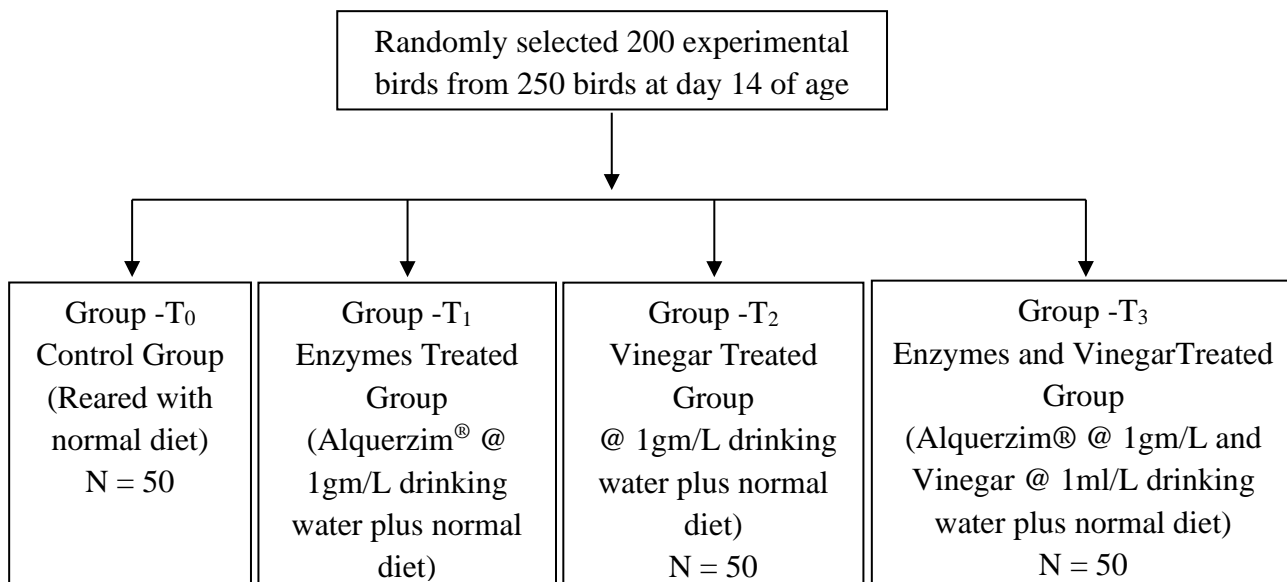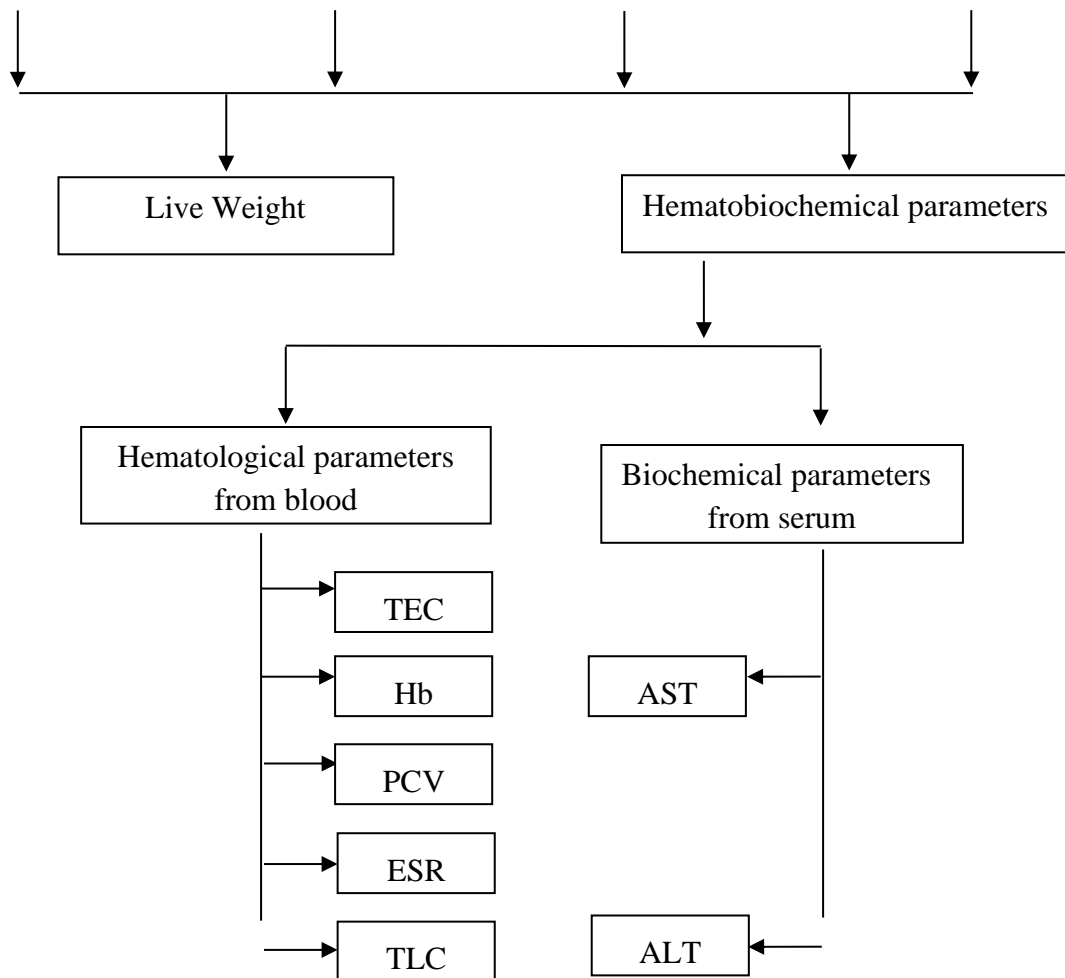
3.1 Experimental duration and place

The present study was conducted during the period from November to December 2018 in Bismillah Broiler Farm, Tongibari, Munshiganj under the supervision of the department of Physiology, Sylhet Agricultural University, Sylhet. Laboratory tests were conducted at the District Veterinary Hospital, Munshiganj and Seba Diagnostic and Clinics (Pvt.) Limited,Tongibari, Munshiganj.

3.2 Experimental birds and 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 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, broiler chicks of group T_1 were treated with enzymes (Alquerzim®) @ 1gm/L drinking water and commercial broiler ration, broiler chicks of group T_2 were treated with vinegar @ 1gm/L drinking water and commercial broiler ration, broiler chicks of group T_3 were treated with enzymes (Alquerzim®) @ 1 gm/L plus vinegar @ 1ml/L drinking water drinking water and commercial broiler ration for 21 days. Initial body weight of each group was recorded just prior to segregation. Body weight was recorded at seven days interval up to the end of 21 days of experimental period and the randomly selected birds from each group were sacrificed to collect blood sample at the 21st day of experiment for hematobiochemical parameters (TEC, Hb, PCV, ESR, AST and ALT).

Layout of the Experiment

Randomly selected 200 experimental birds from 250 birds at day 14 of age

Group -T_0 Control Group (Reared with normal diet) N = 50	Group -T_1 Enzymes Treated Group (Alquerzim® @ 1gm/L drinking water plus normal diet)	Group -T_2 Vinegar Treated Group @ 1gm/L drinking water plus normal diet) N = 50	Group -T_3 Enzymes and VinegarTreated Group (Alquerzim® @ 1gm/L and Vinegar @ 1ml/L drinking water plus normal diet) N = 50

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                    ┌──────────────┐          ┌──────────────────────────────┐
                    │  Live Weight │          │ Hematobiochemical parameters │
                    └──────────────┘          └──────────────────────────────┘

        ┌─────────────────────────┐          ┌──────────────────────────┐
        │ Hematological parameters│          │ Biochemical parameters   │
        │      from blood         │          │      from serum          │
        └─────────────────────────┘          └──────────────────────────┘

                    ┌────────┐                        
                    │  TEC   │                        
                    └────────┘                        
                    ┌────────┐              ┌────────┐
                    │   Hb   │              │  AST   │
                    └────────┘              └────────┘
                    ┌────────┐                        
                    │  PCV   │                        
                    └────────┘                        
                    ┌────────┐                        
                    │  ESR   │                        
                    └────────┘                        
                    ┌────────┐              ┌────────┐
                    │  TLC   │              │  ALT   │
                    └────────┘              └────────┘
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3.3 Experimental diets

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

3.4 Preparation of experimental house

An open sided house was used for experimental purpose. Four pens of equal sizes were made by wire-net and bamboo materials. A service area was running along the middle of the pens. It was brushed, swiped properly and cleaned up with fresh water then washed with disinfectant. Feeders, waterers, buckets and all other necessary equipments were properly cleaned, washed and disinfected thoroughly with a disinfectant.

3.5 Routine management

The commercial management procedures were followed during the whole experimental period.

3.5.1 Litter management

Fresh and dry rice husks were used as litter materials at a depth of about 2cm. It was stirred three times daily from 14 days to prevent cake formation and minimize dampness. Litters were replaced by new ones when found damp.

3.5.2 Floor space

Each pen was 10 ft x 6 ft and was allotted 40 birds. Therefore, the space given for each bird was 1.5 square feet.

3.5.3 Brooding

One 100-watt electric bulb hanging at chick level at the center of each pen was used to maintain brooding temperature. The brooding temperature was kept 95ºF at the beginning of the first week of age and decreased gradually (5ºF/week) in subsequent week until adjusted to the normal environmental temperature. Increasing or decreasing of temperatures were done by lowering or raising the bulbs according to the temperature prevailed and the birds behavior.

3.5.4 Lighting

The birds were exposed to 23 hours of lighting and dark period of 1 hour per day throughout the experimental period. The dark provision was practiced to make the birds familiar with possible darkness due to electricity failure.

3.5.5 Feeding and drinking

The broilers were given starter diet from 0-14 days, grower diet from 15-25 days and finisher diet from 26-35 days. For first 3 days, feeds were given on newspaper and feeder and after that only in feeder. Initially feed was given on tray feeder and thereafter round tube feeders were used for supplying feed. Four feeder and drinker were provided in each pen. Feed was supplied four times daily for the first seven days and after that reduced to three times. Fresh and clean drinking water supplied three times daily. Therefore, the birds had free access to both feeder and waterer. Feeders were cleaned everyday in the morning while drinkers were washed twice daily (morning and evening).

3.5.6 Biosecurity

3.5.6.1 Routine biosecurity

Strict routine biosecurity program was maintained inside and outside the experimental sheds as a most effective part of the disease prevention program. Entry of the visitors to the experimental shed was highly restricted. A foot bath was maintained at the gate of the shed where $KMnO_4$ water was used. All equipments in the experimental house were kept clean. Spraying was done outside the shed twice daily (morning and evening). To prevent rat and wild animals fencing was done and adequate care was taken.

3.5.6.2 Vaccination

During the study period birds were vaccinated against deadly common infectious diseases as a part of disease prevention program. The following vaccination schedule was maintained during the experimental period to prevent the common diseases of broilers and the vaccines were administered as per manufacturer's instructions (Table 4).

Table 1. Vaccination schedule for broiler chicken

S.N.	Age of vaccination	Name of vaccines	Trade Name	Company	Doses	Method of vaccination

1	Day 5	IB + ND	MA5 + Clone30	Intervet International, B.V. BOXMEER, Netherlands	1000 Birds/vial	Eye drop
2	Day 10	IBD	GM97	Hipra, Spain	1000 Birds/vial	Eye drop
3	Day 17	IBD	GM97	Hipra, Spain	1000 Birds/vial	Eye drop
4	Day 21	ND	Clone30	Intervet International, B.V. BOXMEER, Netherlands	1000 Birds/vial	Eye drop

IB=Infectious Bronchitis. ND=Newcastle disease. IBD=Infectious Bursal Disease.
Source: BCRDV, Animal Vaccine Research Center (AVRC), Mohakhali, Dhaka.

3.6 Live weight of broiler chicks

Live weight as gm 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).

3.7 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.

3.8 Separation and preservation of serum for biochemical studies
Principle:
Without anticoagulant all blood cells are clotted and the serum is separated as light yellowish liquid towards the upper level of test tube.
Materials required:
1. Test tube containing blood (without anticoagulant)
2. Syringe and needle
3. Test tube for centrifuge
4. Centrifuge machine
5. Bijou bottle
Method:
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.

3.9 Hematological examination

The following parameters of blood of each group were studied during the experimental period for fulfilling the objectives.

1. Total Erythrocyte Count (TEC)
2. Haemoglobin (Hb) concentration
3. Packed Cell Volume (PCV)
4. Erythrocyte Sedimentation Rate (ESR)
5. Total Leukocyte Count (TLC)

Estimation of Total Erythrocyte Count (TEC), Hemoglobin (Hb) concentration, Packed Cell Volume (PCV), Erythrocyte Sedimentation Rate (ESR) and Total Leukocyte Count (TLC) were performed following the methods described by Lamberg and *Rothstein* (1977).

3.9.1 Total Erythrocyte Count (TEC)

Principle:

Blood is diluted by an isotonic solution. Therefore, the red cells remain intact in the mixture.

Materials required:

A. Hemocytometer

    i. Hemocytometer slide

    ii. Cover glass

    iii. Red blood cell diluting pipette with rubber tube

B. Red blood cell diluting fluid (Hayem's solution)

C. Microscope

D. Cotton

E. Blood sample

Method:

1. Blood was drawn into the diluting pipette up to 0.5 mark.
2. The tip of the pipette was then filled with the fluid (Hayem's solution) up to 101 mark.
3. The pipette was shaken in 8 (eight) knot motion for 1 minute to mix up the contents inside.
4. Then the pipette was placed on the table.
5. The hemocytometer slide was placed under the microscope and the counting chamber was made visible with low power objective (X10).
6. The cover glass was placed over the counting chamber.
7. After discarding 3 drops of the mixture from the pipette, a little drop was placed on the counting chamber under the cover glass (Charging the chamber). 1 minute after placing the drop the distribution of cells was checked by low power objective.
8. The cell count was then started using high power objective (X40). The cells were counted in four corner and one centre secondary squares (A, B, C, D and E).
9. The numbers of cells counted in 5 squares were multiplied by 10,000.

Result:

The numbers of TEC of collected blood samples were expressed in million/mm^3 of blood.

3.9.2 Estimation of Hemoglobin (Hb) concentration

Principle:

Blood is diluted by N/10 HCl which is a hypotonic solution. This results in hemolysis of red cells. The released hemoglobin reacts with acid and produces acid hematin, a tance or deep brown colored compound. The color of this compound is matched with the standard to know the hemoglobin content.

Materials required:

A. Hellige Hemometer

    i. Sahli pipette with rubber tube

    ii. Graduated diluting tube

    iii. Comparator

    iv. N/10 HCl

    v. Dropper

    vi. Stirrer

B. Cotton

C. Blood sampl

Method:

1. N/10 HCl was taken into the diluting tube up to 2gm% mark.
2. Blood was drawn into the Sahli pipette up to 20µl mark.
3. The tip of the pipette was introduced into the tube and the blood was expelled from the pipette into the acid inside the tube.
4. The pipette was taken out of the tube and the mixture was shaken gently.
5. The mixture was allowed to stand for 5 minutes by keeping the tube in the comparator.
6. The tube was taken out and distilled water was added drop by drop and the mixture was stirred after addition of each drop.
7. This was continued until the color of the mixture matched with the standard.
8. The Hb amount was measured by holding the tube against light.

Result:

The amount of Hb (concentration) in the collected blood samples were expressed in gm%.

3.9.3 Estimation of Packed Cell Volume (PCV)

Principle:

The blood is placed in centrifugation to forcefully pack the red cells. The volume of packed red cells is measured in percent.

Materials needed:

1. Wintrobe tube
2. Special loading pipette
3. Centrifuge machine
4. Cotton

5. Blood

6. Method:

Blood was drawn into the loading pipette. The tip of the pipette was introduced into the Wintrobe tube and the tube was filled with blood up to 10 mark of the right sided scale. The wintrobe tube was then placed in the centrifuge machine and was centrifuged @ 3000rpm for 30 minutes. Then the hematocrit or PCV was recorded using the following formula:

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

Result:

The PCV in collected blood samples were expressed in percentage (%).

3.9.4 Estimation of Erythrocyte Sedimentation Rate (ESR) by Wintrobe method

Principle:

Erythrocytes settle down by the force of gravity in a stagnant blood sample. The rate of setting is measured against the particular period of time.

Materials needed:

1. Wintrobe tube
2. Special loading pipette
3. Special rack
4. Cotton
5. Blood

Method:

Blood was drawn into the loading pipette. The tip of the pipette was introduced into the Wintrobe tube and the tube was filled with blood up to 0 mark of the left sided scale. Then it was placed in the rack for 1 hour. ESR were measured by the observation of the upper level of blood was down from the 0 mark of the left sided scale of Wintrobe tube.

Result:

The ESR of the collected blood samples were expressed in mm in first hour.

3.9.5 Total Leukocyte Count (TLC)

Principle:

Blood is diluted by a hypotonic solution which results in hemolysis of red blood cells. The leukocytes are then counted in a counting chamber and their number in undiluted blood calculated.

Materials required:

    A. Hemocytometer:

        i.   Haemocytometer slide (counting chamber)

        ii.   Cover glass

        iii.   White blood cell diluting pipette with rubber tube

    B. Red blood cell diluting fluid.

    C. Compound microscope

    D. Cotton

    E. Blood sample

    F. Filter paper

Method:

1. Blood was drawn into the diluting pipette up to 0.5 mark.
2. Tip of the pipette was wiped carefully with a piece of absorbent cotton.
3. Then the tip of the pipette was then filled with the diluting fluid (N/10 HCl) up to 11 mark.
4. The pipette was shaken in 8 knot fashion for 1 minute to mix up the contents inside.
5. Then the pipette was placed on the table.
6. The hemocytometer slide was placed under the microscope and the counting chamber was made visible with low power objective (X10).
7. The cover glass was placed over the counting chamber.
8. After discarding 3 drops of the mixture from the pipette, a little drop was placed on the counting chamber under the cover glass (charging the chamber). 1-2 minutes after placing the drop the distribution of cells was checked by low power objective.
9. The cell count was then started using high power objective (X40). The cells were counted in four corner big squares (A, B, C and D) outside the ruling area for TEC of the counting chamber. Included all the cells touching the top and left ruled boundary lines.
10. The numbers of cells counted in 4 squares were multiplied by 50. The result of TLC of collected/supplied blood samples were expressed in thousand/µl or thousand/mm^{3}of blood.

Result: The results of TLC of collected blood samples were expressed as thousand/mm^{3}of blood.
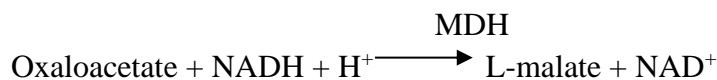
3.10 Biochemical examination

Blood sera biochemical parameters AST and ALT were detected from the bird's serum by the use of specific test kit and analyzer.

3.10.1 Determination of Aspartate Aminotransferase (AST)

Kinetic method for the determination of AST according to the recommendations of the expert panel of the IFCC (International Federation of Clinical Chemistry) without pyridoxalphosphate activation.

Test principle:

$$\text{2-oxoglutarate + L-aspartate} \xrightarrow{\text{AST}} \text{L-glutamate + Oxaloacetate}$$

$$\text{Oxaloacetate + NADH + H}^+ \xrightarrow{\text{MDH}} \text{L-malate + NAD}^+$$

Endogenous oxalate is eliminated in a preliminary reaction (Deneke *et al*., 1985).

Materials required:

   i. Serum
  ii. Specific test kit, liquiUV Test for AST (Trade Worth Lab. Ltd., Germany)
 iii. Pipette
 iv. Cuvette
  v. Hot water bath
 vi. Auto analyzer (Humalyzer 3500)

Contents of the test kit:

1. Substrate composed with 2-oxoglutarate (60mmol/l) and NADH (0.9 mmol/l).

2. Buffer or Enzyme reagent composed with Tris buffer $^{PH\ 7.8}$ (100mmol/l), L-aspartate (300mmol/l), LDH (≥0.9kU/l) and MDH (≥0.6kU/l).

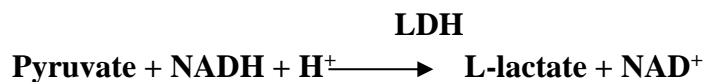**Brief procedure (as per manual of test kit):**

A 2ml substrate was added to 8ml buffer and warmed for 10 minutes at 37^0C to prepare the working reagent. From the working reagent, 1ml was mixed with 100μl previously prepared and stored serum in clean dry test tube. Then the tip of the collecting tube of cuvette of Humalyzer 3500 was introduced quickly into the mixture of serum and working reagent containing test tube. The mixture was automatically collected by the analyzer after pressed the button on "push". The analyzer was set previously at 340nm wave length with 1cm optical path at 37^0C for the detection of selected parameter AST. After 3 minutes the screen (monitor) of the Humalyzer 3500 was showed the result of AST automatically.

Result:

The AST levels of the collected samples were expressed in U/L.

3.10.2 Determination of Alanine Aminotransferase (ALT)

Kinetic method for the determination of ALT is followed the recommendations of the expert panel of the IFCC (International Federation of Clinical Chemistry) without pyridoxalphosphate activation.

Test principle:

$$\text{2-oxoglutarate + L-alanine} \xrightarrow{\text{ALT}} \text{L-glutamate + Pyruvate}$$

$$\text{Pyruvate + NADH + H}^+ \xrightarrow{\text{LDH}} \text{L-lactate + NAD}^+$$

Endogenous pyrovate is eliminated in a preliminary reaction (Deneke *et al.*, 1985).

Materials required:

 i. Serum
 ii. Specific test kit, liquiUV Test for SGPT (Trade Worth Lab. Ltd., Germany)
 iii. Pipette
 iv. Cuvette
 v. Hot water bath
 vi. Auto analyzer (Humalyzer 3500)

Contents of the test kit:

1. Substrate composed with 2-oxoglutarate (60mmol/l) and NADH (0.9 mmol/l).
2. Buffer or Enzyme reagent composed with Tris buffer $^{PH\ 7.5}$ (150mmol/l), L-alanine (750mmol/l) and LDH (≥1.2kU/l).

Brief procedure (as per manual of test kit):

A 2ml substrate was added to 8ml buffer and warmed for 10 minutes at 37^0C to prepare the working reagent. From the working reagent, 1ml was mixed with 100μl previously prepared and stored serum in clean dry test tube. Then the tip of the collecting tube of cuvette of Humalyzer 3500 was introduced quickly into the mixture of serum and working reagent containing test tube. The mixture was automatically collected by the analyzer after pressed the button on "push". The analyzer was set previously at 340nm wave length with 1cm optical path at 37^0C for the detection of selected parameter ALT. After 3 minutes the screen (monitor) of the Humalyzer 3500 was showed the result of ALT automatically.

RESULT

The experiment was conducted to study the effects of enzymes and vinegar with water in broilers on live weight gain and hematobiochemical parameters such as Total Erythrocyte Count (TEC million/mm^3 of blood), Hemoglobin concentration (Hb gm/dl of blood), Packed Cell Volume (PCV%), Erythrocyte Sedimentation Rate (ESR mm in 1st hour), Total Leukocyte Count (TLC thousand/mm^3 of blood), Aspartate Aminotransferase (AST U/L) and Alanine Aminotransferase (ALT U/L). The experiment was started on 14 days of age. Prior to experiment, the chickens were divided into 4 groups having 50 chickens in each group. Group T_0 was kept for control (no treatment), group T_1 was treated with enzymes, group T_2 was treated with vinegar, group T_3 was treated with enzymes and vinegar.

4.1 Effect of enzymes and vinegar on live weight in broiler

Effects of enzymes and vinegar with water on live weight of different groups of broiler chickens are presented at Table 1.

Table 2. Effect of enzymes and vinegar on live weight in gm (Mean ± SE) of different groups of broiler

Group	No.of Birds	Body Weight Gain (gm)				
		0 day	7th days	14th days	21st days	28th days
T_0	50	125.23±2.03	530.58±3.62^d	902.29±12.63^d	1643.27±27.62^d	2123.54±36.74^d
T_1	50	127.62±2.53	550.39±8.21^b	954.55±15.72^b	1713.43±34.01^b	2249.59±53.31^b
T_2	50	130.35±3.51	535.43±5.31^c	947.54±8.31^c	1667.82±49.63^c	2138.61±42.01^c
T_3	50	132.27±3.41	561.59±8.68^a	1061.59±21.53^a	1856.59±27.94^a	2521.38±98.21^a

*Values followed by different superscript in the same column differ significantly p<0.01

Body weight of 7 days of age (day 0 of experiment) was more or less similar. Highest body weight was recorded in group T_3 and lowest in group T_0. The recorded body weight was 125.23± 2.03gm in group T_0, 127.62±2.53gm in group T_1, 130.35±3.51gm in group T_2 and 132.27±3.41gm in group T_3.

On 14 days of age (7th day of experiment) it was observed that the body weight in control group T_0 was 530.58±3.62gm, in group T_1 was 550.39±8.21gm, in group T_2 was 535.43±5.31gm and group T_3 was 561.59±8.68gm. All the data were statistically significant (p<0.01). The highest and lowest body weight was observed as 561.59±8.68gm and 530.58±3.62gm, respectively.

On 21 days of age (14th day of experiment) the body weight in control group T_0 was 902.29±12.62gm and the treated groups were 954.55±15.72gm in group T_1, 947.54±8.31gm in group T_2 and 1061.59±21.53gm in group T_3. The increased body weights rates were statistically significant (p<0.01). From the table we can see that, the highest body weight was recorded in treated group T_3 and lowest in control group T_0. But among the treated groups the lowest body weight was recorded in group T_3.

On 28 days of age (21st day of experiment) the body weight in control group was recorded 1643.27 ± 34.62gm in T_0. In the treated groups were 1713.43 ± 34.01gm in group T_1, 1667.82 ± 49.63gm in group T_2 and 1856.59 ± 27.94gm in group T_3. The average body weight of all treated groups were statistically significant ($p<0.01$) than the average body weight of control group. The highest body weight was recorded in treated group T_3 and lowest in control group T_0.

On 35 days of age (28th day of experiment) the body weight in control group was recorded 2123.54 ± 36.74gm in T_0. In the treated groups were 2249.59 ± 53.31gm in T_1, 2138.61 ± 42.01gm in T_2 and 2521.38 ± 98.21gm in group T_3. The highest body weight was recorded in group T_3 (2521.38 ± 98.21gm). The increased body weights rates were statistically significant ($p<0.01$).

It was observed that increased body weight recorded on 14, 21, 28 and 35 days of age and it showed that body weight was gradually increased in group T_0 but increase in other groups was rapid in contrast to control group.

4.2 Effect of enzymes and vinegar on hematological parameters in broiler

On 21st day of experiment (35 day of age) the effect of enzymes and vinegar with water on hematological parameters of different groups of broiler chickens are presented at Table 6

Table 3. Effect of enzymes and vinegar on hematological parameters (Mean±SE) of different groups of broiler on 21st day of experiment (35 day of age)

Group	No.of Birds	TEC(millions/mm^3)	Hb (gm/dl)	PCV (%)	ESR	TLC(thousand/mm^3)
T_0	10	2.73 ± 0.03^c	7.65 ± 0.17^c	27.93 ± 0.17^c	2.49 ± 0.14^a	24.88 ± 0.41^a
T_1	10	3.00 ± 0.02^b	8.36 ± 0.16^b	30.28 ± 0.29^b	2.01 ± 0.27^b	22.06 ± 0.54^b
T_2	10	3.27 ± 0.21^b	8.73 ± 0.21^b	30.81 ± 0.21^b	1.97 ± 0.18^b	21.14 ± 0.86^b
T_3	10	3.31 ± 0.20^a	9.03 ± 0.41^a	31.07 ± 0.38^a	1.49 ± 0.16^c	19.78 ± 0.41^c

*Values followed by different superscript in the same column differ significantly $p<0.01$

Total Erythrocyte count is presented in Table 3. On the final day of experiment (35 days of age) the values of TEC was 2.73 ± 0.03 millions/mm^3 in control group T_0 and in the treated group T_1 was 3.00 ± 0.02 millions/mm^3, Group T_2 was 3.27 ± 0.21 millions/mm^3 and Group T_3 was 3.31 ± 0.20 millions/mm^3. The highest value of TEC was recorded in the group T_3 (3.31 ± 0.20 millions/mm^3) and the lowest in the control group T_0 (2.73 ± 0.03 millions/mm^3).The value of control group was significantly lower than the treated groups.

The Haemoglobin content of different groups of birds is presented in Table 3. On the final day of experiment (35 days of age) the values of Hb was 7.65 ± 0.17 gm/dl in control group T_0 and in the treated group T_1 was 8.36 ± 0.16 gm/dl, Group T_2 was 8.73 ± 0.21 gm/dl and Group T_3 was 9.03 ± 0.41 gm/dl. The highest value of Hb was recorded in the group T_3 (9.03 ± 0.41 gm/dl) and the lowest in the control group T_0 (7.65 ± 0.17 gm/dl).The values of treated groups were significantly higher than the value of control group T_0.

All the values of packed cell volume (PCV) is presented in Table 3. On the final day of experiment (35 days of age) PCV value of groups T_0, T_1, T_2 and T_3 were 27.93 ± 0.17%, 30.28 ± 0.29%, 30.81 ± 0.21% and 31.07 ± 0.38% respectively. The highest value was recorded in group T_3 (31.07 ± 0.38%) and the lowest

value was recorded in group T_0 (27.93±0.17%). All the values of treated groups were significantly higher than the value of control group T_0.

Erythrocyte sedimentation rate (ESR) is showed in table 3. On the final day of experiment ESR value of groups T_0, T_1, T_2 and T_3 were 2.49±0.14mm, 2.01±0.27mm, 1.97±0.18mm and 1.49±0.16mm in 1st hour respectively. The lowest value was found in group T_3 (1.97±0.18mm in 1st hour) and highest was in control group T_0 (2.49±0.14mm in 1st hour). All the value of treated groups were significantly ($p<0.01$) lower than the control group T_0.

Total Leucocyte Count (TLC) is presented in table 3. On the final day of experiment (35 days of age) TLC value of groups T_0, T_1, T_2 and T_3 were 24.88±0.41thousand/mm^3, 22.06±0.54 thousand/mm^3, 21.14±0.86 thousand/mm^3 and 19.78±0.41 thousand/mm^3. The lowest value was found in group T_3 (19.78±0.41 thousand/mm^3) and highest was in control group T_0 (24.88±0.41thousand/mm^3). All the value of treated groups were significantly ($p<0.01$) decreased than the control group T_0.

4.3 Effect of enzymes and vinegar on biochemical parameters in broiler

On 21st day of experiment (35 day of age) the effect of enzymes and vinegar with water on biochemical parameters of different groups of broiler chickens are presented at Table 4.

Table 4. Effect of enzymes and vinegar on serum biochemical parameters (Mean±SE) of different groups of broiler on 21st day of experiment (35 day of age)

Groups	No of Birds	AST (U/L)	ALT (U/L)
T0	10	41.50±1.23[a]	7.13±0.19[a]
T1	10	37.68±1.78[b]	5.72±0.21[b]
T2	10	38.57±0.98[b]	5.21±0.34[b]
T3	10	29.52±1.29[c]	3.96±0.18[c]

*Values followed by different superscript in the same column differ significantly $p<0.01$

Serum Alanine Aminotransaminase (ALT) is presented in table 4. On the final day of experiment (35 days of age) ALT value of groups T_0, T_1, T_2 and T_3 were 41.50±1.23u/l, 37.68±1.78u/l, 38.57±0.98u/l and 29.52±1.29u/l. The lowest value was found in group T_3 (29.52±1.29u/l) and highest was in control group T_0 (41.50±1.23u/l). All the value of treated groups were significantly ($p<0.01$) decreased than the control group T_0.

Serum Aspertate Aminotransaminase (AST) is observed in table 4. On the final day of experiment (35 days of age) AST value of groups T_0, T_1, T_2 and T_3 were 7.13±0.19u/l, 5.72±0.218u/l, 5.21±0.34u/l and 3.96±0.18u/l. The lowest value was found in group T_3 (3.96±0.18u/l) and highest was in control group T_0 (7.13±0.19u/l). All the value of treated groups were significantly ($p<0.01$) lower than the control group T_0.

SUMMARY AND CONCLUSIONS

The research work was carried out on Cobb-500 broiler chicken to evaluate the effects of enzymes and vinegar on growth performance and hematological parameters on broiler. All the two hundred and fifty (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 of age 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 on day 14 of age. Initial live weight of each bird was recorded on 1st day of experiment (day 14 of age) and kept them into four different

cages. Live weight was recorded at 7 days interval up to the end of 21st day of experimental period (35 day of age). 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) for hematological study.

Live weight was significantly (p<0.01) increased in all treatment groups in comparison with that of the control group T_0 at day 14, 21, 28 and 35 of age. On 21st day of experiment (35 days of age) the highest live weight was recorded in the treatment group T_3 which followed in descending order in groups T_1, T_2 and T_0. Among the treatment groups, combinedly enzymes and vinegar given group T_3 showed the highest result. Blood parameters like TEC, Hb concentration and PCV values were significantly (p<0.01) increased but ESR and TLC decreased significantly (p<0.01) in all treatment groups than the control group T_0 on 21st day of experiment (35 days of age). Among the treatment groups, combinedly enzymes and vinegar supplemented group T_3 showed the better hematological profile. Biochemical parameters like AST and ALT were decreased significantly (p<0.05) in all treatment groups than the control group T_0 on 21st day of experiment (35 days of age). Among the treatment groups, combinedly enzymes and vinegar supplemented group T_3 showed the lowest result.

From the present experiment it can be concluded that the combined supplementation of enzymes and vinegar are highly beneficial for live weight gain, better hematological profile in broiler chickens. However, these are preliminary findings in a small population of broiler chicks. So, further elaborate studies will be required to make final conclusion involving more birds.

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