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Effects of dietary supplementation of Nano zinc and Phytase on haematological parameters of Kadaknath layers

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Abstract
The present investigation was carried out to evaluate the effects of dietary incorporation of Nano zinc and phytase enzyme on haematological parameters of Kadaknath layers. For the study, four hundred Kadaknath layers of twenty nine weeks of age, were selected and randomly distributed into ten dietary treatment groups having four replicates in each group with ten birds per replicate. The experimental Kadaknath layers were fed on different levels of Nano zinc and Phytase enzyme either alone or in combination for a period of twenty weeks. The dietary regimens for different treatment groups were as follows: T_1(control)- Basal diet, T_2- Basal diet+30 mg/kg Nano zinc, T_3- Basal diet+40 mg/kg Nano zinc, T_4- Basal diet+50 mg/kg Nano zinc, T_5- Basal diet+60 mg/kg Nano zinc, T_6- Basal diet+ 500 FTU/kg phytase enzyme, T_7- Basal diet+30 mg/kg Nano zinc+ 500 FTU/kg Phytase enzyme, T_8- Basal diet+40 mg/kg Nano zinc+ 500 FTU/kg phytase enzyme, T_9- Basal diet+50 mg/kg Nano zinc+ 500 FTU/kg phytase enzyme, T_{10}- Basal diet+60 mg/kg Nano zinc+ 500 FTU/kg phytase enzyme. At the end of the experiment one bird from each replicate was sacrificed and blood samples were collected in EDTA coated vials at the time of slaughter. The samples were analyzed by standard procedures for the estimation of haematological parameters such as haemoglobin, packed cell volume, total erythrocyte count, total leukocyte count and differential leukocyte count. The observations recorded in the present study revealed no significant differences in the haematological parameters of Kadaknath layers as a result of dietary addition of Nano zinc and Phytase enzyme. Thus the study concluded that Nano zinc and Phytase enzyme can be safely incorporated in the diet of Kadaknath layers up to the level of 60 mg/kg and 500 FTU/kg, respectively.

Keywords: Nano zinc, Phytase, Kadaknath, Haemato-logical parameters

Introduction
The sustained use of native chickens in the traditional poultry production system showed the need to consider the value of native chickens. Native breeds of chickens are playing an important role in rural economies in most of the developing and underdeveloped countries. Indigenous breeds are well known for their tropical adaptability and disease resistance. Regardless of low output from native chicken in the tropics they can thrive and produce with irregular supply of feed and water and with minimum healthcare (Padhi *et al*., 2016) [13]. Kadknath is an Indian native chicken breed which is known to have high nutritional and medicinal value. Kadaknath meat contains more than 25% protein, less fat (0.73-1.03%), less cholesterol (184.75 mg) and can be easily consumed even by heart patients. The low production performance of native chicken breeds may be improved with the use of some technological interventions like Nanotechnology. Nanotechnology is the promising and emerging technology that has tremendous potential to revolutionize agriculture and livestock sectors globally (Gopi *et al*., 2017) [8]. Supplementation of poultry with Nano minerals in different forms seems promising on the performance, health and immunity as well as reducing pathogen gut load. Zinc is an essential trace element as it plays an important part in growth performance, carcass traits, egg production and egg quality in poultry. In order to find low-cost alternatives, researchers have focused on Nano zinc sources in the animal feed industry (Lee *et al.*, 2017) [11]. Nanoparticles of zinc oxide have attracted more attention recently, primarily on account of their small particle size and increased surface reactivity (Yusof *et al*., 2019) [17]. It can act as antibacterial, antioxidant and improve reproduction of the poultry. The commonly used grains in basal laying hen diets are rich in phytate content that may reduce availability and inhibit absorption of zinc. Phytase is an enzyme that initiates phosphate removal of from phytate and it has been widely used in animal feeding specifically in the poultry.

Supplementation of phytase into the diet of poultry has great impact to the improvement of poultry immune systems and growth performance. Interestingly, phytase is able to improve both quantity and quality of eggs in laying hens (Gao *et al*., 2013).

Material and Methods

A total of 400 Kadaknath layers of 29 weeks of age were selected on body weight basis and reared on deep litter system at poultry farm, CVAS, Bikaner. The birds were randomly assigned to ten different dietary treatment groups. Each treatment group was further subdivided in to four replicates having ten birds in each replicate. Commercially available readymade layer feed in mesh form was used for feeding of experimental Kadaknath birds during the feeding trial. The experimental feed of different treatment groups was supplemented with different levels of zinc oxide Nano powder and phytase enzyme either alone or in combination to each other *viz*. T_1(control)- Basal diet, T_2- Basal diet+30 mg/kg Nano zinc, T_3- Basal diet+40 mg/kg Nano zinc, T_4- Basal diet+50 mg/kg Nano zinc, T_5- Basal diet+60 mg/kg Nano zinc, T_6- Basal diet+ 500 FTU/kg phytase enzyme, T_7- Basal diet+30 mg/kg Nano zinc+ 500 FTU/kg phytase enzyme, T_8- Basal diet+40 mg/kg Nano zinc+ 500 FTU/kg phytase enzyme, T_9- Basal diet+50 mg/kg Nano zinc+ 500 FTU/kg phytase enzyme, T_{10}- Basal diet+60 mg/kg Nano zinc+ 500 FTU/kg phytase enzyme. Experimental Kadaknath layers were reared on deep litter system under standard managemental conditions and fed on different treatment rations for a period of 20 weeks. At the end of the experiment blood samples were collected from experimental birds at the time of slaughter by serving the juglar vein. Blood samples were collected into the EDTA coated tubes for haematological study. Haemoglobin and PCV were determined by Sahli-Hellige haemoglobinometer and microhaematocrit method, respectively. Total erythrocyte count (TEC) and total leukocytes count (TLC) was carried out manually through haemocyometer as per standard method of Benjamin (1978) [3]. Differential leucocyte count (DLC) was carried out as per the standard method described by Jain (1986) [10] and expressed as per cent. The data obtained during the experimental period were analyzed statistically with the help of SPSS software and the results were interpreted.

Results and Discussion

Mean values of haemato-logical parameters including haemoglobin, packed cell volume (PCV), total erythrocytes count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC) of Kadaknath layers have been presented in Table 1. The study reported non significant (p>0.05) effect of Nano zinc and phytase supplementation on all the hemato-logical parameters of Kadaknath layers. The hematological parameters of Kadaknath layers in all the treatment groups lie within the normal range indicating no adverse effect on general health condition due to Nano zinc and phytase enzyme supplementation.

The results of the present study fall in line with the previous investigations conducted by Mishra *et al*., (2014) [12] who reported non significant changes in haemoglobin and PCV of layer chicks supplemented with Nano zinc. Similarly, Fathi *et al*., (2016) [7] observed that the blood parameters including haemoglobin, total erythrocyte count and leukocyte count had not significantly affected by Nano zinc oxide supplementation. El-Hack *et al*., (2021) [5] reported non significant changes in total erythrocyte count, leukocyte count, neutrophils, monocytes and basophiles due to supplementation of zinc oxide Nano particles. In addition, El-Shenawy *et al*., (2022) [6] reported no significant differences in haemoglobin, PCV, RBC and WBC count of broiler chicks by the replacement of organic zinc oxide with Nano zinc oxide. However, significant increased values of haemoglobin, PCV, lymphocytes and eosinophils were recorded in treatment group. According to Reda *et al*., (2021) [14] no significant differences were observed for white blood cell differential counts, including lymphocytes and granulocytes. The same direction was observed for the concentration of haemoglobin and platelet Count. However, the studies reported by Ismail *et al*. (2016) [9] exhibited dissimilarity with the present study as they observed that the concentrations of blood haemoglobin for hens fed diets supplemented with inorganic, organic and Nano forms of zinc were significantly higher (p≤0.05) than those of the controls. Similarly, Sizova *et al*., (2021) reported that the concentration of hemoglobin and the number of red blood cells tended to increase in the experimental group supplemented with Nano zinc at 21, 28 and 35 days of age when compared with the control. Dosoky *et al*., (2022) [4] also reported that the WBC and RBC counts, PCV, lymphocytes (%), heterophils (%), lymphocytes/heterophils ratio, and monocytes were significantly (P≤0.05) affected by zinc oxide Nano particles supplementation.

Regarding phytase supplementation, similar findings were recorded in the previous studies performed by Shehab *et al*., (2012) [15] who showed that heamatological parameters including haemoglobin, PCV, total erythrocyte count and mean corpuscular volume were not significantly affected by dietary supplementation of phytase enzyme. Similarly Al-Harthi *et al*., (2020) [1] indicated that the Haemoglobin, PCV, MCH and MCHV, were not significantly affected by phytase supplementation. On the other hand Baloch *et al*., (2021) [2] observed significant differences in haemoglobin, PCV, RBC and WBC count of broilers fed on dietary phytase enzyme at different levels thus they were in disagreement with the present investigation.

Table 1: Effect of supplementation of Nano-zinc and phytase on haemato-logical parameters of Kadaknath layers

Attributes	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	SEM
Haemoglobin (g/dl)	10.39	10.66	10.20	10.73	10.77	10.00	10.91	10.18	10.72	11.14	0.11
PCV (%)	28.17	30.13	29.06	30.07	29.99	27.39	30.99	28.84	30.50	30.77	0.37
TEC (10^6/cumm)	2.68	2.76	2.73	2.82	2.80	2.65	2.84	2.83	2.85	2.83	0.02
TLC (10^3/cumm)	25.19	24.98	25.74	25.34	25.20	26.01	25.16	25.75	24.91	25.51	0.11
Lymphocytes (%)	65.92	66.31	65.35	66.29	65.98	66.76	65.85	66.22	66.69	66.24	0.13
Monocytes (%)	2.80	2.68	2.93	2.78	2.79	2.91	2.73	2.89	2.50	3.09	0.05
Eosinophiles (%)	3.00	3.07	3.15	2.90	3.20	2.83	2.88	3.01	3.18	2.93	0.04
Basophils (%)	1.91	2.04	1.92	1.94	2.00	2.02	2.02	1.98	1.89	2.05	0.02
Heterophils (%)	25.88	25.82	25.62	25.36	26.17	25.63	25.36	25.77	25.19	25.61	0.09

Conclusion

The outcomes of the present study revealed non significant effect of dietary supplementation of Nano zinc and phytase enzyme on haematological parameters of Kadaknath layers. Thus, the study suggested no adverse effect on health status of experimental Kadaknath layers. It may be concluded that the Nano zinc and phytase enzyme can be used as a supplement in poultry diet up to the level of 60 mg/kg and 500 FTU/kg, respectively.

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