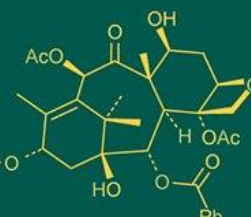
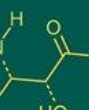
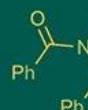


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(9): 30-36
www.biochemjournal.com
Received: 15-06-2026
Accepted: 17-07-2026

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Efficacy of Renonic in Mitigating High-Protein Diet Stress: Insights from Hemato-Serological Parameters, Relative Organ Weights, and Renal Histopathology in Broiler Chickens

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i9a.9822>

Abstract

This study was conducted on 240 day old Vencobb-430 broiler birds for 5 weeks to assess the effect of a renal test product (Renonic)TM procured from M/s. Carus Laboratories Pvt. Ltd., Karnal, Haryana, India on the hemato-biochemical parameters and histopathological changes in kidney in gout prone conditions induced by high protein and water restricted diet. The birds were distributed into 4 treatment groups (A; control with basal diet only, E; negative control with high protein diet (3rd -21st days) and restricted water supply, F; high protein diet (3rd -21st days) with restricted water supply + Renonic @ 1ml/L water, and G; high protein diet (3rd -21st days) with restricted water supply + standard anti-gout solution @5ml/100 birds (3-10 days), 10ml/100 birds (11-21 days) and 15 ml/100 birds (21-28 day) with 4 replicates per treatment having 15 birds in each. All the birds were floor reared under uniform management conditions throughout the whole period and were provided with *ad lib* water, basal diet according to BIS 2007, and vaccination as per routine schedule. High protein diet induced renal challenge did not affect hematological parameters (Hb, PCV, TLC, eosinophils, basophils, monocytes; ($p > 0.05$), but significantly elevated heterophil percentage, reduced lymphocyte count, and increased serum creatinine, BUN, and uric acid ($P < 0.05$) in comparison to control group. Relative weights of liver and kidneys showed no significant differences among treatment groups ($p > 0.05$). Renal histopathology revealed focal congestion, hydropic degeneration, tubular necrosis, and MNC infiltration in negative control group E, while groups F and G showed normal renal architecture comparable to control group A. Also, supplementation with Renonic (group F) and the standard gout solution (group G) significantly attenuated heterophilia, restored lymphocyte counts, and reduced serum nitrogenous wastes to levels comparable to the control (group A; $p > 0.05$), with Renonic demonstrating numerically superior bio-efficacy over standard anti-gout solution (group G). In conclusion, therapeutic supplementation with Renonic effectively protect tubular integrity, enhances nitrogenous waste clearance, and alleviates stress-induced immune shifts in commercial broilers.

Keywords: High protein diet, Broilers, Organ weight, Hemato-biochemical, Renal-histopathology, RenonicTM

Introduction

Nutritional homeostasis is fundamental to maintaining systemic health, immune competence, and metabolic stability in broiler chickens. Dietary manipulations, specifically the inclusion of crude protein levels exceeding physiological requirements, can significantly alter the metabolic environment. Excess protein cannot be stored in the body and must undergo hepatic catabolism and renal excretion as uric acid—a process that incurs a high metabolic energy cost, alters digestive dynamics, impairs intestinal nutrient absorption and can lead to uric acid deposition on organs, leading to gout, a well-known condition in poultry. There are numerous factors associated with avian gout, such as high protein content in feed, excessive salt, dietary calcium, phosphorus imbalance, insufficient vitamin A uptake and lack of drinking water which all take a toll on kidney function. In addition, genetic and disease factors, viral infection and improper use of drugs and feeding can also lead to avian gout. (Bulbule *et al.*, 2014, Alice Mitchell, 2015) ^[1]. Excess amino acid catabolism places severe stress on major metabolic organs, primarily the liver and kidneys, and generates elevated

levels of circulating nitrogenous metabolites such as uric acid, creatinine and blood urea nitrogen (Mir *et al.* 2005; Alice Mitchell, 2015; Kriseldi *et al.* 2018; Xi *et al.* 2020) [6, 1, 4, 11]. The metabolic load of handling excess dietary nitrogen directly impacts organ development and morphology. The liver catabolizes surplus amino acids, while the kidneys filter and excrete elevated levels of nitrogenous waste, frequently altering relative organ weights and inducing physiological strain.

Evaluating hematological and serum biochemical profiles provides crucial, non-invasive insights into the physiological, nutritional, and health status of birds. Elevated dietary protein intake and associated dehydration or nutrient imbalances induce systemic oxidative stress, alter serum key biomarkers indicative of renal cellular integrity (Hong *et al.* 2020) [3]. Persistent metabolic overload from excess protein can ultimately progress to tissue damage, which is reflected at the cellular level by micro-architectural alterations in renal histology.

While dietary protein excess and water restriction impair metabolic homeostasis and induce microscopic renal damage, specific protective feed additives offer a means to preserve organ microstructure and blood biochemistry. The present investigation aimed to determine the extent to which high-protein diets and water deficiency related stress impact the hemato-serological parameters, organ weights, and kidney histopathology of broiler chickens, and to assess whether therapeutic test product administration can mitigate

these systemic alterations. The following parameters were studied in this research trial-

1. Hematological parameters - Hb, PCV, TLC and DLC
2. Serological parameters-Creatinine, BUN, uric acid and glucose
3. Organ weights- liver and kidney relative weight to live body weight
4. Renal histopathology- Gross and histomorphological

Material and methods

This trial was conducted on 240, day old Vencobb-430 broiler birds for 5 weeks at broiler unit (deep litter system) of Department of Poultry Science, College of Veterinary & Animal Sciences, MAFSU, Parbhani, Maharashtra to assess the effect of Renonic™ (procured from M/s. Carus Laboratories Pvt. Ltd., Sector 3, HSIIDC, Karnal, Haryana, India) on the hemato-biochemical parameters and kidney histopathology. The birds were distributed into 4 treatment groups (A; control with basal diet only, E; negative control with high protein diet (3rd -21st days) and restricted water supply, F; high protein diet (3rd -21st days) with restricted water supply + Renonic @ 1ml/L water, and G; high protein diet (3rd -21st days) with restricted water supply + standard solution for gout @5ml/100 birds (3-10 days), 10ml/100 birds (11-21 days) and 15 ml/100 birds (21-28 day) with 4 replicates per treatment having 15 birds in each. All the birds were provided with standard and uniform managemental conditions throughout the experimental period. All the birds were

Table 1: Ingredients and nutrient composition of pre-starter, starter and finisher of control group and treatment groups

Sr. No.	Ingredients (%)	Control: Pre-starter	Control: Starter	Control: Finisher	Treatment: Pre-starter	Treatment: Starter	Treatment: Finisher
1	Maize	53.00	54.00	58.22	39.10	37.10	58.22
2	Soybean meal	40.20	37.80	32.60	46.50	46.55	32.60
3	Maize gluten	0.00	0.00	0.00	8.00	8.00	0.00
4	Vegetable oil	2.77	4.15	5.10	2.50	4.50	5.10
5	Dicalcium phosphate (DCP)	1.80	1.80	1.90	1.80	1.80	1.90
6	Limestone powder (LSP)	1.20	1.20	1.20	1.20	1.20	1.20
7	Salt (NaCl)	0.25	0.25	0.25	0.25	0.25	0.25
8	Trace mineral premix*	0.10	0.10	0.10	0.10	0.10	0.10
9	Vitamin premix**	0.05	0.05	0.05	0.05	0.05	0.05
10	DL-Methionine	0.16	0.19	0.15	0.10	0.05	0.15
11	L-Lysine	0.07	0.06	0.03	0.00	0.00	0.03
12	Choline chloride 60%	0.10	0.10	0.10	0.10	0.10	0.10
13	Toxin binder	0.10	0.10	0.10	0.10	0.10	0.10
14	Coccidiostat	0.05	0.05	0.05	0.05	0.05	0.05
15	Sodium bicarbonate	0.15	0.15	0.15	0.15	0.15	0.15
	Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient composition (Calculated basis)							
Sr. No.	Parameters	Control: Pre-starter	Control: Starter	Control: Finisher	Treatment: Pre-starter	Treatment: Starter	Treatment: Finisher
1	ME (Kcal/kg)	3000.33	3101.54	3200.44	2947.85	3059.55	3200.44
2	Crude Protein (%)	23.02	22.04	20.03	29.30	29.12	20.03
3	Ether Extract (%)	5.18	6.57	7.64	4.57	6.47	7.64
4	Crude Fiber (%)	4.20	4.08	3.87	4.40	4.34	3.87
5	Calcium (%)	1.01	1.00	1.01	1.04	1.04	1.01
6	Total Phosphorus (%)	0.70	0.69	0.68	0.76	0.75	0.68
7	Available Phosphorus (%)	0.42	0.42	0.43	0.42	0.42	0.43
8	Total Lysine (%)	1.30	1.23	1.08	1.47	1.47	1.08

* **Trace Mineral Mixture:** Each 1kg contains: Copper-15g, Iron-80g, Manganese-100g, Zinc-80g, Iodine-2g, Cobalt-0.5g, Selenium-0.5g, Chromium 10mg and Molybdenum 50mg.

Vitamin Premix: Each 500g contains: Vit. A-12.50MIU, Vit. D3-2.50 MIU, Vit. E-12g, Vit. K-1.50g, Thiamine (B1)-1.50g, Riboflavin (B2)-5g, Pyridoxine (B6)-2g, Cyanocobalamin (B12)- 0.015g, Niacin-15g, Cal D Pantothenate-10g and Folic acid-0.50g vaccinated as per routine vaccination schedule. The control group was fed with basal diet which was prepared as per Bureau of Indian Standards (BIS 2007) [2]. However, other treatment groups were fed high protein diet (30% CP) during days 3rd to 21st of age and after 21st day of age normal basal diet as per BIS 2007 [2] was fed. The feed formulation and composition for control and other treatment groups are shown in Table 1.

Eight birds from each treatment group (2 birds per replicate) were randomly selected at the end of 2nd and 3rd week of age and about 2 ml blood from each bird via wing vein was taken in EDTA vials and serum vacutainers for studying hematological and serological parameters, respectively. The same birds were slaughtered using the standard procedure in the department of pathology for histopathological examination and weight recording of liver and kidneys. The results of the trial were statistically analyzed for any significant differences as per methods described by Snedecor and Cochran (1994). The treatment means were compared by critical differences (CD) and analysis of variance.

Results and Discussion

Hematological and Differential Leucocyte counts:

Data pertaining to hematological and DLC parameters of broilers is represented in Table 2. The hemoglobin (Hb), packed cell volume (PCV), and total leukocyte count (TLC) showed no significant differences ($p > 0.05$) among treatment groups at either the 2nd or 3rd week of age. Similarly, granular leukocyte percentages (eosinophils and

basophils) as well as monocytes remained unaltered ($p > 0.05$) across all groups. However, significant variations ($P < 0.05$) were observed in Heterophil and Lymphocyte percentages. At both intervals, Group E exhibited the highest heterophil percentage (28.75 ± 0.75 at week 2; 29.50 ± 0.19 at week 3) and a concomitant reduction in lymphocyte percentage (63.50 ± 0.73 at week 2; 62.88 ± 0.44 at week 3). Supplementation with Renonic and gout solution (Groups F and G, respectively) significantly ameliorated these shifts, bringing heterophil and lymphocyte proportions numerically back toward control (Group A) levels by the 3rd week ($p > 0.05$).

The hematological data of this study indicated that neither high protein diets nor test product supplementation altered ($p > 0.05$) the total leucocyte count and erythrocyte indices (Hb, PCV) in comparison to control group; showing a normal similar hemopoiesis, oxygen carrying capacity, fluid balance, blood viscosity and overall cellular immunity across all treatment groups. In avian species, heterophil to lymphocyte ratio is a classic indicator of stress and immune function and the group E shows the highest physiological stress as evident by the result data. Supplementation of test product RenonicTM (group F) and gout solution (group G) effectively restored lymphocyte populations and attenuated heterophilia, suggesting anti-stress and immunomodulatory properties. Similar to our findings, Sanjeev Kumar *et al.* (2024) [5] reported highest heterophil to leucocyte ratio in gout induced group with no antigout drug supplementation when compared to control and groups with test drug therapy. The authors reported that inclusion of therapeutic drugs alleviated the renal stress factors, evidently noted by decrease in TLC, heterophil, monocyte count in comparison to gout induced group and brought the values closer to control group.

Table 2: Haematological parameters of broilers at the end of 2nd and 3rd week in different treatment groups

Treatments	Hb (g/dl)		PCV (%)		TLC (/dl)	
	2 nd week	3 rd week	2 nd week	3 rd week	2 nd week	3 rd week
A	10.95 ± 0.34	11.56 ± 0.38	32.81 ± 1.05	34.44 ± 0.98	10483.75 ± 379.33	11546.75 ± 386.02
E	10.98 ± 0.59	11.25 ± 0.30	32.93 ± 1.78	34.50 ± 1.30	11350.63 ± 471.43	12097.00 ± 256.21
F	11.10 ± 0.37	11.70 ± 0.58	33.00 ± 1.07	33.85 ± 1.44	10626.25 ± 541.22	11643.25 ± 558.90
G	10.73 ± 0.55	11.19 ± 0.44	31.88 ± 1.81	34.00 ± 0.78	10521.25 ± 563.33	11225.50 ± 314.81

*Means bearing different superscripts in a row differ significantly ($p < 0.05$).

Table 3: Haematology DLC parameters of broilers at the end of 2nd and 3rd week in different treatment groups

Treatments	Heterophils (%)		Eosinophils (%)		Basophils (%)		Lymphocytes (%)		Monocytes (%)	
	2 nd week	3 rd week	2 nd week	3 rd week	2 nd week	3 rd week	2 nd week	3 rd week	2 nd week	3 rd week
A	26.50 ^b ± 0.42	26.25 ^c ± 0.37	2.13 ± 0.23	2.00 ± 0.27	0.88 ± 0.23	0.88 ± 0.23	66.63 ^a ± 0.53	66.88 ^a ± 0.40	3.88 ± 0.30	4.00 ± 0.27
E	28.75 ^a ± 0.75	29.50 ^a ± 0.19	2.13 ± 0.30	1.88 ± 0.30	1.00 ± 0.27	1.25 ± 0.25	63.50 ^b ± 0.73	62.88 ^c ± 0.44	4.63 ± 0.38	4.50 ± 0.19
F	26.88 ^b ± 0.40	27.88 ^b ± 0.40	2.00 ± 0.27	2.25 ± 0.25	1.00 ± 0.27	1.00 ± 0.27	66.13 ^a ± 0.52	65.00 ^b ± 0.46	4.00 ± 0.27	3.88 ± 0.30
G	27.25 ^b ± 0.37	28.50 ^b ± 0.33	2.00 ± 0.19	2.38 ± 0.26	0.88 ± 0.30	0.75 ± 0.25	66.00 ^a ± 0.38	65.00 ^b ± 0.71	3.88 ± 0.23	3.38 ± 0.38

*Means bearing different superscripts in a row differ significantly ($p < 0.05$).

Serum biochemical parameters

The data pertaining to serological parameters is depicted in Table 4. The renal function markers like creatinine, blood urea nitrogen (BUN) and uric acid were found to be significantly ($P < 0.05$) elevated in group E in comparison to control group A. In contrast, glucose level remained statistically similar across all treatment groups ($p > 0.05$)

during both 2nd and 3rd week of age. The test product and standard gout solution supplemented groups (groups F and G, respectively) ameliorated the stress effects of high protein diets coupled with restricted water supply by limiting the rise in levels of creatinine, BUN and uric acid comparable ($p > 0.05$) to control group A.

Table 4: Serum biochemical parameters of broilers at the end of 2nd and 3rd week in different treatment groups

Treatments	Creatinine (mg/dl)		BUN (mg/dl)		Uric Acid (mg/dl)		Glucose (mg/dl)	
	2 nd week	3 rd week	2 nd week	3 rd week	2 nd week	3 rd week	2 nd week	3 rd week
A	0.57 ^b ± 0.04	0.33 ^b ± 0.04	5.35 ^c ± 0.08	4.59 ^b ± 0.31	4.68 ^a ± 0.30	4.46 ^b ± 0.83	178.24 ± 6.22	219.66 ± 7.28
E	0.99 ^a ± 0.09	0.49 ^a ± 0.03	6.36 ^a ± 0.17	6.07 ^a ± 0.26	8.43 ^b ± 0.54	6.66 ^a ± 0.23	163.44 ± 3.38	216.30 ± 4.93
F	0.78 ^{ab} ± 0.11	0.36 ^b ± 0.01	5.97 ^b ± 0.13	4.98 ^b ± 0.30	5.49 ^a ± 0.23	5.67 ^{ab} ± 0.19	169.99 ± 5.21	213.32 ± 4.63
G	0.80 ^{ab} ± 0.07	0.37 ^b ± 0.04	5.86 ^b ± 0.08	5.02 ^b ± 0.25	5.71 ^a ± 0.32	5.71 ^{ab} ± 0.22	172.32 ± 6.31	210.09 ± 8.33

*Means bearing different superscripts in a row differ significantly ($p < 0.05$).

Excessive dietary protein increases the metabolic burden on the avian kidney, as avian species excrete excess nitrogen primarily as uric acid. The significant surge in serum creatinine, BUN, and uric acid in Group E reflects hyperuricemia and transient renal overload. Renonic supplementation successfully reduced these metabolite concentrations toward basal control levels by the end of the 3rd week. Additionally, the effects of Renonic were numerically better than standard gout solution used in group G. This renal protective effect highlights the test product's potential to enhance nitrogenous waste clearance, protect tubular integrity, and alleviate dietary protein induced nephrotoxicity in commercial broilers. Similar to our findings, a phytogenic renal formulation having *piper betle* leaf extract (20 g/kg) reduced the uric acid content progressively from d 17 (19.4 ± 0.62 mg/dl) to d 21 (9.81 ± 0.3 mg/dl) in gout challenged broilers (Vikrama Chakravarthi *et al.* 2022) [10]. The authors also reported enhanced antioxidant activity and production performance over conventional antigout drug therapy, highlighting phytogenic preparations as effective alternatives for managing avian renal health. Our observations align with Kumar *et al.* (2024) [5], who noted peak serum creatinine and uric acid concentrations in gout-challenged birds, whereas therapeutic intervention successfully restored these

metabolic markers close to baseline values appeared in control group.

Organ weights

The mean values of liver and kidney weight percent relative to the live body weight at the end of 2nd and 3rd week are presented in Table 5. The statistical analysis revealed that all treatment groups showed significant variations in organ weight proportions at the end of 2nd and 3rd week of age. The E group supplemented with high protein diet with restricted water supply showed significantly higher ($p < 0.05$) relative kidney weight at the end of 2nd week in comparison to control group A and group F supplemented with high protein diet with restricted water supply and Renonic @1ml/L. However, the statistical difference was non-significant in groups A, F, and G.

At the end of 3rd week, the E group had the significantly higher relative weight percent of kidney than control group A. The relative kidney weight in groups supplemented with high protein diet with Renonic and antigout solution (Groups F and G, respectively) numerically decreased ($p > 0.05$) in comparison to group E fed high protein diet with restricted water supply. The relative weight of the liver was comparable and did not show any significant variation ($p > 0.05$) among all experimental groups during both the 2nd and 3rd weeks of age.

Table 5: Mean kidney and liver weight as a percentage of live body weight of broilers in different treatment groups at the end of 2nd and 3rd weeks of age

Treatments	At the end of 2nd week: Avg. body weight (g)	At the end of 2nd week: Kidney (%)	At the end of 2nd week: Liver (%)	At the end of 3rd week: Avg. body weight (g)	At the end of 3rd week: Kidney (%)	At the end of 3rd week: Liver (%)
A	453.88 ± 13.41	0.82 ^b ± 0.03	3.48 ± 0.03	974.38 ± 35.46	0.72 ^b ± 0.01	2.71 ± 0.15
E	435.88 ± 13.47	0.92 ^a ± 0.02	3.55 ± 0.15	912.50 ± 50.21	0.76 ^a ± 0.01	2.64 ± 0.16
F	479.63 ± 15.02	0.83 ^b ± 0.01	3.52 ± 0.06	926.00 ± 34.89	0.74 ^{ab} ± 0.01	2.53 ± 0.05
G	461.75 ± 8.86	0.85 ^{ab} ± 0.02	3.54 ± 0.07	966.25 ± 46.24	0.74 ^{ab} ± 0.01	2.62 ± 0.06

*Means bearing different superscripts in a row differ significantly ($p < 0.05$).

Numerous previous studies have documented enlarged kidney and liver size of birds when gout was induced by high protein diets, driven by hyperuricemia and inflammation (Mir *et al.* 2005; Patel *et al.*, 2007; Singh *et al.*, 2013; Alice Mitchell, 2015; Hong *et al.*, 2020; Sandhyarani K. *et al.* 2022) [6, 7, 9, 1, 3, 8]. The increased relative weight of organs in groups E, F and G in comparison to control group A might be due to digestive and nephropathic alterations caused by high protein diet along with restricted water supply. However, the treatment group F supplemented with Renonic in water might have protected the renal function on high protein diet as evident by significantly and numerically lower mean values of kidney weight during 2nd and 3rd week end, respectively, in comparison to negative control group E.

Histopathology: The histopathological observations of kidney tissues of different treatment groups at week 2nd and

3rd were studied for the cellular architecture, gross pathological signs, gout lesions or any congestion, inflammation and degenerative changes (Figure plates 3-10).

Gross examination at the end of the 2nd week revealed minimal to mild focal renal congestion in birds from the negative control group (E). Kidneys from all other experimental groups exhibited normal morphologic appearance without any appreciable gross pathological lesions. By the end of the 3rd week, renal gross architecture appeared healthy and normal across all groups, with no recognizable gross changes in birds of all groups. However, histomorphological evaluation at week 2 age demonstrated minimal to mild, focal to multifocal congestion, hydropic and vacuolar degeneration, cystic degenerative changes, cellular swelling, focal mononuclear cell (MNC) infiltration, and focal tubular necrosis in the renal tissue of group E. Conversely, kidney sections from the control (Group A) and

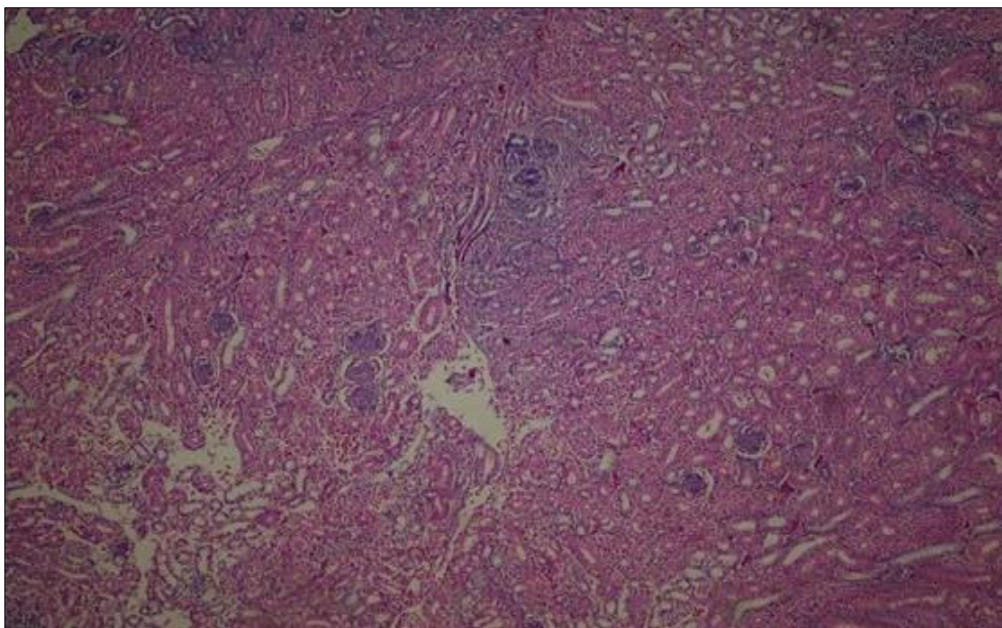
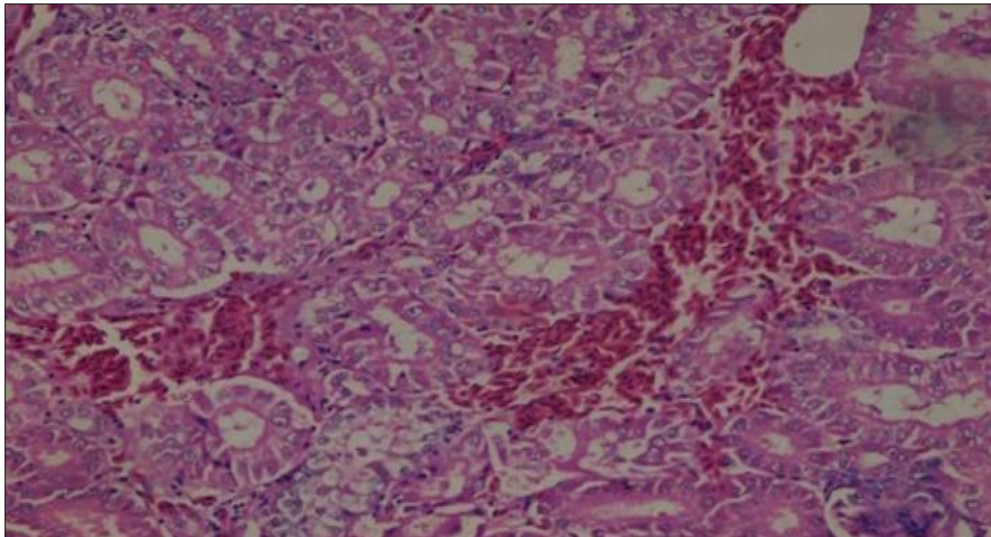
product supplemented groups (Groups F and G) displayed well preserved histoarchitecture without significant pathological alterations. Similar observations were made at week 3 age also. Notably, renal tissues from the Renonic and competitor product treated groups (F and G, respectively) exhibited substantially healthier tissue architecture relative to Group E, reflecting progressive recovery and tissue protection over time.

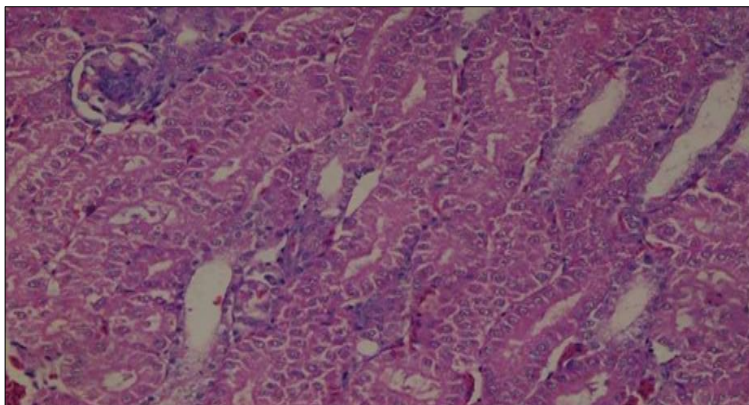
The highest gross and histopathological alterations found in group E in comparison to control group A indicated renal overload and nephropathy due to high protein diet coupled with water deficiency. Feeding high-protein rations accelerates purine catabolism, elevating systemic nitrogenous waste clearance and driving intraglomerular hypertension and hyperfiltration. Over time, this elevated metabolic strain induces tubular epithelial degeneration, localized ischemia (manifesting as focal congestion), cellular swelling, and focal inflammatory white blood cells infiltration within the renal parenchyma. The high levels of uric acid in systemic circulation subsequently precipitates as monosodium/calcium urate crystals on various organs, particularly in the kidneys (Zhang *et al.* 2025) [12]. These histopathological lesions align directly with the renal

degenerative changes and elevated serum creatinine, BUN, and uric acid concentrations observed in Group E.

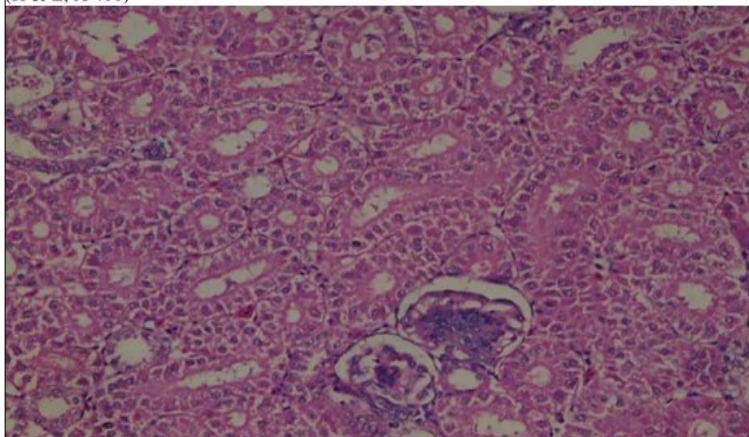
Supplementation with Renonic (group F) and the competitor product (group G) effectively attenuated these high-protein-induced pathological changes. The preservation of normal tubular epithelium, absence of significant focal necrosis, and reduction in inflammatory cellular infiltration indicate a strong nephroprotective effect. These morphological findings provide structural evidence supporting the observed recovery in kidney tissue architecture and validate the therapeutic efficacy of test product (Renonic) in mitigating renal stress in broiler chickens. In agreement with our histopathological findings, Kumar *et al.* (2024) [5] reported severe histoarchitectural disruption in gout induced groups; including renal tubular necrosis, urate crystal depositions, cellular swelling, and interstitial nephritis in hyperuricemic broilers, whereas therapeutic intervention effectively attenuated these pathological changes and preserved overall renal tissue structure.

Figure plates showing renal histomorphology of treatment groups at different week age

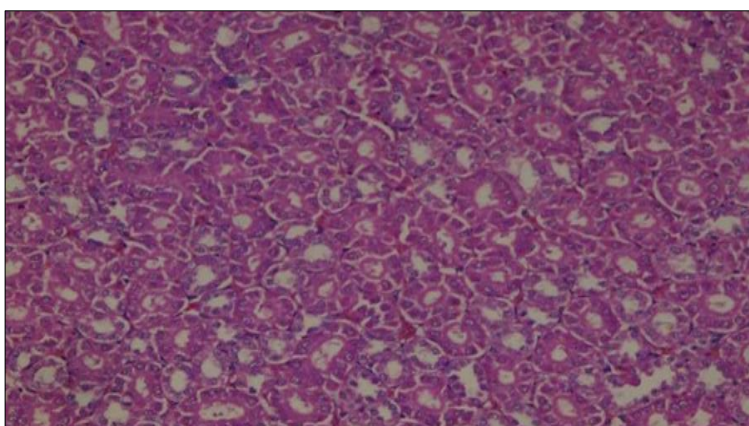




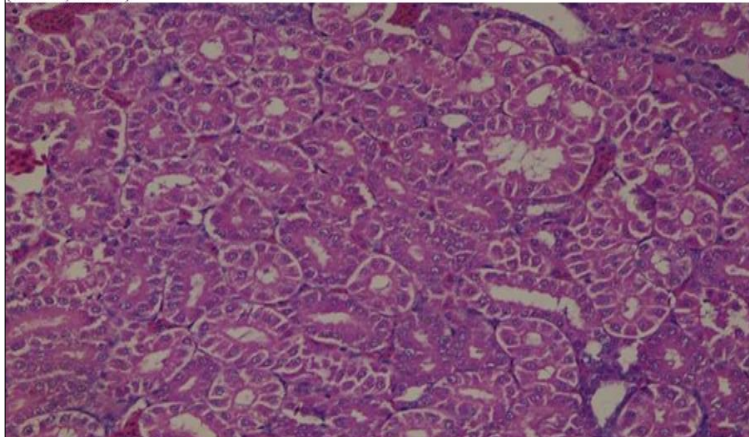
- 05: Section of kidney from bird of group F with apparently normal histoarchitecture (H & E, X 400)



- 06: Section of kidney from bird of group G with apparently normal histoarchitecture (H & E, X 400)



- 09: Section of kidney from bird of group F with apparently normal histoarchitecture (H & E, X 400)



-10: Section of kidney from bird of group G with apparently normal histoarchitecture (H & E, X 400)

Conclusion

Overall, the present study confirms that high dietary protein intake induces metabolic strain on the avian renal system, manifesting as altered biochemical profiles, enlarged organ weights, and histopathological lesions. However, timely therapeutic interventions such as Renonic™ supplementation can effectively mitigate these metabolic and nephrotoxic effects, restoring key biochemical markers and preserving renal architecture integrity. Implementing such targeted therapeutic formulations can be a reliable strategy to alleviate dietary and environmental renal stress in commercial broiler production.

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