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ARTICLE

Lactate dehydrogenase activities, performance indices, Immunity and Histopathological assessment of Broiler birds fed with varying concentrations of Taurine-supplemented diets

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Abstract
The study aimed to evaluate the effects of taurine supplementation on the performance, muscle lactate dehydrogenase (LDH) activities, immunity, and histological evaluation of broiler chicks. One hundred and seventy-five one-day-old un-sexed broiler birds were divided into five treatment groups with varying levels of taurine supplementation (0.00 % to 0.04 %) in their diets. The experiment lasted 49 days, during which the birds were vaccinated as required. Results showed that LDH activities in serum, breast, and thigh muscles did not significantly differ between the control group and birds supplemented with 0.01 % to 0.04 % taurine. Similarly, there were no significant differences in weight gain and feed conversion ratio between birds fed the control diet and those supplemented with 0.01 % taurine. However, carcass yield increased significantly in all treated groups compared to the control. Immunoglobulin A concentrations increased significantly at 0.04 % taurine supplementation, while Immunoglobulin G concentrations decreased at the same level of supplementation. No significant changes were observed in the pH of breast and thigh muscles, and histological assessment of organs showed no notable differences across groups. In conclusion, taurine supplementation had no adverse effects on LDH activities, growth performance, immunity, or organ histology in broiler birds. These findings suggest that taurine can be safely used as a dietary supplement in broiler production without compromising bird health or performance.

Keywords: Taurine, Lactate dehydrogenase, Growth performance, Immunity, Histopathology, Broiler birds

Introduction

The global demand for poultry meat is rising, and broiler production is essential to supplying it. Many methods have been used to improve the growth performance of broilers in order to increase productivity and profitability. Antibiotics and feed additives are examples of the growth-promoting additives used in these methods. Traditionally, feed has been prepared with the ideal amounts of protein, energy, vitamins, and minerals - all of which are crucial for optimizing growth potential. However, restrictions on their use have been placed in place due to concerns about antimicrobial resistance and food safety (Leeson and Summers, 2001). Therefore, there is increased interest in utilizing alternative feed ingredients in broiler diets due to concerns about sustainability and environmental effect. These consist of single-cell proteins, amino acids, plant-based proteins, and insect meal, which have the potential to serve as sustainable protein sources while lowering dependency on conventional feed components. (Adeola and Cowieson, 2011). In broiler production methods, intense selection for quick development and high stocking densities might jeopardize the health and welfare of the animals. Sustainable broiler production is severely hampered by problems such skeletal deformities, metabolic disorders, and disease susceptibility (Julian, 2005). The nutritional value of meat can also be improved and increased through the proper management of broiler feed (Barteczko and Lasek, 2008), where ssential and non-essential amino acid supplementation were reported to affect meat nutrient content (Lilly, 2010; Namroud et al., 2010; Zeweil et al., 2011). The chemically uncomplicated molecule 'taurine' has a significant impact on cells. This has led to the hypothesis that taurine is a nutrient that many mammals require on a semi-essential or essential basis (Schaffer et al, 2010).

Lactate dehydrogenase (LDH), a key enzyme in the anaerobic metabolic cycle, is indispensable. It is an oxidoreductase with enzyme commission number EC 1.1.1.27. The enzyme is in charge of catalyzing the reversible conversion of lactate to pyruvate in tandem with the reduction of NAD+ to NADH (Farhana and Lappin, 2023). An organism's lactate dehydrogenase activity and isozyme content are not constant values; they change during metabolism and as a result of a variety of influences. (Khan et al., 2020). Taurine, a free amino acid that contains sulphur and controls Ca2+ homeostasis and carbohydrate metabolism, is one of the elements that can affect their content. (Ostapiv et al., 2015 Taurine facilitates calcium-dependent excitation-contraction processes and reduces fatigue, increasing fat burning and decreasing muscle damage (Lawrence and Jamie, 2014).

Growth performance study reflects the feeding behaviour (feed consumption rate), feed utilization and efficacy (ratio of feed to weight gain and weight gain) of animal/human (Carco et al., 2018). Inclusion of sulphur-containing amino acids like taurine in broiler bird diets are of paramount importance to growth performance, immunity and carcass yield (Oguntoye et al., 2018). Taurine appears to be an essential nutrient for chicks due to effects on muscle improvement and its enhancement of immunity. Immunoglobulins (Ig) or antibodies are glycoproteins produced by plasma cells (Justiz et al., 2023). As a component of the adaptive immune response, antibodies originate mainly to mediate the immunological response against invading organism (Aziz et al., 2023).

The anti-apoptotic, antioxidant, and osmoregulatory effects of taurine are demonstrated by its inhibition of oxidative stress and inflammation, induction of the conjugation of xenobiotics with bile acids, stabilization of cell membranes, control of hepatic glutathione levels, and suppression of certain cytochrome isozymes. The information provided suggests that taurine could help avoid hepatotoxicity due to its special cytoprotective capabilities, protecting the organs' histopathology without affecting their structural integrity. (Nikkhah et al., 2021).

The effects of taurine supplementation on broiler growth and performance have been the subject of numerous studies. For instance, dietary taurine supplementation enhanced body weight increase, feed conversion ratio, and carcass features in broilers, according to Wu et al. (2017). In a similar vein, Zhao et al. (2019) showed that supplementing broilers exposed to heat stress conditions with taurine reduced oxidative stress and enhanced growth performance. According to these results, supplementing with taurine may be able to boost growth in broiler production systems. Taurine's diverse physiological effects and positive effects on growth performance and health make it a promising growth enhancer in broiler production. To maximize the growth-promoting advantages of taurine supplementation in broiler diets while maintaining animal welfare and environmental sustainability, more research is necessary to determine the ideal dosage, timing, and duration of this practice. The goal of this study was to assess how taurine supplementation affected the activities of lactate dehydrogenase, growth performance, immunity, and histopathological architecture of the liver and kidney in broiler chicks given varied concentrations of taurine-supplemented diets.

Materials and methods

Experimental Design and Birds Management

The birds' pens were washed and fumigated before the arrival of the birds. The wooden pens have adequate lighting and ventilation to allow for free airflow, hence preventing the likelihood of respiratory infections. All established norms and regulations pertaining to ethics and animal experimentation in research were adhered to. Fountain University Osogbo's ethical review board granted ethical permission with approval number, FUCRIT/BCH/2023/001.

For the experiment, 175 un-sexed one-day-old broiler birds purchased from Zartech hatchery, Ibadan were utilized with five feeding treatments, five replicates, and seven birds per replication. Treatment 1 (T1- 0.00 % Taurine supplementation) was used as the control while treatments two to five (T2- T5) represented graded level taurine supplementation at 0.01 %, 0.02 %, 0.03 % and 0.04 % respectively in broiler diets ration. The experiment ran for forty-nine (49) days and the birds were fed ad-libitum. The birds were thereafter slaughtered and samples were collected and preserved for analysis.

Lactate dehydrogenase (LDH) activities

The procedure used for Lactate dehydrogenase activities measurement was described by Weisshaar et al. (1975) where the technique measures the simultaneous reduction of nicotinamide adenine dinucleotide (NAD) and oxidation of L-lactate to pyruvate. Since other reactants are present in non-rate limiting quantities, the change in absorbance at 340 nm caused by the presence of reduced NAD (NADH) is directly proportional to the LDH activity and is evaluated using a bichromatic (340 nm) rate approach.

1 ml of the reconstituted reagent (comprising 3 ml of Buffer with 1 vial of NADH; Rla and Rlb) was added to 0.2 ml of sample. The blank was constituted by replacing the sample with distilled water. The mixture was mixed properly and initial absorbance

was read at 340 nm after 0.5 min and at 1, 2 and 3 min. Activity of LDH was calculated as follows:
LDH activity (U/L) = 8095×ΔA340 nm/min.

Performance indices

Feed conversion rate was calculated as the total feeds consumed in gram per bird per day divided the weight gain also in in gram per bird per day (Carco et al., 2018). According to Oladimeji et al., 2020, carcass yield is the percentage of the carcass that actually ends up as meats. Excessive fat in animals tend to have a lower cutting yields because fat is removed and discarded and the procedure followed was in accordance to this.

The formulas are as follows:

Weight gain = Final weight – Initial weight.

Feed conversion ratio = Feed consumed / Weight gain

Carcass cutting yield = Pound of meat / Carcass weight ×100

Organ to body weight ratio = weight of organ / weight of animal

Muscle potential hydrogen (pH) was determined using a portable pH meter at 45 min postmortem as described by Zuber et al., (2021). Through the help of standard buffer solution, the pH meter was calibrated. The pH level was expressed as the average of the 2 measurements.

Immunoglobulin evaluation

The IgA and IgG test kits (Euroimmun kits orduct), a highly sensitive enzyme linked immunoassay (ELISA) kits was used to measure IgA and IgG in biological samples of broiler birds. The procedure of the kits manuals was followed.

Histopathological assessment

This was done using a slightly modified version of the Ibrahim et al. (2018) technique. Formalin was used to fix the samples for histological examination. The liver and kidney fixed specimens were treated overnight in a tissue processor for dehydration, cleaning, and impregnation. Serial sections of 4mm thickness were cut from the specimens using a microtome after they had been fixed in paraffin blocks using an embedding station. Hematoxylin and eosin staining of the sections was performed using an autostainer. Light microscopy was used to examine the mounted specimens.

Data Analysis

Graph pad Prism 6.0, a statistical software for biomedical sciences, was used to do statistical analysis on the data from the assays. The data were analysed by One way ANOVA and were presented as an average ± standard error of mean (SEM) of each group data. And a P value of < 0.05 was considered statistically significantly different.

Results

Effects of taurine supplemented diets on lactate dehydrogenase activities in muscle and serum of broiler birds

The effects of taurine supplementation on the activities of lactate dehydrogenase in broiler chicken thighs, breasts, and serum are as shown in figures 1, 2 and 3 respectively. When comparing the serum lactate dehydrogenase activity of birds across various taurine (TAU) supplemented diets with those on the control diet (0.00% TAU concentration), no statistically significant changes were observed (p>0.05). The thigh lactate dehydrogenase activities of the birds on the control diets did not differ significantly (p > 0.05) from those of the birds on the 0.03 and 0.04% taurine-supplemented diets, but they did differ significantly (p<0.05) from those of the birds on the 0.01 and 0.02% taurine-supplemented diets. The enzymatic activities of the birds breast lactate dehydrogenase differed substantially (p < 0.05), with the 0.02% diet having the most significant (p < 0.05) and the control diet having the least significant (p < 0.05) enzymatic activities

Performance indices of broiler birds on taurine supplemented diets

The pH concentrations of broiler birds' thigh and breast muscle, when subjected to varying TAU supplemented diets (ranging from 0.01% to 0.04%), exhibited no significant differences (p>0.05) compared to those of birds on the control diet (0.00%), as illustrated in figures 4 and 5, respectively.Additionally, there were no significant (p > 0.05) variations in the Feed Conversion Ratio (FCR) between broiler chickens fed diets supplemented with 0.01% taurine and those fed the control diet (0.00%). On the other hand, broiler chickens fed diets supplemented with 0.02 to 0.04% taurine showed significantly (p < 0.05) different FCRs (Figure 6).

The final weight increase (gram/bird/day) of the birds fed the 0.01 and 0.03% TAU-supplemented meals was not significantly (p>0.05) different from that of the birds on the control diet. The aforementioned were significantly different from the final weight gain (gram/bird/day) of the birds on the 0.02 and 0.04% TAU-supplemented diets (Figure 7).

Comparing the breast carcass yield of the birds fed the 0.01 and 0.02% taurine-supplemented diets to the birds fed the control diets, no differences were found that were statistically (p>0.05) significant. The aforementioned were noticeably (p < 0.05) higher than the breast yield of chickens fed diets supplemented with taurine at levels of 0.03 and 0.04%.

In the same vein, while the thigh carcass yield of the birds on the 0.02 to 0.04 % TAU-supplemented diets was significantly (p<0.05) higher than that of the birds on the 0.01% TAU-supplemented diet, there were no significant (p>0.05) differences between the thigh carcass yield of the birds on the control and those of the birds on the 0.02 to 0.04 % TAU supplemented diets.

The wing carcass yield of the birds fed the 0.01 to 003% TAU-supplemented meals did not differ statistically (p>0.05) from that of the birds fed the control diets, but it was all substantially (p<0.05) greater than that of the birds fed the 0.04% TAU-supplemented diets.

The carcass drumstick yield was statistically (p<005) substantially higher in the birds fed the various TAU-supplemented diets than it was in the birds fed the control diets. The organ to body ratios of the birds fed the 0.01 and 0.04%TAU supplemented meals did not differ significantly (p>0.05) from those of the birds fed the control diet. These were noticeably different (p<0.05) from those of the birds on the 0.02 and 0.03% TAU supplemented meals.

Immunoglobulin A and G concentrations of broiler chickens fed diets containing taurine

Birds placed on 0.04% TAU-supplemented diet possessed a marked significant increment (p<0.05) in serum immunoglobulin A concentration when compared with other TAU-supplemented diets, control inclusive (Figure 15).

Birds placed on 0.04% TAU-supplemented diet possessed a marked significant reduction (p<0.05) in immunoglobulin G concentration when compared with other TAU-supplemented diets, control inclusive (Figure 16).

Histopathological assessment of the tissues of broiler birds fed varying percentages of taurine-supplemented diet

When compared with the same organs from birds on the control diet, no significant histologic differences (p>0.05) were found in the histological evaluation of the liver and kidney samples (Plates 1 and 2) of birds on various TAU-supplemented diets. The liver tissues were with preserved structure, comprising of

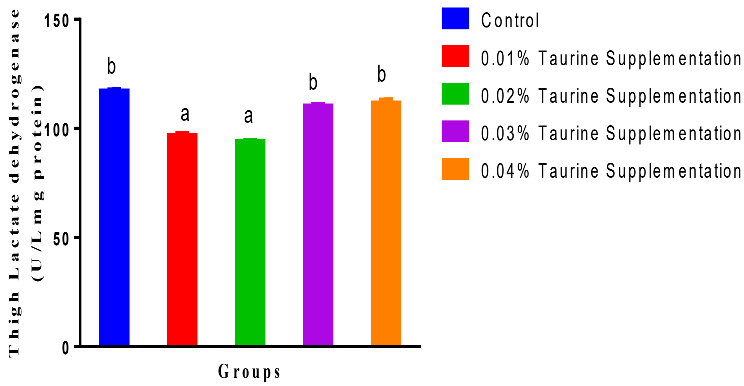

Figure 1: Activities of lactate dehydrogenase (U/L) in the thigh muscles of broiler chicks fed diets supplemented with various amounts of taurine; *[ab] are means with various superscripts across the group which differ greatly. P< 0.05. SEM: Standard Error of Mean.*

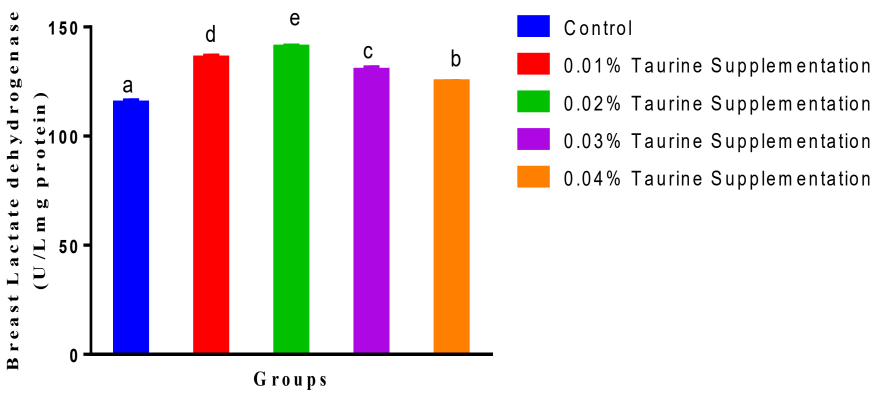

Figure 2: Activities of lactate dehydrogenase (U/L) in the breast muscle of broiler chicks fed diets supplemented with various amounts of taurine. *[abcde] are means with various superscripts across the group which differ greatly. P< 0.05. SEM: Standard Error of Mean.*

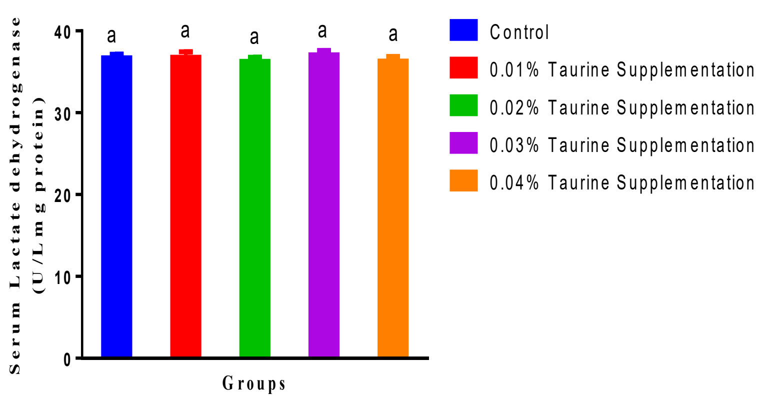

Figure 3: Serum lactate dehydrogenase activity (U/L) in broiler chicks fed various taurine supplementation levels. [a] is a mean with identical superscript across the group that are not that different at all. P≤ 0.05. SEM: Standard Error of Mean.

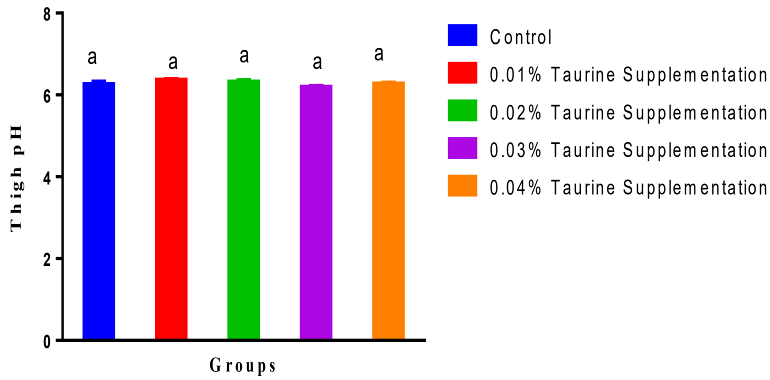

Figure 4: Potential of hydrogen (pH) concentration of broiler birds' thigh muscle fed varying concentrations of taurine supplemented diets.
[a] *is a mean with identical superscript across the group which do not differ significantly. P> 0.05. SEM: Standard Error of Mean.*

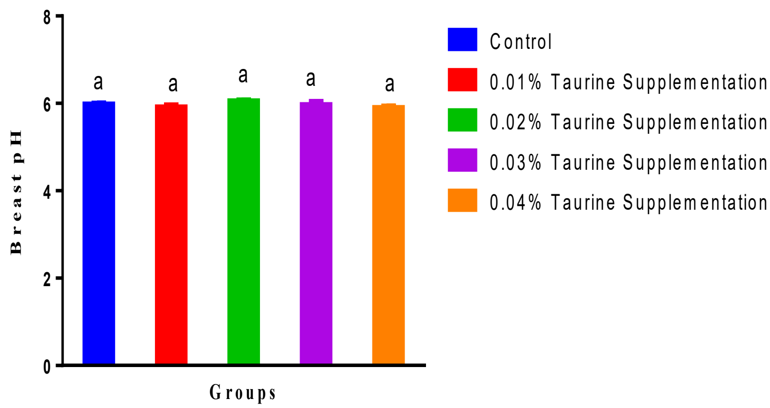

Figure 5: Potential of hydrogen (pH) concentration of broiler birds' breast muscle fed varying concentrations of taurine supplemented diets. [a] *is a mean with same superscripts across the group that are not significantly different.* (p>0.05)*. SEM: Standard Error of Mean.*

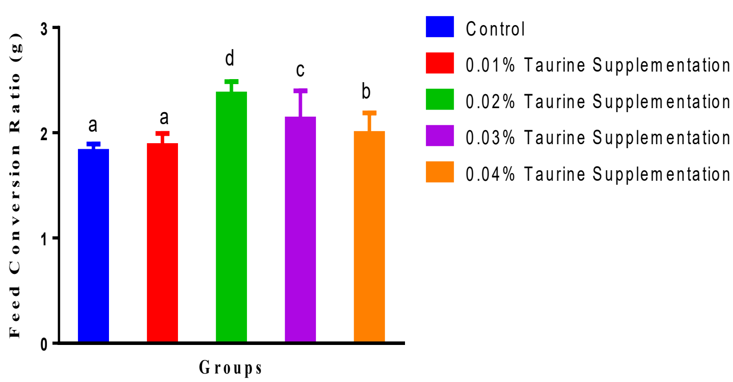

Figure 6: Performance characteristics of broiler chicks given various doses of taurine-supplemented diets (feed conversion ratio -%).
[abcd] *are means with varying superscripts across the group which differ significantly* (p<005).

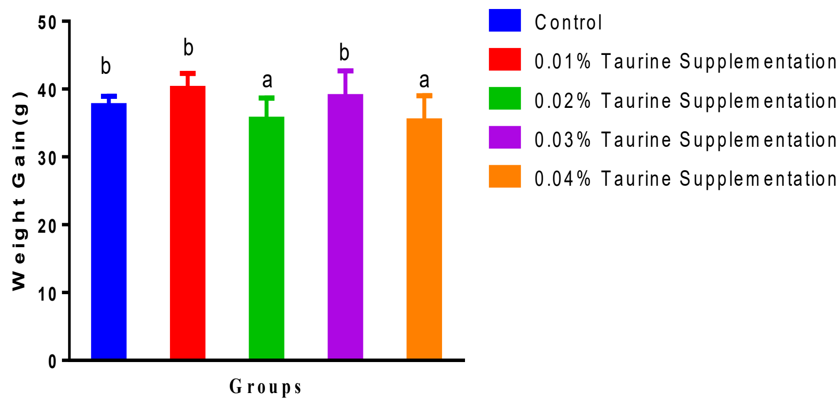

Figure 7: Performance characteristics of broiler chicks fed varied amounts of taurine-supplemented diets (final weight gain - g); [ab] *are means with different superscripts across the group that are significantly different* (p<005).

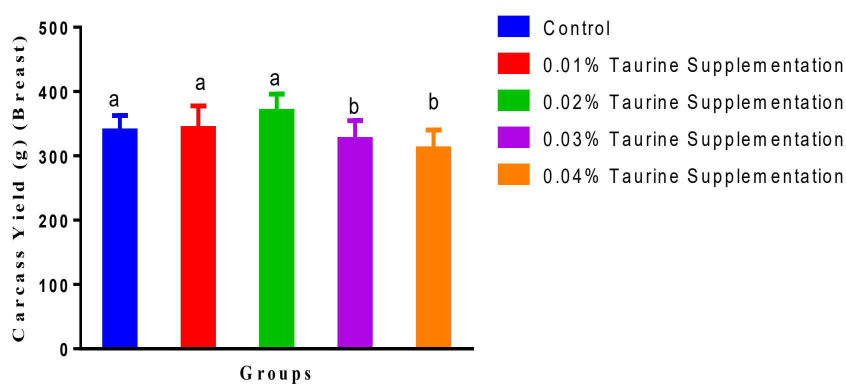

Figure 8: Carcass characteristics - breast (g) as an index of performance study in broiler chicks fed various taurine supplementation levels. [ab] *are means with distinct superscripts across the group that are significantly different. (P< 0.05). SEM: Standard Error of Mean.*

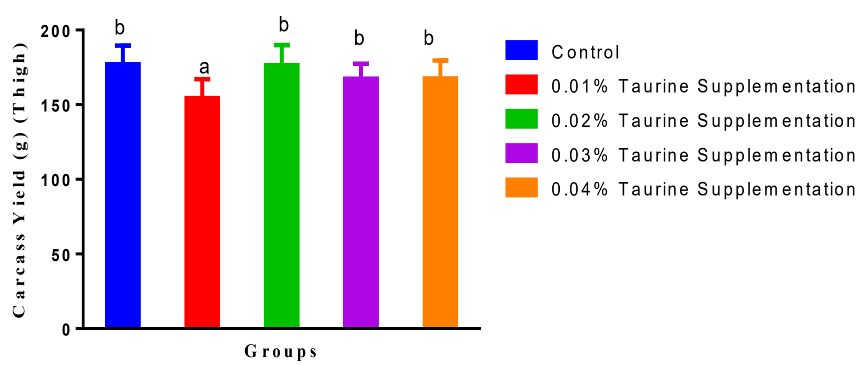

Figure 9: Carcass characteristics - thigh (g) as an index of performance study in broiler chicks fed various taurine supplementation levels.
[ab] *are means with distinct superscripts across the group that are significantly different. (P< 0.05). SEM: Standard Error of Mean.*

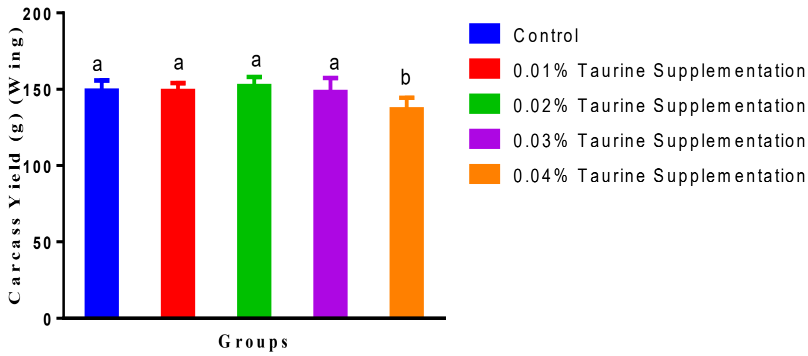

Figure 10: Carcass characteristics -wing (g) as an index of performance study in broiler chicks fed various taurine supplementation levels.
ab are means with distinct superscripts across the group which differ significantly (P< 0.05). SEM: Standard Error of Mean.

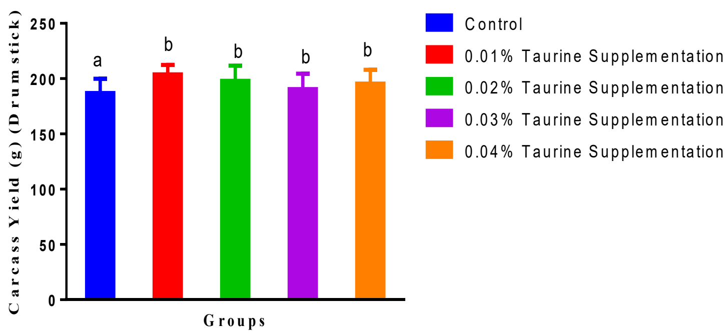

Figure 11: Carcass characteristics - drumstick (g) as an index of performance study in broiler chicks fed various taurine supplementation levels.
ab are means with distinct superscripts across the group which differ significantly. (P< 0.05). SEM: Standard Error of Mean.

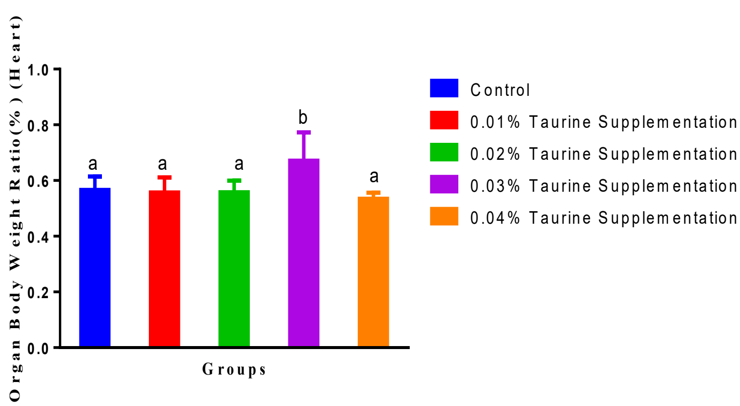

Figure 12: Ratio of organ to body weight - heart (%) of broiler birds fed various doses of taurine-supplemented meals.
ab are means with different superscripts across the group that are significantly different. (P< 0.05). SEM: Standard Error of Mean.

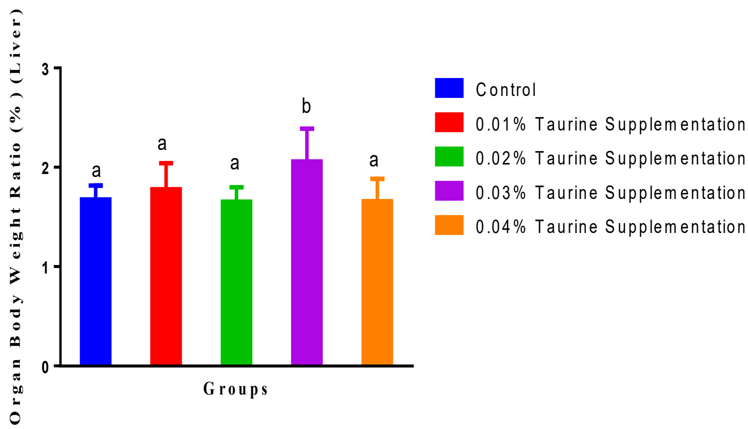

Figure 13: Ratio of organ to body weight - liver (%) of broiler birds fed various doses of taurine-supplemented meals. ab are means with distinct superscripts across the group which differ greatly. (P< 0.05). SEM: Standard Error of Mean.

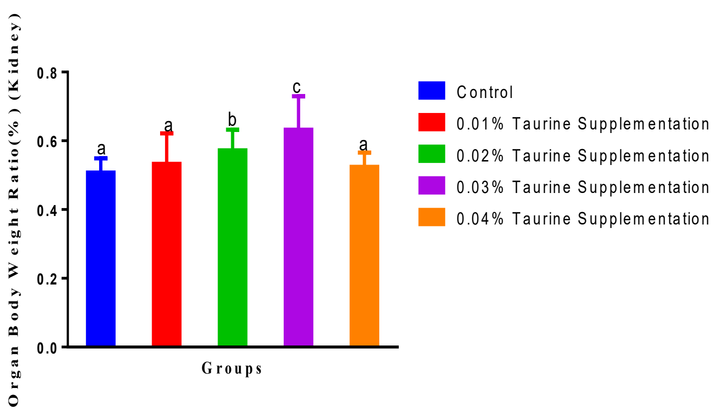

Figure 14: Ratio of organ to body weight - kidney (%) of broiler birds fed various doses of taurine-supplemented meals. abc are means with distinct superscripts across the group which differ greatly. (P< 0.05). SEM: Standard Error of Mean.

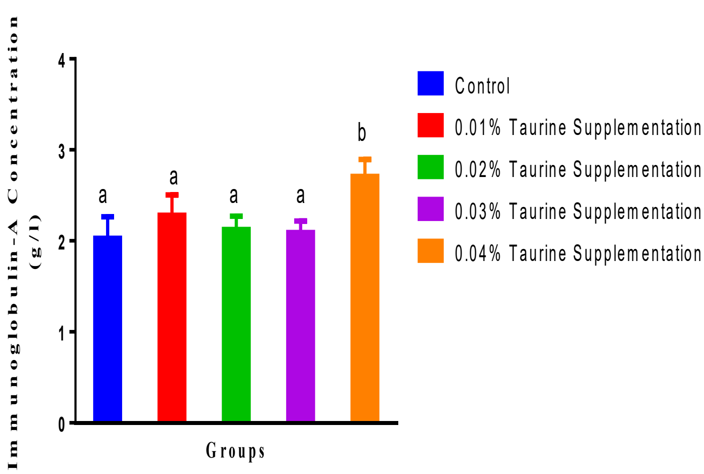

Figure 15: Immunoglobulin-A concentration (g/l) of broiler birds fed varying percentages of taurine supplemented diet. ab are means with distinct superscripts across the group which differ significantly (P< 0.05). SEM: Standard Error of Mean.

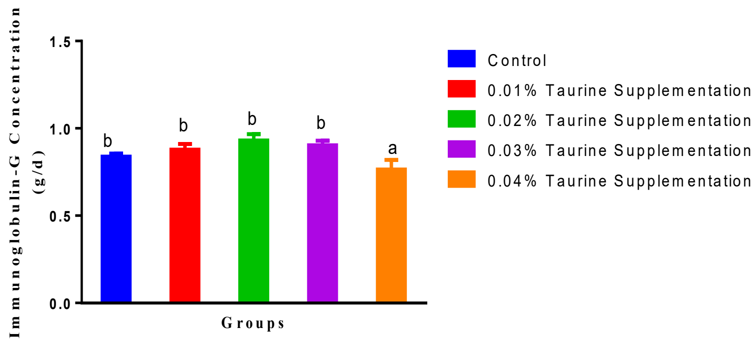

Figure 16: Immunoglobulin-G concentration (g/l) of broiler birds fed varying percentages of taurine supplemented diets. ab are means with distinct superscripts across the group which differ significantly (P< 0.05). SEM: Standard Error of Mean.

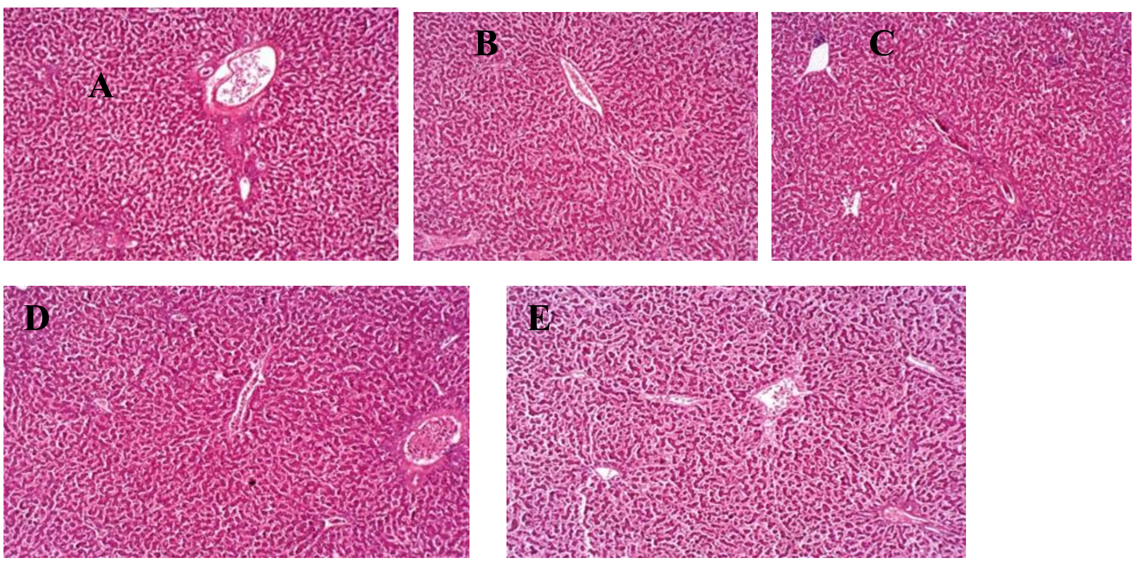

Plate 1: (Liver section (A – 0.00%, B – 0.01%, C – 0.02%, D – 0.03%, E – 0.04% TAU supplementation)).

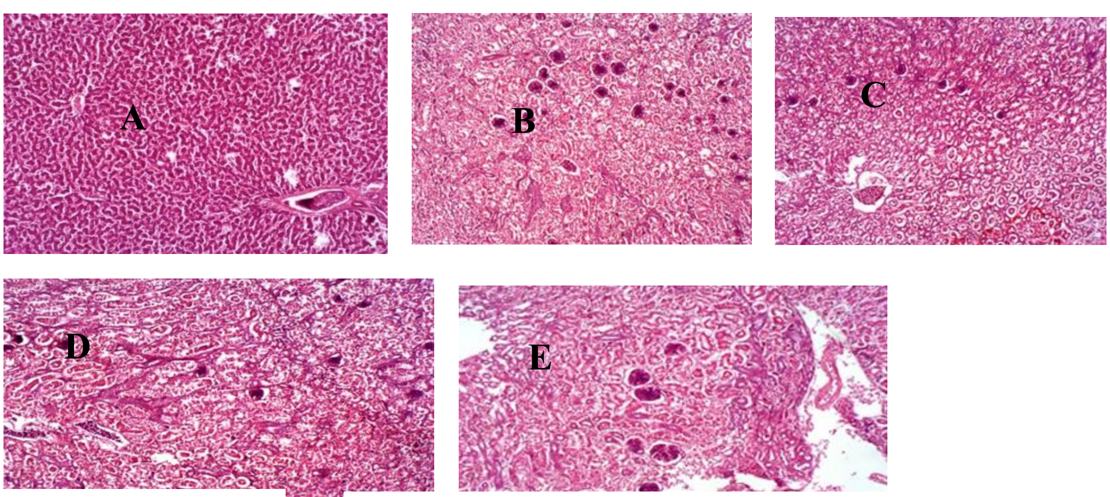

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Plate 2: (Kidney section (A – 0.00%, B – 0.01%, C – 0.02%, D – 0.03%, E – 0.04% TAU supplementati

anastomosing cords of hepatocytes with normal hepatic vessels. The kidney section across board equally showed renal tissue composed of normal glomeruli, proximal and distal tubules with no features of acute or chronic damage.

Discussion

Taurine, a non-proteinogenic amino acid, has garnered attention for its potential beneficial effects on animal health and performance. Studies have suggested its involvement in various physiological processes, including antioxidation, osmoregulation, and modulation of immune function.

According to Vijayakumar et al. (2016), lactate dehydrogenase is a tetrameric enzyme that is present in a variety of tissues and organs, including red blood cells, muscle, liver, heart, and kidneys. Catalyzing the interconversion of pyruvate and lactate, it is an essential enzyme in anaerobic metabolism. Particularly in situations involving oxygen deprivation or vigorous physical exercise, LDH is essential for preserving cellular energy metabolism (Saxena et al., 2004). Moderate level of lactate dehydrogenase activities in this study might indicate normal glucose metabolism and formation of pyruvate. This contradicts the findings of Ostapiv et al. (2015), who found that taurine increases lactate dehydrogenase activity in the blood, liver, skeletal muscle, brain, and testes of rats. This might probably be due to the differences in the route and duration of the administration.

Taurine has been reported to improve performance of poultry. Taurine had earlier been used as a feed supplement to confer better growth and health of common carp fry (Abdel-Tawwab and Monier, 2018). Taurine improved the growth performance of broilers, which mainly benefited from its contribution to stimulating the appetite and increasing palatability cum utilization of the feed (Koven et al., 2016). The statistically ($p<0.01$) similar weight gain obtained for birds placed on 0.01 and 0.03% as compared with the control is in tandem with the finding of Ogunbode et al. (2020) which reported that taurine supplementation at 0.002 to 0.008% improves growth performance of broiler birds. It also corresponds with the results of Abdul Hafeez et al. (2021), who found that taurine supplemented birds performed better in terms of growth performance during heat stress regardless of the inclusion levels. However, it contradicts the reports of Yuan and Wang (2010) which reported that taurine supplementation had no significant effect on the growth performance of broiler birds. The variances in the research findings could be attributed to the amount of taurine present in the varied diets, the makeup of those diets, or possibly environmental factors.

The sulfur-containing amino acid taurine has a variety of physiological uses, one of which is growth promotion. The exact processes underlying its growth-promoting qualities remain unclear despite the publication of several ideas. One explanation is that taurine may enhance the release of growth hormone, which promotes development and growth. Moreover, taurine may facilitate the absorption and utilization of nutrients, leading to improved growth in general. Additionally, taurine possesses antioxidant properties that may protect cells from damage and encourage healthy cell division. Considering everything, taurine appears to be critical for development and growth, yet further study is needed to fully comprehend the mechanisms.

Immunoglobulin A (IgA), a type of antibodies that protects the mucous membranes of the respiratory, genitourinary, and digestive tracts, is crucial for mucosal immunity. It is essential for protecting against infections at these entry sites since it is the immunoglobulin that is most prevalent in mucosal secretions (American Academy of Allergy, Asthma and Immunology 2019). The most prevalent class of antibodies in the bloodstream and a vital part of the immune system is immunoglobulin G, or IgG. The initial and secondary immune responses to infections depend on IgG. It aids in phagocytosis, neutralizes poisons, and confers protection against a range of pathogenic agents (Kuby et al., 2007). Immunity is conferred by taurine as it maintained concentrations of immunoglobulin (antibodies) A and G except at high amount of taurine as observed in 0.04% taurine supplementation for IgA. According to a paper by Ogunbode et al. (2021), taurine has immune-related properties since it increases and usually improves antibody titres against several viral infections (such as Newcastle disease and infectious bursal disease). Since IgA is mostly present in mucosal secretions including saliva, tears, and fluids from the gastrointestinal and respiratory system, elevated levels of IgA frequently signify mucosal immune activation (Brandtzaeg, 2013). The mucosal surfaces' inflammatory states, allergies, or infections may be the cause of this rise in IgA production. For instance, because of the ongoing inflammation in the gut mucosa, higher levels of IgA are frequently seen in gastrointestinal illnesses such as inflammatory bowel disease (IBD) (Macpherson et al., 2018).

Conversely, increased IgG levels usually signify systemic immunological activation and might be a sign of chronic inflammatory disorders, autoimmune diseases, or recent or continuing infections. The most prevalent antibody in the bloodstream, IgG, neutralizes infections and aids in immune cells' removal from the body (Ackerman et al., 2018). Because autoantibodies against self-antigens are present in autoimmune illnesses such rheumatoid arthritis and systemic lupus erythematosus, elevated levels of IgG are frequently seen (Liu et al., 2021).

Liver and kidney have designated structures, and the study of the microscopic organization and architecture of these essential organs is known as liver and kidney histology which provides insight into their functions and associated illnesses (Junqueira et al., 2018). Taurine supplementation posed no negative effect on the architecture of the liver and kidney, it enhances the cellular structure of the studied organs. This is consistent with the review of Chesney et al. (2010), which observed that taurine protects the renal tissues as well as the experimental liver's tissues from injury. Abdul Hafeez et al. (2021) also reported an enhanced histological features of broiler birds fed varying taurine supplemented diets after being subjected to heat stress. Guroy et al. (2022) equally reported a non-negative pathological assessment of fish organs when fed taurine as a fish feed supplement.

Limitations of the study

Unavailability of equipment like colorimeter to analyze the quality of the meat as an index of performance study to ascertain the improvement of overall health of birds which indirectly will impact human health.

Conclusion

The findings suggest that taurine is a safe supplement for broiler birds' diets for improved growth and quality of meat for consumers in terms of feed conversion rate, carcass yield, organ to body weight ratio, final weight gain and degree of acidity/alkalinity (pH). It equally maintains immunity as regards immunoglobulin A and G concentrations, as well as having no negative effects on histopathological assessment of the organs of broiler birds. Hence, safe for human consumption.

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Conflicts of interest

Authors declared no conflicts of interest

AUTHORS' CONTRIBUTION
Conception: (OSM)
Design: (OSM, ORO)
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Interpretation: (OSM, MHO)
Writing of manuscript: (OSM, ORO)
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