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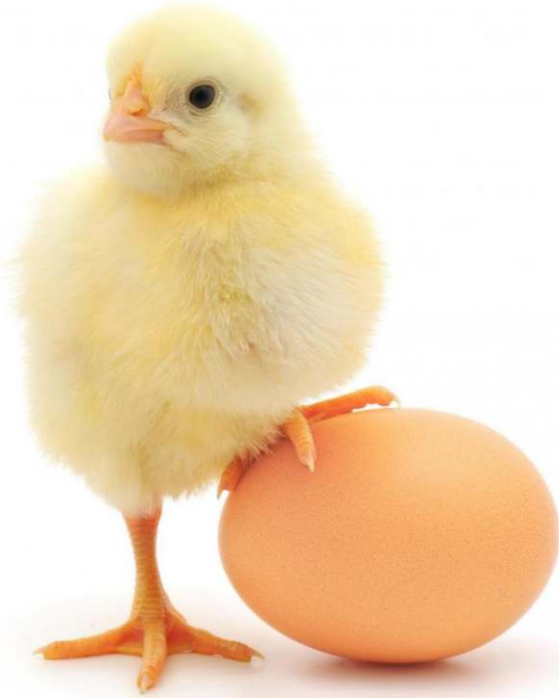

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Research Article
Influence of Dietary Vitamin A, Zinc and Copper on Productive and Reproductive Performance of Broiler Breeders

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Abstract

Objective: The study was designed to investigate the impact of selected essential micronutrients, vitamin A, zinc (Zn) and copper (Cu), on productive and reproductive performance of broiler breeders from 53-72 weeks of age. **Materials and Methods:** Total number of 96 broiler breeders (72 ♀ and 24 ♂) at 53 weeks of age, were randomly assigned to 8 equal groups of 9 hens each, divided into 3 replicate. The experiment was conducted in a 2×2×2 factorial arrangement of the dietary treatments. Eight experimental diets were formulated using two levels of vitamin A (12500 and 25000 IU kg^{-1}), two levels of Zn (132 and 264 mg kg^{-1}) and two levels of Cu (15.7 and 31.4 mg kg^{-1}) in this study. **Results:** The diet containing 12500 vitamin A IU kg^{-1}+264 Zn mg kg^{-1}+15.7 Cu mg kg^{-1} resulted in the best productive (egg production, egg mass and feed conversion ratio) and reproductive performance (fertility, hatchability and day-old chick weight). The optimal level of vitamin A, Zn and Cu resulted 12500 vitamin A IU kg^{-1}, 264 Zn mg kg^{-1} and 15.7 Cu mg kg^{-1}, respectively. **Conclusion:** Feeding diet containing 12500 vitamin A IU kg^{-1}, 264 Zn mg kg^{-1} and 15.7 Cu mg kg^{-1} would produce best productive and reproductive performances of Cairo B-2 broiler breeders.

Key words: Broiler breeders, copper, vitamin A, zinc, egg production

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

One of the main prerequisite for efficient and profitable chicken breeding is to produce fertile eggs and to obtain the highest hatchability. The production of fertile eggs is affected by many factors related to both parents and surrounding environment[1]. Moreover, a good nutritional status of the parent birds is crucial for transfer to the egg of an adequate, balanced supply of nutrients required for normal development of the embryo[2,3]. In general, the composition of macronutrients in eggs is more constant than those elements usually found at lower amounts in the diets, such as micronutrients[4]. Several micronutrients had some interrelationships with other nutrients and may affect the productive and reproductive performance of hens, especially during the last period of production[5].

Vitamin A has essential role in vision, bone and muscle growth, reproduction and maintenance of healthy epithelial tissue[6]. Vitamin A contents in the egg yolk remained stable throughout egg production when hens were supplemented with 9,000 of vitamin A IU kg^{-1} of feed, whereas the levels declined and were not able to sustain egg production in non-supplemented hens[7]. Thus, vitamin A or any of its precursors must be provided in the diet.

The Zn has a unique and extensive role in key biological processes, including immune function, growth, development and reproduction[8]. Providing maize-soybean meal diets to hens with supplemental Zn enhances progeny cellular immune status, humoral immune status and livability[9]. Inadequate Zn intake by broiler breeders results in low hatchability and poor chick quality[2]. Also, Zn plays a synergistic role on vitamin A and affects transporting, absorption and accumulating of Zn[10]. Thus, the absorption, transportation and utilization of vitamin A are influenced by Zn status[11].

Copper (Cu) is an essential trace element that plays a vital role in the physiology of animals such as fetal growth and early post-natal development, for hemoglobin synthesis, connective tissue maturation, especially in the cardiovascular system and in bones, for proper nerve function and bone development and in the inflammatory processes[6]. Copper is often added to poultry diets at prophylactic concentrations for its growth promoting effects[12]. Pearce *et al.*[13] demonstrated that pharmacological levels of Cu (>250 mg kg^{-1} diet) caused changes in 17 beta-estradiol and enzymes involved in carbohydrate, lipid and amino acid metabolism in mature laying hens and suggested that Cu supplements can affect reproductive physiology and lipid metabolism. Therefore, the main goals of this study were to investigate the effects of vitamin A, Zn and Cu on productive and reproductive performance in Cairo B-2 broiler breeders.

MATERIALS AND METHODS

Birds and management: The present study was carried out at the poultry farm and laboratory of Animal Nutrition Branch, Faculty of Agriculture, Cairo University. The experiment was designed in a 2×2×2 factorial arrangement using 96 Cairo B-2 (a new strain of the native Egyptian breed white baladi chickens females which were crossed with arbor acres grandparent female line males[14]) broiler breeders (72 ♀ and 24 ♂) that were randomly divided into 8 equal groups of 9 hens and 3 roosters each, in 3 replicates, from 53-72 weeks of age and fed two levels of vitamin A (12500 and 25000 IU kg^{-1}), two levels of Zn (132 and 264 mg kg^{-1}) and two levels of Cu (15.7 and 31.4 mg kg^{-1}). The control diet was contained 12500 vitamin A IU kg^{-1}, 132 Zn mg kg^{-1} and 15.7 Cu mg kg^{-1} as shown in Table 1. Hens and roosters were individually kept

Table 1: Composition and calculated analysis of the basal diets#

Ingredients	Female	Male
Yellow corn	69.65	66.35
Soybean meal	20.26	09.43
Wheat bran	01.00	20.40
Lime stone	06.89	01.62
Di-calcium phosphate	01.25	01.25
Vitamin premix[1]	0.200	0.200
Mineral premix[2]	0.300	0.300
Salt	0.400	0.400
Dl. Methionine	0.05	0.05
Total	100.00	100.00
Chemical composition (calculated)*		
Metabolizable energy (Kcal kg^{-1})	2800.00	2700.00
Crude protein (%)	15.00	13.00
Crude fiber (%)	3.06	4.36
Ether extract (%)	2.84	3.21
Calcium (%)	3.00	1.00
Available phosphorus (%)	0.35	0.35
Sodium (%)	0.16	0.16
Meth (%)	0.30	0.27
Meth+cysteine (%)	0.57	0.52
Lysine (%)	0.73	0.55
Vitamin A (IU kg^{-1})	12533.00	12461.00
Zinc (mg kg^{-1})	132.00	146.00
Copper (mg kg^{-1})	15.70	15.90

#Chemical compositions of feed stuffs were calculated according to NRC (1994). Vitamin A (as retinol where each gram contains 1 million IU of vitamin A), Zn oxide (75%) and Cu sulphate (25%) were purchased from local market in Egypt (Misr for Feed Additives). [1,2] Each 1 kg diet contains vitamin A 11000 IU, vitamin D3 3500 IU, vitamin E 100 mg, vitamin K3 4.4 mg, vitamin B_1 6.6 mg, vitamin B_2 12 mg, vitamin B_6 4.4 mg, vitamin B_{12} 0.022 mg, pantothenic acid 15.5 mg, nicotinic acid 50 mg, folic acid 2 mg, biotin 0.22 mg, choline chloride 2.422 gm, manganese 120 mg, zinc 110 mg, iron 44 mg, copper 9 mg, iodine 1.2 mg, selenium 0.4 mg and cobalt 0.30 mg. *Kcal: Kilocalorie, kg: Kilogram, mg: Milligram and IU: International unit in 2-deck batteries of clean, wire-mesh cages, with cage dimensions of 25×45 cm in open system housing. Mash feed form was offered at constant amount (125 g/hen/day and 130 g/rooster/day). Water was offered *ad libitum* over the experimental period (20 weeks), under a total of 16 h light per day regimen.

Measurements: Hen-day egg production (HD) percentage was calculated every 4 weeks of the total experimental period (20 weeks). Eggs were collected and weighed every 4 weeks during the experimental periods. Records of egg production [EP(HD)] and egg weight (EW) were used to calculate egg mass (EM, g/hen/day). The EM and feed intake (FI) were used to calculate the amount of feed (kg) which was required to produce one kg of eggs per hen or to calculate feed conversion ratio (FCR) during specific period. Shell thickness was determined using a dial pipe gauge. Haugh units (HU) were calculated based upon the height of albumen determined by a micrometer and EW[15]:

$$\text{Albumen index (AI)} = \frac{\text{Height}}{\text{Diameter mean}} \times 100$$

Dry shell weighed to the nearest 0.10 g:

$$\text{Egg shell percentage (ESP)} = \frac{\text{Egg shell weight}}{\text{EW}} \times 100$$

Egg content weight, was calculated by the difference between EW and egg shell weight[16]:

$$\text{Egg content percentage (ECP)} = \frac{\text{Egg content weight}}{\text{EW}} \times 100$$

Heamagglutination inhibition (HI) titers against Newcastle disease virus (NDV) were determined according to the method described by Van der Zijpp *et al.*[17]. Blood hemoglobin was measured according to El-Husseiny *et al.*[5]. Reproductive performance including fertility, hatchability and one-day chick's weight were calculated.

Statistical analysis: Data pooled through the experiment were proceeded by General Linear Model procedures (GLM) described in SAS User's Guide (SAS, Institute[18]). The significant mean differences among treatments means were separated by Duncan's multiple range-test[19] (differences were considered significant at $p<0.05$), using factorial analysis model as follows:

$$Y_{ijkl} = \mu + A_i + Z_j + C_k + (AZC)_{ijk} + e_{ijkl}$$

Where:

Y_{ijkl}	=	Observed value of a given dependent variable
μ	=	Overall adjusted mean
A_i	=	Fixed effect of vitamin A levels
i	=	1, 2
Z_j	=	Fixed effect of Zn levels
j	=	1, 2
C_k	=	Fixed effect of Cu levels
k	=	1, 2
AZC_{ijk}	=	Fixed effect of interaction between vitamin A, Zn and Cu
e_{ijkl}	=	Random error associated to each observation

RESULTS AND DISCUSSION

Table 2 shows the effect of experimental treatments on HD egg production, egg weight (EW), egg mass (EM) and FCR. No significant ($p<0.05$) difference between each levels of vitamin A, Zn and Cu regarding all the productive performance parameters were observed. The results agree with Coskun *et al.*[20], who found that vitamin A supplementation at levels of 0, 4000, 12000 and 24000 IU kg^{-1}

Table 2: Effect of experimental treatments on productive performance[1]

Experimental treatments	EP (%)	EW (g)	EM (g/h/day)	FCR
Effects				
Vitamin A (A) effect				
12500 IU kg^{-1} (A1)	54.4	58.6	31.9	3.93
25000 IU kg^{-1} (A2)	54.0	57.4	31.0	4.04
SEM	0.77	0.76	0.66	0.08
p-value	0.65	0.15	0.22	0.25
Zinc (Zn) effect				
132 mg kg^{-1} (Zn1)	52.9^{b}	57.9	30.6^{b}	4.09^{b}
264 mg kg^{-1} (Zn2)	55.6^{a}	58.1	32.3^{a}	3.88^{a}
SEM	0.62	0.80	0.58	0.06
p-value	0.02	0.80	0.02	0.03
Copper (Cu) effect				
15.7 mg kg^{-1} (Cu1)	55.0	57.8	31.8	3.94
31.4 mg kg^{-1} (Cu2)	53.4	58.3	31.1	4.03
SEM	0.76	0.80	0.67	0.08
p-value	0.12	0.49	0.36	0.38
A×Zn×Cu interactions				
[2]T1 (A1+Zn1+Cu1)	54.4	58.0	31.5	3.97
T2 (A1+Zn1+Cu2)	50.5	58.4	29.5	4.25
T3 (A1+Zn2+Cu1)	58.0	58.8	34.1	3.67
T4 (A1+Zn2+Cu2)	54.9	59.4	32.6	3.84
T5 (A2+Zn1+Cu1)	53.9	56.9	30.7	4.09
T6 (A2+Zn1+Cu2)	52.6	58.4	30.8	4.08
T7 (A2+Zn2+Cu1)	53.8	57.3	30.9	4.06
T8 (A2+Zn2+Cu2)	55.6	57.1	31.7	3.95
SEM	0.99	1.59	1.11	0.12
p-value	0.22	0.84	0.29	0.34

[a,b]Means in the same column, within each factor with different superscripts are significantly ($p\leq 0.05$) different. [1]EP: Egg production percentage, EW: Egg weight, EM: Egg mass, FCR: Feed conversion ratio (g feed g^{-1} egg). [2] T1 is considered as control

to layer diets had no significant effect on egg yield. Kaya *et al.*[11] reported that adding vitamin A to hen's diet resulted in no significant differences in egg production, egg weight, egg mass and FCR, but Abdalla *et al.*[21] concluded that increasing high level of vitamin A (24000 IU kg^{-1} diet) significantly increased egg production. On the other hand, inclusion different levels of vitamin A (5000, 10000,15000, 20000, 25000, 30000 or 35000 IU kg^{-1}) did not affect laying performance of broiler breeders[22], while inclusion of 10800 vitamin A IU kg^{-1} in native Chinese broiler breeders diet improved egg production and egg mass[3]. Birds received 120, 180 and 210 Zn mg kg^{-1} DM in their diets laid 1.21, 1.64 and 1.76% more eggs than those in the control[23]. Egg production increased by inclusion high level of Zn (175 mg kg^{-1})[5]. Hen-day egg production was the greatest when diets were supplemented with Zn[8]. Also, adding 100 Zn mg kg^{-1} in organic form for 12 weeks improved laying performance[24], whereas, Abedini *et al.*[25] observed that 80 Zn mg kg^{-1} in the form of nano Zn enhanced egg production and egg mass. Broiler breeders showed improvements in egg production, egg weight and FCR as a result of adding 80 mg organic Zn kg^{-1} [2].

In laying hens, adding 100, 200 or 300 Cu mg kg^{-1} had no effect on egg rate, egg mass, egg weight or FCR[26]. Similar results were obtained in laying ducks that fed diets supplemented with 4, 8, 12, 16, 20 or 24 mg Cu kg^{-1}[27]. Furthermore, in breeders elevating Cu level from 10-120 mg kg^{-1} diet had no effect on laying performance (egg production, egg weight, egg mass and FCR)[28]. Conversely, egg production was significantly increased in the second 4 weeks period by supplementing high level of Cu (250 Cu mg kg^{-1}) in laying hen diets[29]. Also, adding high level of Cu (125 or 250 mg kg^{-1}) declined egg production[30]. Hens fed diets supplemental with 250 Cu mg kg^{-1} showed improvements in egg production but egg weight significantly decreased and egg mass and FCR did not alter[31].

Table 3 shows the effects of experimental treatments on egg quality and blood parameters of the hens. Neither egg quality parameters nor blood parameters were significantly affected ($p \leq 0.05$) by levels of vitamin A, Zn and Cu or their combinations levels, except for yolk color index. In yellow feather broiler breeders, increasing level of vitamin A had no effect on eggshell quality[3], whereas, in Ross broiler breeders, yolk color and eggshell thickness negatively affected by increasing vitamin A levels[22]. Eggshell quality negatively affected by increasing Zn level in laying hen's diet[5]. Adding 40 Zn mg kg^{-1} diet in the form of nano-Zn increased eggshell quality without effect on yolk color score compared with the control that contained 27 mg Zn from ingredients[25]. Adding 100 Zn mg kg^{-1} increased eggshell thickness compared with

Table 3: Effect of experimental treatments on egg quality

Experimental treatments	Egg shell thickness (mm)	Eggshell (%)	Egg contents (%)	Haugh units (%)	Yolk color index
Effects					
Vitamin A (A) effect					
12500 IU kg^{-1} (A1)	0.379	9.50	90.50	72.50	7.40^{b}
25000 IU kg^{-1} (A2)	0.392	9.70	90.30	70.40	8.00^{a}
SEM	0.01	0.19	0.19	1.32	0.22
p-value	0.20	0.72	0.72	0.34	0.04
Zinc (Zn) effect					
132 mg kg^{-1} (Zn1)	0.382	9.60	90.40	72.30	7.50
264 mg kg^{-1} (Zn2)	0.389	9.60	90.40	70.50	7.90
SEM	0.01	0.19	0.19	1.27	0.23
p-value	0.49	0.72	0.72	0.42	0.19
Copper (Cu) effect					
15.7 mg kg^{-1} (Cu1)	0.390	9.70	90.30	71.40	7.50
31.4 mg kg^{-1} (Cu2)	0.382	9.50	90.50	71.50	7.90
SEM	0.01	0.19	0.19	1.35	0.24
p-value	0.43	0.63	0.63	0.96	0.27
A×Zn×Cu interactions					
[1]T1 (A1+Zn1+Cu1)	0.385	9.70	90.30	70.70	7.00
T2 (A1+Zn1+Cu2)	0.376	9.30	90.70	73.50	7.60
T3 (A1+Zn2+Cu1)	0.383	9.40	90.60	73.30	7.30
T4 (A1+Zn2+Cu2)	0.373	9.80	90.20	72.50	7.50
T5 (A2+Zn1+Cu1)	0.375	9.40	90.60	73.10	7.90
T6 (A2+Zn1+Cu2)	0.392	9.80	90.20	72.00	7.40
T7 (A2+Zn2+Cu1)	0.416	10.10	89.90	68.40	7.90
T8 (A2+Zn2+Cu2)	0.386	9.30	90.70	68.00	8.90
SEM	0.01	0.31	0.31	2.21	0.39
p-value	0.44	0.22	0.22	0.75	0.34

[a,b]Means in the same column, within each factor with different superscripts are significantly ($p \leq 0.05$) different. [1]T1 is considered as control

Table 4: Effect of experimental treatments on reproductive performance and blood parameters

				Blood parameters	
Experimental treatments	Fertility (%)	Hatchability (%)	Chick weight (g)	HI titers against NDV	Hemoglobin content
Effect					
Vitamin A (A) effect					
12500 IU kg^{-1} (A1)	0.379	9.50	90.50	8.30	15.20
25000 IU kg^{-1} (A2)	0.392	9.70	90.30	8.30	15.10
SEM	0.01	0.19	0.19	0.18	0.38
p-value	0.20	0.72	0.72	0.89	0.88
Zinc (Zn) effect					
132 mg kg^{-1} (Zn1)	0.382	9.60	90.40	8.20	15.8^{a}
264 mg kg^{-1} (Zn2)	0.389	9.60	90.40	8.30	14.6^{b}
SEM	0.01	0.19	0.19	0.18	0.34
p-value	0.49	0.72	0.72	0.76	0.03
Copper (Cu) effect					
15.7 mg kg^{-1} (Cu1)	0.390	9.70	90.30	8.30	15.10
31.4 mg kg^{-1} (Cu2)	0.382	9.50	90.50	8.30	15.30
SEM	0.01	0.19	0.19	0.18	0.38
p-value	0.43	0.63	0.63	0.89	0.69
A×Zn×Cu interactions					
[1]T1 (A1+Zn1+Cu1)	0.385	9.70	90.30	8.10	15.90
T2 (A1+Zn1+Cu2)	0.376	9.30	90.70	8.20	15.40
T3 (A1+Zn2+Cu1)	0.383	9.40	90.60	8.50	14.00
T4 (A1+Zn2+Cu2)	0.373	9.80	90.20	8.30	15.50
T5 (A2+Zn1+Cu1)	0.375	9.40	90.60	8.40	15.80
T6 (A2+Zn1+Cu2)	0.392	9.80	90.20	8.20	15.90
T7 (A2+Zn2+Cu1)	0.416	10.10	89.90	8.10	14.40
T8 (A2+Zn2+Cu2)	0.386	9.30	90.70	8.30	14.40
SEM	0.01	0.31	0.31	0.39	0.72
p-value	0.44	0.22	0.22	0.95	0.65

[a,b]Means in the same column, within each factor with different superscripts are significantly ($p \le 0.05$) different. [1]T1 is considered as control, NDV: Newcastle disease virus

the control that contained 50 mg Zn[24]. In broiler breeders adding 80 Zn mg kg^{-1} in the form of organic Zn improved eggshell quality compared with the control that did not contain supplemental Zn[2]. Adding 250 Cu mg kg^{-1} did not affect egg shell quality in laying hens and breeders[28,30,31]. Also, in laying ducks, dietary Cu had no effects on yolk color, eggshell thickness or strength[27].

The effect of the experimental treatments on reproductive performance and hemoglobin are presented in Table 4. Vitamin A levels showed no significant effect on fertility and one-day old chick weight, while low level resulted in significantly ($p \le 0.05$) better hatchability. The higher level of Zn improved the one-day old chick weight, while fertility and hatchability were not significantly affected by Zn level. Copper levels had no significant effect on one-day old chick weight. Low Cu level recorded better ($p \le 0.05$) fertility and hatchability values compared to the higher level. The diet contained 12500 vitamin A IU kg^{-1}, 264 Zn mg kg^{-1} and 15.7 Cu mg kg^{-1} recorded the better ($p \le 0.05$) fertility and hatchability values, followed by diet contained 25000 IU vitamin A kg^{-1}, 132 mg Zn kg^{-1} and 15.7 mg Cu kg^{-1}. Vitamin A is required for several critical life-sustaining processes, including reproduction, metabolism, differentiation, hematopoiesis, bone development and pattern formation during embryogenesis[32]. In the testis, vitamin A is required for the normal production of spermatozoa[33]. Fertility percentage was significantly ($p < 0.01$) affected by supplementation of vitamin A, where the best percentage was recorded for the layer fed 12000 vitamin A IU kg^{-1} diet and 4000 vitamin A IU kg^{-1} compared with 0 and 24000 IU kg^{-1}[21]. Increasing vitamin A from 5000-135000 IU kg^{-1} had no effect on fertility or hatchability in Ross broiler breeders[22]. Also, hatchability and fertility were not affected by increasing vitamin A levels from 170-21600 IU kg^{-1} but 10800 vitamin A IU kg^{-1} improved hatchling weight of Chinese native broiler breeders[3]. Zinc is recognized as the most critical trace mineral for male sexual function[34]. Zinc helps in protecting the structure of the genetic material or the DNA chromatin in the sperm nucleus. This structure is important for successful fertilization[34]. Feeding broiler breeders diets contained 80 Zn mg kg^{-1} in the form of organic Zn improved fertility, hatchability and qualified chicks compared with the control that did not contain supplemental Zn[2], whereas, dietary Cu had no effect on fertility or hatchability of hen breeders when levels of Cu increased from

10-120 mg kg^{-1} in their diets[28]. In laying hens, Skrivan *et al.*[35] showed antagonism among Zn, Cu and iron, which negatively affect their absorption and utilization. It has been reviewed that Zn affects poultry immunity[36]. Thus, it is determined haemoglobin and immunoglobulin levels in this experiment. Previous study, in laying hens, showed that dietary Zn supplementation improved haemoglobin concentrations and immunoglobulin[5], whereas, dietary Cu supplementation had no effect on globulin levels in breeders[28]. Also, dietary vitamin A supplementation did not affect Newcastle disease virus antibody titer in broiler breeders[22]. Generally, adding different nutrients to the birds that received enough amounts of nutrients in their diets do not affect their performance[19].

CONCLUSION

It is concluded that feeding diet containing 12500 vitamin A IU kg^{-1}, 264 Zn mg kg^{-1} and 15.7 Cu mg kg^{-1} would produce best productive and reproductive performances of Cairo B-2 broiler breeders.

SIGNIFICANCE STATEMENT

This study discovers the possible synergistic effect of vitamin A, Zn and Cu combination that can be beneficial for productive and reproductive performances of Cairo B-2 broiler breeders. This study will help the researchers and producers to fulfill the genetic potential of Cairo B-2 broiler breeders. Thus, a new theory on these micronutrients combination and possibly other combinations, may be arrived at.

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