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Effect of Zinc Supplementation and Varietal Different Cereal based Diet on Blood Chemistry and Tissue Changes in Rhode Island Red Chicken

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ABSTRACT

Background: Black wheat is rich in polyphenols and anthocyanin pigments which causes health benefits. Zinc mineral is important for protein synthesis and has major role to play in disease resistance and reproductive health improvement in poultry.

Methods: The present study was aimed to assess the effects of zinc supplementation through diverse cereal-based diets on blood chemistry and tissue changes in Rhode Island Red (RIR) chickens. Total 270-day-old RIR chicks were divided randomly in nine Treatment groups. Group T1 was kept as control while in T2 Nano zinc @30 ppm, T3 inorganic zinc @30 ppm, T4 black wheat @ 10 kg/100 kg of feed, T5 black wheat @ 10 kg/100 kg of feed + nano zinc @30 ppm, T6black wheat @ 10 kg/100 kg of feed + inorganic zinc @30 ppm, T7 wheat cultivar @ 10 kg/100 kg of feed, T8wheat cultivar @ 10 kg/100 kg of feed + Nano zinc @30 ppm and T9 wheat cultivar @ 10 kg/100 kg of feed + inorganic zinc @30 ppm was supplemented. Blood samples were collected at 90thdays post treatment and histopathological changes were noted

Result: Significantly increased (Pd"0.05) blood glucose was recorded with no significant difference in the haematological values in treatment groups. Gross lesions in liver, were seen only in T2 and T3 while in kidney these were seen only in T6 and T3. Histopathological lesions in liver and intestine were severest in group T2 while in kidney in T6. Supplementation of zinc-cereal enriched diets can have impact on avian health, highlighting their crucial significance in poultry nutrition.

Key words: Blackwheat, Blood chemistry, Pathology.

INTRODUCTION

Whole grain supplementation with compound feeds for broilers results in cost effective production. The feeding of wheat in poultry diet has been practiced in broiler production (Cumming, 1992) particularly with appropriate enzymatic combination to enhance the chicken performance (Boros *et al.,* 2004). Black wheat has been developed through normal plant breeding to increase its utility for humans (Kumari and Tzudir, 2021). Black wheat is known to have higher levels of phenolic compounds, amninoacid content(Joshi *et al.,*2024) anthocyanin and anti-oxidant potential than white wheat cultivar (Sharma *et al.,* 2020). Zinc is an essential micro-mineral required by the anti-oxidant system of the body. The bio-availability of organic Zinc is higher than that of inorganic Zinc, but the application of organic Zinc in diet is limited owing to its higher cost (Bratz *et al.,* 2013; Deori *et al.,* 2023). Zinc oxide nanoparticles (NP) have stronger chemical activity and undergo oxidation reactions with a variety of organic compounds compared to Zinc oxide. Zinc oxide NPs have improved feed conversion ratio and anti-oxidative properties in broilers (Akhavan-Salamat and Ghasemi, 2019, Arulnathan *et al.,* 2023). There is paucity of literature on how Zinc from different origins when incorporated into a range of cereal based diets affects different production variables, deposition of Zinc in tissues and their bio-availability in birds. Therefore, the present investigation was planned to evaluate the hemato-biochemical and tissue changes in RIR birds.

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MATERIALS AND METHODS

Present work was conducted at the Instructional Poultry Farm, G. B.Pant University of Agriculture and Technology,

Pantnagar. A total of 270 Rhode Island Red (RIR) birds of both the sexes were randomly divided into 9 groups of 30 birds each. Treatment 1 group was kept as control and fed basal standard diet, Treatment 2 birds were fed nanozinc@ 30 ppm in basal diet, Treatment 3: inorganic zinc @ 30 ppm, Treatment 4: black wheat @10 kg/100 kg of feed, Treatment 5: black wheat @ 10 kg/100 kg of feed + nanozinc@ 30 ppm, Treatment 6: black wheat @ 10 kg/ 100 kg of feed + inorganic zinc @ 30 ppm, Treatment 7: wheat cultivar @ 10 kg/100 kg of basal diet, Treatment 8 : wheat cultivar @ 10 kg/100 kg of basal diet+ nanozinc@ 30 ppm and Treatment 9: wheat cultivar @ 10 kg/100 kg of basal diet+ inorganic zinc @ 30 ppm. Nano ZnO with particle size less than 100 nm was procured from Sigma Aldrich, USA while inorganic ZnO with a molecular weight of 81.39 was procured from Glasil, USA. Black wheat was obtained from local market and the wheat cultivar was procured from the University farm. Standard managemental practices were used for maintaining the birds throughout the experimental period of 90 days. The experiment was conductedfromAugust to October, 2022. The research proposal was approved by the Institutional Animal Ethic Committee, Pantnagar. The standard basal diet was formulated by blending ingredients to meet the nutrient requirements outlined by BIS (2007).

Haemato-biochemical studies

At the end of the experiment i.e. 90th DPT, 6 birds per group were sacrificed humanely and the blood was collected for hemato-biochemical studies. Haematological parameters viz Haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) were evaluated. Biochemical parameters studied were serum glucose, total protein, albumin, globulin, ALT, cholesterol, triglyceride, HDL,LDL, VLDL and creatine.

Pathological studies

All the 6 sacrificed birds were subjected to detailed post mortem examination and the gross lesions were duly recorded. For histopathological studies, representative samples from liver, kidney, intestine and spleen were collected in 10% neutral buffered formalin and processed as per the standard protocol (Luna, 1968).

RESULTS AND DISCUSSION

The results of the haematological and biochemical studies are presented in Table 1 and 2, respectively. The results indicated no significant difference in the values of haematological parameters *viz.* Hb, PCV, TEC, TLC, MCV, MCHC and MCH, total protein, albumin, globulin, ALT, cholesterol, triglyceride,HDL and LDL,VLDL,creatinine levels between any treatment groups at 90th DPT. There was significant difference($P \leq 0.05$) in blood glucose concentration among different treatment groups. Significant difference ($P \leq 0.05$) was observed between T1 and T2; T1

Table 1: Haematological parameters in chicken of various groups fed varietal cereal based diet.

Attributes	Treatments										
	T1	T2	T3	T4	T5	T6	T7	T8	T9	SEm	P value
Hb (g/dl)	11.60±0.34	11.72±0.28	11.86±0.42	10.93±0.19	11.62±0.36	11.84±0.73	11.67±0.56	11.18±0.58	11.15±0.18	0.14	0.789
PCV (%)	37.40±0.46	36.02±0.95	35.77±1.26	35.80±0.35	36.44±0.73	36.19±0.97	36.22±0.59	36.53±0.81	35.80±0.89	0.25	0.910
TEC (10^6/uL)	2.31±0.06	2.21±0.11	2.32±0.02	2.15±0.03	2.18±0.12	2.15±0.2	2.16±0.05	2.18±0.11	2.18±0.06	0.08	0.610
TLC (10^3/uL)	21.44±0.43	22.23±0.06	20.85±0.56	22.10±0.58	21.47±0.84	20.81±0.74	21.75±0.28	21.60±0.76	21.50±0.41	0.18	0.669
MCV (fl)	137.05±0.79	137.05±0.74	136.55±0.45	136.50±0.36	135.95±0.89	136.98±1.39	137.13±0.69	136.71±0.43	138.18±0.45	0.22	0.953
MCH (pg)	42.35±0.61	42.39±0.17	42.23±0.19	42.24±0.50	42.44±1.28	41.99±0.24	42.62±0.26	42.11±0.54	42.95±0.44	0.17	0.976
MCHC (%)	31.07±0.9	31.57±0.37	31.09±0.59	31.13±0.49	31.35±1.06	31.86±0.59	31.60±0.97	31.02±0.61	32.19±0.60	0.19	0.918

[T1: Standard diet; T2: Standard diet +NanoZn@30ppm; T3:Standarddiet +InorganicZn@30ppm; T4:Incorporationof black wheat 10kg/100kgfeed;T5: Incorporation of black wheat 10kg/100 kg feed+ Nano Zn@30 ppm; T6: Incorporation of black wheat 10kg/100 kg feed+ Inorganic Zn@30 ppm; T7:Incorporation of wheat 10kg/100 kg feed; T8: Incorporation of wheat 10kg/100 kg feed+ Nano Zn@30 ppm; T9: Incorporation of wheat 10kg/100 kg feed+ Inorganic Zn@30 ppm.

Table 2: Serum biochemical parameters in chicken during1 14th weeks as affected by zinc supplementation on varietal cereal based diet.

Attributes	Treatments										
	T1	T2	T3	T4	T5	T6	T7	T8	T9	Sem	P value
Glucose (mg/dl)	211.13 ±2.64c	220.63 ±1.38b	216.21 ±2.02bc	216.28 ±1.29bc	229.11 ±0.27a	216.64 ±0.76bc	220.69 ±5.41b	227.58 ±0.51a	216.15 ±0.36bc	1.23	0.000
Total protein (g/dl)	6.53± 0.05	6.53 ±0.06	6.51 ±0.05	6.48 ±0.09	6.48 ±0.03	6.45 ±0.09	6.46 ±0.08	6.45 ±0.09	6.50 ±0.06	0.02	0.988
Albumin (g/dl)	4.17 ±0.07	4.36 ±0.17	4.29 ±0.08	4.32 ±0.14	4.32 ±0.16	4.43 ±0.10	4.64 ±0.11	4.44 ±0.09	4.33 ±0.20	0.04	0.493
Globulin (g/dl)	2.29 ±0.07	2.36 ±0.03	2.31 ±0.16	2.30 ±0.06	2.32 ±0.13	2.33 ±0.06	2.58 ±0.08	2.40 ±0.07	2.40 ±0.18	0.03	0.670
ALT (U/L)	14.73 ±0.65	15.25 ±0.66	14.65 ±0.34	15.03 ±1.06	15.30 ±0.62	15.38 ±0.17	15.50 ±0.17	15.56 ±1.09	14.55 ±0.63	0.2	0.950
Cholesterol (mg/dl)	135.56 ±1.83	135.98 ±1.31	135.87 ±0.87	137.06 ±0.59	135.85 ±0.70	136.18 ±1.40	135.90 ±0.65	136.19 ±1.14	135.23 ±1.55	0.34	0.991
Triglyceride (mg/dl)	117.44 ±0.59	117.41 ±1.09	115.95 ±1.25	116.20 ±0.11	117.10 ±2.41	116.35 ±0.27	117.04 ±1.17	115.64 ±0.43	116.17 ±1.38	0.35	0.947
HDL (mg/dl)	57.49 ±0.69	57.86 ±0.26	57.11 ±0.47	57.92 ±0.81	58.28 ±1.43	56.22 ±1.35	56.78 ±1.94	57.90 ±0.83	57.50 ±150	0.34	0.951
LDL (mg/dl)	91.89 ±0.26	91.54 ±0.61	92.91 ±0.38	92.51 ±0.39	92.00 ±1.19	92.78 ±0.82	92.66 ±0.09	92.42 ±0.19	92.66 ±1.90	0.33	0.49
VLDL (mg/dl)	23.48 ±0.12	23.48 ±0.22	23.19 ±0.25	23.24 ±0.02	23.42 ±0.4	23.27 ±0.05	24.11 ±0.23	23.12 ±0.09	23.23 ±0.28	0.07	0.947
Creatine (mg/dl)	0.65 ±0.05	0.62 ±0.02	0.65 ±0.03	0.67 ±0.02	0.66 ±0.02	0.67 ±0.03	0.69 ±0.03	0.67 ±0.03	0.66 ±0.03	0.01	0.884

and T5; T1 and T8; T2 and T5; T2 and T8 but no significant difference was found between T1, T3, T4, T6 and T9 groups and also between T5 and T8.

High glucose level found in the present study is in agreement with Mishra *et al.* (2014). The results indicated no significant difference in the values of haematological parameters *viz.* Hb, PCV, TEC, TLC, MCV, MCHC and MCH which are in agreement with Fathi *et al.* (2016)and Hidayat *et al.* (2021) and Adesua and Onibi (2014). The results indicated no significant difference in the values of total protein, albumin, globulin, ALT, cholesterol, triglyceride, HDL and LDL, VLDL, creatinine levels and this is in corroboration with the findings of Sahoo *et al.* (2014); Mishra *et al.* (2014); Abdel-Monem *et al.* (2021); Uyanik *et al.* (2001) and El-Shenawy *et al.* (2022).

Pathological studies

Gross Pathological studies

The grading and comparison of gross lesions of different groups of experimental birds are given in Table 3. Liver of birds of groups T1, T4, T5, T6, T7, T8 and T9 did not reveal any gross lesions while liver of group T2 and T3 were pinkish in discoloration. The lesions were more severe in group T2 than group T3. Kidneys of birds of groups T1, T2, T4, T5, T7, T8 and T9 did not reveal any gross lesions while kidneys of group T3 and T6 were pinkish in discoloration. The lesions were more severe in group T6 than group T3. Intestine and spleen of birds of any of the treatment groups did not reveal any gross lesion.

Histopathological studies

The grading and comparison of histopathological lesions of different groups of birds of experiment are given in Table 4.

Liver

Liver of birds of groups T1 and T4 did not show any lesion. Liver of birds of group supplemented with nano Zn (T2) showed marked congestion of large blood vessels (Plate 1) throughout the parenchyma. There was presence of necrotic foci at few places along with mononuclear cells (MNCs) infiltration (Plate 2). At few places there was presence of fibrous connective tissue and MNCs infiltration around the blood vessels. Pseudo bile duct was also present around some of the blood vessels (Plate 1). Similar lesions were seen in the liver of birds of other groups as observed in the group T2 but there was variation in the intensity of these lesions. In group T3, these lesions were less severe than groups T2. In groups T5 and T9, these lesions were of similar intensity but lesser than the intensity

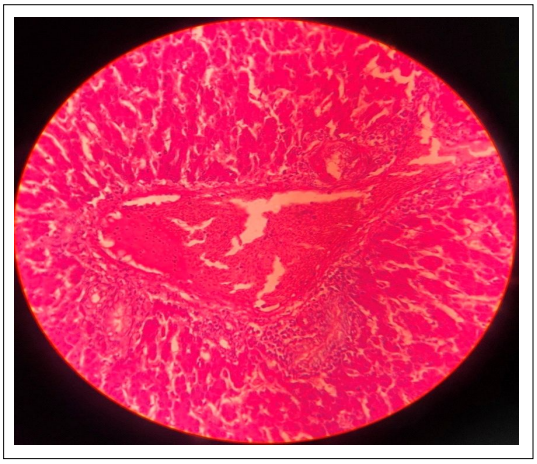

Plate 1: Liver showing severe congestion of large blood vessel and presence of pseudobile ducts around the vessel (Group T2, HE, × 400).

Table 3: Gross lesion grading in different organs of RIR chicken of different experimental groups.

Organ \ Treatment	T1	T2	T3	T4	T5	T6	T7	T8	T9
Liver	-	+++	++	-	-	-	-	-	-
Kidney	-	-	++	-	-	+++	-	-	-
Intestine	-	-	-	-	-	-	-	-	-
Spleen	-	-	-	-	-	-	-	-	-

-Nochanges. +++Severe
+Mild.
++Moderate.

Table 4: Histopathological lesionscore indifferent organs of RIR chickens of different experimental groups.

Organ \ Treatment	T1	T2	T3	T4	T5	T6	T7	T8	T9
Liver	-	++++	+++	+	++	+	+	+	++
Kidney	-	++	+++	+++	+	++++	+	++	++
Intestine	-	++++	+/-	+/-	+++	++	-	++	++
Spleen	-	-	-	-	-	-	-	-	-

-No changes; +++Severe; +Mild; ++++Very severe; ++Moderate.

of group T2 and T3. In groups T6, T7 and T8, these lesions were of similar intensity but lesser than the intensity of group T2, T3, T5 and T9. In this study, liver of birds of group supplemented with nano Zn (T2) showed marked congestion of large blood vessels, presence of necrotic foci along with mononuclear cells (MNCs) infiltration, presence of fibrous connective tissue and MNCs infiltration around the blood vessels and presence of pseudo bile duct.

Kidney

Kidney of birds of groups T1 and T5 did not reveal any histopathological lesions. Severest kidney lesions were seen in group T6. Lesions in this group were severe congestion of large blood vessels (Plate 3) throughout the parenchyma, interstitial haemorrhages at several places (Plate 4), congestion of glomeruli throughout the parenchyma at majority of places. Degeneration and necrosis of tubular epithelial cells at many places throughout the parenchyma (Plate 4). Similar lesions were seen in the kidney of birds of other groups as observed in the group T6 but there was variation in the intensity of these lesions. Compared to group T6, these lesions were less severe in group T3 followed by groups T4, T2, T8, T9 and the mildest lesions were recorded in group T7. In addition to the above-mentioned lesions, there was swelling of glomeruli at many places in groups T2, T3 and T4. While in group T8 and T9, mild to moderate shrinkage of glomeruli was observed in addition to the lesions observed in group T6.Kidney of birds of group T5 did not show any pathological lesion.

Intestine

Intestine of birds of groups T1 and T7 did not reveal any histopathological lesions. Severest lesions were seen in

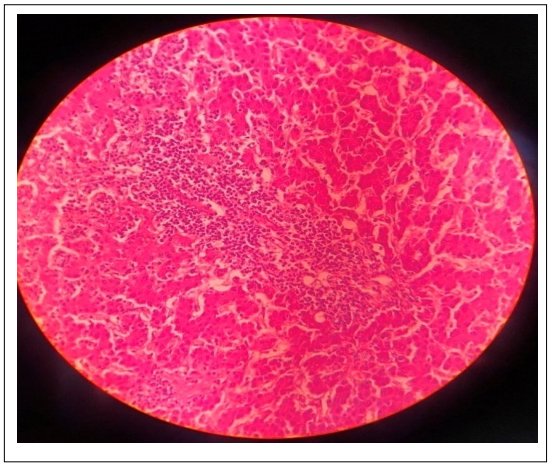

Plate 2: Liver depicting presence of necrotic foci with mononuclear cells (MNCs) infiltration (Group T2, HE, × 400).

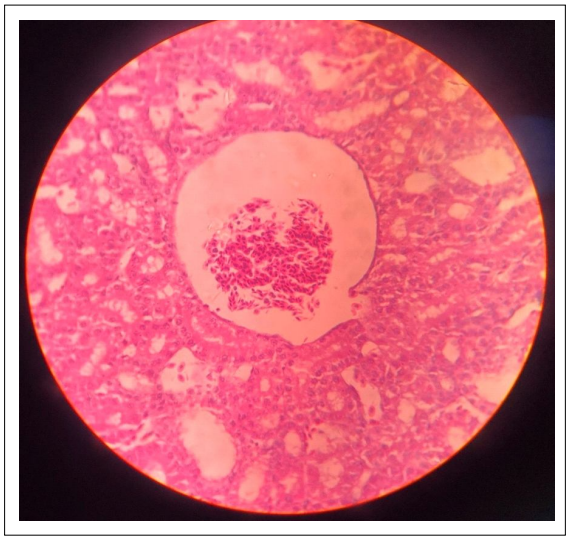

Plate 3: Kidney showing congestion of large blood vessel (Group T6, HE, × 100).

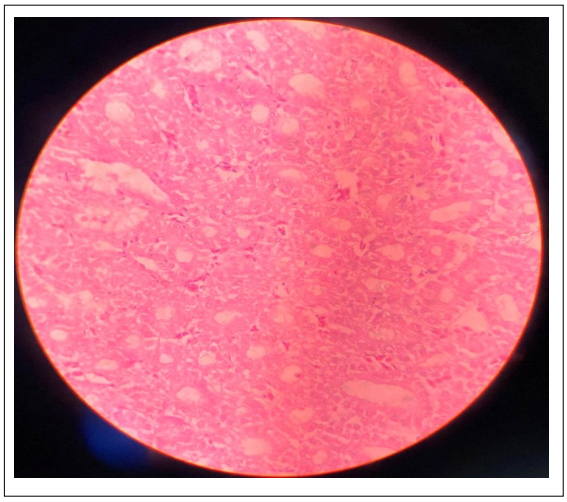

Plate 4: Kidney depicting interstitial hemorrhages and degeneration and necrosis of kidney tubular epithelial cells in majority of tubules (Group T6, HE, × 400).

Plate 5: Intestine showing severe increase in length of villi (Group T2, HE, × 100).

group T2. Lesions in this group were severe increase in length (Plate 5) as well as thickening of many villi throughout the length of intestine (Plate 6). Severe increase in number of goblet cells throughout the length of villi (Plate 7) was noted. This change was seen throughout the length of intestine, atrophy of intestinal villi at places, presence of lymphoid follicles in the mucosa of some of the villi (Plate 8) and infiltration of mononuclear cells in the mucosa of intestine. In groups T3 and T4, the only lesion that could be recorded was damage to the tip of the villi at few places and slight increase in number of intestinal villi respectively. Similar lesions like fusion of villi as well as increase in number of goblet cells were observed in group T5, T6, T8 and T9 but severity was lesser compared to group T2. Intestine of bird of group T7 did not reveal any histopathological lesions. Similar results were recorded by Omar *et al.* (2016). Severest intestine lesions were seen in group T2. Lesions were severe increase in length as well as fusion of villi throughout the length of intestine. There was recorded severe increase in number of goblet cells in the villi. Atrophy of intestinal villi at placeswas also observed. There was also presence of lymphoid follicles in the mucosa of some of the villi.

Spleen

Spleen tissue in all treatment groups showed non-significant histopathological changes. There were no degenerative or necrotic changes throughout the spleen tissue. Spleen in all the treatment groups did not reveal any histopathological changes.

The histopathological lesions observed, in the liver; in the present study align with that of Radi *et al.* (2021**).** Liver lesions were less severe in the zinc oxide-administered group (T3), which could be attributed to potentially greater toxicity of zinc oxide nanoparticles (ZnONPs) compared to zinc oxide (ZnO). The solubility of ZnONPs, leading to elevated intracellular Zn ion concentrations, may contribute to their heightened toxic effects (Saman *et al.,* 2013) and inflammation in various organs (Hatab *et al,* 2022) in thegroups (T2, T5 and T8). Group T5 exhibited milder lesions, possibly due to black wheat's antioxidant properties. Similar mitigated findings were observed in T6 and T9, attributed to the antioxidant characteristics of black wheat or wheat cultivar combined with ZnO or ZnONPs. Conversely, group T4 showed no lesions, potentially due to the enhanced nutritional value of black wheat. These findings align with Kumari *et al.* (2020) who highlighted black wheat's antioxidative effects, enhancing anti-inflammatory activity, serum antioxidant status and reducing lipid peroxidation.

Kidney in group T5 displayed no pathological lesions. Similarresult was observed by Hatab *et al.* (2022). This protection could be attributed to the supportive role of black wheat supplementation. The most severe kidney lesions were evident in T6, possibly due to lower intracellular zinc oxide levels compared to nano zinc oxide particles. These lesions were less severe in T3 and T9, possibly due to

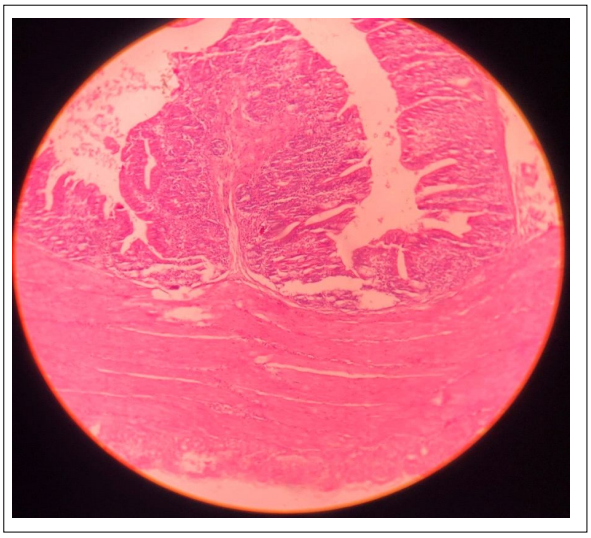

Plate 6: Intestine showing thickening of villi (Group T2, HE, × 100).

Plate 7: Intestine showing increase in number of goblet cells throughout the length of villi (Group T2, HE, × 100).

Plate 8: Intestine showing presence of lymphoid follicles in the mucosa of (Group T2, HE, × 100).

similar mechanism. Supplementation of nano zinc in T2 and T8 resulted in mild kidney lesions. Similar observation was found by AbdAlaziz and Albaker (2021). Group T4 exhibited the mildest kidney lesions, potentially due to beneficial effects of black wheat, which are in line with Dhua *et al.* (2021).

Conversely, the most severe intestine lesions were identified in T2, characterized by increased villus length, villus fusion, elevated goblet cell count, villus atrophy and mucosal lymphoid follicles. This agrees with finding of Hatab *et al.* (2022). Group T3 and T4 exhibited milder intestine lesions which are supported by the findings of Qu *et al.* (2023) suggesting potential toxic effects. Comparable outcomes were seen in T5 and T8, where ZnONPs were supplemented alongside black wheat or wheat cultivar, although to a lesser extent. Group T4 exhibited mild increase in intestinal villi, which might be due to protective impact of black wheat. In T6 and T9, the increased rate of intestinal passage might have contributed to the observed outcomes. No histopathological alterations were observed in spleen tissues across all treatment groups which are inagreement with El-Shenway *et al.* (2022).

CONCLUSION

It is concluded that **s**upplementation of zinc from different sources with/without different cultivar of wheat affects blood chemistry and pathological condition in RIR birds.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarilyrepresent the views of their affiliated institutions. The authors are responsible for the accuracy andcompleteness of the information provided, but do not accept any liability for any direct or indirect lossesresulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal careand handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. Nofunding or sponsorship influenced the design of the study, data collection, analysis, decision to publish,or preparation of the manuscript.

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