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Evaluating the Effect of Different Sources of Zinc on Egg Production, Egg Quality, and Hatchability Traits in Chickens

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ABSTRACT

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Reports on feeding of zinc not only improved the health status of the chicken but also improved egg production, hatchability, and fertility and quality parameters. Different sources of zinc are available in the market for feeding. The current study aimed to assess the effect of dietary supplementation of inorganic, organic, and nano zinc on egg production, egg quality, and hatchability traits in white Plymouth Rock chickens. A total of 252 wings banded White Plymouth Rock birds of 33 weeks age were randomly assigned in seven groups. The diet was supplemented with inorganic, organic @ 30 and 60 mg/kg while nano zinc @ 15, 30 and 60 mg/kg. Egg production, fertility, hatchability, egg quality parameters changes were recorded at different phases. It was suggestive that a higher level of nano zinc supplementation @ 60mg/kg can be considered as a replacement of both inorganic and organic sources of zinc supplementation with better results.

Introduction

Zinc is a component of the uterine enzyme carbonic anhydrase, which supports eggshell formation (Nys *et al.*, 2011). Zinc indirectly affects the quality of the epithelium due to its role in protein synthesis. The deficiency of Zinc in the diet decreases egg production and increases the chances of reproductive disease conditions. Zinc is a component of Carbonic anhydrase, which is an essential enzyme for eggshell formation. Three different sources of zinc are available for the feed industry *viz* inorganic, organic, and nano zinc. Due to the

higher bioavailability of Zn from nano-zinc than inorganic and organic sources, they have better absorption than the other two sources resulting in better growth Pathak *et al.*, (2020). Nano zinc (nZn) represents one of the nanotechnological approaches to prepare mineral salt having a particle size of 1 to 100nm Feng *et al.*, (2009). Keeping these findings in mind, a study was framed to assess the effect of dietary supplementation of inorganic, organic, and nano zinc on egg production, egg quality, and hatchability traits in White Plymouth Rock chickens.

Materials and Methods

A total of 252 wings banded White Plymouth Rock birds of 33 weeks age were randomly assigned to seven groups with four replicates in each group having 09 birds in each replicate (36 birds per treatment) in a deep litter system with all standard managerial practices were used for the study up to 53 weeks of age. The duration was divided into four phases viz. Phase I from 33rd to 37th week, Phase II 38th to 42nd week, Phase III 43rd to 47th week, Phase IV 48th to 52nd week. Birds were immunized as per the standard vaccination schedule and were fed with layer mash with one of the experimental diets. The experimental diets (mash form) were formulated as per BIS (2007) specifications. The ingredient composition, calculated nutrient values, and analyzed nutrient values of the basal (Table 1). Experimental feed samples were analyzed for the proximate composition according to the AOAC (2012) with a Zinc concentration of 30 ppm by atomic absorption spectrophotometry (ICP-OES; Perkin Elmer Optima 8000) in the basal diet. Dietary additions of Zn were made with Zn being provided with one of the following seven diets. To formulate T₁ and T₂, zinc sulphate at 30 and 60 mg/kg of diet respectively was added. To formulate T₃ and T₄, zinc methionine at 30 and 60 mg/kg of diet respectively. For T₅, T₆, and T₇, nano zinc was added to the diet to contain 15mg, 30mg, and 60mg levels of zinc respectively. Each bird received 125g/day at the beginning of the trial and the feed allocation was gradually increased as the egg production increased. Based on the number of eggs produced in each phase, the hen housed egg production (HHEP) and hen day egg production (HDEP) were calculated. Eggs collected from the experimental hens were tested for their fertility as well as hatchability percent set during each period. Eggs collected during 37th, 42nd, 47th, and 52nd week were analyzed

for egg quality parameters. Two eggs from each replicate and eight eggs from each treatment group were taken for the study. The eggs collected for quality analysis were weighed, measured, and broken on the same day to assess the following external and internal quality parameters. The experiment was conducted upon approval of Institutional Ethics Approval Committee guidelines. Data on various parameters obtained during the trial were analyzed statistically by ANOVA using SPSS 20 statistical software. Differences between the means were tested using Duncan's Multiple Range Test Duncan (1995) at $P < 0.05$.

Results and Discussion

Egg production

During the phase, I, the HHEP percent, and HDEP percent significantly ($P \leq 0.05$) differed among different treatment groups. HHEP ranged from 71.34 percent in the T₁ group to 83.01 percent in T₇ (Nano zinc, 60 mg/kg). HHEP was significantly ($P \leq 0.05$) more in all groups when compared to ZnSO₄ groups (Table 2). Among Zn-Met treatment groups, the difference was comparable, the HHEP in T₄ (Zn-Met, 60 mg/kg) was comparable to that of T₅ (Nano zinc, 15 mg/kg). The effect of different treatments on the HDEP percent was similar to that of HHEP. The HDEP ranged from 71.34 percent (T₁) to 83.01 percent in the T₇ group. A significantly ($P \leq 0.05$) higher percent of HDEP was recorded in all groups when compared to the T₁ group. The differences in Zn-Met treated groups were comparable to that of T₅ nano zinc supplemented group T₅ (nano zinc, 15 mg/kg). Among ZnSO₄ groups, the difference was insignificant and was comparable for HDEP percent. The results obtained are under the findings of (Klecker *et al.*, 2002; Fakler *et al.*, 2002) who reported an increase in egg production by supplementing organic zinc.

These results are also similar to the reports of (Amen and Daraji 2012; Yang *et al.*, 2012) reported an increase in egg production by supplementation of zinc. The findings have contradicted the reports of Guo *et al.*, (2002) reported no effect on egg production in laying hens fed diets supplemented with inorganic zinc (zinc sulfate). The significant increase in egg production of the T₇ group might be due to the important role of Zn in the synthesis and secretion of LH and FSH hormones (Bedwal and Bahuguna, 1994). Dietary zinc may influence egg production by interacting with the endocrine system since the hen is changing the production and secretion of reproductive hormones during sexual maturation Renema *et al.*, (1999).

During the rest of the phases (II-IV), HHEP and HDEP percent were significantly better in T₇ (Nano zinc, 60 mg/kg) when compared to ZnSO₄ groups (T₁ and T₂). But the difference was insignificant ($P \leq 0.05$) when compared to the rest of the treatment groups. Among ZnSO₄ groups and Zn-Met groups, the difference was insignificant ($P \leq 0.05$) respectively. The HHEP and HDEP percent in T₁ was comparable to that of T₂ and T₃ with T₄. Findings of Kout El-Kloub *et al.*, (2004) are also in line with the present findings, displayed that supplementing the diet of Egyptian laying hens with zinc oxide or Zn-methionine at two levels of 100 or 150 ppm resulted in significantly greater egg production and are comparable. Similar reports are presented by Hudson *et al.*, (2004) in broiler breeders reported partial (50%) or complete substitution of zinc sulphate with zinc amino acid complexes increased hen-day production, but only in the early phase of laying cycle (24 to 37 weeks of age). The findings of the present study are not in line with the findings of Drumus *et al.*, (2004), who reported a non-significant effect of increasing the zinc level in the diet with different sources of zinc. The increase in egg

production in zinc treatment groups' may be attributed to the increase in blood plasma sex hormones concentrations as was found a significant positive correlation among these hormones concentrations in blood plasma and egg production Durmus *et al.*, (2004).

Fertility

During the I phase, the mean fertility percentage was non-significant between the treatment groups, however, a comparably higher percentage of fertility was recorded for the T₇ group (Table 3). Similar findings are also reported by (Kidd *et al.*, 1992), wherein a non-significant difference was recorded in hens fed with ZnO and Zn-Met.

During the rest of the three phases (II-IV) for fertility percentage, a significantly ($P \leq 0.05$) higher percent of fertility was recorded for the T₇ group when compared between the treatment groups. However, the difference was comparable with the Zn-Met treatment group (T₄) and Nano zinc supplemented group at the levels of 15 and 30 mg/kg. The reason for the comparable fertility percentage between Zn-Met at the level of 60 mg/kg with nano zinc at the level of 15 mg/kg might be attributed to similarity in bioavailability. The findings for increased fertility in the T₇ group are also supported by the findings of Kout El-Koub *et al.*, (2004) who reported the addition of a higher level of Zn-Met increased fertility percentage. The reason could be attributed to the higher bioavailability of nano zinc particles compared to other sources of zinc supplementation. Zinc is involved in the production, storage, and secretion of reproductive hormones and affects receptor sites (McDowell, 1992). Among ZnSO₄ groups, the difference was comparable between them with the lowest value (87.05) recorded for the T₁ group. Similar findings are also reported by Slamony *et al.*, (2015), wherein an increase of fertility percent was

recorded in 125mg than 100 mg/kg of Zn-Gly supplementation. The findings have contradicted the reports of (Kidd *et al.*, 1992), wherein diet supplemented with Zn-oxide and Zn-Met did not differ the fertility percentage significantly.

Hatchability

A significantly ($P \leq 0.05$) higher hatchability on TES was recorded for the T₇ group between the treatment groups which was persistent throughout different phases (Table 4). However, the difference was comparable

to Zn-Met groups and other two nano zinc fed treatment groups. The difference for ZnSO₄ groups was comparable to Zn-Met groups and other two nano zinc fed treatment groups at the level of 15 and 30 mg/kg. The Zn-Met treatment groups were comparable to that of nano zinc supplemented groups. King'ori (2011) reported that among the most influential egg parameters that influence hatchability is weight, shape index and shell thickness. The reason might be attributed to higher egg weight of the T₇ treatment group was significantly higher than other treatment groups.

Table.1 Ingredient composition and nutrient composition of the basal diet

Ingredients	Percentage
Yellow maize	63.35
Soyabean meal (46%)	23.00
DORB	1.50
Vegetable oil	1.50
Shell grit	8.40
Dicalcium phosphate	1.00
Mineral mixture *	0.55
Vitamin premix **	0.10
DL-methionine	0.10
Common salt	0.40
TOTAL	99.967
Nutrient Composition	
ME (Kcal/kg) ^a	2834
Crude protein (%) ^b	16.5
Calcium (%) ^a	3.62
Phosphorous (%) ^a	0.42
Lysine (%) ^a	0.87
Methionine (%) ^a	0.47

* Mineral mixture: Each 100 g contains Magnesium oxide- 1.48g, Ferrous sulphate- 6.0 g, copper sulphate- 0.05g, Manganese Sulphate-0.04 g, Potassium Iodide- 0.001g, Potassium Chloride-17.09g and Sodium selenite- 0.001g.

** Vitamin-mineral Premix: Each 100g contains Vitamin AD3 (Vitamin A-10,00,000 IU/g, Vitamin D- 200000 IU/g)- 0.165g, Vitamin K3-0.103g, Vitamin E- 2.4g, Thiamine Mononitrate- 0.206 g, Riboflavin- 0.513g, Pyridoxine hydrochloride- 0.309g, Cyanocobalamine- 0.00031g, Folic acid- 0.103g, Niacin-4.124 g, Ca-D-Pantothenate- 1.031g, Biotin- 1.5g, Maltodextrine- 89.545g.

^a calculated values; ^b analyzed values

Table.2 Effect of supplementing ZnSo₄, Zn-Met and nano zinc on egg production (%) of White Plymouth Rock bird

Treatment	Zinc source	Level (mg/kg)	Phase I		Phase II		Phase III		Phase IV	
			HHEP (%)	HDEP (%)						
T1	ZnSo ₄	30	71.34±1.42 ^a	71.34±1.42 ^a	67.85±1.13 ^a	69.79±1.16 ^a	66.03±0.88 ^a	67.91±0.90 ^a	64.68±1.42 ^a	66.53±1.46 ^a
T2	ZnSo ₄	60	73.49±1.13 ^a	73.49±1.13 ^a	70.79±1.27 ^{ab}	72.81±1.30 ^{ab}	67.77±0.89 ^a	69.71±0.92 ^a	66.03±1.10 ^{ab}	67.91±1.13 ^{ab}
T3	Zn-Met	30	75.00±1.13 ^{ab}	75.00±1.13 ^{ab}	72.53±2.07 ^b	74.61±2.13 ^b	69.36±1.24 ^a	71.34±1.28 ^a	68.73±1.07 ^b	68.73±1.07 ^b
T4	Zn-Met	60	77.38±1.46 ^b	77.38±1.46 ^b	74.44±1.45 ^{bc}	74.44±1.45 ^{bc}	73.49±1.15 ^b	75.59±1.19 ^b	72.53±1.43 ^c	74.61±1.47 ^c
T5	NanoZinc	15	78.09±1.44 ^{bc}	78.09±1.44 ^{bc}	74.75±1.39 ^{bc}	74.75±1.39 ^{bc}	73.65±1.31 ^b	75.75±1.34 ^b	72.38±1.37 ^c	74.44±1.41 ^c
T6	Nano Zinc	30	81.26±1.05 ^{cd}	81.26±1.05 ^{cd}	76.82±1.38 ^c	76.82±1.38 ^c	75.39±1.17 ^{bc}	77.55±1.21 ^{bc}	74.20±1.12 ^c	76.32±1.15 ^c
T7	Nano Zinc	60	83.01±1.17 ^d	83.01±1.17 ^d	78.49±1.45 ^c	78.49±1.45 ^c	77.22±1.29 ^c	79.42±1.33 ^c	73.65±1.32 ^c	75.75±1.36 ^c

Means having same superscript do not differ significantly

Table.3 Effect of supplementing ZnSo₄, Zn-Met and nano zinc on fertility (%) of White Plymouth Rock bird

Treatment	Zinc source	Level (mg/kg)	Phases			
			I	II	III	IV
T1	ZnSo ₄	30	87.68±2.89	87.05±1.60 ^a	87.32±1.77 ^a	84.68±0.63 ^a
T2	ZnSo ₄	60	89.24±2.64	88.21±0.95 ^a	88.30±0.85 ^{ab}	87.51±1.11 ^{ab}
T3	Zn-Met	30	90.29±1.88	90.34±1.13 ^{ab}	89.58±1.93 ^{abc}	88.94±1.61 ^{bc}
T4	Zn-Met	60	91.34±1.83	92.38±0.94 ^{bc}	91.16±1.88 ^{abc}	90.38±1.09 ^{bc}
T5	Nano Zinc	15	91.13±1.31	93.20±1.50 ^{bc}	91.53±0.72 ^{abc}	90.68±1.27 ^{bc}
T6	Nano Zinc	30	92.25±1.59	93.67±0.94 ^{bc}	92.32±0.47 ^{bc}	91.21±0.58 ^{cd}
T7	Nano Zinc	60	94.25±1.21	94.21±0.85 ^c	93.46±0.73 ^c	93.97±0.78 ^d

Means having same superscript do not differ significantly

Table.4 Effect of supplementing ZnSo₄, Zn-Met and nano zinc on hatchability percentage of White Plymouth Rock bird

Treatment	Zinc source	Level (mg/kg)	Total Egg Set (TES)/ Fertile Egg Set (FES)			
			Phases			
			I	II	III	IV
T1	ZnSo ₄	30	74.19±3.94 ^a	74.29±1.57 ^a	74.70±1.16 ^a	71.85±1.21 ^a
			84.42±2.31 ^a	85.41±1.97	85.60±0.96 ^a	84.86±1.57
T2	ZnSo ₄	60	76.01±3.16 ^{ab}	75.44±0.86 ^{ab}	76.10±0.73 ^{ab}	75.02±2.44 ^{ab}
			85.09±1.68 ^a	85.53±0.52	86.18±0.42 ^a	85.71±2.50
T3	Zn-Met	30	77.52±2.02 ^{abc}	78.66±0.89 ^{bc}	77.44±0.96 ^{ab}	76.00±0.92 ^{bc}
			85.84±1.11 ^{ab}	87.15±1.88	86.51±0.89 ^a	85.52±1.32
T4	Zn-Met	60	79.98±1.45 ^{abc}	80.91±1.91 ^{cd}	79.39±1.87 ^{bc}	78.58±1.01 ^{bc}
			87.58±0.62 ^{abc}	87.55±1.48	87.06±0.55 ^{ab}	86.97±1.20
T5	Nano Zinc	15	80.39±0.96 ^{abc}	80.84±1.37 ^{cd}	79.92±1.37 ^{bc}	78.36±1.17 ^{bc}
			88.24±0.98 ^{abc}	86.73±0.82	87.29±1.13 ^{ab}	86.52±2.20
T6	Nano Zinc	30	83.03±2.47 ^{bc}	82.64±1.64 ^{cd}	81.77±1.75 ^{cd}	80.12±1.21 ^c
			89.94±1.32 ^{bc}	88.22±1.59	88.55±1.59 ^{ab}	87.85±1.42
T7	Nano Zinc	60	85.05±0.98 ^c	84.70±1.16 ^d	84.19±1.18 ^d	84.78±0.94 ^d
			90.26±0.82 ^c	89.94±1.66	90.01±0.93 ^b	90.24±1.15

Means having same superscript do not differ significantly

Table.5 Effect of supplementing ZnSo₄, Zn-Met and nano zinc on external egg quality parameters of White Plymouth Rock bird

Treatment	Zinc source	Level (mg/kg)	Egg weight (g)/shape index			
			Weeks			
			37 th	42 nd	47 th	52 nd
T1	ZnSo ₄	30	59.62±0.47 ^a	58.98±0.69 ^a	58.07±0.64 ^a	57.10±0.87 ^a
			75.09±0.61 ^a	74.87±0.45 ^a	73.83±0.65 ^a	72.74±0.90 ^a
T2	ZnSo ₄	60	61.52±0.88 ^{ab}	60.84±0.58 ^{ab}	59.85±0.54 ^b	58.68±0.54 ^{ab}
			77.92±1.72 ^{ab}	75.98±0.90 ^{ab}	74.94±0.85 ^{ab}	73.78±0.86 ^{ab}
T3	Zn-Met	30	62.98±0.72 ^{bc}	61.42±0.85 ^b	60.66±0.64 ^{bc}	59.74±0.66 ^{bc}
			78.00±1.14 ^{ab}	76.04±0.48 ^{ab}	75.16±0.97 ^{ab}	74.81±0.90 ^{ab}
T4	Zn-Met	60	63.12±0.91 ^{bc}	62.24±0.95 ^{bc}	61.74±0.73 ^{cd}	61.62±0.69 ^{cd}
			79.08±1.38 ^b	77.37±0.90 ^b	76.28±0.57 ^{bc}	75.35±0.65 ^{bc}
T5	Nano Zinc	15	63.54±0.61 ^{bc}	62.93±0.68 ^{bc}	61.76±0.64 ^{cd}	61.52±0.63 ^{cd}
			79.32±1.51 ^b	77.31±0.94 ^b	77.57±0.93 ^{cd}	75.76±0.94 ^{bc}
T6	Nano Zinc	30	64.05±1.32 ^{bc}	64.14±0.50 ^c	62.87±0.62 ^{de}	62.27±0.76 ^{de}
			81.27±0.66 ^{bc}	80.85±0.87 ^c	79.23±0.72 ^d	77.68±0.65 ^{cd}
T7	Nano Zinc	60	65.26±0.36 ^c	66.25±0.64 ^d	64.35±0.43 ^e	64.15±0.61 ^e
			83.04±0.89 ^c	82.60±0.92 ^c	81.56±0.41 ^e	79.35±0.57 ^d

Means having same superscript do not differ significantly

Table.6 Effect of supplementing ZnSO₄, Zn-Met and nano zinc on yolk and albumen index of White Plymouth Rock bird

Treatment	Zinc source	Level (mg/kg)	Yolk index/albumen index			
			Weeks			
			37 th	42 nd	47 th	52 nd
T1	ZnSO ₄	30	0.35±0.009 ^a 0.06±0.001 ^a	0.351±0.004 ^a 0.060±0.002 ^a	0.348±0.005 ^a 0.059±0.001 ^a	0.340±0.006 ^a 0.057±0.002 ^a
T2	ZnSO ₄	60	0.36±0.005 ^{ab} 0.06±0.001 ^a	0.361±0.005 ^{ab} 0.061±0.001 ^{ab}	0.355±0.004 ^{ab} 0.061±0.001 ^{ab}	0.350±0.004 ^{ab} 0.058±0.009 ^a
T3	Zn-Met	30	0.37±0.008 ^{abc} 0.06±0.001 ^a	0.370±0.006 ^{ab} 0.063±0.001 ^{abc}	0.361±0.007 ^{abc} 0.060±0.001 ^{abc}	0.366±0.008 ^{abc} 0.059±0.001 ^{ab}
T4	Zn-Met	60	0.39±0.008 ^{bcd} 0.06±0.001 ^{ab}	0.381±0.007 ^{bc} 0.064±0.001 ^{abc}	0.372±0.007 ^{bc} 0.06±0.001 ^{abc}	0.374±0.007 ^{bcd} 0.061±0.002 ^{ab}
T5	Nano Zinc	15	0.39±0.01 ^{bcd} 0.06±0.002 ^{ab}	0.38±0.011 ^{bc} 0.064±0.002 ^{abc}	0.373±0.011 ^{bc} 0.063±0.002 ^{abc}	0.37±0.01 ^{bcd} 0.061±0.001 ^{ab}
T6	Nano Zinc	30	0.40±0.06 ^{cd} 0.07±0.001 ^{bc}	0.392±0.006 ^{cd} 0.066±0.001 ^{bc}	0.383±0.006 ^{cd} 0.064±0.001 ^{bc}	0.381±0.011 ^{cd} 0.063±0.001 ^{bc}
T7	Nano Zinc	60	0.42±0.01 ^d 0.073±0.001 ^c	0.410±0.007 ^d 0.067±0.04 ^c	0.398±0.008 ^d 0.067±0.001 ^c	0.39±0.01 ^d 0.066±0.001 ^c

Means having same superscript do not differ significantly

Hatchability for small eggs is lower than medium and large eggs, which is following the present results of the nano zinc supplemented group which was having a higher egg weight as compared to other groups. The hatchability on TES was recorded highest in T₇ (Nano zinc, 60 mg/kg) which differed significantly when compared to other treatment groups.

The lowest hatchability on the TES percentage of 72.85 was recorded for the T₁ group. The hatchability on TES percentages of Zn-Met treatment groups was comparable to that of nano zinc fed groups (T₅ and T₆) groups. Among ZnSO₄ groups at different levels were comparable for hatchability on TES percentage. This conclusion regarding the improvement of hatchability is supported by the reports Hassan *et al.*, (2003), wherein an increase in hatchability percentage was recorded from organic trace minerals supplementation.

The Hatchability on FES in the first phase of the study was significantly ($P \leq 0.05$) highest 90.26 in the T₇ group when compared to other treatment groups. But, the difference was comparable with nano zinc supplementation at the levels of 15 and 30 mg/kg and also with the Zn-Met treatment group at the level of 60 mg/kg. Among ZnSO₄ groups, the difference was comparable between them with the lowest value recorded for the T₁ group.

The higher percentage of hatchability on FES in the case of T₇ can substantiate by a higher egg weight in the respective treatment group. (Abiola *et al.*, 2008; Malago and Baitilwake, 2009) showed that there was a close correlation between egg size and chick hatching weight. Breeder factors that affect hatchability include nutrition, health, egg size, egg weight, and quality King'ori (2011). The results are contradictory to the findings of Hudson *et al.*, (2004) reported a significant reduction in early embryonic mortality when breeder hens were fed a Zn-amino acid

complex as the only source of Zn supplementation in the diet.

During phase II-IV, the value for hatchability on FES was statistically insignificant ($P \leq 0.05$) in all the treatment groups. The findings of the present study are in line with King'ori (2011) reported that supplementation of zinc had little or no effect on the hatchability. Age is one of the factors which could affect the percent hatchability. Hatchability for eggs of older breeders decreases because of a change in egg quality and failure to adjust the incubation condition. It was further stated that as the hen ages, the albumen quality, which is the main source protein to the embryo development deteriorates Slamony *et al.*, (2015).

External egg quality parameters

In the eggs collected during different weeks of egg production, the egg weight was recorded significantly higher in T₇ (Nano zinc, 60 mg/kg) with the lowest egg weight in T₁ (ZnSO₄, 30 mg/kg) between different treatment groups depicted (Table 5). The differences among the ZnSO₄ groups were insignificant ($P \leq 0.05$) with a higher weight in T₂ (ZnSO₄, 60 mg/kg). The differences in egg weights of Zn-Met treated groups were comparable to that of nano zinc supplemented groups. The egg weight in Zn-Met supplemented at the level of 60 mg/kg (T₄) was comparable to that of nano zinc supplemented groups at the level of 15 and 30 mg/kg. Similar findings were also reported by (Slamony *et al.*, 2015; Durmus *et al.*, 2004), wherein dietary supplementation with zinc resulted in a significant increase in egg weight and the concentration of zinc in eggs. The finding is also supported by the results of (Amem and Daraji 2011) reported an increase in egg weight by increasing the Zn concentration in the diet. The better changes observed with nano zinc treated groups at the

level of 60 mg/kg in egg weight of this study may be attributed to that both of nano zinc improved nutrient digestibility and metabolism. While Kidd *et al.*, (1992) found that there was no significant effect on egg weight upon addition of zinc to the diet of broiler parent stock.

A significantly ($P \leq 0.05$) higher shape index value was recorded for T₇ (Nano zinc, 60 mg/kg) in comparison to other treatment groups with the lowest value of shape index in T₁ group which was supplemented with ZnSO₄ at the level of 30 mg/kg in all the eggs collected during different weeks of the study period. The shape index values for T₅ and T₆ were comparable with a higher value at the level of 30 mg/kg nano zinc in the diet. Among ZnSO₄ treatment groups, the shape index values were comparable at all the weeks of the study period. In general, a long and narrow egg of any size would have a low index and a short and broad egg (whether large or small) would have a high index. The higher shape index value of indigenous chicken might be due to the broad and short size and shape of the eggs Pathak *et al.*, (2016). In the present study eggs of T₇ were found to be broader with a relatively shorter length contributing to a higher shape index. The result of the present study is in contrast to the reports of (Slamony *et al.*, 2015; Bahakaim *et al.*, 2014) exhibited that no significant differences in egg shape index with the dietary organic zinc supplementation of Golden Montazah hens.

Internal egg quality

The yolk index was highest (0.422) in T₇ and lowest (0.35) in the T₁ group supplemented with ZnSO₄ at the level of 30 mg/kg (Table 6). The difference was comparable among the Zn-Met treatment groups at the levels of 30 and 60 mg/kg. The yolk index values of ZnSO₄ treatment groups were comparable to

that of the Zn-Met group at the level of 30 mg/kg. Among ZnSo₄ treatment groups, the values were comparable. The reports of the present study are in line with the reports of (Lim and Paik 2003; Kout El-Koub *et al.*, 2004) reported zinc methionine chelate supplementation, did not affect internal egg quality. (Slamony *et al.*, 2015) and (Bahakaim *et al.*, 2014) also exhibited no significant differences in the yolk index with the dietary organic zinc supplementation of Golden Montazah hens.

During the 37th week, the albumen index ranged from (0.061) in T₁ and (0.073) in T₇ groups. The height of the albumen was increased significantly in the eggs of the T₇ treatment group. Albumen index was significantly ($P \leq 0.05$) different in the T₇ group when compared to other groups except in T₆. Among the ZnSo₄ supplemented groups at a different level and Zn-Met groups the differences in albumen indices were comparable. The albumen index value of T₄ (Zn-Met, 60 mg/kg) was comparable to that of nano zinc supplemented groups in T₅ and T₆. The present findings are corroborated with the reports of (Slamony *et al.*, 2015; Bahakaim *et al.*, 2014) exhibited that no significant differences in the albumen index with the dietary organic zinc supplementation of Golden Montazah hens. The findings are in contrast to the reports of (Tabatabaie *et al.*, 2007) reported an increase of albumen height in the eggs of the hens supplemented with organic zinc. The reason for the increase in the albumen index might be attributed to increased albumen height in the T₇ treatment. Another reason might be attributed as Zinc plays a role in the magnum during the deposition of albumen and in the isthmus where eggshell membranes are produced. Findings for the increase in height of albumen in the treatment group compared to the control group were also reported by (Tabatabaie *et al.*, 2007) who supplemented

the diet with organic zinc at a different level.

A significantly ($P \leq 0.05$) higher eggshell thickness (0.42) was recorded for T₇ in comparison to other treatment groups. The eggshell thickness was found to be lowest (0.36) in the T₁ group. The value of eggshell thickness in T₁ and T₂ were comparable. A comparable value of eggshell thickness was observed between Zn-Met treated groups and Nano zinc supplemented groups at the level of 15 and 30 mg/kg. Among ZnSo₄ treatment groups, the values were comparable to each other. The findings of the present study are correlated with the reports of Bahakaim *et al.*, (2014) exhibited shell thickness was significantly improved as a result of using organic Zn in the diet of Golden Montazah birds. The findings of Klecker *et al.*, (2002) also supported present findings, who reported that the substitution of 20-40% supplemental inorganic Zn with their chelates increased eggshell thickness.

Among different sources used in the study, nano zinc particles are the source in which bioavailability is highest, due to their extremely small size and unique physical properties Sahoo *et al.*, (2014). The results for increase shell thickness might be attributed to the importance of zinc function in the formation of the egg. Zinc supplementation also has been reported to improve eggshell quality because it is a component of the carbonic anhydrase enzyme, which supplies the carbonate ions during eggshell formation Innocenti *et al.*, (2004).

The Haugh unit value ranged from 72.41 in T₁ to 78.44 in T₇. The difference was recorded significant ($P \leq 0.05$) in T₇ when compared to other treatment groups except with T₆. Among Zn-Met groups, the differences were statistically insignificant and were comparable to that of T₅ and T₆. The increase

in the Haugh unit in the present study is in agreement with the report of Sahin and (Kucuk 2003; Idowu *et al.*, 2011) who reported that zinc supplementation positively affected the Haugh unit. Concerning the interaction between Zn levels and sources, it was observed that the Haugh unit was significantly affected. Supplementation of nano zinc at 60 mg/kg Zn gave the preferable value in the Haugh unit. The reason might be attributed to an increase in albumen height in the T₇ group.

In conclusion the Hen House and Hen Day Egg production were increased with nano zinc supplementation at a higher level of @60mg/kg. The fertility, hatchability on Total Egg set, and Fertile Egg Set were improved on an increasing level of zinc supplementation in all the sources of zinc; however, a better percentage of the aforementioned parameters were with nano zinc supplementation at a higher level. The egg quality traits were also improved with a higher level of nano zinc supplementation.

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Authors' contributions

Siddhartha Shankar Pathak and Biju Borah were involved in conduct of the trial while Prasoon Sagunan helped in preparation of manuscript.

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