Veterinary and Animal Science 22 (2023) 100325

Contents lists available at ScienceDirect

Veterinary and Animal Science

journal homepage: www.elsevier.com/locate/vas

# Effects of antibiotic, acidifier, and probiotic supplementation on mortality rates, lipoprotein profile, and carcass traits of broiler chickens

Sabuj Kanti Nath [a,*], Md Taslim Hossain [a], Mahfuza Ferdous [a], Mst. Assrafi Siddika [a], Amir Hossain [b], Amim Al Maruf [c], Ahanaf Tahmid Chowdhory [c], Tilak Chandra Nath [d]

[a] Department of Animal Nutrition, Faculty of Veterinary, Animal and Biomedical Sciences, Khulna Agricultural University, Khulna-9100, Bangladesh
[b] Department of Poultry Science, Faculty of Veterinary, Animal and Biomedical Sciences, Khulna Agricultural University, Khulna-9100, Bangladesh
[c] Faculty of Veterinary, Animal and Biomedical Sciences, Khulna Agricultural University, Khulna-9100, Bangladesh
[d] Department of Parasitology, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Sylhet-3100, Bangladesh

ARTICLE INFO

*Keywords:*
Hubbard classic broiler
Blood lipoprotein
Body weight gain
Feed conversion ratio

ABSTRACT

Antimicrobial resistance is a significant issue, therefore it's relevant to assess the effects of antibiotics, acidifiers, and probiotic supplementation finding a good alternative to reduce the use of antibiotics in broiler production in rural areas of Bangladesh. Using randomized control trial, this 28-day study evaluated 360 Hubbard Classic broiler chicks divided into four groups: oxytetracycline-treated, acidifier-treated, Lactobacillus-based probiotic-treated, and control (no antibiotics, acidifiers, or probiotics). Each group was replicated three times with 30 birds each with adlibitum feeding. Body weight and feed intake were recorded weekly, and on 28th day, carcass traits and blood lipoprotein levels were evaluated. Results showed that in first and fourth weeks, the body weight gain significantly varied in probiotics and acidifier-treated birds than the control group ($P < 0.001$). The probiotic group had gained considerable increase in body weight (185.0 g vs 161.7 g and 1745.0 g vs 1592.7 g) than the control group. Notably, in the first week, the feed conversion ratio for the probiotic group was 0.76, but the antibiotic group's was 0.96 ($P < 0.001$). The weights of the drumstick (88.33 g) and liver (61.0 g) having probiotic supplements were substantially higher than those in the control group (77.0 g and 51.33 g, respectively) ($P < 0.001$). According to serum lipoprotein analysis, the probiotic and acidifier groups exhibited lower LDL levels (71.1 mg/dl and 69.8 mg/dl, respectively) and higher triglyceride levels (122.9 mg/dl and 135.4 mg/dl). These findings highlight the potential of probiotics and acidifiers as effective antibiotic alternatives, promoting carcass traits and lowering LDL levels in broilers in Bangladesh.

## 1. Introduction

In order to supply the expanding need for high-quality protein sources all across the world, broiler chicken production is essential. As the world's population is expanding quickly and dietary preferences are changing, poultry meat has become an essential part of diets because it is inexpensive, versatile, and has a significantly smaller environmental impact in comparison to other meat sources (Alexandratos & Bruinsma, 2012; Herrero et al., 2015; Nkukwana, 2018). The poultry industry, especially the raising of broiler chickens, makes a substantial contribution to rural livelihoods and food security while also promoting economic growth through trade and the creation of jobs (Pica-Ciamarra & Otte, 2010; Pius et al., 2021; Vaarst et al., 2015). However, optimizing production effectiveness, disease control, and meat quality are critical concerns as the demand for broiler meat keeps rising. It is, therefore, crucial to conduct research to improve these features of broiler farming in order to sustainably address the problems with global food security (Wong et al., 2017).

The poultry industry faces a variety of difficulties. Disease outbreaks, such as those brought on by infectious agents like *Salmonella* sp. and *Clostridium perfringens*, not only impair flock health but also result in financial losses and raise questions about the safety of the food being produced (Mak et al., 2022). Furthermore, variables such as inadequate diet, environmental stresses, and microbial imbalances in the gut might contribute to suboptimal development rates in broilers (Ahmad et al., 2022; Lauridsen, 2019). Moreover, Because of growth constraints, the industry is unable to satisfy the rising demand for protein Hafez and Attia (2020). Consumer satisfaction and the viability of broiler products

* Corresponding author.
*E-mail address:* sabuj.vet@gmail.com (S.K. Nath).

https://doi.org/10.1016/j.vas.2023.100325

Available online 28 November 2023

are challenged by variations in carcass meat quality and production, which are impacted by genetics, nutrition, and management (Mir et al., 2017; Petracci & Cavani, 2011).

From day-old chicks (DOC) to finished goods, the majority of rural poultry producers in Bangladesh frequently use antibiotics to reduce pathogenic infections and boost production (Haque et al., 2020). Antibiotic use is currently discouraged and is already prohibited in the European Union due to its detrimental impact on public health (Castanon, 2007). In the near future, antibiotic use may be restricted globally. Drug-resistant bacteria spread due to the prolonged misuse of antibiotics in chicken farms (Egbule, 2022).

Although, animal feeds used to contain antibiotic supplements as a growth booster, as a successful method of lowering the population of pathogenic bacteria present in the gut, and to improve immunological responses (Yaqoob et al., 2022). Antibiotics also perform a variety of physiological functions, including nutrient absorption and feed intake (Abd El-Hack et al., 2022), energy and nitrogen retention (Lee et al., 2023), metabolic functions like liver protein synthesis (Miller & Singer, 2022), and so on. Despite having favorable effects antibiotics also exhibit some side effects for instance slowed feed transit times (Wallace et al., 2023), decreased gut wall thickness and diameter (Pothineni & Keller, 2023), reduced energy and vitamin synthesis in the gut (Voland et al., 2022), and toxic ammonia production (Li et al., 2022).

In recent years, alternative feed additives like organic acids, prebiotics, probiotics, enzymes, and their derivatives which have the potential to improve growth performance and promote animal health while avoiding the negative effects associated with antibiotic use, have seen a noticeable increase in interest (Ayalew et al., 2022). Besides, acidifiers have the potential to improve nutrition utilization, alter gut pH, and cease the growth of dangerous microorganisms in the digestive system (Hamidifard et al., 2023; Melaku et al., 2021; Okey, 2023). Recent research demonstrates that probiotics and prebiotics may accelerate the growth rate of broiler chickens (Kong et al., 2022). They may also be able to improve the antibody titer against IBD in broilers given diets devoid of antibiotics. Furthermore, by promoting beneficial bacteria communities in the gut, probiotics have been shown to improve digestion, promote immune response, and reduce the risk of antibiotic resistance (Bahaddad et al., 2023; Timothy & de la Fuente, 2023; Wong-Chew et al., 2022).

One notable research gap in broiler production in Bangladesh is the dearth of extensive research investigating the individual effects of probiotic, antibiotic, and acidifier supplementation on numerous parameters. Extensive research has been conducted on the effects of individual feed additives; however, less focus has been on the combined impact of these compounds on carcass characteristics, disease resistance, productivity, and lipoprotein profiles. By focusing on the particular context of poultry production in Bangladesh, this study attempts to close these knowledge gaps and provide an in-depth overview of the potential benefits of probiotic, antibiotic, and acidifier supplements to promote sustainable broiler farming practices in the region. The aim of the study is to investigate the potential benefits of antibiotic, acidifier, and probiotic supplements by investigating how these variables affect broiler chickens' productivity, lipoprotein profile, disease prevalence, and carcass traits.

## 2. Materials and methods

This study protocol was reviewed and approved by the Parasite Research Center, International Parasite Resource Bank, South Korea, and the Department of Parasitology, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Bangladesh (2020EC-71).

### 2.1. Selection criteria of the experimental birds

A total of 360 Hubbard Classic strain day-old chicks of mixed sex (male and female) were purchased from a reliable agent in Khulna, Bangladesh. The purchased chicks were 44 gs on average in body weight. To guarantee uniform size and the lack of any apparent deformities, strict selection criteria were used prior to purchase. These standards included, but were not restricted to, assessing physical attributes including the feather's condition, the structure of the legs, and overall alertness. The study excluded any chicks displaying indications of illnesses, deformity, or aberrant development. Establishing a homogeneous baseline for the experimental trial and minimizing any confounding variables that might alter the study's results were the goals of this meticulous pre-selection process.

### 2.2. Experimental design and treatment groups

A randomized control trial was used to perform the experiment. Randomization was applied to select the birds for the treatment and control groups in the experiment. The incorporation of three concentration levels of the treatments in order to determine a potential dose-response relationship and identify the appropriate dosage for maximal efficacy without side effects. A total of 360 broiler chickens were divided evenly and randomly into the $T_0$, $T_1$, $T_2$, and $T_3$ treatment groups. Each treatment group containing 90 birds was divided equally among the three replications, with 30 birds per replication. Throughout the four-week rearing period, the birds were closely monitored to assess a number of crucial variables.

The experimental design involved the following four treatment groups:

**Control Group ($T_0$):** This group was given only the basic diet without any supplements.

**Acidifier Group ($T_1$):** Acidifiers were mixed with drinking water in all replicates at concentrations of 0.5 ml/Liter, 1.0 ml/Liter, and 2.0 ml/Liter.

**Probiotics Group ($T_2$):** Probiotics were administered to drinking water at concentrations of 0.5 gm/Liter, 1.0 gm/Liter, and 2.0 gm/Liter.

**Antibiotics Group ($T_3$):** In this group, Tetracycline was added to the drinking water. Tetracycline replicates were given to subjects in drinking water at concentrations of 0.5 gm/Liter, 1.0 gm/Liter, and 2.0 gm/Liter.

The design of the experiment is depicted in Fig. 1.

### 2.3. Experimental acidifier, probiotics, and antibiotic

Antibiotic: Oxytetracycline, which is marketed under the trade name Pulv.Vetomycin® was the experimental antibiotic used in this investigation. Oxytetracycline Hydrochloride USP is present in this antibiotic in a 20 % concentration.

Acidifier: The company name of the acidifier was Liq. NovoVital, which contains monosters of butyric acid, caprylic acid, capric acid, and lauric acid was used as the acidifier in this study. These compounds are recognized for having the potential to affect gut health and enhance favorable conditions within the digestive tract.

Probiotics: Pulv. Grobio-Pro Vet, a probiotic supplement, was used in this investigation. With a concentration of 2 billion CFU (colony-forming units), this powder comprises a mixture of advantageous microorganisms, including *Bacillus subtilis, Bacillus licheniformis, Bacillus coagulans,* and *Saccharomyces boulardii*. Various enzymes, including amylase, beta-glucanase, beta-xylanase, lipase, pectinase, and protease, are also included in the supplement. The formulation also contains a variety of important vitamins and minerals, such as Zinc Sulfate, Manganese Sulfate, Cobalt Sulfate, and Vitamins A, D3, E, and K. It also contains amino acids, for instance, DL-Methionine.

### 2.4. Housing and brooding

First, a small poultry shed with 360 birds was chosen and set up for the rearing of broilers. The shed was carefully cleaned using tap water,

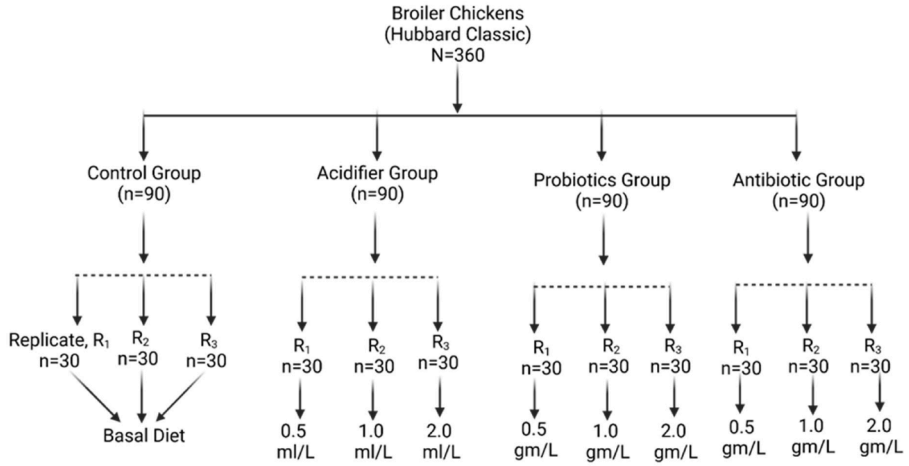


**Fig. 1.** The design of the experiment.

caustic soda, and tincture iodine. In accordance with the manufacturer's instructions, phenyl solution was also applied to the floors, ceiling and corners to kill microorganisms. Then, brushing was done with a steel brush and fresh water. In a similar way, brooding boxes and broiler cages were cleaned using tap water, caustic soda, and phenyl solution. The shed was left for a week to dry after cleaning and disinfection. To ensure adequate airflow, all windows were opened. For rigorously preserving biosecurity, the lime was placed on the shed's floor and surrounding area after one week.

Following thorough cleaning and drying, the brooding boxes were prepared for raising broiler chicks. The brooding box's floor was covered with dry, clean newspaper, which was changed every four hours throughout the whole brooding time. In each brooding box, 100, 50, and 25-watt incandescent lamps were used to maintain the brooding temperature. The broilers were continually lighted. Chicks were raised throughout the brooding phase at temperatures of 95°F, 90°F, 85°F, and 80°F for the first, second, third, and fourth weeks, respectively.

### 2.5. Feeding and watering

Birds were provided feed and water on paper throughout the early stages of brooding, as well as tiny, round, plastic feeders and drinkers with a 1.5-liter capacity per brooding box. For every ten birds, there was around one drinker available. Small liner feeders (2.21 ft. ˣ 0.25 ft.) were installed in each box to replace the tiny circular feeders after the seventh day. Larger liner feeders (3.5 ft. ˣ 0.38 ft.) and round drinkers with a three-liter capacity were employed during cage rearing.

The broilers were supplied with ready feed which was purchased from the local feed dealer. During the rearing period broiler starter feed was supplied from 1 to 13 days and broiler grower was used for 14–28 days. Table 1, depicts the Proximate Composition of the Broiler Starter and Broiler grower.

### 2.6. Immunization

According to treatments and replications, arrangements for broiler rearing were made. In order to ensure that the distribution of chicks was uniform, the compartments were unbiasedly chosen. All birds were appropriately immunized against New Castle Disease (ND) at 4th days and Infectious Bursal Disease (IBD) at 10th days, with booster doses administered at 21th days for ND and 17th days for IBD.

### 2.7. Assessment of productivity

Body weight gain: Weigh each chicken at the beginning and end of each week to calculate the weekly body weight gain. The calculated weight gain was determined by subtracting the two readings. Live weight was measured by using a digital weighing scale, the live weight of the broiler chickens was determined weekly for every treatment group.

Feed Intake: Each chicken's daily feed intake was measured and recorded to determine its weekly feed intake.

Feed Conversion Ratio: Each week's total weight gain was divided by the total amount of feed consumed to determine the feed conversion ratio. A higher feed conversion ratio is an indicator of reduced efficiency, meaning that broilers require more feed to gain one unit of body weight.

**Table 1**
Ration formulation for broiler starter and broiler grower.

| Name of the ingredients | Starter | Grower |
|---|---|---|
| Maize | 58.65 | 60 |
| Rice polish | 3 | 4.9 |
| Vegetable oil | 1.8 | 2.6 |
| Molasses | 0.5 | 0.4 |
| Soybean meal | 28.55 | 25 |
| Fishmeal | 5.6 | 5.3 |
| Meat & Bone meal | 0.3 | 1.05 |
| Limestone | 1 | 0.45 |
| Di-calcium phosphate | 0.1 | 0 |
| V M premix | 0.25 | 0.15 |
| Common salt | 0.25 | 0.15 |
| **Total** | **100.00** | **100.00** |
| **Nutrients (%)** | | |
| ME (Kcal/Kg) | **3002.91** | **3100.59** |
| CP% | 22.02 | 21.00 |
| CF% | 3.72 | 3.78 |
| EE% | 5.06 | 5.06 |
| Ca% | 1.10 | .90 |
| P% | 0.79 | .79 |
| Lysine% | 1.36 | 1.27 |
| Methionine% | 0.37 | 0.36 |
| Tryptophan% | 0.26 | 0.24 |
| Cysteine% | 0.87 | 0.82 |

ME = Metabolizable Energy, CP = Crude Fiber, EE = Ether Extract, Ca = Calcium, *P* = Phosporus.

### 2.8. Monitoring mortality of the birds

To monitor the mortality associated with each dietary intervention of the birds, we concentrated on identifying specific symptoms associated with relevant diseases. The health examination was carried out through the observation of systematic physical examinations, clinical signs, and behavioral monitoring to ascertain the mortality of the birds.

### 2.9. Carcass characteristics

After the broiler chicks were dissected at 28 days of age, the weight of each internal organ—such as the drumstick, breast, liver, colon, gizzard, proventriculus, and heart—was measured using a digital weight balance.

### 2.10. Collection of blood and serum samples analysis

On day 28, five birds were randomly chosen for blood collection from each replication. Blood samples were collected from the brachial vein. Each bird had about 3.0 cc of blood drawn from it using a sterile syringe, which was then placed vertically in the refrigerator. Serum was collected in a sterile plastic container after six hours to estimate serum parameters. Centrifugation was performed for 15 min at 3000 rpm to separate the serum. Several blood parameters (cholesterol, triglyceride, LDL, and HDL) were evaluated using standard kits (BioMereux, France) and an automatic analyzer (Humalyzer 300, Merck®, Germany) according to the manufacturer's instructions (FVMAAU; Addis Abeba, Ethiopia).

### 2.11. Statistical methods

Data that were obtained from the experiment such as, live weight, weight gain, feed intake feed consumption, feed conversion ratio, carcass parameters, blood parameters, and mortality were entered into MS Excel spreadsheet. The dataset was checked for missingness and integrity by careful visual examination of the spreadsheet before entering into IBM SPSS Statistics (version 28.0.1.1) for statistical analysis. The continuous variables were checked for normal distribution using the visual observation of the histogram. The effects of the treatments on the measured traits were analyzed using a univariate generalized linear model. The model included the outcome of interest as the dependable variable, treatments as the fixed effect, and replication as the random effect. A separate model was constructed for each week for body weight gain and feed intake. Only one model was constructed separately for the feed conversion ratio, carcass parameters, and blood parameters. The mean was reported as the least squares means and their standard error. Differences between treatment means were tested for statistical significance and were adjusted according to the multiple comparison test using Bonferroni corrections. The 95 % confidence intervals and P values were estimated from the regression model as described above.

## 3. Results

### 3.1. Body weight gain, feed intake and feed conversion ratio per week

According to the results, broilers in treatment group $T_2$ gained their greatest body weight, averaging 185.0 g, 537.0 g, 1131.7 g, and 1745.0 g in the first, second, third, and fourth weeks, respectively. Conversely, broilers in group $T_3$ recorded values of 155.0 g, 450.3 g, 1015.7 g, and 1504.7 g in the corresponding weeks, indicating the least amount of body weight gain Table 2. The initials (a, b, c, d) next to each treatment stand for the statistically highly significant differences between them ($P < 0.001$). For instance, based on multiple comparisons with the Bonferroni correction, values with different superscripts in a similar row are different significantly from one another ($P < 0.05$) (Table 2).

Similarly, $T_0$ showed the highest feed intake values every week (147.7 g, 517.0 g, 1292.3 g, and 2410.0 g for the first, second, third, and fourth weeks, respectively), whereas $T_3$ showed the lowest feed intake values. For every week, statistically significant differences in feed consumption are seen ($P < 0.05$), indicating that the dietary treatments have an impact on how much the broiler chickens consume (Table 2).

Group $T_2$ exhibited the most efficient performance in terms of feed conversion ratio, with ratios for the corresponding weeks of 0.76, 0.95, 1.13, and 1.38. The p-values show that the variations in feed conversion ratios between treatments have substantial statistical significance ($P < 0.001$) (Table 2).

### 3.2. Carcass traits

At 28 days of age, the weight of a number of internal organs, including the heart, proventriculus, gizzard, liver, and drumstick, was determined. Significant differences were also seen in the weights of several internal organs among the treatment groups. For instance, group $T_2$ broilers, had the largest drumstick weight at 28 days, weighing 88.33 g, whereas group $T_3$ broilers had the lowest weight, weighing 69.00 g. Additionally, group $T_2$ had the largest liver weight (61.00 g), whereas group $T_3$ had the lowest liver weight (44.33 g). (Table 3) shows that these differences were similar in other internal organs.

### 3.3. Lipoprotein and glucose profile

Significant variations in the lipid profile between the treatment groups were found in the serum analysis conducted after 28 days. Triglyceride levels, for example, were greater in groups $T_1$ and $T_2$ than in $T_0$

**Table 2**
Univariable association of weekly body weight gain, feed intake, and feed conversion ratio of 360 broiler chicken with three separate replications ($n = 30$) under four dietary treatments.

| Items | Treatments* | | | | SEM | *P* |
|---|---|---|---|---|---|---|
| | T0 | T1 | T2 | T3 | | |
| ***Body Weight Gain*** | | | | | | |
| 1st week | $161.7^{a}$ | $171.3^{b}$ | $185.0^{c}$ | $155.0^{d}$ | 0.938 | <0.001 |
| 2nd week | $480.3^{a}$ | $504.0^{b}$ | $537.0^{c}$ | $450.3^{d}$ | 1.427 | <0.001 |
| 3rd week | $1053.7^{a}$ | $1101.0^{b}$ | $1131.7^{c}$ | $1015.7^{d}$ | 2.560 | <0.001 |
| 4th week | $1592.7^{a}$ | $1662.3^{b}$ | $1745.0^{c}$ | $1504.7^{d}$ | 2.612 | <0.001 |
| ***Feed Intake*** | | | | | | |
| 1st week | $147.7^{a,b,d}$ | $147.0^{a,b,d}$ | $140.0^{c}$ | $148.7^{a,b,d}$ | 1.427 | 0.018 |
| 2nd week | $517.0^{a,b,c}$ | $515.7^{a,b,c}$ | $512.0^{a,b,c}$ | $501.3^{d,c}$ | 2.277 | 0.010 |
| 3rd week | $1292.3^{a,b,d}$ | $1292.0^{a,b,d}$ | $1275.0^{c,d}$ | $1283.0^{a,b,c,d}$ | 2.977 | 0.017 |
| 4th week | $2410.0^{a,b,c}$ | $2383.0^{a,b,c,d}$ | $2402.3^{a,b,c,d}$ | $2377.3^{b,c,d}$ | 5.412 | 0.015 |
| ***Feed conversion ratio*** | | | | | | |
| 1st week | $0.91^{a}$ | $0.86^{b}$ | $0.76^{c}$ | $0.96^{a,d}$ | 0.010 | <0.001 |
| 2nd week | $1.08^{a}$ | $1.02^{b}$ | $0.95^{c}$ | $1.11^{d}$ | 0.006 | <0.001 |
| 3rd week | $1.22^{a}$ | $1.17^{b}$ | $1.13^{c}$ | $1.26^{d}$ | 0.004 | <0.001 |
| 4th week | $1.51^{a}$ | $1.43^{b}$ | $1.38^{c}$ | $1.58^{d}$ | 0.005 | <0.001 |

* Each treatment contains three replicates having 30 birds.

Note: Treatments $T_1$ and $T_2$ showed significantly higher body weight growth than treatments $T_0$ and $T_3$ over the study period of four weeks ($P < 0.001$). Based on multiple comparisons using the Bonferroni correction, the letters "a," "b," "c," and "d" denote significant differences within rows ($P < 0.05$).

**Table 3**
Univariable association of weight of carcass traits of internal organs of broilers at 28 days of 360 broiler chicken with three separate replications ($n = 30$) under four dietary treatments.

| Traits | Treatments | | | | SEM | *P* |
|---|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | $T_3$ | | |
| Drumstick (gm) | 77.00[a] | 82.33[b] | 88.33[c] | 69.00[d] | 0 0.674 | <0.001 |
| Breast (gm) | 377.00[a] | 386.67[b] | 411.67[c] | 333.33[d] | 1.08 | <0.001 |
| Liver (gm) | 51.33[a] | 55.67[a,b] | 61.00[c] | 44.33[d] | 0.938 | <0.001 |
| Intestine (gm) | 92.00[a] | 97.67[b] | 114.00[c] | 81.00[d] | 0.441 | <0.001 |
| Gizzard (gm) | 33.00[a] | 39.67[a,b] | 47.33[c] | 30.67[a,d] | 1.411 | <0.001 |
| Proventriculus (gm) | 13.33[a] | 14.00[a,b] | 16.33[c] | 11.33[a,d] | 0.471 | 0.002 |
| Heart (gm) | 11.00[a] | 12.00[a,b] | 14.33[c] | 10.33[a,d] | 0.536 | 0.008 |

[a, b, c, d] means with various superscripts in a row differ from one another significantly ($P < 0.05$) in multiple comparison by Bonforoni correction. $T_0$ = control feed; $T_1$ = water contains organic acid at the rate of 0.5 ml/L, 1.0 ml/L, and 2.0 ml/L; $T_2$ = feed contains probiotic inclusion level 0.5 gm/L, 1.0 gm/L, 2.0 gm/L; $T_3$ = feed containing antibiotic at the rate of 0.5 gm/L, 1.0 gm/L, 2.0 gm/L; SEM = Standard Error of Mean; Significant ($p \leq 0.05$).

and $T_3$, with values of 135.37 mg/dl and 122.87 mg/dl, respectively. Furthermore, the LDL levels in groups $T_1$, $T_2$, and $T_3$ were consistently lower than those in $T_0$, with respective values of 69.800 mg/dl, 71.133 mg/dl, and 66.433 mg/dl (Table 4).

### 3.4. Mortality rates

Mortality rates were kept track of during the entire experiment. The control group ($T_0$) had the highest death rate (8.92 %), followed by the probiotic ($T_2$), acidifier ($T_1$), and antibiotic ($T_3$), with the lowest rate (2.58 %) (Fig. 2).

## 4. Discussion

The study revealed substantial variations in body weight gain, feed intake, and feed conversion ratio across treatment groups. When compared to the control ($T_0$) and antibiotic ($T_3$) groups, broilers fed with acidifiers and probiotics ($T_1$ and $T_2$) showed greater gains in body weight and increased feed conversion ratios. This is in line with the body of research showing that acidifiers and probiotics can have a good effect on the intestinal environment, nutrient uptake, and growth performance in chicken (Aliverdi-Nasab et al., 2023; Leone & Ferrante, 2023; Mantzios et al., 2023; Vimon et al., 2023).

According to the study, during the experimental period (1–42 days of age), the probiotic complex's addition had no significant impact on the growth performance or carcass characteristics of AA+ broilers. On the other hand, the probiotic complex's dietary addition dramatically raised the AA+ broilers' thymus index, decreased the amount of *E. Coli* and Salmonella in their feces, and decreased the amounts of NH3 and H2S emissions in their feces (Zou et al., 2022). In addition, Probiotics like *Bacillus subtilis* were commonly used because of their many beneficial properties, which included controlling intestinal microecological balance, enhancing nutrition utilization, and promoting animal growth and development in broilers (Gao et al., 2017; Sen et al., 2012). In contrast,

**Table 4**
Univariable association of Serum lipid profile and glucose constituents' level of broilers at 28 days of age of 360 broiler chicken with three separate replications ($n = 30$) under four dietary treatments.

| Parameter | Serum constituents' level (mg/dl) | | | | SEM | *P* |
|---|---|---|---|---|---|---|
| | $T_0$ | $T_1$ | $T_2$ | $T_3$ | | |
| Cholesterol | 118.17 | 130.37 | 119.63 | 112.10 | 3.71 | 0.064 |
| Glucose | 10.53 | 10.53 | 10.13 | 10.17 | 0.17 | 0.249 |
| Triglyceride | 123.43 | 135.37 | 122.87 | 106.33 | 4.99 | 0.034 |
| LDL | 78.200 | 69.800 | 71.133 | 66.433 | 1.044 | 0.001 |
| HDL | 60.167 | 66.033 | 63.767 | 57.567 | 2.27 | 0.135 |

Here, $T_0$ = control feed; $T_1$ = water contains organic acid at the rate of 0.5 ml/L, 1.0 ml/L, and 2.0 ml/L; $T_2$ = feed contains probiotic inclusion level 0.5 gm/L, 1.0 gm/L, 2.0 gm/L; $T_3$ = feed containing antibiotic at the rate of 0.5 gm/L, 1.0 gm/L, 2.0 gm/L; SEM = Standard Error of Mean; Significant ($p \leq 0.05$).

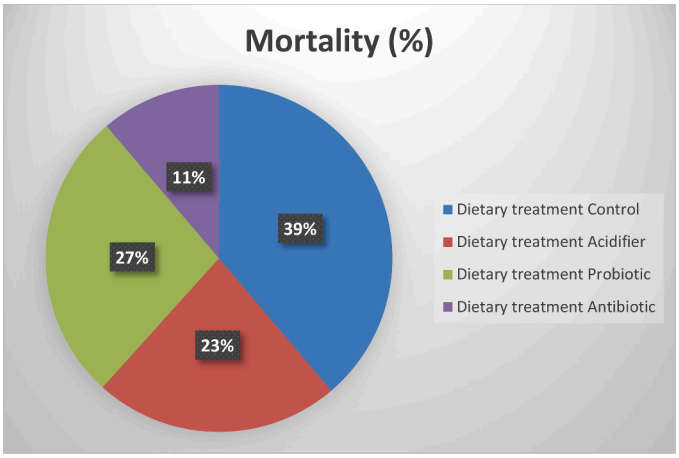


**Fig. 2.** Univariable association of mortality rate of broilers at day old chick to 28 days of 360 broiler chickens with three separate replications ($n = 30$) under four dietary treatments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

in some studies, there was a noticeable effect of the probiotic supplementation on carcass attributes, and higher body weights were seen in broilers fed with probiotics (Aksu et al., 2005; Sumanu et al., 2021; Tayeri et al., 2018). The supplementation also affected the weights of the liver, gut, and gizzard, with the probiotic group frequently displaying the highest values (Joysowal et al., 2018). These results are consistent with earlier studies that showed probiotics could improve overall carcass quality. The addition of an acidifier had advantageous effects on gizzard weight as well, which may indicate enhanced digestion (Sadati et al., 2022).

In comparison to the control ($T_0$) and antibiotic ($T_3$) groups, the acidifier and probiotic groups ($T_1$ and $T_2$) had lower Low-density lipoprotein (LDL) levels. LDL cholesterol is sometimes referred to as "bad cholesterol" because of its connection to the emergence of atherosclerosis and coronary artery disease (Li et al., 2023; Moghadasian, 2002; Papp et al., 2023). Research has shown a correlation between decreased levels of LDL cholesterol in broilers and the usage of probiotics and acidifiers. Based on studies, probiotics may alter the composition of the gut microbiota, improving intestinal health and enhancing nutrient absorption, which could have an impact on cholesterol metabolism (Ebeid et al., 2021; Gao et al., 2022; Lye et al., 2010). On the other hand, acidifiers have been demonstrated to make the stomach more vulnerable to harmful bacteria, promoting a healthier microbial balance and possibly influencing the synthesis and absorption of cholesterol (Qiu et al., 2023; Sohail et al., 2015). Our observed reduction in LDL levels produced by the addition of probiotics and acidifiers may lower the possibility of lipid-related disorders.

During the study mortality rate were recorded, the main reason for the broilers' deaths was suffocation from too much litter, which is common in commercial chicken production systems. The control group's somewhat higher mortality rate highlights the vulnerability of broilers raised without supplements, possibly as a result of their increased vulnerability to diseases and environmental stressors (Hosseini-Vashan et al., 2016). Moreover, the lower death rate in the antibiotic group indicates that antibiotics are effective in controlling bacterial risks that frequently contribute to disease outbreaks in broiler flocks (Cervantes, 2015). The probiotic and acidifier groups' relatively low death rates highlight the possible benefits of these dietary supplements for promoting disease resistance and broiler health (Sarangi et al., 2016). This implies that these alternate dietary supplementation strategies also contribute to the health and disease resistance of broilers. Acidifiers can improve intestinal pH and produce an atmosphere that is less favorable for the development of dangerous microbes (Suiryanrayna & Ramana, 2015).On the other hand, Probiotics can improve the balance of bacteria in the gastrointestinal tract, support the immune system, and destroy pathogenic bacteria (Patil et al., 2023). The lower mortality rates in these groups indicates that probiotics and acidifiers may have anti-infective effects. Further research on the causes of mortality is required to give more comprehensive understanding of the health of broiler chickens. Moreover, the limitations of this study are the short rearing period, small sample size, emphasis on a particular region and broiler strain, exclusion of external factors, limited blood parameters, a single supply of chicks, and potential confounding factors.

## 5. Conclusions

In summary, this study emphasizes the potential advantages of using probiotics and acidifiers in broiler diets in place of antibiotics. Acidifier and probiotic administration enhanced body weight gain, feed conversion ratio, and carcass characteristics while showing favorable effects on serum lipoprotein profiles. In addition, these additions helped the death rates be lower compared to control group. These results indicate that probiotics and acidifiers may provide feasible options for boosting up broiler health and performance without the use of antibiotics, consequently supporting sustainable chicken production.

## Ethical statement

This study protocol was reviewed and approved by the Parasite Research Center, International Parasite Resource Bank, South Korea, and the Department of Parasitology, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Bangladesh (2020EC-71).

## CRediT authorship contribution statement

**Sabuj Kanti Nath:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Md Taslim Hossain:** Investigation, Supervision, Validation, Visualization. **Mahfuza Ferdous:** Writing – original draft, Writing – review & editing. **Mst. Assrafi Siddika:** Visualization, Writing – original draft, Writing – review & editing. **Amir Hossain:** Conceptualization, Investigation, Methodology. **Amim Al Maruf:** Investigation, Resources. **Ahanaf Tahmid Chowdhory:** Investigation, Methodology, Resources. **Tilak Chandra Nath:** Methodology, Validation, Visualization, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

This research was funded by R & D project, Ministry of Science and Technology, Bangladesh. We would like to thank all faculty members, technical officers, and Lab attendants of the department of Animal Nutrition, Faculty of Veterinary, Animal and Biomedical Sciences, Khulna Agricultural University, Bangladesh.

## References

Abd El-Hack, M. E., El-Saadony, M. T., Salem, H. M., El-Tahan, A. M., Soliman, M. M., Youssef, G. B., Taha, A. E., Soliman, S. M., Ahmed, A. E., & El-Kott, A. F (2022). Alternatives to antibiotics for organic poultry production: Types, modes of action and impacts on bird's health and production. *Poultry Science, 101*(4), Article 101696.

Ahmad, R., Yu, Y. H., Hsiao, F. S. H., Su, C. H., Liu, H. C., Tobin, I., … Cheng, Y. H. (2022). Influence of heat stress on poultry growth performance, intestinal inflammation, and immune function and potential mitigation by probiotics. *Animals, 12*(17), 2297.

Aksu, M. I., Karaoğlu, M., Esenbuğa, N., Kaya, M., Macit, M., & Ockerman, H. W. (2005). Effect of a dietary probiotic on some quality characteristics of raw broiler drumsticks and breast meat. *Journal of Muscle Foods, 16*(4), 306–317.

Alexandratos, N., & Bruinsma, J. (2012). World agriculture towards 2030/2050: The 2012 revision.

Aliverdi-Nasab, K., Zhandi, M., Yousefi, A. R., Zahedi, V., & Rafieian-Naeini, H. R. (2023). The effect of acidifier supplementation on egg production performance and intestinal histology of Japanese quail (Coturnix japonica). *Veterinary Medicine and Science, 9*(1), 263–271.

Ayalew, H., Zhang, H., Wang, J., Wu, S., Qiu, K., Qi, G., Tekeste, A., Wassie, T., & Chanie, D. (2022). Potential feed additives as antibiotic alternatives in broiler production. *Frontiers in Veterinary Science, 9*, Article 916473.

Bahaddad, S. A., Almalki, M. H., Alghamdi, O. A., Sohrab, S. S., Yasir, M., Azhar, E. I., & Chouayekh, H. (2023). Bacillus Species as direct-fed microbial antibiotic alternatives for monogastric production. *Probiotics and Antimicrobial Proteins, 15*(1), 1–16.

Castanon, J. (2007). History of the use of antibiotic as growth promoters in European poultry feeds. *Poultry Science, 86*(11), 2466–2471.

Cervantes, H. M. (2015). Antibiotic-free poultry production: Is it sustainable? *Journal of Applied Poultry Research, 24*(1), 91–97.

Ebeid, T. A., Al-Homidan, I. H., & Fathi, M. M. (2021). Physiological and immunological benefits of probiotics and their impacts in poultry productivity. *World's Poultry Science Journal, 77*(4), 883–899.

Egbule, O. S. (2022). Occurrence of extended spectrum beta–lactamases and sul 1 in multi-drug resistant Escherichia coli and Salmonella isolated from poultry feeds. *Scientific African, 18*, e01362.

Gao, J., Wang, R., Liu, J., Wang, W., Chen, Y., & Cai, W. (2022). Effects of novel microecologics combined with traditional Chinese medicine and probiotics on growth performance and health of broilers. *Poultry Science, 101*(2), Article 101412.

Gao, Z., Wu, H., Shi, L., Zhang, X., Sheng, R., Yin, F., & Gooneratne, R. (2017). Study of Bacillus subtilis on growth performance, nutrition metabolism and intestinal microflora of 1 to 42 d broiler chickens. *Animal Nutrition, 3*(2), 109–113.

Hafez, H. M., & Attia, Y. A. (2020). Challenges to the poultry industry: Current perspectives and strategic future after the COVID-19 outbreak. *Frontiers in Veterinary Science, 7*, 516.

Hamidifard, M., Khalaji, S., Hedayati, M., & Rajaei sharifabadi, H. (2023). Use of condensed fermented corn extractives (liquid steep liquor) as a potential alternative for organic acids and probiotics in broiler ration. *Italian Journal of Animal Science, 22*(1), 418–429.

Haque, M. H., Sarker, S., Islam, M. S., Islam, M. A., Karim, M. R., Kayesh, M. E. H., Shiddiky, M. J., & Anwer, M. S. (2020). Sustainable antibiotic-free broiler meat production: Current trends, challenges, and possibilities in a developing country perspective. *Biology, 9*(11), 411.

Herrero, M., Wirsenius, S., Henderson, B., Rigolot, C., Thornton, P., Havlík, P., De Boer, I., & Gerber, P. J. (2015). Livestock and the environment: What have we learned in the past decade? *Annual Review of Environment and Resources, 40*, 177–202.

Hosseini-Vashan, S., Golian, A., & Yaghobfar, A. (2016). Growth, immune, antioxidant, and bone responses of heat stress-exposed broilers fed diets supplemented with tomato pomace. *International Journal of Biometeorology, 60*, 1183–1192.

Joysowal, M., Saikia, B., Dowarah, R., Tamuly, S., Kalita, D., & Choudhury, K. D. (2018). Effect of probiotic Pediococcus acidilactici FT28 on growth performance, nutrient digestibility, health status, meat quality, and intestinal morphology in growing pigs. *Veterinary World, 11*(12), 1669.

Kong, Q., Liu, T., & Xiao, H. (2022). *Effects of probiotics and prebiotics on gut pathogens and toxins, 13*. Frontiers Media SA, Article 856779.

Lauridsen, C. (2019). From oxidative stress to inflammation: Redox balance and immune system. *Poultry Science, 98*(10), 4240–4246.

Lee, J. T., Rochell, S. J., Kriseldi, R., Kim, W. K., & Mitchell, R. D. (2023). Functional properties of amino acids: Improve health status and sustainability. *Poultry Science, 102*(1), Article 102288.

Li, S., Peng, L., Yang, C., Song, S., & Xu, Y. (2022). Cometabolic biodegradation of antibiotics by ammonia oxidizing microorganisms during wastewater treatment processes. *Journal of Environmental Management, 305*, Article 114336.
Li, Y., Mei, H., Liu, Y., Li, Z., Qamar, H., Yu, M., & Ma, X. (2023). Dietary supplementation with rutin alters meat quality, fatty acid profile, antioxidant capacity, and expression levels of genes associated with lipid metabolism in breast muscle of qingyuan partridge chickens. *Foods (Basel, Switzerland), 12*(12), 2302.
Lye, H.-S., Rusul, G., & Liong, M.-T. (2010). Removal of cholesterol by lactobacilli via incorporation and conversion to coprostanol. *Journal of Dairy Science, 93*(4), 1383–1392.
Mak, P. H., Rehman, M. A., Kiarie, E. G., Topp, E., & Diarra, M. S. (2022). Production systems and important antimicrobial resistant-pathogenic bacteria in poultry: A review. *Journal of Animal Science and Biotechnology, 13*(1), 1–20.
Mantzios, T., Tsiouris, V., Papadopoulos, G. A., Economou, V., Petridou, E., Brellou, G. D., Giannenas, I., Biliaderis, C. G., Kiskinis, K., & Fortomaris, P. (2023). Investigation of the effect of three commercial water acidifiers on the performance, gut health, and campylobacter jejuni colonization in experimentally challenged broiler chicks. *Animals, 13*(12), 2037.
Melaku, M., Zhong, R., Han, H., Wan, F., Yi, B., & Zhang, H. (2021). Butyric and citric acids and their salts in poultry nutrition: Effects on gut health and intestinal microbiota. *International Journal of Molecular Sciences, 22*(19), Article 10392.
Miller, M., & Singer, M. (2022). Do antibiotics cause mitochondrial and immune cell dysfunction? A literature review. *Journal of Antimicrobial Chemotherapy, 77*(5), 1218–1227.
Mir, N. A., Rafiq, A., Kumar, F., Singh, V., & Shukla, V. (2017). Determinants of broiler chicken meat quality and factors affecting them: A review. *Journal of Food Science and Technology, 54*, 2997–3009.
Moghadasian, M. H. (2002). Experimental atherosclerosis: A historical overview. *Life Sciences, 70*(8), 855–865.
Nkukwana, T. (2018). Global poultry production: Current impact and future outlook on the South African poultry industry. *South African Journal of Animal Science, 48*(5), 869–884.
Okey, S. N. (2023). Alternative feed additives to antibiotics in improving health and performance in poultry and for the prevention of antimicrobials: A review. *Nigerian Journal of Animal Science and Technology (NJAST), 6*(1), 65–76.
Papp, R.-E., Hasenegger, V., Ekmekcioglu, C., & Schwingshackl, L. (2023). Association of poultry consumption with cardiovascular diseases and all-cause mortality: A systematic review and dose response meta-analysis of prospective cohort studies. *Critical Reviews in Food Science and Nutrition, 63*(15), 2366–2387.
Leone, F., & Ferrante, V. (2023). Effects of prebiotics and precision biotics on performance, animal welfare and environmental impact. A review. *Science of the Total Environment, 901*, Article 165951.
Patil, A., Kotekar, D., & Chavan, G. (2023). Knowing the Mechanisms: How Probiotics Affect the Development and Progression of Cancer.
Petracci, M., & Cavani, C. (2011). Muscle growth and poultry meat quality issues. *Nutrients, 4*(1), 1–12.
Pica-Ciamarra, U., & Otte, J. (2010). Poultry, food security and poverty in India: Looking beyond the farm-gate. *World's Poultry Science Journal, 66*(2), 309–320.
Pius, L. O., Strausz, P., & Kusza, S. (2021). Overview of poultry management as a key factor for solving food and nutritional security with a special focus on chicken breeding in East African countries. *Biology, 10*(8), 810.
Pothineni, B. K., & Keller, A. (2023). Nanoparticle-based formulations of glycopeptide antibiotics: A means for overcoming vancomycin resistance in bacterial pathogens? *Advanced NanoBiomed Research, 3*(4), Article 2200134.
Qiu, K., Chen, Z., Zheng, A., Chang, W., Cai, H., Zhang, X., et al. (2023). Augmentation of performance, carcass trait, biochemical profile and lipid metabolism concerning the use of organic acidifier in broiler chickens. *Agriculture, 13*(9), 1765.
Sadati, M. M., Rezaeipour, V., & Abdullahpour, R. (2022). Efficacy of whole wheat grain in combination with acidified drinking water on growth performance, gizzard development, intestinal morphology, and microbial population of broiler chickens. *Livestock Science, 259*, Article 104911.
Sarangi, N. R., Babu, L., Kumar, A., Pradhan, C., Pati, P., & Mishra, J. (2016). Effect of dietary supplementation of prebiotic, probiotic, and synbiotic on growth performance and carcass characteristics of broiler chickens. *Veterinary World, 9*(3), 313.
Sen, S., Ingale, S., Kim, Y., Kim, J., Kim, K., Lohakare, J., Kim, E., Kim, H., Ryu, M., & Kwon, I. (2012). Effect of supplementation of Bacillus subtilis LS 1-2 to broiler diets on growth performance, nutrient retention, caecal microbiology and small intestinal morphology. *Research in Veterinary Science, 93*(1), 264–268.
Sohail, M. U., Hume, M. E., Byrd, J. A., Nisbet, D. J., Shabbir, M. Z., Ijaz, A., et al. (2015). Molecular analysis of the caecal and tracheal microbiome of heat-stressed broilers supplemented with prebiotic and probiotic. *Avian Pathology, 44*(2), 67–74.
Suiryanrayna, M. V., & Ramana, J. (2015). A review of the effects of dietary organic acids fed to swine. *Journal of Animal Science and Biotechnology, 6*(1), 1–11.
Sumanu, V. O., Aluwong, T., Ayo, J. O., & Ogbuagu, N. E. (2021). Effect of probiotic and fisetin supplementation on performance, carcass characteristics and small intestinal morphology in broiler chickens. *Open Veterinary Science, 2*(1), 23–32.
Tayeri, V., Seidavi, A., Asadpour, L., & Phillips, C. J. (2018). A comparison of the effects of antibiotics, probiotics, synbiotics and prebiotics on the performance and carcass characteristics of broilers. *Veterinary Research Communications, 42*, 195–207.
Timothy, B., & de la Fuente, J. (2023). Exploring the potential of probiotics in boosting the immune system's response to reduce the severity of malaria. *Journal of Cellular Immunology, 5*(2), 36–40.
Vaarst, M., Steenfeldt, S., & Horsted, K. (2015). Sustainable development perspectives of poultry production. *World's Poultry Science Journal, 71*(4), 609–620.
Vimon, S., Angkanaporn, K., & Nuengjamnong, C. (2023). Microencapsulation of Lactobacillus plantarum MB001 and its probiotic effect on growth performance, cecal microbiome and gut integrity of broiler chickens in a tropical climate. *Animal Bioscience, 36*(8), 1252.
Voland, L., Le Roy, T., Debédat, J., & Clément, K. (2022). Gut microbiota and vitamin status in persons with obesity: A key interplay. *Obesity Reviews, 23*(2), e13377.
Wallace, M. W., Danko, M. E., Zamora, I. J., Morris, E. A., Li, J., Froehlich, M., & Robinson, J. R. (2023). Infectious complications and antibiotic use in gastroschisis. *Surgical Infections, 24*(5), 405–413.
Wong, J., de Bruyn, J., Bagnol, B., Grieve, H., Li, M., Pym, R., & Alders, R. (2017). Small-scale poultry and food security in resource-poor settings: A review. *Global Food Security, 15*, 43–52.
Wong-Chew, R. M., de Castro, Morelli, L., Perez, M., & Ozen, M. (2022). Gut immune homeostasis: The immunomodulatory role of Bacillus clausii, from basic to clinical evidence. *Expert Review of Clinical Immunology, 18*(7), 717–729.
Yaqoob, M. U., Wang, G., & Wang, M. (2022). An updated review on probiotics as an alternative of antibiotics in poultry—A review. *Animal Bioscience, 35*(8), 1109.
Zou, Q., Meng, W., Wang, T., Liu, X., & Li, D. (2022). Effect of multi-strain probiotics on the performance of AA+ male broilers. *Frontiers in Veterinary Science, 9*, Article 1098807.