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Dietary acidifier and lysozyme improve growth performances and hemato-biochemical profile in broiler chicken

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ABSTRACT: Acidifier and lysozyme may have a significant impact on growth performance and body defense in broilers. The experiment was conducted to evaluate the effects of dietary acidifier and lysozyme on physiognomies (live body weight and body weight gain), feed conversion ratio (FCR) and selected hemato-biochemical parameters in broiler chicken. A total of 60, day old Lohman broiler chicks were collected and reared up to 28 days with timely vaccination. At day 14, the chicks were randomly divided into four equal groups: A, B, C and D (n=15). Group A was considered as non-treated control; group B was supplemented with acidifier, group C with lysozyme and group D with both acidifier and lysozyme through drinking water. Results showed that acidifier, lysozyme and combined groups had significantly higher live body weight and lower feed conversion ratio, FCR ($p<0.05$) compared to the control group. Total erythrocyte count (TEC), hemoglobin concentration and packed cell volume (PCV) were significantly higher ($p<0.05$) especially in lysozyme supplemented group. Serum lipid profile (total cholesterol, triglyceride and LDL cholesterol) were significantly decreased in the acidifier supplemented group, whereas HDL cholesterol was increased ($p<0.05$). Lysozyme supplemented birds showed an increased level of total cholesterol and significant ($p<0.05$) decrease in triglyceride and LDL level. Combined supplementation showed almost similar results as lysozyme. Serum alanine transaminase (ALT), aspartate transaminase (AST) and creatinine concentration didn't differ significantly upon supplementations. It is concluded that acidifier and lysozyme could be used in broiler feed for better growth performance and hemato-biochemical profile.

KEYWORDS: Acidifiers; lysozymes; growth performance; hemato-biochemical parameters; broilers.

INTRODUCTION

The broiler industry is becoming a prominent field day by day due to its low cost of startup and quicker return of the investment. Although poultry farming has become popular day by day, there are some constraints which cannot be overlooked. Newer approaches are followed with different considerations, such as the use of antibiotics as a growth promoter, use of probiotics, prebiotics and synbiotics, use of phytobiotics or enzymes or other feed additives to increase the profitability from broiler farming. Acidifiers are being used in poultry nutrition in different forms and combinations. Besides, one strategy for replacing antibiotics in animal diets is to employ antibacterial molecules normally found along the digestive tract such as Lactoferrin and lysozyme which are present in mucosal secretions and in milk where they provide defense against bacteria along epithelial surfaces, and to use the exogenous lysozyme for preventive purpose in chicken is thought to be safer than antibiotics [1, 2]. The evaluation and significance of undertaking any feeding protocol for broiler chicken should be done beforehand from the physiological point of view to assure the actual surety of their effectiveness.

Acidifiers are organic acids which reduce pH value of food, therefore act as conserving agents. It prevents microbial contamination of food and this effect is also exhibited in digestive tract of poultry [3]. Organic acids contributed tremendously to the profitability in the poultry husbandry and providing healthy and nutritious poultry products to the people [4]. Feed acidifiers inhibit

the growth of pathogenic intestinal microflora and results in better growth and performance of the chicken [3]. They also act as mold inhibitors. Acidifiers improve protein and energy digestibility by decreasing microbial competition with the host for nutrients and endogenous nitrogen losses by lowering the incidence of subclinical infections and secretion of immune mediators. Acidifiers involve in reducing production of ammonia and other growth-depressing microbial metabolites. In presence of organic acids, pH of digesta decreases, increase pancreatic secretion and also have an effect on intestinal mucosa [5]. Acidifiers have benefits related to uncontrolled variables such as the buffering capacity of dietary ingredients, presence of other antimicrobial compounds, cleanliness of the production environment, and heterogeneity of gut microbiota. Microbes compete with the host for nutrients and nutrient absorption increases by reducing the microbial population with the help of acidifier, so the digestion of protein and energy improve [6]. Various acidifier preparations are available in the market at present and their indiscriminate uses are in practice without much scientific information. However, information on the use of acidifiers, their levels in broiler diets in our country condition and its effects on growth performance and hematology is yet to be studied further.

Again, lysozyme is a common enzyme that is commercially obtained from avian egg white and is widespread in many tissues and secretions of animals [7]. Chemically it is an antimicrobial enzyme that takes part in the innate immune system. Lysozyme is a glycoside hydrolase that catalyzes 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in peptidoglycan of gram-positive bacterial cell wall [8]. Some studies have reported significant function of lysozyme in various organisms as a defender against bacteria [9]. So, levels of lysozyme are most effective, as well as determining which critical periods of the broiler growth cycle lysozyme may have the greatest impact on growth performance and microbial populations of the gastrointestinal tract (GIT) of broiler chickens. Research regarding the use of lysozyme as an alternative to antibiotics for poultry is limited. Therefore, the usefulness of supplementation of acidifier and lysozyme in addition to commercial feed, with revealing their effects on hemato-biochemical parameters in broiler, is yet to be investigated. The research work was aimed to know the effects of dietary acidifier, lysozyme and their combined supplementation on growth performance, hemato-biochemical profile and on liver and kidney function test.

MATERIALS AND METHODS

Statement of the experiment

The research was conducted at the experimental shed of the Department of Physiology, Faculty of Veterinary Science of Bangladesh Agricultural University, Mymensingh during the period from 1st November 2018 to 28th November 2018. A total of sixty healthy day-old Lohman broiler chicks were purchased from Agro-Industrial Trust Hatchery Ltd, Digharkanda, Mymensingh.

Ethical approval

The present research work and all experimental protocols were approved and performed according to the guidelines for the care and use of animals as established by Animal Welfare and Experimentation Ethics Committee, Bangladesh Agricultural University, Mymensingh, Bangladesh [AWEEC/BAU/2020-15].

Experimental design

The experiment was conducted in a completely randomized design. On the 14th day of age, broiler chicks were randomly divided into the four equal groups; each group contained 15 birds receiving the following treatment, i) Group A - untreated control and received non-medicated water, ii) Groups B - acidifier - 1 ml/L through drinking water, iii) Group C - lysozyme at the rate of - 1 g/4 L drinking water, and iv) Group D- both acidifier and lysozyme. Initial body weight of each bird was recorded and kept them separately. Bodyweight was recorded at seven days interval up to the end of the experimental period and the birds were sacrificed to collect a blood sample for hematological studies (TEC, Hb and PCV) and serum sample for biochemical studies (Lipid profile, AST, ALT and creatinine). All parameters were analyzed and compared with each other.

Management of the experimental birds

Broiler starter, grower pellet and broiler finisher feeds were obtained from ACI Godrej Agrovet Feeds Ltd, Dhaka, Bangladesh. From 12 to 23 days grower feed and 23 to 30 days finisher feed were supplied to the birds twice in a day. Acidifier used in this study was ‘Fra® AC 34’ marketed by ACI Animal Health Ltd, Bangladesh. It is a well-balanced mixture of Monoglycerides of propionic acid (Monopropionin), Monoglycerides of butyric acid (Monobutyrin) and essential oils. As a lysozyme preparation ‘AciGuard WS’ was used, which was also supplied by ACI Animal Health Limited,

Bangladesh. Feed supplementations were prepared on a daily basis. Birds received their freshly prepared daily medication in the morning hour of each day. The concentration of medications in the water, to give the required dose per kilogram of body weight was calculated by determining the water consumption and body weight of each bird on the day of medication. Managements and rearing of birds were strictly followed according to the standard broiler farming system. Birds were vaccinated against common infectious diseases as per manufacturers' instructions. Strict biosecurity measures were taken during the experimental periods.

Measurements of body weight and feed conversion ratio (FCR)

The bodyweight of each bird was measured in a weekly basis at 14th, 21th and 28th days of age using digital balance and total body weight gain were calculated (body weight gain= final body weight – initial body weight). FCR was determined by the formula: total feed consumed by birds, divided by total body weight gain. Feed consumption is the amount of feed consumed by the birds in a period of time. Feed intake was calculated as the difference between the amount of feed supplied to the birds and the amount of feed that remained at the end of each feeding period. At the end of the week, the total amount of feed was calculated by the sum of several days' consumed feed.

Blood collection

After completion of the experimental period, blood samples were collected at slaughter. About 10 mL of blood was collected from each bird. Half of the blood was kept in a sterile test tube containing anticoagulant (3.8% Trisodium citrate solution) at a ratio of 1:10 and remaining half of the blood was taken in another sterile test tube without any anticoagulant for preparing serum [10].

Preparation of serum

Test tubes containing blood without anticoagulant were kept in a slanting position at room temperature. These samples were refrigerated overnight at 4°C. The separation of serum from the clotted blood was achieved following centrifugation at 1000 rpm for 15 min. These cell-free serum samples were preserved at –20°C for further biochemical analysis.

Hemato-biochemical analysis

The estimation of selected hematological parameters (Hb, TEC and PCV) were performed within two hours of the collection according to the standard procedure [10]. The biochemical tests were performed in collaboration with Professor Muhammad Hossain Central Laboratory, Bangladesh Agricultural University, Mymensingh. The serum total cholesterol, triglycerides, high density lipoproteins (HDL), creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were performed according to standard method [11] using a UV spectrophotometer T 80, PG instruments, Great Britain. Specific reagents from High Technology Incorporation (HTI), USA were used for each test.

Statistical analysis

The data obtained in the laboratory was stored in Microsoft Excel-2013 and imported to the software Graph Pad Prism 8 for analysis. Results are expressed as the mean +SEM. One-way analysis of variance (ANOVA) was used for data analysis [12]. Significant differences between the groups were detected in the ANOVA using Tukey's multiple comparison test. $P<0.05$ was considered as statistically significant.

RESULTS

Effects on the physiognomies (body weight, body weight gain and FCR) of broilers

On the 14th day of age, the mean body weights of different groups of broiler birds prior to treatment were more or less similar and not statistically significant ($p>0.05$). On the 21st day of age, the highest body weight was recorded in group C (lysozyme) and the lowest body weight was in group A (control). All the data were statistically significant ($p<0.05$) (Table 1). Again, on the 28th day of age, the highest body weight was recorded in group C (lysozyme) followed by group D and group B and the lowest body weight was in group A (control). The body weight increased slowly in the control group A in respective days of the experiment but much rise in body weight was noticed in the treated groups (B, C and D) (Table 1). It was highest in group C in comparison with control group A. Although body weight on 1st day of the experiment (14th day) was more or less similar, a distinct fluctuation was observed with the advancement of age among different groups (Table 1). In parallel, there was a significant increase of body weight gain and % body weight gain among all treated groups compared to the control group ($p<0.05$) and maximum growth was observed in lysozyme treated group (Figure 1). The

highest FCR was recorded in group A (control) and decreased FCR was found in group B, C and D (Figure 1).

Table 1. Effects of dietary acidifier and lysozyme on live body weight (g) in broiler chicken at different time intervals

Groups	Body weight (g) (Mean ± SEM)		
	14th day	21st day (g)	28th day (g)
Control	524.00 ± 7.92	1049.17 ± 37.00^{a}	1566.67 ± 37.12^{a}
Acidifier	505.00 ± 8.16^{ns}	1290.83 ± 27.28^{b}	1895.00 ± 22.77^{b}
Lysozyme	510.50 ± 20.84^{ns}	1281.67 ± 67.46^{b}	1973.33 ± 50.24^{c}
Combined	511.50 ± 21.92^{ns}	1302.50 ± 63.02^{b}	1943.33 ± 41.77^{c}

^{a,b,c} different superscript letters in a column denote significant difference at p<0.05 level, ns - Not significant (p>0.05)

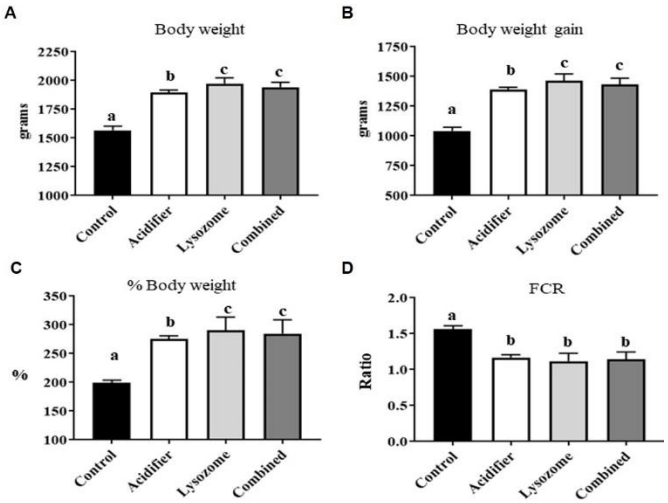

Figure 1. Effects of dietary acidifier and lysozyme on physiognomies (mean ± SEM) in broiler chicken at 28th day of age. Broilers birds were reared with supplemented acidifier, lysozyme and both (details in materials and methods). A, body weight; B, body weight gain; C, percentage of body weight gain; D, feed conversion ratio (FCR). Different letters: a,b and c over a bar graph denote significant difference at p<0.05.

Table 2. Effects of dietary acidifier and lysozyme on hematological parameters in broiler chicken

Groups	Control	Acidifier	Lysozyme	Combined
Hb (g/dL)	7.98 ± 0.09^{a}	8.11 ± 0.09^{a}	9.52 ± 0.17^{b}	8.10 ± 0.12^{a}
TEC (million/uL)	2.33 ± 0.06^{a}	2.44 ± 0.04^{a}	2.95 ± 0.06^{b}	2.32 ± 0.10^{a}
PCV (%)	31.04 ± 0.73^{a}	32.00 ± 0.64^{a}	37.76 ± 0.35^{b}	31.68 ± 1.23^{a}
MCV (fl)	133.37 ± 3.47^{ns}	131.40 ± 2.21^{ns}	132.62 ± 3.39^{ns}	136.92 ± 3.36^{ns}
MCHC (%)	25.77 ± 0.53^{ns}	25.39 ± 0.51^{ns}	25.22 ± 0.61^{ns}	25.09 ± 0.78^{ns}
MCH (pg)	34.32 ± 0.78^{ns}	33.32 ± 0.52^{ns}	33.36 ± 0.46^{ns}	34.35 ± 1.37^{ns}

^{a,b,c}different superscript letters in a row denote significant difference at p<0.05 level, Mean ± SEM, ns - Not significant (p>0.05).

Effects on hematological parameters

The mean values of hematological parameters (Hb, TEC, PCV and erythrocyte indices) in different treatment groups of broilers are presented in Table 2. The birds fed with the diet containing lysozyme had higher Hb, TEC and PCV values than birds fed with acidifier, combined and control groups. The data were statistically significant between lysozyme versus other groups. In case of erythrocyte indices including mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) didn't differ significantly (p>0.05) between the control and treatment groups and among the treatment groups.

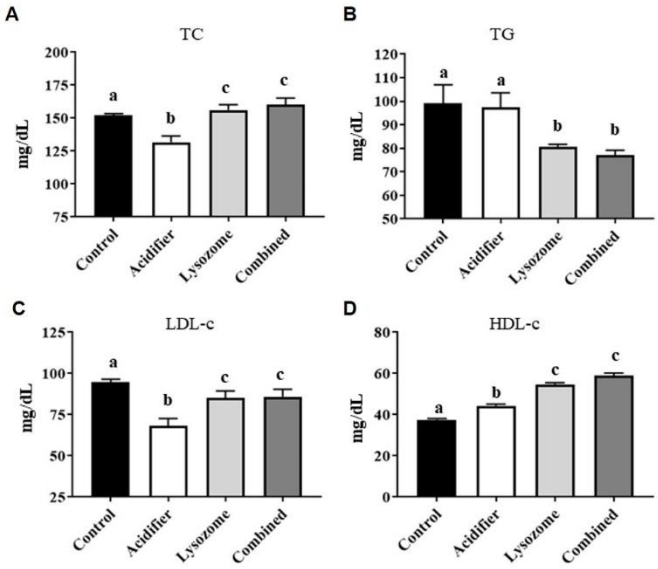

Figure 2. Effects of dietary acidifier and lysozyme on lipid profile (mean ± SEM) in broiler chicken at 28th day of age. Broilers birds were reared with supplemented acidifier, lysozyme and both (see Materials and Methods for details). Blood samples were collected, and sera were separated and analysed for lipid profile. A, TC, total cholesterol; B, TG, triglycerides; C, LDL-c, low density lipoprotein cholesterol and D, HDL-c, high density lipoprotein cholesterol. Different letters: a,b, and c over a bar graph denote significant difference among the group at p<0.05 level.

Effects on biochemical parameters

The result showed that dietary acidifier with drinking water decreased the serum total cholesterol and LDL cholesterol level and improved HDL-cholesterol without altering the triglyceride values when compared to control (Figure 2). Birds of lysozyme treated, and combined group had lower triglyceride levels and higher serum total cholesterol and HDL cholesterol values without significant variation of LDL-cholesterol values. The mean values of liver and kidney function tests (ALT, AST and creatinine) in broilers supplemented with acidifier

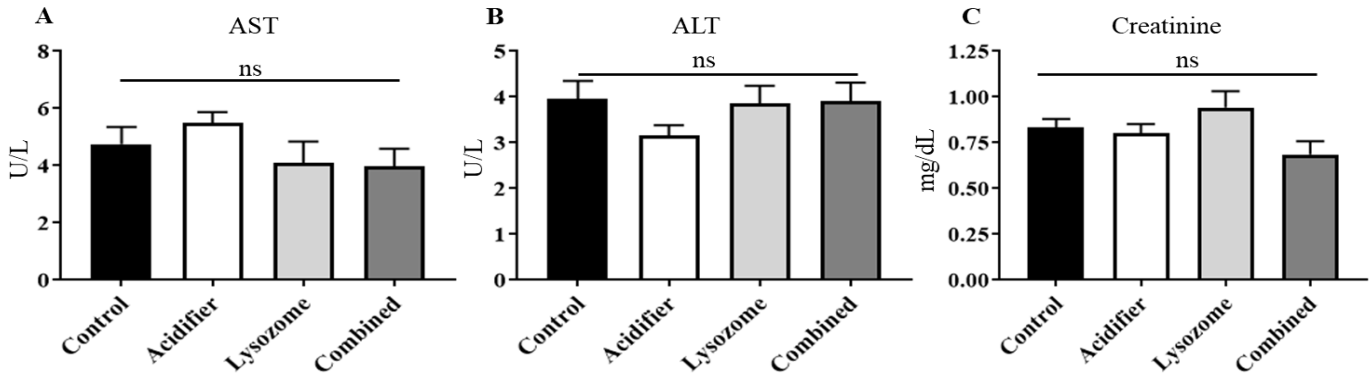

Figure 3. Effects of dietary acidifier and lysozyme on ALT, AST and creatinine (mean ± SEM) in broiler chicken of the experimental groups. Broilers birds were reared with supplemented acidifier, lysozyme and both (see Materials and Methods for details). Blood samples were collected, and sera were separated and analyzed. A, AST, aspartate amino transferase; B, ALT, alanine amino transferase; and C, creatinine. ns - Not significant (p>0.05)

and lysozyme were slightly higher, but the values were within normal ranges and statistically non-significant (Figure 3).

DISCUSSION

The experiment was conducted to study the effects of dietary acidifier and lysozyme on growth performance, hematological parameters (TEC, Hb, PCV, MCV, MCHC and MCH) and biochemical parameters (Total cholesterol, triglyceride, HDL-c, LDL-c, AST, ALT and creatinine) in broiler chicken. The increased rate of body weight gain was found in the treated group of broilers which might be due to an increased feed utilization, digestion, absorption and metabolism of supplied feed nutrients due to addition of acidifier and lysozyme. The result of the present study is similar with the findings of previous reports even though the doses, duration and route of administration were varied [13, 14, 15, 16], which stated that mean body weight and daily live weight gain were higher ($p<0.05$) in the chickens fed with acidified ration. Previously it was found that addition of transgenic rice that expresses lysozyme improved feed efficiency and the birds had increased villi height in the small intestine compared to birds fed with conventional rice [17]. There was a significant increase of body weight gain and % body weight gain among all treated groups compared with the control group ($p<0.05$) and maximum growth was observed in lysozyme treated group (figure 1). This may be due to decreased nutrient utilization by gut microflora, which is decreased in number by the action of lysozyme. This fact is supported by previous reports [18] that, weight gain by birds fed the 50 ppm lysozyme were greater ($p<0.05$) as it caused a negative effect on the growth of intestinal microbiota of broiler chickens from the cage trial. Supplementation of acidifier with drinking water was also found to be useful for growth in broiler rearing. This result is in line with the previous findings [19] that dietary inclusion of citric acid and avilamycin showed the highest carcass weight in broilers which was significantly ($p<0.05$) higher than control group whereas, carcass weight of citric acid treated group was higher than control and avilamycin groups ($p<0.05$). The present investigation showed that acidifier and lysozyme both have a significant ($p<0.01$) lowering effect on FCR in broiler chicks and especially lysozyme treated birds achieved minimum FCR than other groups. Similar decrease in FCR was reported by other investigators [20, 21, 22].

The present study showed that dietary acidifier and lysozyme had positive effects on hematological parameters in broiler chicken. Hematological indices including WBC, RBC and hematocrit (Hct) were significantly increased in juvenile rainbow trout fish fed on varying levels of dietary lysozyme [23]. Improvement of erythrocyte count and the increasing of the hemoglobin concentration after the administration of lysozyme may be explained by an increased number of cells in the bone marrow. Lysozymes are associated with the development of monocyte-macrophage system and immunoglobulin. The acidifier supplementation improves blood cell producing organs (increased tibia weight and relative liver weight) in broilers [24]. But, the minimum hematological value in combined supplementation group may be due to the decreased lysozyme activity by relatively low pH and salts of acidifier. These findings are similar with previous findings [15, 24]. The present experiment revealed that lysozyme supplementation increased total cholesterol level, but insignificantly decreased in triglyceride and LDL-cholesterol level. On the other hand, acidifier supplementation decreased the total cholesterol and LDL-cholesterol. This may be due to the better utilization of supplied feed nutrients as a result of the decreased loss of nutrients by microbes and an increased anabolic activity occurring from increased villi height in ileum. Again, the combination of acidifier and lysozyme showed more or less similar activity like lysozyme alone, with slight decreased rate which may be

due to some inhibitory effects of lysozyme at lower pH resulted by acidifier and environmental condition. It was previously reported that significant reduction in serum level of cholesterol, total lipid or low-density lipoprotein (LDL) was achieved due to dietary acidification. Acidifier stimulated immune response and reduced gut pH to make balanced gut microflora [25]. But, another report [26] differed that blood total protein and cholesterol values were not affected in the treatment groups of broilers by the use of acidified ration. In case of enzymes involved in liver and kidney function test (AST, ALT and creatinine), the results suggested that the use of acidifier, lysozyme or both might not influence the liver and kidney function of birds. This result coincides with the findings [15, 21] but differed the findings [24], which showed that there was increased serum AST and ALT activities concentration upon acidifier supplementation.

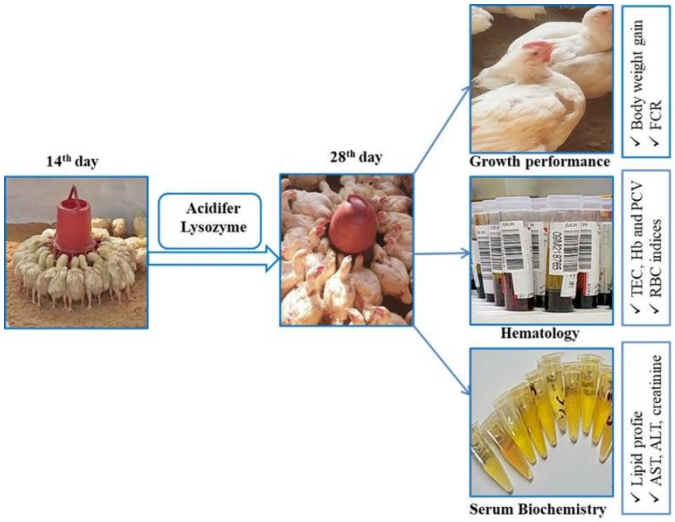

Figure 4. Schematic view of the experiment revealing promoting effects of dietary acidifier and lysozymes in broiler chickens

CONCLUSION

It could be concluded from this research finding that the use of acidifiers, and lysozyme as feed supplement significantly ($p<0.01$ and $p<0.05$) increased weight gain, feed conversion ratio (FCR) and certain hematological parameters (Hb, TEC and PCV) probably due to their positive effects on feed utilization by increasing digestion, absorption and metabolism (Figure 4). But, when both are used simultaneously, the effect of lysozyme is reduced by lowered pH of acidifier. Although the cholesterol level increases in lysozyme supplementation, LDL-cholesterol is not increased significantly. So dietary acidifier and lysozymes can be used as an alternative to antibiotic growth promoter, individually for maximum broiler production. The broiler chickens supplemented with acidifier and lysozyme are safe and economic compared to those supplemented with antibiotic growth promoter. Humans can consume safer animal protein to boost up the body defense system. Further study is needed to investigate the molecular mechanism of action of acidifier and lysozyme as well as their tolerance level.

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AUTHOR CONTRIBUTIONS

MAM designed the experiment, analyzed data and wrote the final draft of the manuscript. KKIK performed the experiment, analyzed data and wrote the draft of the manuscript; MAI, performed the experiment. KMJ, AM and NA critically revised the manuscript.

CONFLICTS OF INTEREST

The author declares no conflict of interest.

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