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Impact of incremental zinc sulphate monohydrate on productive performance, blood chemistry and egg quality dynamics of bovan black strain

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ABSTRACT

This study evaluated the effect of zinc sulphate monohydrate on performance characteristics and blood chemistry of hens. A total of 100 Point of Lay (PoL) Bovan Black hens were randomly assigned to four dietary treatments: control or T_1 (0mg/kg zinc), and treatments T_2 (30mg/kg zinc), T_3 (60mg/kg zinc), and T_4 (90mg/kg). Birds were allowed access to feed and water *ad libitum* for 12 weeks. Parameters assessed included: feed proximate composition (%), Hen-Day Production (HDP), feed intake, egg weight, feed efficiency, percentage mortality, and blood biochemical indices. Dry matter of diet ranged from 85.07% (T_1) to 82.39% (T_4), as crude protein increased negligibly from 16.68.58% (T_1) to 16.94% (T_4). While zinc levels peaked in T_4 (22.59 mg/kg), calcium (1.17%) and phosphorus (0.64–0.65%) remained constant across treatments. HDP was significantly ($p<0.05$) higher in T_3 (70.13%) than T_4 (62.52%) as feed efficiency improved in T_2 (3.17%). Total antioxidant capacity was significantly higher ($p<0.05$) in T_2 (14.34 mM) than T_1 (7.37 mM), as T_3 (9.79 mM) and T_4 (11.72 mM) showed intermediate values. Glutathione peroxidase activity was also significantly higher ($p<0.05$) in T2 (36.01 U/L) compared to T1 (25.13 U/L), T_3 (28.88 U/L), and T_4 (24.38 U/L). Percentage mortality and egg quality were unaffected. Moderate zinc supplementation (30-60 mg/kg) enhanced laying performance and oxidative status without any adverse effects. Therefore, it may be concluded that incorporation of zinc in hen's diets enhances growth performance, improved egg quality parameters, and positively influenced health indicators and antioxidant status, highlighting its potential role in optimizing poultry nutrition and enhancing health management practices.

Keywords: Feed efficiency, Hen-day production, Immunoglobulin, Mortality, Pullets

The dietary inclusion of Zinc (Zn) has been shown to significantly enhance the productivity and health of laying hens. Zinc functions as a vital cofactor for numerous enzymes and plays a key role in biological processes such as DNA synthesis, cell division, and protein metabolism essential for optimal growth and egg production in poultry (Li *et al.* 2019). Additionally, zinc is crucial in antioxidant defense mechanisms, mitigating oxidative stress and thereby improving the overall well-being and longevity of laying birds (Ali *et al.* 2021). Several studies have examined the effects of different zinc sources, both organic and inorganic, on laying performance. Organic sources like zinc methionine or zinc proteinate tend to be more bioavailable than inorganic forms, resulting in better absorption and utilization in poultry systems (Chen *et al.* 2022; Aguihe *et al.* 2024).

This improved bioavailability has been linked to enhancements in egg production and egg shell quality, both of which are critical indicators in the commercial poultry industry. Abou-Kassem *et al.* (2020) reported that hens fed diets enriched with organic zinc exhibited significantly higher egg weight and shell thickness compared to those receiving inorganic zinc. Improvements in egg shell quality are crucial for reducing economic losses associated with cracked or broken eggs during handling, packaging, and transport (Elnesr *et al.* 2024). Moreover, zinc supplementation has been associated with better feed conversion ratios, indicating more efficient nutrient utilization and feed resource management in laying birds (Saleh *et al.* 2022). Zinc also plays a vital role in modulating immune function. Zinc deficiency in poultry is known to compromise immune responses, rendering birds more susceptible to infections and disease outbreaks (Huang *et al.* 2019).

Zinc-supplemented diets have been shown to bolster immune status, reduce mortality rates, and enhance resistance against pathogens. In reproductive physiology,

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Table 1. Gross composition (g/Kg) of experimental diet

Ingredient	T1	T2	T3	T4
Maize	58.65	57.00	57.87	56.78
Wheat bran	8.00	8.00	8.00	8.00
Groundnut cake	7.50	7.50	7.50	7.50
Palm kernel cake	7.00	7.00	7.00	7.00
Soybean meal	10.00	10.00	10.00	10.00
Bone meal	2.00	2.00	2.00	2.00
Limestone	7.00	7.00	7.00	7.00
Methionine	0.15	0.15	0.15	0.15
Lysine	0.15	0.15	0.15	0.15
Salt	0.30	0.30	0.30	0.30
Vitamin premix	0.50	0.50	0.50	0.50
Total (%)	100.00	100.00	100.00	100.00
Zinc (mg/kg)	0.00	30.00	60.00	90.00
CP (%)	16.68	16.73	16.86	16.94
ME (kcal/kg)	2742.50	2743.61	2749.21	2752.66

zinc contributes to the proper development and function of reproductive organs, thereby influencing laying performance and egg fertility (Niu *et al.* 2021). Thus, zinc not only supports current laying cycles but also positively impacts the birds' future reproductive potential. Zinc supplementation in the diets of laying birds offers a multitude of benefits, including improved egg production, enhanced egg quality, better feed efficiency, and stronger immune response (Niknia *et al.* 2022). Therefore, the present study explored optimal zinc levels for physiological responses in animal nutrition (Idahor *et al.* 2018), contributing to sustainable egg production systems (Osayande and Ilaboya, 2024, Obajuluwa *et al.* 2024).

MATERIALS AND METHODS

Study location and animals management: The experiment was conducted in the poultry unit of the Teaching and Research Farm, Edo State College of Agriculture and Natural Resources, Iguoriakhi, Edo State. A total of 100 day-old Bovan Black birds were purchased from a reputable farm in Benin City and housed in a battery cage system.

A composite diet was formulated to meet NRC (1994) nutrient requirements and analyzed using AOAC (1990) procedures. Hens were randomly allotted to four treatment groups (T_1–T_4) with three replicates of 25 birds each in a completely randomized design. Zinc sulphate monohydrate was added at 0, 30, 60, and 90 mg/kg in T_1–T_4, respectively. Birds received grower mash before 10% lay and layer mash during the 10-week trial.

Data collection: Parameters evaluated were feed intake (g/bird/day), hen day production (%), egg mass (g), feed conversion (kg feed/kg egg produced) and feed conversion ratio (kg feed/dozen eggs). Eggs were collected thrice daily at 9:00-10:00am and 3:30 to 4:00pm, and carefully labelled for evaluation. Percentage hen day production (HDP %) was calculated as the percentage of the ratio of the total number of eggs and the total number of days by the number of hens.

$$\text{HDP (\%)} = \frac{\text{Number of hen-days}}{\text{Number of eggs produced}} \times 100$$

Egg Mass (g/hen/day) = HDP (%) × Average egg weight (g)

Haematology and Serum analysis: To ensure bird welfare, 5ml of blood was collected from the brachial vein using sterile syringes into EDTA bottles. Birds were gently restrained to reduce stress-induced variations. Whole blood was stored at 4 °C, mixed gently, and analyzed within 24 hours. For serum, blood was collected in plain tubes, allowed to clot for 30–60 minutes, then centrifuged at 3,000 revolution per minute for 15 minutes. Serum was aspirated and stored at 4 °C. Serum assays for total protein, albumin, globulin, and uric acid were performed using Fortress Diagnostics kits. Antioxidant indices (TAC, SOD, GPx, catalase, and lipid peroxidation) were analyzed following standard procedures.

Statistical Analysis: Data were subjected to ANOVA and significant treatments means were separated using SAS (2002)

RESULTS AND DISCUSSION

The experimental diets (T_1 to T_4) were formulated to evaluate the impact of incremental zinc supplementation while maintaining consistency in other essential nutrients. As presented in Table 2, the nutritional composition of the diets, including dry matter, crude protein, ether extract, and crude fiber. Dry matter content reduce from 85.07% (T_1) to 82.39% (T_4). Crude protein content showed a slight reduction from 21.58% (T_1) to 21.27% (T_4), indicating negligible effects of zinc on protein availability, critical for egg production and somatic growth (Ogbuewu and Mbajiorgu, 2022). Calcium and phosphorus levels remained stable across all treatments at 1.17% and 0.64–0.65% respectively, isolating zinc's effects and minimizing confounding influences from other minerals. Zinc content varied significantly ($p<0.05$), increasing progressively from 8.21 mg/kg (T_1) to 22.59 mg/kg (T_4). This controlled

Table 2. Constituents analysis of experimental diet

Ingredients	T1	T2	T3	T4
Dry Matter %	85.07	84.15	83.27	82.39
Crude Protein (%)	16.68	16.73	16.86	16.94
Ether Extract %	3.94	3.9	3.86	3.82
Crude Fibre (%)	3.62	3.6	3.58	3.56
Calcium (%)	1.17	1.17	1.17	1.17
Zinc	8.21	19.63	20.11	22.59
Available Phosphorus (%)	0.65	0.64	0.64	0.64

Table 3. Performance Characteristics of Bovan Black layers fed graded levels of zinc supplement

Parameters	T1 (Control)	T2 (30mg/kg ZINC)	T3 (60mg/kg ZINC)	T4 (90mg/kg ZINC)	SEM
HDP (%)	69.50^{a}	69.73^{a}	70.13^{a}	62.52^{b}	1.11
Feed Intake (g/bird/day)	112.40^{b}	121.27^{b}	129.20^{a}	115.30^{c}	1.26
Water Intake (ml)	227.55^{b}	230.23^{bc}	245.34^{ab}	252.77^{a}	3.77
Egg Weight (g)	54.73^{a}	55.46^{a}	54.53^{a}	54.89^{a}	0.51
Mortality (%)	0.04	0.02	0.07	0.00	1.33
Egg Mass (g)	38.13^{c}	46.01^{bc}	48.29^{a}	47.13^{ab}	0.19
FCR	2.05^{b}	2.19^{b}	2.37^{a}	2.10^{b}	0.06

a, b, c: Means with different letters on the same row are significantly different at $P<0.05$

dietary design strengthens the attribution of physiological and production outcomes to zinc supplementation.

Performance characteristics, assessed in Table 3, revealed significant effects of zinc on laying hens, with Hen-day production (HDP) being highest in T_3 at 70.13%, followed by T_2 at 69.73%, both were significantly higher ($p<0.05$) than the control (T_1) at 69.50%. However, T_4 exhibited a significantly lower ($p<0.05$) HDP of 62.52% suggesting potential zinc toxicity that might have disrupted metabolic and reproductive pathways as reported for zinc supplementation for broiler chickens and young chicks (Ilaboya *et al.* 2024; Zhang *et al.* 2022; Batal *et al.* 2001). Feed intake was highest in T_3 (129.20 g/bird/day), followed by T_2 (121.27 g/bird/day), with significant differences ($p<0.05$) when compared to T_4 (115.30 g/bird/day), and T_1 (112.4 g/bird/day). This high intake in T_3 aligned with its superior HDP, indicating enhanced appetite and nutrient utilization at moderate zinc levels, while reduced intake in T_4 might be due to impaired palatability or appetite suppression due to excessive zinc as reported earlier in laying hens and straight run broiler chickens (Saleh *et al.* 2020, Hudson *et al.* 2004). Feed efficiency, a key economic parameter, was optimized in T_2 (3.17%) and T_3 (3.41%), with birds requiring less feed per unit of egg produced compared to T_1 (3.32%) and T_4 (3.32%); though this differences were not significant ($p>0.05$) across all treatment effects. This improvement is likely to have stemed from zinc's role in enhancing nutrient absorption and metabolic efficiency (Bao *et al.* 2007). However, there seem to be poor feed efficiency in T_4 which suggested that high zinc levels might impair digestion and metabolism, negating potential benefits in young broiler chickens (De-Grange *et al.* 2020). Egg weights remained consistent across all treatments, indicating that zinc supplementation within the tested range did not influence ($p>0.05$) egg size, thereby consistent with the report that zinc supports metabolic and health functions rather than egg mass (Shao *et al.* 2023). Therefore, these performance metrics highlighted the importance of moderate zinc supplementation (30–60mg/kg) for optimizing egg production and feed efficiency without compromising egg quality. Mortality rates, a direct measure of bird welfare were low across all groups. This study reported tolerable mortality levels across all groups, even at the highest zinc dose (T4). Layer chickens on T_4 diets recorded no mortality (0.00%), followed by T_2 (0.02%), T_1 (0.04%), and T_3 (0.07%). The absence of mortality in T_4 suggested a protective effect of high zinc levels, possibly mediated by enhanced immune function or antioxidant capacity (Rath *et al.* 2022; Zaghari *et al.* 2022). Water intake increased significantly ($p<0.05$) with zinc supplementation, ranging from 230.23–245.34 ml/bird/day (T_2 and T_3) to 252.77 ml/bird/day (T_4), reflecting a clear response to zinc levels. Body weight gain showed no significant differences ($p>0.05$), with T_3 recording the highest (54.76 g), followed by T_4 (53.23 g), T2 (52.10 g), and T_1 (50.23 g), suggesting a positive influence of moderate zinc supplementation.

Hematological parameters as illustrated in Table 4 showed stable packed cell volume (PCV; 27.65–29.87%), hemoglobin (HB; 9.47–9.72 g/dL), erythrocyte, and leukocyte counts across treatments, indicating no hematological disorders as reported for broiler chickens on phytase supplementation (Ilaboya *et al.* 2024). Lymphocyte percentages ranged from 59.33–61.3%, with

Table 4. Haematological indices of Boran Black layers fed graded levels of zinc supplement of

	T1	T2	T3	T4	SEM
Packed cell volume (%)	29.33	27.65	28.33	29.87	0.99
Haemoglobin (g/dL)	9.54	9.72	9.47	9.64	0.11
Erythrocytes ($x10^6$/L)	3.00	2.71	2.75	3.07	0.18
Leukocytes ($x10^8$/L)	20.38	19.88	20.12	20.18	0.21
Thrombocytes ($x10^7$/L)	1.22	1.33	1.23	1.25	0.05
Lymphocyte (%)	59.33	60.68	60.49	61.3	0.82
Heterophils (%)	28.63^{b}	30.33^{a}	33.25^{a}	30.67^{b}	0.41
Monocyte (%)	3.11^{b}	3.96^{a}	3.82^{b}	2.99^{b}	0.29
Eosinophils (%)	3.22^{b}	3.09^{b}	4.84^{a}	3.67^{b}	0.27
Basophils (%)	0.69	0.52	0.77	0.4	0.26
Heterophil: Lymphocyte Ratio	0.48^{b}	0.50^{a}	0.55^{a}	0.50^{ab}	0.50

a, b, c: Means with different letters on the same row are significantly different at $P<0.05$

Table 5. Serum protein of Bovan Black layers fed graded levels of zinc supplement

	T1	T2	T3	T4	SEM
Protein (g/dL)	29.62[b]	34.99[a]	33.49[a]	34.33[a]	1.19
Glucose (g/dl)	133.21[a]	129.44[b]	133.06[a]	130.87[b]	0.15
Globulin (g/dl)	1.68[b]	1.90[a]	1.93[a]	1.96[a]	0.04
Albumin (g/dL)	1.37[b]	1.89[a]	1.42[ab]	1.49[ab]	0.09
Uric Acid (mg/dl)	12.60[b]	16.53[a]	16.88[a]	17.11[a]	1.66
Zinc (mg/dl)	8.21[c]	19.63[b]	20.11[b]	22.59[a]	1.43

a, b, c: Means with different letters on the same row are significantly different at P<0.05

a slight elevation in T_4, possibly indicating a compensatory adaptive immune response (Rao *et al.* 2013).

The biochemical parameters illustrated in Table 5, revealed significant trends with total protein levels higher ($p<0.05$) in T_2, T_3, and T_4 compared to T_1, reflecting zinc's role in enzyme activation and protein synthesis (Qi *et al.* 2019). The astronomical decline in glucose levels in T_2 (129.44 g/dL) and T_4 (130.87 g/dL) compared to T_1 (133.21 g/dL) and T_3 (133.06 g/dL) suggests a hypoglycemic effect (Min *et al.* 2019), but were in line with that reported for vitamin C supplementation in layer chickens reared on commercial layer chickens reared in Mizoram, India (Debbarma *et al.* 2023). Albumin was highest in T_2 (1.89 g/dL) compared to T_1 (1.37 g/dL; $p<0.05$), indicating improved liver function, while globulin levels were elevated ($p<0.05$) from T_2–T_4 (1.90–1.96 g/dL) compared to T_1 (1.68 g/dL), reflecting enhanced immune function as reported for different zinc sources to low crude protein diets in quality chicks (Aya *et al.* 2021). Uric acid concentrations were lower in T_1 (12.60 mg/dL) compared to T_2-T_4. This might be linked to increased protein metabolism (Khondowe *et al.* 2021), where zinc concentrations is said to have increased significantly ($p<0.05$) from T_1 (8.21 mg/dL) to T_4 (22.59 mg/dL). These values were higher than those reported for broiler chickens fed snot apple powder (Jimoh *et al.* 2025), values however remained within physiological range for laying hens, underscoring species-specific responses to zinc.

Oxidative markers as shown in Table 6 showed that T_2 exhibited highest ($p<0.05$) total antioxidant capacity (TAC; 14.34 mM) compared to T_1 (7.37 mM), with T_3 (9.79 mM) and T_4 (11.72 mM) showing intermediate values. Superoxide dismutase (SOD) activity was significantly higher ($p<0.05$) in T_2 (2.83 U/mL), T_3 (2.59 U/mL), and T_4 (2.78 U/mL) compared to T_1 (1.16 U/mL). Glutathione peroxidase (GPx) activity was highest in T_2 (36.01 U/L), followed by T_3 (28.88 U/L) and T_4 (24.38 U/L), indicating that moderate zinc supplementation bolstered antioxidant defenses as reported in organic and inorganic zinc supplementation in layer chickens and Pekins duck (Yu *et al.* 2020; Attia *et al.* 2013). Lipid peroxidation (MDA) was higher in T_2 (1.00 µM) and T_3 (0.92 µM) compared to T_1 (0.87 µM) and T_4 (0.86 µM; $p<0.05$), these values were in line with the values obtained for leachates in African giant land snail in South Western Nigeria (Jimoh *et al.* 2024). Catalase activity was highest in T_1 (148.63 u/ml/min) and significantly lower in T_2 (106.98 u/ml/min), T_3 (102.75 u/ml/min), and T_4 (103.08 u/ml/min; $p<0.05$), suggesting that excessive zinc might disrupt specific antioxidant pathways as reported for zinc oxide concentration in hens in late laying phases (Abedini *et al.* 2018). This observations aligned with reports that high zinc levels can perturb the balance between reactive oxygen species (ROS) and antioxidant enzymes (Hidayat *et al.* 2020; Wang *et al.* 2019). Moderate zinc supplementation (30–60 mg/kg) optimized egg production, feed efficiency, and health outcomes in laying hens, while excessive levels (90mg/kg) may induce toxicity and impair performance.

This study provided valuable insights into the effects of graded zinc supplementation on the performance, hematological, biochemical, and oxidative stress markers of Bovan Black laying birds. Moderate zinc supplementation (30 to 60 mg/kg) enhanced immune function, and antioxidant capacity without negatively affecting egg quality or bodyweight gain. However, high zinc levels (90 mg/kg) led to reduced egg production and feed intake, highlighting the need for careful supplementation strategies to avoid toxicity. These findings have important implications for commercial poultry production and underscored the critical role of zinc in optimizing layer performance and health.

Table 6. Oxidative Stress markers of Bovan Black layers fed graded levels of zinc supplement

	T1	T2	T3	T4	SEM
Total antioxidant capacity (mM)	7.37[b]	14.34[a]	9.79[ab]	11.72[ab]	1.04
Lipid Peroxidation (MDA uM)	0.87[b]	1.00[a]	0.92[a]	0.86[b]	0.03
Superoxide dismutase (U/mL)	1.16[b]	2.83[a]	2.59[a]	2.78[a]	0.29
Catalase (u/ml mins)	148.63[a]	106.98[b]	102.75[b]	103.08[b]	10.86
ALP	109.92[c]	119.23[b]	123.06[b]	132,66[a]	2.76
Glutathione peroxidase (U/L)	25.13[b]	36.01[a]	28.88[b]	24.38[b]	2.39

a, b, c: Means with different letters on the same row are significantly different at P<0.05

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