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The Effect of Different Sources of Dietary Zinc on Egg Production, Egg Quality, Sperm Quality, and Immunological Response in Laying Chickens Exposed to High Temperature

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A TOTAL of 300 hens and 60 males of (the Bandarah strain at 30 weeks old) were chosen at random allocated to 3 groups, with 5 replicates of 20 hens and 4 cocks/each housed in individual cages in a semi-closed house. Birds received one of three dietary treatments content zinc sulfate or organic zinc as zinc methionine in two levels of 40 or 60 mg/kg, the experiment lasted for 20 weeks from 30-50 weeks of age. Results showed that a dietary Zn-Met 40 or 60 mg/kg diet boosted egg production and improved feed conversion ratio, egg weight, and egg mass. Zinc methionine showed distinctions between external and internal egg quality, Zn content of the egg, and carbonic anhydrase activity compared with zinc sulfate. A high level of Zn-Met had increased estrogen, progesterone, and T_3. Also, Humoral, and cellular immunity were increased, and improved semen quality. It could be concluded that dietary Zn-Met at 60 mg/kg improved egg generation, egg value, semen quality, and immune status of laying Bandarah strain.

Keywords: Zinc methionine, Laying rate, Egg quality, Immunity, Semen quality.

Introduction

Zinc acts as a necessary microelement in the feed industry as an additive, whether organic or in organic used forms. Inorganic zinc is relatively inexpensive, whereas organic zinc is easier to absorb than inorganic sources. The NRC. [1] recommends a supplemental dose of 50 mg Zn/kg for laying hens.

Organic Zn plays as an essential trace mineral for metabolic functions associated with normal growth and maintenance, including bone mineralization, feather formation, enzymatic structuring, and appetite regulation [2]. Zn has a function in the synthesis of eggshell membranes [3]. Also, Martin. [4] reported that zinc activates the carbonic anhydrase enzyme which is essential for eggshell formation [5] Organic-Zn showed a There is a big difference in internal and external egg quality.

Zn plays a key role in the immune system [6]. Dietary Zn improved the antibody synthesis [7] and improved the performance of the non-specific immunity system.

Surai et al., and Gallo et al. [8,9], noted dietary Zn for broiler breeder males, improved the antioxidant enzymes in semen, increased semen volume, and improve semen quality.

The goal of this study was to compare the effects of two levels of Zn methionine versus zinc sulfate on the rate of egg-laying, egg quality, Zn content in eggs, immunity status, and sperm quality.

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Material and Methods

The present study was carried out at the El-Sabahia poultry Research station in Alexandria belonging to Animal Production Research Institute. There are a total of 300 females and 60 males of Bandarah layer strain at 30 ages in weeks were used. Birds were weighed and randomly allocated to three treated groups with 5 replicates of 20 hens and 4 cocks/ each, housed in individual cages in semi-closed house, maintained 16hrs light-8hrs dark, the average temperature in the summer season ranged between(26-30^{o}C) with (45-55%) RH.

Birds were received one of the three experimental diets as follows:

The first group of the birds was fed a standard diet supplemented with 50 mg Zn-SO4/kg. (Control group), second group 40 mg zinc methionine and (the third group), 60 mg Zn-Met the experiment lasted for 20 wks. The experimental diets' chemicals, the structure, and assessment are displayed in (Table 1) Zinc methionine complex (zinpro®180) zinc concentration 18% characteristics investigated:

The feed conversion ratio was recorded every 4weeks. Egg collection daily to calculate egg mass, and each replicate was weighed separately. Egg production rate: The percentage of egg production (%) for each laying hen for each treated group was calculated.

Egg mass (g/hen/d) = Egg weight on average (g) x egg number each 4weeks/hen. At 35, 43 and 50wk of age, 10 eggs from each replicate were randomly chosen from the same day of production to evaluate egg quality. External egg quality: The egg shape index was calculated according to Romanoff and Romanoff [10].

Shell percent: (shell weight/ egg weight) x100. Shell thickness without membranes was measured by a micrometer to the nearest 0.01mm.

Internal egg quality: yolk, albumen, and shell weights were recorded separately and calculated as a percentage of egg weight. The Haugh unit was determined by calculating according to Haugh [11] Yolk index: The index according to Funk [12].

Egg zinc concentration was measured using a commercial kit. At the conclusion of the experiment, five samples taken from each replicate were drawn from the brachial vein and placed in heparinized tubes to be counted as white blood cells (WBC's) plasma was obtained by centrifugation of the remaining blood at 3000 rpm for 20 minutes for biochemical determination (estrogen, progesterone, triiodothyronine, and carbonic anhydrase. Birds were injected with (SRBC's) into the brachial vein with 1.0 ml of a suspension of (SRBC's) 7% in sterile saline [13]. On the 7th day of injection blood samples were drowned in each bird's brachial vein and intended to allow it to clot in order to provide serum for antibody titer (HI) response to SRBC's was measured using the micro hemagglutination technique. Immunoglobulin fractions were measured according to Saif and Dohms [14]. Carbonic anhydrase was determined using a commercial kit. Plasma estradiol-17(E_2), progesterone (P_4), and T_3 were assayed by radioimmunoassay assay (RIA).

At 38 weeks of age, semen samples were collected from 20 cocks from each treatment once weekly by abdominal massage technique. Ejaculate volume (ml), sperm mortality (%), sperm abnormality (%) and sperm concentration x10^9 cell/ml were determined.

Statistical analysis:

The data were statistically analyzed using one-way ANOVA of SAS® version 9.4 (SAS Institute Inc., Cary, North Carolina, USA). The statistical model used in this study was:

$Y_{ij} = \mu + t_i + e_{ij}$

Where: Yij= an observation from the j^{th} bird, μ: general mean, t_i: effect of treatment, in the random error. The statistical significance of the effects was assessed at a P-value of 0.05. Mean was separated using SNK (Student Now man keuls).

Results and Discussion

No mortality rate was recorded during the experimental period. The dietary supplementation of organic Zinc on feed intake, egg weight, egg production (EP), and egg mass are all factors to consider under hot ambient temperature are shown in Table (2). Data concerning the effect of dietary supplementation of organic Zinc on feed consumption (FI). Recorded that the groups of 40 and 60 mg Zn-Met increased higher FI by 3.48% and 3.88%, respectively compared with the treated group fed a diet containing Zn level 50 mg Zn-sulphate/kg.

TABLE 1. Calculated composition and chemical analysis of an experimental layer diet (kg/Ton).

Feedstuffs	Control Kg/ton	Cont+40mg Zn-Met Kg/ton	Cont+ 60 mg Zn-Met Kg/ton
Yellow Corn	636.0	636.0	636.0
Wheat Bran	15.37	15.38	15.30
Soybean	246.0	246.0	246.0
Vit+Min premix[1]	3.00	3.00	3.00
NaCl	4.00	4.00	4.00
Di. Ca. Phosphate.	15.00	15.00	15.00
Limestone	80.00	80.00	80.00
Methionine	0.50	0.40	0.38
$ZnSO_4$(Kg)	0.13	00	00
Zn+Met (18%Zn)	0.00	0.22	0.33
Total	1000	1000	1000
Calculated composition, %			
CP%	16.27	16.27	16.27
ME%	2714	2714	2714
Ca%	3.08	3.08	3.08
P%	0.41	0.41	0.41
Ly%	0.81	0.81	0.81
TSAA%[2]	0.58	0.58	0.59
Supplemented Zn mg/kg	50	40	60
Total Zn mg/kg diet	75	65	85

Per kilogram of diet (free Zn), 1Vit+Min mixture provides: vitamin A, 12000 IU, vitamin E, 10 IU, menadione, 3 mg, Vit. D3, 2200 ICU, riboflavin, 10 mg, Ca pantothenate, 10 mg, nicotinic acid, 20 mg, choline chloride, 500 mg, vitamin B12, 10 g, vitamin B6, 1.5 mg, vitamin B1, 2.2 mg, folic Mn, 55, Fe, 30, Cu, 10, Se, 0.10, Antioxidant, 3 mg (milligrams per kilogram of diet). 2. Total sulpher amino acids (TSAA)

TABLE 2. Effect of dietary supplementation organic Zinc on productive performance of Bandarah laying hens under hot ambient temperature.

Criteria	Control 50mg $ZnSO_4$	Zn-Met 40mg	Zn-Met 60mg	SEM	P- value
Feed intake, g/h/d	119.57^b	120.07^b	124.40^a	0.273	0.0001
FCR, g feed/g egg	5.59^a	3.53^b	3.35^b	0.139	0.0001
Egg weight, g	47.79^b	51.49^a	51.58^a	0.299	0.0001
Egg production rate%	49.67^b	67.47^a	70.72^a	1.821	0.0001
Egg mass, g/h/d	23.66^b	34.77^a	36.42^a	0.912	0.0001

a,b,c means having different superscripts in the same row are significantly different ($P <0.05$)
SEM : standard error of means, P value : probability level, FCR: feed conversion ratio.

The results of FC were improved by increasing dietary Zn-Met level to 60 mg/kg in comparison to a control group [15] reported that the addition of Zn to the diet increased feed intake and FCR. In contrast, Prabakar et al. [5] stated that zinc methionine (Zn-Met) supplementation on feed intake has no effect. Results of egg weight indicated that EW was increased by different levels of Zn-Met (40 and 60 mg) by 7.18 and 7.35%, respectively compared with the control group. Yu et al. [16] noted that hens receiving 70 mg organic Zn/kg increased egg weight.

Results of egg production showed that there was a high difference between the treated groups and the control group. Hens received 40 and 60 mg Zn-Met/kg were recorded with the highest egg production by 26.39% and 29.77%, respectively compared with the control group. The results of Tabatabaie et al. [17] are like our results involved that by regulating the secretion of reproductive hormones, dietary Zn supplementation improved egg production performance. (Estrogen and progesterone see Table 5) during sexual development and protein synthesis epithelium during the formation of eggs. In accordance with the previous research, Abedini et al. [18] found that the addition of 80 mg/ kg Zn-Met to the basal diet and control (without Zn supplementation) of laying hens improved egg production. Lio et al. [19] found that high temperature decreased egg weight and laying rate.

While increased feed: egg ratio: in laying broiler breeders fed organic Zinc diet. Results showed that egg mass was affected by different levels of Zn-Met supplementation during the period of our studies. Results indicated that increasing dietary Zn-Met to layers diet to 60 mg Zn-Met/kg diet increased the EM by compared with control. The current results are consistent with those obtained by Chen et al. [20] showed that dietary supplementation with a 70 or 140 mg Zn-Met/kg diet significantly increased average daily egg mass. Esfahani et al. [21] found that the effect of supplemented organic Zn increased egg mass compared to ZnO. Results, Huang et al. [22] found that in poultry breeder birds, Zn deficiency reduced egg production by 3-10 g/d/bird egg mass.

The effect of organic Zinc dietary supplementation on egg quality traits of laying hens under hot ambient temperatures during different periods is shown in Table (3). Results showed that yolk weight%, yolk index, Haugh units, and shell weight% had significantly greater values in the supplementary Zn-Met fed groups compared with the control group. While egg shape index and shell thickness were higher in values with 60mg zinc compared with other treated groups. While the control group recorded lower significantly compared with Zn-Met supplemented groups. The control group recorded an increase in albumin weight% compared to other groups.

Zinc in egg yolk concentration and carbonic anhydrase (CAA) recorded higher values in the supplementary 60 mg/kg Zn-Met compared with other treated groups. Our findings are consistent with those of others obtained by, Alm El-Dein et al., and Zhang et al., [23,24] noted that dietary Zn-Met improved shell thickness, and shell weight percentage and enhance carbonic anhydrase activity also, and thicker eggshells were observed in organic Zn added groups. In contrast, Abedini et al. [18] found that the addition of 80 mg/ kg diet Zn-Met the eggshell thickness, yolk weight, Zn-Met had no effect on albumen weight, albumen height, Haugh unit, or shell weight. Similarly, shell strength and CAA activity were better in the test group when compared to the control group According to [25, 26], increasing Zn levels from 0.0 to 150 mg/kg as inorganic or organic significantly improved egg Zn concentration, with the highest concentration of 150 mg being best for layers.

The impact of dietary supplements of organic Zinc on White blood cells, lymphocytes, heterophils, and H/L of laying hens under hot ambient temperature are shown in Table (4). The data recorded that Zn-Met increased the number of WBC's count, and lymphocyte percentage. While the heterophil% and H/L ratio of the ZnSO4 group were highest in value compared to Zn-Met groups. Whereas the 60 mg Zn-Met group showed a lower mean value compared to the 40 mg/kg Zn-Met fed group. These results are supported by those obtained by, Tolba et al. [27] they noted that Zn supplementation 60 mg Zn/kg diet improved WBC 's, lymphocyte% and decreased H/L ratio of Japanese quail hens under hot environments. Also, Abedini et al. [18] found that Zn (80 mg /kg diet) increased the SRBC's antibody titer.

Furthermore, the heterophil percentage increased in the Zn-Met but, Monocyte, lymphocyte, and H/Percentages were not affected by the treatment Zn. In contrast, Chen et al. [20] studied that high-dose Zn-Met diet WBC's weren't affected.

Results of IgA, IgM, IgG, and SRBC's were significantly decreased in ZnSO4 compared with Zn-Met. While there was no difference noticed between the levels of Zn-Met with respect to IgM and IgG, IgA and SRBC's were higher at 60 mg/kg Zn-Met than 40 mg. The present results agree with Feg et al. [28] who noted that supplemented 90 mg Zn-Gly on Broiler chicks increased immunoglobulin levels (IgA, IgM, and IgG). The effect of dietary supplementation of organic Zinc on reproductive hormones and T_3 of laying hens under hot ambient temperatures are summarized in Table (5).

Results recorded that hen fed diet containing 60 mg Zn-Met/kg recorded the highest in values (E_2), (P_4), and T_3 compared to hens fed either 40 mg Zn-Met/kg or 50 mg ZnSO4. The results are consistent with those achieved by Tolba et al. [27] and Alm El-Dein et al. [23] noted that Zn supplementation increased the progesterone hormone of laying hens in hot environments. Enhancement of CA activity and elevated E_2 and P_4 in the plasma can improve the eggshell, thickness, and egg production in organic zinc than inorganic so zinc methionine was more effective in this study than zinc sulfate.

Data noticed the impact of organic Zinc dietary supplementation on semen quality of cocks under hot ambient temperatures are summarized in Table (6).

TABLE 3. Effect of dietary supplementation organic Zinc on egg quality of Bandarah laying hens under hot ambient temperature.

Criteria	Control 50mg $ZnSO_4$	Zn-Met 40mg	Zn-Met 60mg	SEM	P- value
Yolk weight,%	36.04^b	38.04^a	38.57^a	1.852	0.03671
Yolk index	139.10^b	140.30^a	140.51^a	74.41	0.0327
Albumin weight, %	50.81^a	48.38^b	47.47 ^{b}	2.319	0.0362
Haugh units	81.14 ^{b}	88.36^a	90.92 ^{a}	1.479	0.0015
Shell weight,%	13.15 ^{b}	13.59^a	13.96^a	0.784	0.0603
Egg shape index, %	75.46^b	76.56^b	80.74^a	0.949	0.0048
Shell thickness (mm)	33.72^c	37.70^b	38.74^a	0.172	0.0001
Zinc egg mg/100g	1.24^c	1.70^b	1.94^a	0.043	0.0001
Carbonic anhydrase, (U/ml)	50.09 ^{c}	70.32^b	77.62^a	0.498	0.0001

a,b,c means having different superscripts in the same row are significantly different (P <0.05) SEM : standard error of means, P value : probability level.

TABLE 4. The impact of dietary supplements organic Zinc on the immune status of Bandarah laying hens under hot ambient temperature.

Criteria	Control 50mg $ZnSO_4$	Zn-Met 40mg	Zn-Met 60mg	SEM	P-value
WBCs, x10^3/mm^3	21.33^b	25.00^a	25.65 ^{a}	0.384	0.0001
Lymphocyte, %	39.60^c	44.60^b	46.00^a	0.200	0.0001
Heterophil, %	26.00^a	24.33^b	22.40^c	0.383	0.0001
H/L	65.14^a	54.47^b	48.65^c	0.799	0.0001
IgA, (mg/ml)	0.17^c	0.41^b	0.52^a	0.007	0.0001
IgM, (mg/ml)	0.84^b	1.41^a	1.53^a	0.078	0.0001
IgG, (mg/ml)	3.22^b	4.66^a	4.71^a	0.104	0.0001
SRBC' s	4.55^c	7.06^b	8.12^a	0.141	0.0001

a,b,c denotes that having various superscripts in the same row is significant (P >0.05).

SEM stands for the standard deviation of the mean and P-value stands for probability level. H/Ratio: Heterophil/lymphocyte ratio, IgA: Alpha immunoglobulin, IgM: Mu immunoglobulin, IgG: Gamma immunoglobulin, SRBC, s: Sheep red blood cells

TABLE 5. Effect of dietary supplementation organic Zinc on estrogen, progesterone and T_3 hormones of Bandarah laying hens under hot ambient temperature.

Criteria	Control 50mg $ZnSO_4$	Zn-Met 40mg	Zn-Met 60mg	SEM	P value
Estrogen, ng/ml	533.18^c	571.34^b	626.60^a	4.848	0.0001
Progesterone, pg/ml	5.49^c	7.28^b	9.22^a	0.179	0.0001
Triiodothyronine, µg/dl	15.08^c	18.83^b	21.71^a	0.535	0.0001

a,b,c denotes that having various superscripts in the same row is significant (P >0.05).

SEM stands for the standard deviation of the mean, and P-value stands for probability level.

TABLE 6. Effect of dietary supplementation organic Zinc on semen quality of Bandarah cockers under hot ambient temperature.

Criteria	Control 50mg $ZnSO_4$	Zn-Met 40mg	Zn-Met 60mg	SEM	P value
Ejaculate volume, ml	0.33^b	0.44^a	0.45^a	0.005	0.0001
Sperm concentration $x10^9$cell/ml	1.97^c	2.29^b	2.51^a	0.036	0.0001
Sperm motility, %	87.66^c	92.00^b	94.40^a	0.569	0.0001
Sperm abnormality, %	12.34^a	8.00^b	6.00^c	0.581	0.0001
Sperm alive, %	78.08^c	86.88^b	89.86^a	0.597	0.0001
Dead, %	9.63^a	5.12^b	4.48^c	0.081	0.0001

a,b,c denotes that having various superscripts in the same row is significant (P >0.05).SEM stands for the standard deviation of the mean, and P-value stands for probability level.

Results recorded that, semen volume, sperm concentration, motility, and viability were higher in cocks fed with 60 mg Zn-Met level than in other treated groups and lower decreased sperm abnormality. Similar results were obtained by Alm El-Dein et al, and Amen and Al-Daraji [23, 29] they noted that Zn supplementation to diet, increased some semen quality traits (ejaculate volume, sperm concentration, live sperm percentage, sperm motility) in males. Prabakar at al. [5] reported that Zn-Met supplementation had significantly (P<0.05) Male breeders have higher sperm volume, motility, concentration, and live sperm count. Furthermore, Huang et al. [22] Zn deficiency in poultry breeder birds was found to result in poorer sperm performance (reducing around 10 percent sperm motility).

Conclusion

It could be recommended that supplementing Zinc methionine to Bandarah chicken's diet with a 60 mg/kg diet was more effective than 40 mg Zn-Met and 50 mg ZnSO4 for improving productive, reproductive performance, Zn content of the egg, immunological response, and semen quality under hot ambient temperature.

Conflicts of interest
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تأثير مصادر مختلفة من الزنك فى العلائق على إنتاج البيض وجودته وجودة السائل المنوي والاستجابة المناعية في الدجاج البياض المعرض لدرجات حرارة عالية

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تم اختيار مجموعه من ٣٠٠ دجاجة و ٦٠ ذكراً من سلالة البندرة عند عمر ٣٠ أسبوعاً عشوائياً وتم تقسيمهم إلى ٣ مجموعات، مع ٥ مكررات من ٢٠ دجاجة و ٤ ديكة لكل منها سكنوا في أقفاص فردية في بيت شبه مغلق. تلقت الطيور إحدى ثلاث علائق مختلفة المحتوى من كبريتات الزنك أو الزنك العضوي على شكل زنك ميثيونين بمستويين ٤٠ أو ٦٠ ملجم/كجم، استمرت التجربة لمدة ٢٠ أسبوعاً من عمر ٣٠-٥٠ أسبوعاً. أظهرت النتائج أن العليقة المحتوية على زنك ميثيونين ٤٠ أو ٦٠ ملجم/كجم عززت إنتاج البيض وحسنت معدل التحويل الغذائي، وزن البيضة، وكتلة البيض. أظهر الزنك ميثيونين اختلافات بين الصفات الخارجية والداخلية لجودة البيضة، محتوى البيضة من الزنك، ونشاط أنهيدراز الكربون مقارنةً بكبريتات الزنك. كما أن المستوى العالي من زنك ميثيونين زاد من هرموني الإستروجين والبروجسترون والثايروكسين T_3. كما تحسنت المناعة الخلطية والخلوية، وتحسنت جودة السائل المنوي. يمكن استنتاج أن العليقة المحتوية على ٦٠ ملجم زنك ميثيونين /كجم حسنت إنتاج البيض، قيمة البيضة، جودة السائل المنوي، والحالة المناعية لسلالة البندرة.

الكلمات الدالة: زنك ميثيونين ، انتاج البيض ، جودةالبيض ، المناعة ، جودة السائل المنوى.