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Supplementation effect of zinc on varietal different cereal based diet on haemato biochemical parameters and egg composition of Rhode Island Red chicken

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Abstract
New advances in poultry sector like use of nanoparticle in diet highlights beneficial effect on poultry health. The present study was conducted to assess the effect of supplementation of zinc with different sources and wheat on haemato biochemical and egg quality parameters of Rhode Island Red (RIR) chicken. In total 270 RIR birds were selected, randomly divided into nine treatment groups. Treatments included T_1: control, T_2: Standard diet + nano Zn @ 30 ppm, T_3: Standard diet + Inorganic zinc @ 30 ppm, T_4: Standard diet + incorporation of black wheat (10 kg/100 kg of feed), T_5: Standard diet + incorporation of black wheat (10 kg/100 kg of feed) + Nano zinc @ 30 ppm, T_6: Standard diet + incorporation of black wheat (10 kg/100 kg of feed) + inorganic zinc @ 30 ppm, T_7: Standard diet + incorporation of wheat cultivar (10 kg/100 kg of feed), T_8: Standard diet + incorporation of wheat cultivar (10 kg/100 kg of feed) + Nano zinc @ 30 ppm and T_9: Standard diet + incorporation of wheat cultivar (10 kg/100 kg of feed) + inorganic zinc @ 30 ppm. Haemoglobin and MCHC were significantly higher ($p<0.05$) in T_5, T_6 and T_8 treatment groups. Treatment groups T_4, T_5 and T_6 had significantly higher ($p<0.05$) total protein. Serum cholesterol and triglycerides were significantly lowered in T_2, T_4, T_5 and T_8 treatment groups. Egg protein was significantly higher ($p<0.05$) in T_4, T_5 and T_6 group compared to other treatment groups. Egg cholesterol was lowered in T_2, T_4, T_5 and T_8 treatment group. It was concluded that incorporation of black wheat along with Zn supplementation could exert beneficial effect on RIR chicken.

Keywords: Black wheat, Inorganic zinc, Nano zinc, Rhode Island Red layer

Introduction
In India, poultry is one of the quickest growing sectors which gave fine outputs to the farmers. According to the 20th Livestock Census, there are currently 851.81 million chickens in our nation, and in the years 2020–21, 122.05 billion eggs were produced. In 2020–21, there were around 90 eggs available per person (Annual Report DAHD, 2020–21). Previously, all chicken farm activities were performed manually but with passage of time new technologies were introduced like nanotechnology (Ben *et al.*, 2016) [6] which helped in increasing egg production.
Zinc is one of the essential microelements for regular animal function, including physical development of muscles and growth, as well as reproduction and the production of eggs. Zinc plays an important role in cell division and the production of healthy sperm. This element is also needed for progesterone synthesis, and its deficiency can excessively increase the prolactin secretion. Additionally, heavy Zn supplementation may reduce the stability of vitamins and other minerals and alter the balance of other trace elements in the body. It is well demonstrated that trace elements can affect eggshell quality either by their catalytic properties as key enzymes involved in the process of membrane and eggshell formation or by interacting directly with the calcite crystals in the forming of eggshells. Zinc plays an important role in carbonic anhydrase enzymatic reaction. Deficiency of this enzyme can lead to a reduction of the bicarbonate ion secretion, which eventually causes reduced eggshell quality. Mabe *et al.* (2003) [19] reported that trace elements like Zn, Mn, and Cu may have an impact on the mechanical properties of eggshells. It shows impact on eggshell by altering the production of calcite crystals and altering the crystallographic structure of eggshells.
According to Zhang *et al.* (2001) [31], nanoparticles exhibit new traits such high surface activity, large specific surface area, high catalytic efficiency, and strong adsorption capacity. The chicken sector may undoubtedly benefit from the prospective uses of nanoparticles (NPs)

for improving meat quality and quantity while lowering disease burden (Anwar *et al.*, 2019) [3]. As a result, novel nanoscale processes and characteristics may have useful future uses. The first nanoscale material utilized in commercial and industrial products was manufactured zinc oxide nanoparticles (ZnO-NPs) (Tan *et al.*, 2013) [30]. ZnO-NPs have more chemical activity as compared to ZnO and can oxidize a range of organic substances. Additionally, ZnO-NPs' permeability can enhance drug absorption and minimize unfavourable gastrointestinal effects (Lucas, 2010) [18].

The whole grain cereals inclusion in the poultry feed help in reducing feed cost. In recent years, the practice of giving whole compound feed to poultry has changed in several locations in recent years. For small-scale poultry production, feeding whole grains like sorghum or maize along with a protein concentrate has an apparent financial benefit because it eliminates the need for grinding (Cumming, 1992) [9]. Feeding entire grains of cereal has typically been done in broiler production using wheat. Inclusion of wheat grain with appropriate enzymatic combination could enhance the chicken performance (Boros *et al.*, 2004) [8]. Black wheat was created by standard plant breeding, a method of altering a plant's genetic makeup to make it more useful to people (Kumari and Tzudir, 2021) [16]. Black wheat was developed in India at NABI, Mohali, using exotic material (EC866732) that was acquired from Japan and crossed with a typical high yielding and disease-resistant wheat cultivar (PBW621). Compared to white wheat, black wheat has higher concentrations of anthocyanin and other phenolic chemicals. According to Li *et al.* (2006) [17], black grain wheat meal had a higher protein content (17.71%) and a lower gluten index than white wheat strains. The use of nano zinc supplementation in layer birds have limited studies. Additionally, there was no evidence of study that black wheat was used in poultry feed. Therefore, the objective of this study was to evaluate the effect of zinc with different cereal based diet on the haemato biochemical parameters and egg quality traits in layer birds.

Materials and Methods

Birds, Design and Management

The experiment was performed at the Instructional Poultry Farm, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar, Uttarakhand. All procedures on birds were approved by the Institutional Animal Ethics Committee (IAEC) and CPCSEA, New Delhi (IAEC/CVASc/ANN/468). Two hundred and seventy Rhode Island Red (RIR) birds were randomly allocated to 9 dietary treatments of three replicates of each group in a factorial arrangement (3 X 9). The given treatment groups were: T_1-standard diet, T_2- T_1+Nano zinc @ 30 ppm, T_3- T_1+inorganic zinc @ 30 ppm, T_4- Standard diet + incorporation of black wheat (10 kg/100 kg of feed), T_5- T_4+ Nano zinc @ 30 ppm, T_6- T_4+ inorganic zinc @ 30 ppm, T_7- standard diet + incorporation of wheat cultivar (10 kg/100 kg of feed), T_8-T_7+ Nano zinc @ 30 ppm and T_9- T_7+ inorganic zinc @ 30 ppm. The nano ZnO was procured from Sigma Alorich having <100 nm particle size. Inorganic ZnO was procured from Glasil having molecular weight 81.39. Black wheat was procured from local farmers and wheat cultivar was supplied from University farm. All the layers were kept under standard and uniform management conditions throughout the experimental period. All the layers were offered *ad-libitum* feed and water throughout experimental trial period. 18 hours light period was provided to them. The birds were vaccinated as per the routine vaccination schedule and dose. Standard basal layer diet was prepared by mixing the ingredients to meet the nutrient requirements as per recommendations of BIS (2007) [7] and shown in Table No. 1

Table 1: Composition of Standard diet

Ingredients	Content (%)
Yellow Maize	57
Deoiled rice bran	6.5
Groundnut cake-solvent extracted	09
Rice polish	4.5
Soyabean meal	18
Dicalcium Phosphate	01
Marble powder	03
DL- methionine	0.15
Choline Chloride	0.10
Hepatocare	0.10
Mineral mixture	0.10
Common salt	0.40
Vitamin Premix	0.10
Toxin binder	0.05
Total	100.00

Haemato biochemical parameter

At the end of the feeding trial, two birds per replicate (six birds per treatment) were selected. Approximately 2 ml of blood sample was collected into the EDTA coated vial for analysis of haematological parameters. Furthermore, approx. 3 ml blood sample was collected into plain vials (without anticoagulant) from the wing vein. Plain vials were kept at room temperature in slanting position for 3 to 4 hours to drawn the clotting of blood. After clotting of blood, serum samples were separated by centrifugation at 3000 rpm for 10-15 min which was collected into Eppendorf tubes and stored at -20 °C in a deep freeze with date and sample number until biochemical estimations were completed. Haemato biochemical parameters like Haemoglobin, Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Glucose, Total protein, Cholesterol and Triglycerides were evaluated. Hb (by cyanomethaemoglobin method; Drabkin and Austin, 1932), PCV (by microhematocrit method; Martha *et al.*2012) and TEC (by hemocytometer method; Natt and Herrick 1952) were analysed using their respective standard methods. Serum biochemical parameters, serum glucose, total protein, choles were analysed by using a standard kit supplied by ERBA Mannheim diagnostic centre, following the manufacturer's instructions.

Egg Composition

All eggs produced during the last week of the experiment were collected. The collected eggs were broken gently by using a scalpel and its contents were taken on the glass plate. The egg content was filtered through filter paper and yolk was separated from albumen. The known weight of yolk was taken in petridish and kept in hot air oven at 70±2°C for 48 hours to obtain dried egg components which were mixed together and dried (as whole). The dried yolk samples along with other egg components were grounded and kept in moisture free bags for further chemical analysis to access protein, fat and total ash. The egg composition was accessed by following standard procedure of AOAC (2000) [4]. Egg yolk cholesterol was estimated by following a method described by Folch *et al.* (1957) [14].

Statistical analysis

Statistical analysis of the data obtained during the experiment was conducted using the appropriate method of analysis described by Snedecor and Cochran (1994) [28] and the IBM SPSS Statistics 22 software package. The variance ratio (F-values) was found to be significant at a probability of 5%. Duncan's New Multiple Range Test (Duncan's Range Test) as modified by Kramer (1957) [33] was used to assess the significance of the mean differences.

Results and Discussions

The effect of supplementation of zinc with different sources and wheat on haemato biochemical and egg quality parameters of RIR layer birds are summarized in Table No. 2 and 3, respectively.

Table 2: Effect of supplementation of zinc with different sources and wheat on haemato biochemical parameters in RIR layers

Parameters/ Treatment	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
Haemoglobin%	8.49^{c}±0.31	9.08^{ab}±0.37	8.47^{c}±0.24	10.15^{b}±0.09	12.29^{a}±0.62	12.19^{a}±0.34	8.83^{c}±0.36	11.99^{a}±0.46	8.86^{c}±0.28
PCV	30.47±0.68	29.03±1.92	31.72±1.16	29.95±0.38	29.27±0.83	31.48±0.37	29.88±0.66	31.97±0.81	31.83±0.86
TEC 10^6 (U/L)	3.24±0.30	2.90±0.35	3.33±0.25	3.15±0.17	3.03±0.19	2.99±0.16	2.11±1.07	2.87±0.21	3.31±0.51
MCV (fl)	120.11±1.06	118.58±1.38	118.78±1.95	122.97±1.41	118.69±0.93	117.86±1.44	117.87±1.85	116.08±3.48	119.59±4.44
MCH (pg)	30.06^{c}±1.24	32.10^{bc}±1.03	29.80^{c}±0.47	32.36^{bc}±0.89	36.03^{a}±1.70	34.51^{ab}±0.55	31.41^{bc}±0.86	35.93^{a}±1.53	30.63^{c}±0.79
MCHC %	25.38^{c}±0.36	27.97^{b}±0.59	26.48^{bc}±0.85	28.43^{b}±0.70	30.67^{a}±0.50	32.28^{a}±0.80	25.12^{c}±0.69	31.73^{a}±0.68	24.80^{c}±0.54
Glucose (mg/dl)	218.13±2.12	208.47±3.57	209.24±1.07	209.64±6.52	215.24±8.03	211.71±3.28	203.08±8.76	206.46±5.53	204.61±7.54
Total Protein (g/dl)	4.87^{c}±0.33	4.72^{c}±0.15	5.16^{bc}±0.17	6.01^{a}±0.06	6.01^{a}±0.16	5.58^{b}±0.14	4.59^{c}±0.30	5.19^{bc}±0.32	4.86^{c}±0.07
Cholesterol	162.66^{abc}±1.85	156.39^{cde}±2.63	164.25^{ab}±2.94	145.82^{f}±2.36	150.62^{def}±0.35	159.50^{bc}±2.00	168.14^{a}±2.04	149.86^{ef}±1.44	157.83^{bcd}±4.01
Triglyceride	243.35^{a}±6.66	184.39^{c}±3.97	236.62^{a}±6.01	209.75^{b}±3.91	195.55^{bc}±3.18	240.63^{a}±8.45	228.11^{a}±6.53	208.03^{b}±2.93	239.22^{a}±5.31

The haematological parameters like PCV, TEC and MCV did not reveal any statistical difference among the different treatment groups. Similar findings were observed by Mishra *et al.* (2014) [21] reported that PCV concentration in layer chicks supplemented with nano zinc and inorganic zinc. But, contradictory results were observed about Hb, glucose, total protein, cholesterol and triglycerides. But, mean values of Hb, MCH and MCHC% were significantly different (p<0.05) among different treatment groups. Also, findings by Srivastav *et al.* (2023) [29] observed similar as they concluded that PCV and total erythrocyte count was similar in all groups including nano zinc supplemented group in kadaknath chickens. Our results are contrary to Dosoky *et al.*, (2022) [10] who reported that PCV and total erythrocyte count was significantly (p≤0.05) affected by zinc oxide nano particles supplementation. The mean values of Hb (%) was 8.49±0.31, 9.08±0.37, 8.47±0.24, 10.15±0.09, 12.29±0.62, 12.19±0.34, 8.83±0.36, 11.99±0.46, 8.86±0.28 in T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8 and T_9 treatment groups, respectively. While mean values of MCH and MCHC (%) were 30.06±1.24 and 25.38±0.36 in T_1, 32.10±1.03 and 27.97±0.59 in T_2, 29.80±0.47 and 26.48±0.85 in T3, 32.36±0.89 and 28.43±0.70 in T_4, 36.03±1.70 and 30.67±0.50 in T_5, 34.51±0.55 and 32.28±0.80 in T_6, 31.41±0.86 and 25.12±.69 in T_7, 35.93±1.15 and 31.73±0.68 in T_8, 30.63±0.79 and 24.80±0.54 in T_9, during the feeding trial, respectively. Similar results shown by Ismail *et al.* (2016) [15] in which concentrations of blood haemoglobin for hens fed diets supplemented with inorganic, organic and Nano forms of zinc were significantly higher (p≤0.05) compared to control. In a same manner, Sizova *et al.*, (2021) [27] reported that the supplementation of nano zinc at 21, 28 and 35 days in birds improved haemoglobin concentration. Ayoub *et al*. (2020) found similar results in case of both TEC as well as Hb concentration in control and nano zinc supplemented group of broiler chickens.

Serum total protein was significantly improved (p<0.05) in black wheat supplemented groups compared to other treatment groups. Blood glucose was statistically similar in different treatment groups. Salgueiro *et al.* (2001) [26] reported that there is a close connection between the content of Zn, insulin activity, and glucose metabolism. Thus, supplementation of zinc did not reveal any changes in serum glucose concentration. Abedini *et al.* (2018) [1] observed similar result as they found total protein concentration was increased over supplementation of Zn-methionine and ZnO nanoparticles in laying hens. However similar result was also found by earlier author regarding serum glucose concentration. The results were aggred with Sahin *et al.* (2005) [25] as they found the serum total protein concentration of birds increased when their diet was supplemented with ZnSO4. However, Prasad (2014) [24] reported that Zn deficiency may cause abnormalities in nucleic acid synthesis and the activity of many enzymes. Therefore, the higher serum proteins in the groups receiving Zn could be due to the role of Zn in protein synthesis. Serum cholesterol and triglycerides were significantly lowered in T_2, T_4, T_5 and T_8 treatment groups compared to other treatment groups. El-Katcha *et al.* (2018) [12] found dissimilar results as they found serum glucose, total protein, cholesterol and triglyceride concentrations were non-significant in layer birds which is supplemented with nano zinc, organic zinc and inorganic zinc.

Table 3: Effect of supplementation of zinc with different sources and wheat on egg composition in RIR layers

Attributes	Treatments								
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
Dry matter	25.33±0.19	25.83±0.34	26.11±0.58	26.39±0.38	25.48±0.33	26.24±0.56	25.94±0.09	25.67±0.36	25.62±0.35
Crude protein	43.77^{c}±0.65	44.41^{c}±1.16	45.10^{c}±1.01	49.27^{a}±0.33	48.39^{ab}±0.37	48.64^{ab}±0.87	43.62^{c}±1.24	46.06^{b}±0.42	44.52^{c}±1.77
Ether extract	40.03±0.31	38.72±0.72	37.63±1.76	37.17±0.88	39.28±0.42	38.21±1.32	40.14±0.38	37.90±1.29	38.38±1.94
Total ash	5.02±0.05	5.62±0.28	5.15±0.89	5.51±0.45	4.83±0.24	5.77±0.17	5.23±0.56	6.25±0.32	5.85±0.16
Cholesterol	16.62^{ab}±0.64	14.59^{bc}±0.70	16.63^{ab}±0.35	14.02^{c}±1.14	12.87^{c}±0.56	17.90^{a}±0.45	16.95^{ab}±0.54	13.01^{c}±1.24	16.47^{ab}±0.47

Egg composition parameters like dry matter, ether extract and total ash were similar in different groups. But, crude protein and egg cholesterol were significantly different in different treatment groups. The crude protein was improved in black wheat supplemented group while egg cholesterol decreased in the same group. Fawaz *et al*. (2019) [13] observed mixed results as they found that dry matter, crude protein and ether extract contents were non-significant in different sources of zinc supplemented group. Similar finding observed by Zhao *et al*., (2016) [32] which concluded that the egg protein and water contents were not affected in layer fed diet with Nano-ZnO at different doses. The above mention results could be due to synergistic action of black wheat and nano zinc supplementation in layer feed diet which resulted in improvement of crude protein and lowered cholesterol in eggs. Limited systematic research work is available regarding this aspect, this hides the physiological role of supplementation on egg composition.

Conclusion

In conclusion, Nano zinc supplementation with incorporation of black wheat in layer standard diet could exert better result in terms of health and egg composition. This emphasises the synergistic effect of nano zinc and black wheat as it rich in mineral content and its health beneficial properties.

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