iJRASET
International Journal For Research in Applied Science and Engineering Technology

# INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

**Volume:** 14 **Issue:** V **Month of publication:** May 2026

**DOI:** https://doi.org/10.22214/ijraset.2026.81801

www.ijraset.com

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
*ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538*
*Volume 14 Issue V May 2026- Available at www.ijraset.com*

# Impact of Ethanol Consumption on Protein Metabolism and Hyper-prolinemia in Chicken

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***Abstract:*** ***This study investigates the impact of ethanol consumption on protein metabolism and the development of hyperprolinemia in commercial broiler chicks. Utilizing a 21-day experimental protocol, chicks were subjected to varying concentrations of oral ethanol (2.5% and 5% v/v). The research evaluates changes in growth parameters, serum protein profiles, and liver enzyme activities. Results indicated a dose-dependent reduction in body weight and significant alterations in serum total protein, albumin, and globulin levels. Furthermore, ethanol administration triggered hyperprolinemia, characterized by elevated free proline levels in the serum and liver, likely due to the suppression of proline-degrading enzymes. These findings highlight the metabolic risks posed by alcohol as a disruptor of avian nutrient homeostasis and growth efficiency.***
***Keywords: Hyperprolinemia, Ethanol, Protein Metabolism, Broiler Chickens, Liver Enzymes***

## I. INTRODUCTION

### *A. Summary Of Avian Metabolism And Economic Importance*

The poultry sector is a key component of worldwide agriculture, serving as a significant source of quality protein via meat and eggs. As emphasized by Naqi et al. (2011) and Qaid & Al-Garadi (2021), the productivity of this industry significantly depends on the metabolic well-being of the birds. Contemporary broiler breeds have been genetically optimized for swift growth and elevated feed conversion ratios (FCR). Nonetheless, this metabolic activity renders them overly sensitive to nutritional discrepancies, environmental pressures, and chemical harm. The liver, serving as the main center for nutrient processing and detoxification, is crucial in sustaining the homeostatic equilibrium necessary for such swift growth.

### *B. The Significance Of Dietary Protein And Amino Acids Protein Metabolism*

It is fundamental to growth in vertebrates. In bird species, the need for certain amino acids is stricter compared to many mammals. Amino acids function as the fundamental units for muscle tissue and also act as precursors for bioactive compounds, immune components, and enzymes. Proline is a crucial amino acid in avian nutrition that is frequently neglected. In contrast to certain other vertebrates, chicks need a significant amount of proline for ideal growth and feed efficiency (Austic, 1973). Proline metabolism is distinct as it is tightly associated with the metabolism of arginine and glutamate, creating an intricate regulatory system that sustains nitrogen equilibrium. When this equilibrium is disturbed—whether by nutritional lack or chemical disruption—the physiological effects can be widespread.

### *C. Ethanol As A Disruptor Of Metabolism*

Ethanol, commonly examined concerning human health and mammalian models, shows significant effects on bird physiology when present in the avian environment—via fermented feed or experimental delivery. Munir et al. (2021) and Naqi et al. (2011) showed that administering ethanol to broilers results in notable alterations in live body weight, organ weights, and efficiency. The liver serves as the main location for ethanol oxidation, a process that produces reactive oxygen species (ROS) and modifies the cellular redox state (the NAD+/NADH ratio). In mammals, long-term ethanol intake is associated with fatty liver (steatosis) and protein buildup. As investigated by Baraona et al. (1977) and Porta et al. (1968), ethanol-related hepatomegaly is not solely due to fat accumulation but is largely influenced by the buildup of export proteins (such as albumin and transferrin) that the liver can no longer efficiently release into the bloodstream. This implies a "blockage" of the liver's secretion system.

*D. Development Of Hyper-Prolinemia*

A notable metabolic disorder linked to alcohol intake is Hyperprolinemia—characterized by increased proline concentrations in the bloodstream. This condition often indicates impaired "proline-cycle" enzymes. Pradhan's (1970) study on alcoholic hyperprolinemia in vertebrates indicates that ethanol disrupts the enzymatic degradation of proline. In particular, the enzymes Proline Dehydrogenase (PRODH) (alternatively called Proline Oxidase) and P5C dehydrogenase serve as essential regulators. When ethanol inhibits these enzymes, proline builds up, resulting in:

Oxidative Stress: According to Ferreira et al. (2012), inducing hyperprolinemia experimentally leads to slight oxidative stress and metabolic alterations in the liver.

Apoptotic Signaling: Cell survival is associated with proline metabolism; disturbances in this area can activate programmed cell death, as demonstrated in research on PRODH/POX and metformin-induced apoptosis (Oscilowska et al., 2022).

*E. Comparative analysis: mammal vs. Bird models:*

A significant portion of our knowledge regarding alcohol-related protein accumulation originates from rat models. Mustafa et al. (2024) and Porta et al. (1968) demonstrated that dietary protein amounts can influence the harmful impacts of alcohol. Consuming a high protein diet may provide a protective benefit against liver damage caused by alcohol. Nevertheless, using these mammalian results for chickens necessitates careful consideration since the avian liver varies in its ability to synthesize fatty acids and excrete nitrogen. This project seeks to fill the gap in current literature regarding how ethanol affects the specific proline-arginine-glutamate pathway in chicks.

## II. MATERIAL AND METHODS

*A. Experimental Animals And Husbandry*

The study utilized day-old commercial broiler chicks procured from a local hatchery.The chicks were housed in an environmentally controlled facility with standard management protocols for temperature, humidity, ventilation, and lighting.The birds were provided ad libitum access to water and a standard basal diet formulated to meet National Research Council (NRC) nutrient requirements, completely free of antibiotics or growth promoters. Maintaining a balanced baseline diet is essential for accurately evaluating protein and amino acid metabolism prior to introducing environmental or chemical stressors (Qaid & Al-Garadi, 2021).All procedures and experimental protocols strictly adhered to institutional guidelines for the ethical care and use of laboratory animals.

*B. Experimental Design And Treatment Protocol*

After a preliminary brooding phase, 30 chicks were weighed individually with a digital scale to determine baseline measurements and randomly assigned to three separate experimental groups.

The experimental groups were established in this manner: Group I (Control) was given the standard diet and tap water; Group II (Low Ethanol) was administered the standard diet along with a 2.5% (v/v) ethanol solution; and Group III (High Ethanol) was provided the standard diet plus a 5% (v/v) ethanol solution. To properly simulate consumption and assess the negative toxicological impacts of alcohol on overall health, body mass, and organ performance, ethanol was given through the crop route (oral gavage) (Munir et al., 2021; Mustafa et al., 2024; Naqi et al., 2011; Oyedeji et al., 2013). This technique enables accurate dosage regulation, mimicking voluntary alcohol intake models (Eriksson, 1968). Treatments were given daily from day 1 to day 21, using freshly prepared ethanol solutions every 24 hours to ensure precise concentrations. The final individual body weights were noted on the 21st day


Fig:1 Photographic representation of the in vivo chicken model and Experimental setup

*C. Sample Collection And Preparation*

On day 21, the birds were subjected to an overnight fast and subsequently humanely euthanized.Blood samples were collected via jugular vein puncture into plain test tubes and centrifuged to isolate the serum for biochemical profiling.Simultaneously, liver and breast muscle tissues were excised, rinsed with ice-cold saline, blotted dry, and preserved at -4°C or -20°C. Breast muscle collection allows for the potential observation of systemic protein depletion or ethanol-induced skeletal muscle atrophy (Wen et al., 2022).

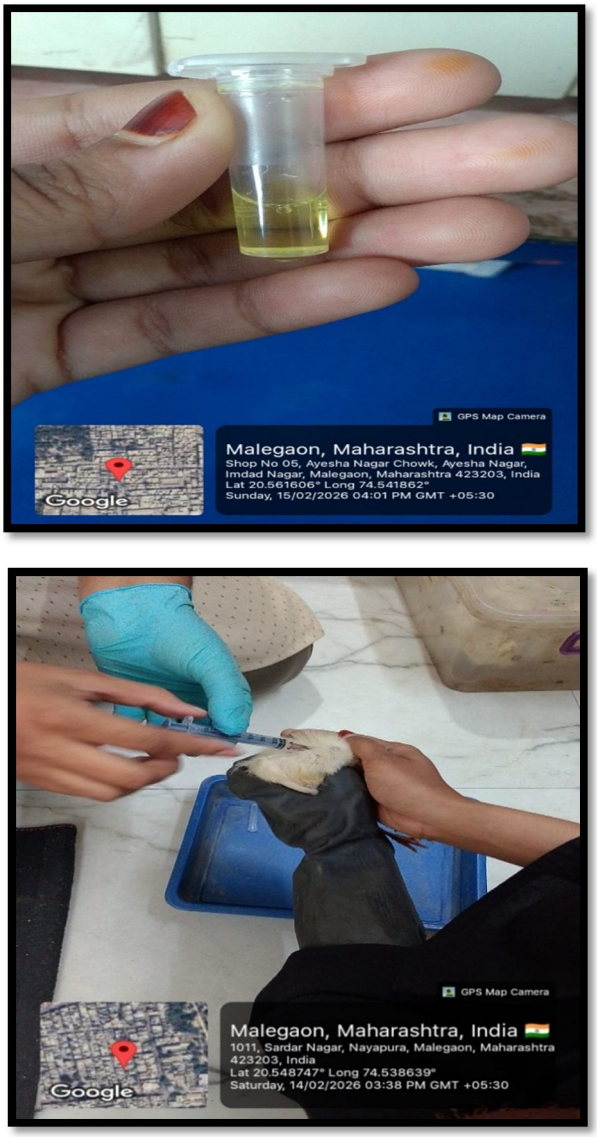



Fig:2 Blood samples collection via jugular vein puncture into plain tubes and centrifuged to isolate serum.

*D. Biochemical Analysis*

*1) Serum Protein, Albumin, And Globulin*

TOTAL SERUM PROTEIN: (Total serum protein levels were measured using the conventional colorimetric Biuret technique (Raghuramulu et al., 1983; Rodkey, 1964).)

*2) Necessary Reagents*

Biuret Reagent: Dissolve 3 g of copper sulfate (CuSO4.5H2O) and 9 g of sodium potassium tartrate in 500 ml of 0.2 mol/liter sodium hydroxide; add 5 g of potassium iodide and dilute to 1 liter with 0.2 mol/liter sodium hydroxide.

2.Standard Protein: 100 µg BSA per ml.

*3) Procedure*

1. Label Tubes: Designate as Blank (B), Standard (S), and Test (T).
2. Pipette:

o Blank: Combine 3.0 mL of Biuret reagent with 0.1 mL of Normal Saline.

o Standard: Combine 3.0 mL of Biuret reagent with 0.1 mL of Standard Solution (6-8 g/dL).

o Test: three categories (Control, 2.5% ethanol, 5% ethanol) with each category including 1-10 test samples, then incorporate 3.0 mL of Biuret reagent plus 0.1 mL of Serum/Plasma.

3. Combine & Incubate: Thoroughly blend and let sit at room temperature for 10–30 minutes.
4. Measurement: Determine the absorbance at 620 nm in comparison to the blank.

*4) Serum Albumin*

Serum Albumin: (The concentration of albumin was measured using the Bromo Cresol Green (BCG) dye-binding technique (Doumas et al., 1971).)

- It was estimated using the Bromo Cresol Green (BCG) reagent method alongside an albumin standard (100 µg/ml), measuring absorbance at 620 nm.

*5) Reagents*

- Bromo Cresol Green reagent
- Albumin standard 100 µg /ml

*6) Procedure*

Take test tubes and mark them as Blank(B), Standard(S), Test 1-10 of each (control, 2.5% ethanol,5%ethanol). Then proceed as follows:

| Sr. No | Reagent | B | S | T |
|---|---|---|---|---|
| 1 | BCG reagent | 1ml | 1ml | 1ml |
| 2 | distilled water | 10µg | 10µg | 10µg |
| 3 | standard glucose | - | 10µg | - |
| 4 | sample | - | - | 10µg |

Mix the contents of the test tubes well and wait for 10 minutes. Read absorbance at 620nm in the colorimeter.

*a)* SERUM GLOBULIN: Calculated by subtracting serum albumin from total serum protein: *Globulin (µg/ml) = Total Protein (µg/ml) − Albumin (µg/ml).*


Fig:3 Biuret method and bromocresol green reagent

*b)* LIVER FUNCTION AND METABOLIC MARKERS:

The activities of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP), along with serum levels of urea and uric acid, were assessed according to standard protocols at an accredited external laboratory.

*c)* ESTIMATION OF PROLINE AND HYPERPROLINEMIA:

To assess the precise effect of ethanol on the proline cycle and the emergence of hyperprolinemia, which is associated with enzymatic inhibitions of Proline Dehydrogenase/Proline Oxidase (PRODH/POX) (Oscilowska et al., 2022), free proline concentrations were measured in liver tissues and serum. This examination is essential, as disruptions in avian proline metabolism may result in significant oxidative stress and changes in tissues (Austic, 1973; Ferreira et al., 2012; Savio et al., 2012).

- 1.Tissue specimens (100 mg) were homogenized in 6% trichloroacetic acid (TCA) and centrifuged at 3000 rpm for 10 minutes to yield a clear supernatant.
- Employing the ninhydrin-based spectrophotometric technique for enzymatic research (Pradhan, 1970), a 2 mL sample of the extract was combined with 2 mL of ninhydrin reagent and 2 mL of glacial acetic acid.
- The solution was heated in a boiling water bath for 1 hour, quickly cooled in an ice bath to stop the reaction, and the chromophore was extracted with 4 mL of toluene.
- The absorbance of the top layer was recorded at 520 nm, and proline levels were determined using an L-proline standard curve, reported as µmol/g fresh weight

## III. RESULTS

### *A. Statistical Analysis*

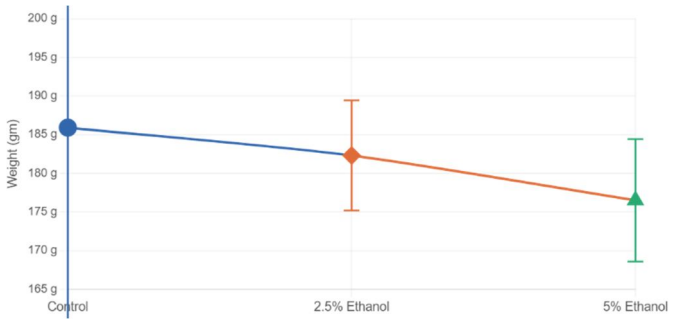
All analyses were carried out utilizing the Statistical Package for the Social Sciences (SPSS) software on a PC-compatible computer. Data were evaluated using the Mann-Whitney U-test for unpaired samples, with P values < 0.05 deemed significant. Descriptive data were presented as mean ± SD.

The experimental results show that ethanol intake considerably affects growth efficiency, protein metabolism, and enzyme profiles in broiler chickens during a 21-day span.

*1) Impact On Growth Parameters*

- Effect on Growth Aspects
- The administration of ethanol resulted in a significant decrease in the body weight of the chicks when compared to the control group.
- Control Group: Preserved the highest average body weights, with multiple birds achieving between 200g and 240g.
- 2.5% Ethanol Group: Displayed a small weight reduction, with the majority of birds grouping between 170g and 190g.
- 5% Ethanol Group: Showed the greatest reduction in growth, with weights generally remaining between 170g and 189g, demonstrating that increased alcohol levels hinder normal growth rates in broilers

| Sr. No. | Groups | Weight in gm |
|---|---|---|
| 1 | Control | 185.9 ± 32.09 |
| 2 | 2.5% Ethanol | 182.3 ± 7.12 |
| 3 | 5% Ethanol | 176.5 ± 7.92 |



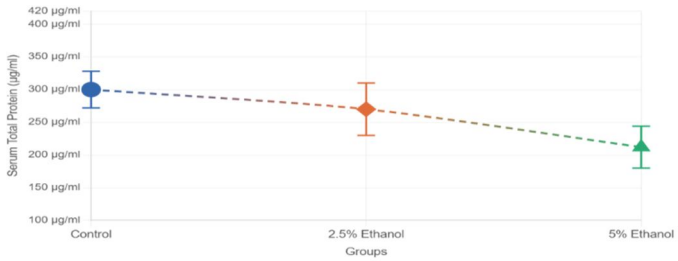
*2) Levels Of Serum Protein And Albumin*

The research noted a decline in total serum protein with rising ethanol levels, indicating a disturbance in the liver's capacity to uphold protein balance.

• Total Serum Protein: The control group exhibited levels reaching up to 425 µg/ml. In contrast, the group with 5% ethanol experienced levels decreasing to as low as 150 µg/ml, suggesting reduced protein synthesis or elevated degradation

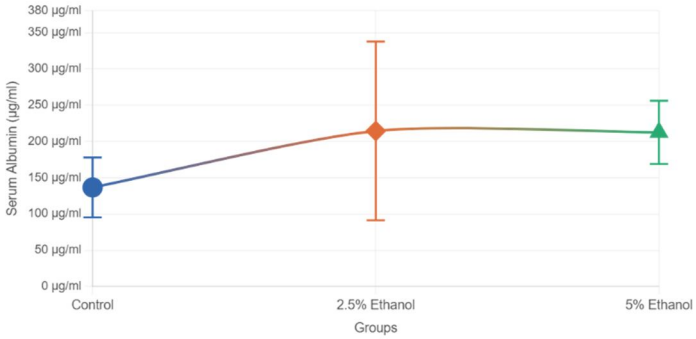
- SERUM TOTAL PROTEIN:

| Sr. No. | Groups | Serum total protein(µg/ml) |
|---|---|---|
| 1 | control | 302.5 ± 85.43 |
| 2 | 2.5% Ethanol | 278 ± 54.04 |
| 3 | 5% Ethanol | 212.5 ± 43.50 |



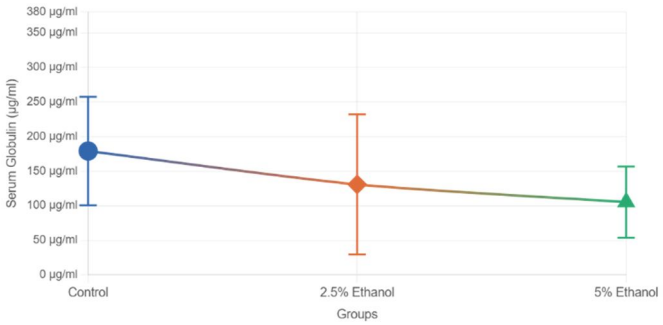

- SERUM ALBUMIN: Like total protein, albumin levels dropped with alcohol exposure. For example, sample 8 in the control group measured 66.6 µg/ml, while multiple samples in the 2.5% ethanol group significantly decreased (e.g., sample 3 at 3 µg/ml).

| Sr. No. | Groups | Serum Albumin (µg/ml) |
|---|---|---|
| 1 | Control | 136.63 ± 41.19 |
| 2 | 2.5% Ethanol | 214.46 ± 123.12 |
| 3 | 5% Ethanol | 212.5 ± 43.50 |




- SERUM GLOBULIN: Determined by subtracting albumin from total protein, globulin levels varied, typically showing lower values in the ethanol-treated groups (e.g., dropping to 8.4 µg/ml in the 5% group) when contrasted with the control

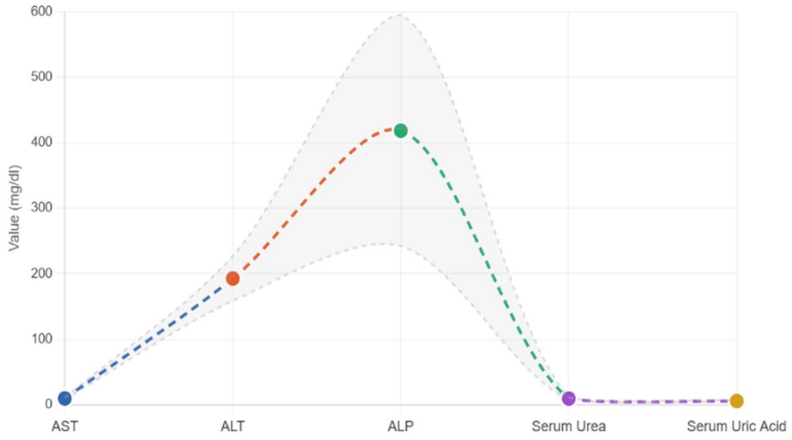
| Sr. No. | Groups | Serum Globulin (µg/ml) |
|---|---|---|
| 1 | Control | 179.16 ± 78.38 |
| 2 | 2.5% Ethanol | 130.86 ± 101.26 |
| 3 | 5% Ethanol | 105.30 ± 51.54 |




*3) Liver Function And Enzyme Activity*

- The liver enzymes' biochemical profile indicates the toxic effects of ethanol on liver tissues.
- AST and ALT: These enzymes indicate the condition of the liver. In the group with 5% ethanol, AST levels were significantly reduced (between 4 and 7.1 mg/dl) when contrasted with the control group (8.2 to 11 mg/dl).
- Alkaline Phosphatase (ALP): ALP levels displayed notable fluctuations, with the 5% ethanol group typically showing reduced values (usually ranging from 109 to 201 mg/dl) compared to the control group, which reached maximum levels of 562.8 mg/dl.
- Serum Urea and Uric Acid: A general reduction in urea and uric acid levels was observed in the ethanol-treated groups, indicating changes in nitrogen excretion pathway.

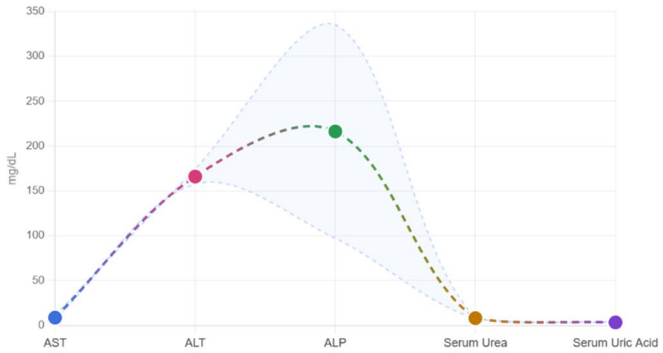
| Sr. No | Control Group (mg/dl) | |
|---|---|---|
| | Parameter | Standard Deviations |
| 1 | AST | 9.52 ± 1.08 |
| 2 | ALT | 192.77 ± 34.37 |
| 3 | ALP | 418.52 ± 176.62 |
| 4 | Serum Urea | 9.30 ± 1.91 |
| 5 | Serum Uric Acid | 5.51 ± 1.38 |



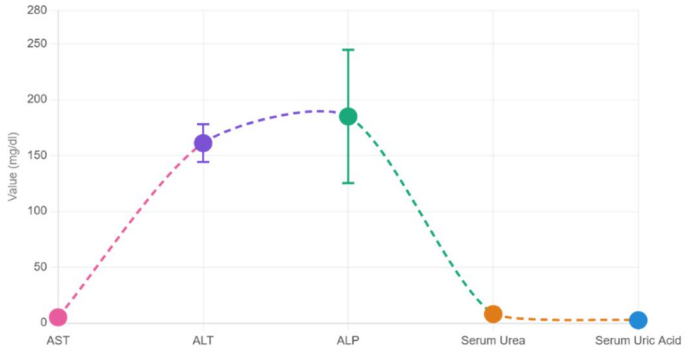
| Sr.no | 2.5% Ethanol Group (mg/dl) | |
|---|---|---|
| | Parameter | Standard Deviations |
| 1 | AST | 8.64± 2.42 |
| 2 | ALT | 165.99 ± 8.6 |
| 3 | ALP | 216.21 ± 119.17 |
| 4 | Serum Urea | 8.06 ± 0.10 |
| 5 | Serum Uric Acid | 3.29 ± 0.30 |

| Sr.no | 5% Ethanol Group (mg/dl) | |
|---|---|---|
| | Parameter | Standard Deviations |
| 1 | AST | 5.23± 0.99 |
| 2 | ALT | 161.24 ± 16.92 |
| 3 | ALP | 185.11 ± 59.69 |
| 4 | Serum Urea | 8.12 ± 1.16 |
| 5 | Serum Uric Acid | 1.62 1.0 |



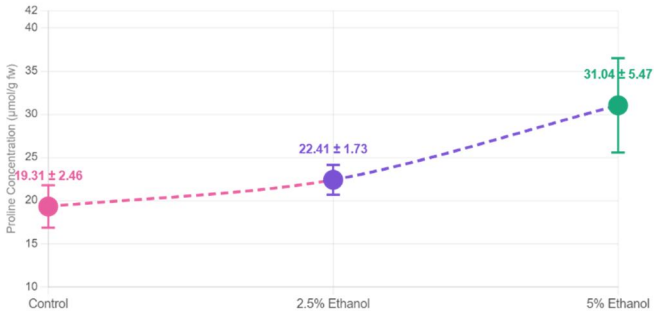

*4) Progression Of Hyperprolinemia*

- The most notable metabolic alteration detected was the increase in proline concentrations in the serum and liver, validating the onset of Hyperprolinemia.
- Proline Concentration: A noticeable dose-dependent rise in proline levels was observed.




- o Control Group: Mean concentrations ranged from about 14.58 to 21.87 µmol/g FW.

- o 5% Ethanol Group: Proline concentrations increased markedly, reaching a peak of 38.26 µmol/g FW.
- Mechanism: This buildup indicates that ethanol inhibits crucial enzymes such as Proline Oxidase and P5C Dehydrogenase, hindering the efficient degradation of
- proline, resulting in its buildup in the bloodstream and tissues




## IV. CONCLUSION

The research shows that alcohol (ethanol) intake notably interferes with protein metabolism and growth performance in broiler chickens. Through the assessment of physiological and biochemical reactions throughout a 21-day span, the subsequent conclusions are reached:

### *A. Growth Suppression And Metabolic Health*

Ethanol administration serves as a powerful metabolic disruptor, resulting in a dose-dependent decrease in body weight. Control birds attained weights of up to 240g, whereas those subjected to 5% ethanol exhibited considerable growth restriction, mainly remaining within the range of 170g to 189g. This suggests that even minimal levels of alcohol negatively affect the feed conversion efficiency required for swift avian growth

| Sr.no | Groups | Proline Concentrations (µmol/g fw) |
|---|---|---|
| 1 | Control | 19.31 ± 2.46 |
| 2 | 2.5% Ethanol | 22.41 ± 1.73 |
| 3 | 5% Ethanol | 31.04 ± 5.47 |

### *B. Disrupted Protein Balance*

Ethanol exposure disrupts the liver's capacity to sustain systemic protein levels.

- Total Serum Protein and Albumin: Both exhibited a significant decrease as ethanol levels rose. Total protein decreased from a peak of 425 µg/ml in controls to as low as 150 µg/ml in the group exposed to 5% ethanol.
- Hepatic Retention: Histopathological findings indicate that this reduction is a result of a "blocking" of the hepatic secretion system. Instead of being released into the bloodstream, export proteins such as albumin are kept within the liver, which contributes to hepatotoxicity.

### *C. Induction Of Elevated Proline Levels*

The most precise biochemical observation is the emergence of Hyperprolinemia.

- Enzymatic Inhibition: Ethanol seems to inhibit important enzymes—Proline Oxidase (PRODH) and P5C Dehydrogenase—that are involved in proline degradation.
- Proline Accumulation: This inhibition led to a notable increase in proline concentrations, attaining 38.26 µmol/g FW in the high-ethanol group, while the control had a peak of 21.87 µmol/g FW.

## V. ACKNOLEDGEMENT

Authors are thankful to Management of Mahatma Gandhi Vidymandir, Principal M. S. G. College, (Autonomous) Malegaon for providing research facilities and continuous guidance a well a encouragement.

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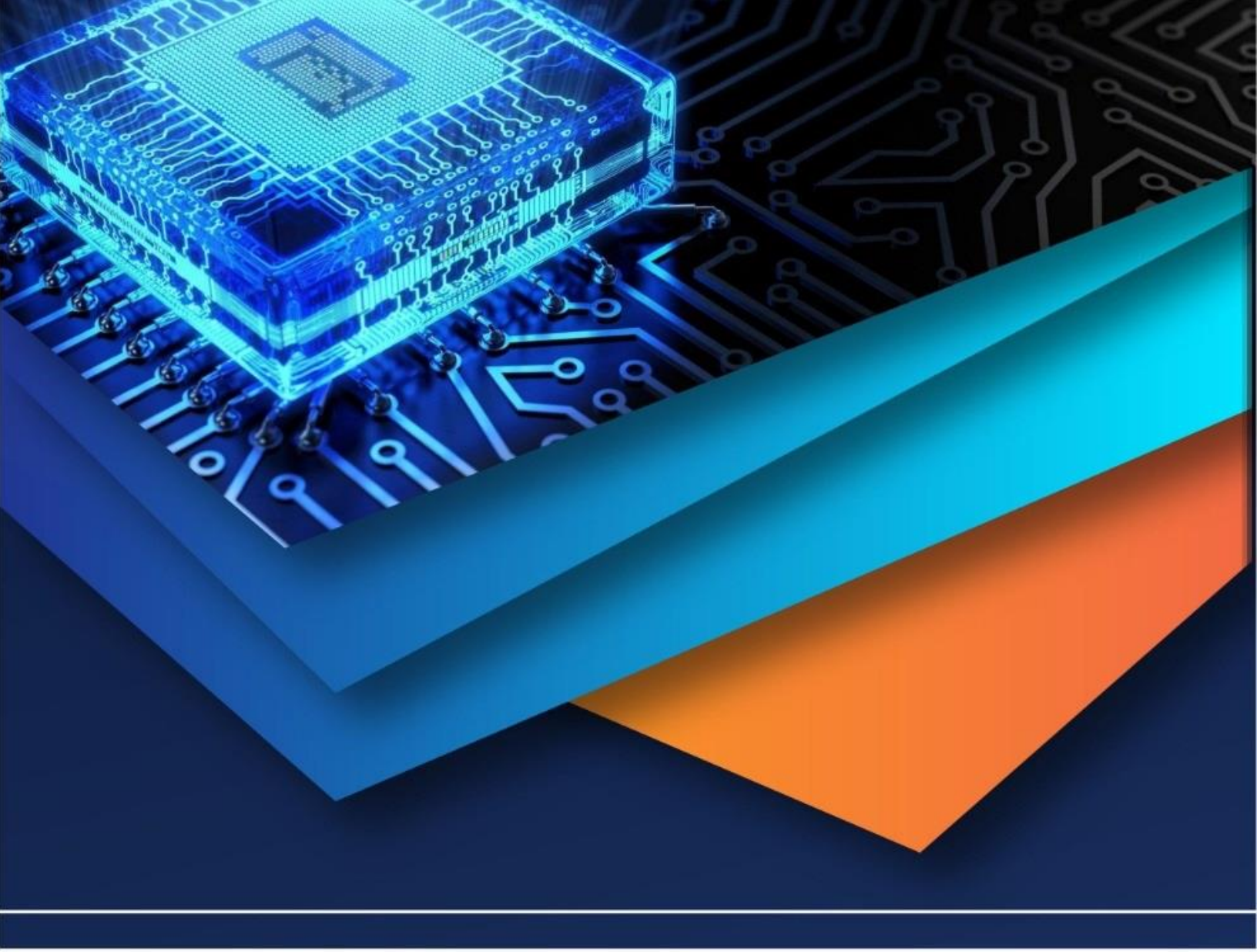

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