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Effect of Using Different Levels of Iron with Zinc and Copper in Layer's Diet on Egg Iron Enrichment

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Abstract: One hundred and eighty Mandarah laying hens (Egyptian local strain) at 24 wks of age were equally divided into twelve treatments to study the effect of iron, Copper and Zinc supplementation on egg iron concentration, performance and egg quality of laying hens. The experimental treatments included three levels of supplemental iron (Fe) 0, 100 and 200 mg/kg diet, in combination with 0, 20 mg Copper (Cu), 45 mg Zinc (Zn) and 20 mg Cu+45 mg Zn /kg diet in factorial arrangement 3 x 4 during three interval periods (4 weeks each). The highest concentration of egg iron was observed for hens fed diet supplemented with 200 mg Fe/kg in combination with Cu and Zn. Followed by those fed 100 mg Fe/kg with the same previous elements being 3.85 and 3.59 mg/100 gm egg respectively. However, supplementation of Cu and Zn individually without iron gave the lowest values of iron in the egg. Iron supplementation either at the level of 100 or 200 mg/kg improved feed conversion and egg mass. The best value of feed conversion and egg mass was detected for those fed 100 mg Fe/kg with Zn supplementation. Egg quality was not affected by iron supplementation. Zn supplementation resulted in the highest value of shell percentage, while Zn + Cu supplementation showed the best value of Haugh Units. Blood hemoglobin g/100 ml and PCV % increased significantly ($p \leq 0.05$) with increasing iron level. Iron addition with Cu or/and Zn improved the economical efficiency as compared to the control.

Key words: Layer's diet, egg iron, hemoglobin

INTRODUCTION

Eggs consider a good source of the three main nutritional requirements: energy, protein and essential accessory factors (vitamins, carotenoids, minerals and certain fatty acids). Add to this, the fact that eggs are economical and you have a near perfect food. Eggs satisfy the needs of poor and busy Egyptians. Nowadays nutritionists pay an attention for increasing the nutritive value of eggs through altering the nutritional composition of the diet fed to chickens. Enriched eggs with iron will resulted in eggs with superior biologic and nutritional value for human consumption.

Iron is an integral part of many proteins and enzymes that maintain good health. In humans, iron is an essential component of proteins involved in oxygen transport (Dallman, 1986). It is also essential for the regulation of cell growth and differentiation (Bothwell *et al*., 1979; Andrews, 1999). A deficiency of iron limits oxygen delivery to cells, resulting in fatigue, poor work performance and decreased immunity (Haas and Brownlie, 2001) and (Bhaskaram, 2001).

The trace elements Fe, Cu and Zn are essential for human and animal nutrition. Thus they are supplemented in animal and poultry diets, but these supplements frequently exceed nutritional requirements. Higher doses of trace elements for animals and poultry are also designed to enrich the product from the aspect of human nutrition. In this respect Skrivan *et al*. (2005) found that supplementation of the basal diet with 120 mg Fe/kg diet increased Fe concentration in egg yolk and white, however the combination of Fe with Zn and Cu improved these increments. Recently Paik *et al*. (2009) found strong interactions between Fe, Zn and Cu. Also Arredondo *et al*. (2006) reported that Fe was affected by dietary Fe and the interaction between Cu and Zn. Moreover Hoffman *et al*. (2000) demonstrated that Supplemental iron is available in two forms: ferrous and ferric, Ferrous iron salts are the best absorbed forms of iron supplements. In this respect Youjin *et al*. (2004) indicated that a concentration of 80 mg added Fe as Fe SO_4/kg diet significantly increased the iron level by 43.95% in yolk and by 72.01% in albumen. Also (Biehl *et al*., 1997) found that weight gain, hemoglobin and hematocrit were markedly improved when supplemental iron level as ferrous sulfate increased from 0.0-80 mg/kg in chicks diet.

According to the Egyptian policy for facing the anemia crisis especially in children, this study is an attempt to solve this problem by increasing egg iron content using different levels of iron in layers diet with or without some

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minerals which work synergistically to improve the utilization of iron.

MATERIALS AND METHODS

This study was conducted at Inshas poultry research station (El-Sharkia Governorate), Animal Production Research Institute, Agriculture Research Center.

A total number of 180 Mandarah laying hens (Egyptian local strain) 24-Wk-old were randomly selected from the farm flock, to be similar in weight and productivity. They were randomly distributed into 12 treatments each of 15 layers (3 replicates in each). All birds were kept individually in layer's cages under the same managerial condition. Feed and water were available *ad libitum*. They received the basal layer diets (as shown in Table 1) which consisted primarily of corn and soybean meal. The basal layer diets covered all the essential nutrients according to the ministerial decree No. 1498. The analytical values of the basal diet were tabulated in Table 1.

Table 1: Composition and calculated analysis of the experimental diets

Ingredients	%
Corn yellow	61.17
Soybean meal 44%	16.70
Wheat bran	6.70
Corn gluten (60) %	4.70
DL-Methionine (99%)	0.51
Calcium carbonate	8.13
Di. Calcium. Phosphate.	1.42
NaCl	0.37
Vitamin and Minerals premix*	0.30
Total	100.00
ME (kcal/kg)	2699.00
CP (%)	16.02
CF (%)	3.47
Ca (%)	3.45
Av. P (%)	0.40
Lysine	0.73
Methionine (%)	0.34
Methionin + Cystine (%)	0.62
Na (%)	0.19

*Supplied per kg of diet: Vit. A, 12000 IU; Vit. D3, 2200 ICU; Vit. E, 10 mg; Vit K3, 2 mg; Vit. B1, 1 mg; Vit. B2 5 mg; B6 1.5 mg; B12 10 mcg; Nicotinic acid 30 mg; Folic acid 1 mg, Pantothenic acid 10 mg; Biotein 1.5 mcg; Choline 250 mg; Copper 10 mg; Iron 30 mg; Manganse 60 mg; Zinc 50 mg; Iodine 1 mg; Selenium 0.1 mg; Cobalt 0.1 mg

The experimental period lasted for 12 weeks in three subsequent interval periods each of four weeks. One treatment of layers received the basal diet, which assigned as the control treatment. Other layers of different treatments received diets supplemented with $FeSO_4$ to add Fe concentration to 100 and 200 mg Fe/kg diet over the requirements, Also $CuSO_4.5H_2O$ and $ZnSO_4.7H_2O$ to add Cu and Zn concentration to 20 and 45 mg/Kg diet, respectively over the requirement in factorial arrangement (3 x 4) as shown in Table 2.

Table 2: Experimental design

Dietary treatments	
Copper and zinc supplementation (mg/kg)	Iron supplementation (mg/kg)
0.0	0.0
20 mg Cu	
45 mg Zn	
20 mg Cu + 45 mg Zn	
0.0	100
20 mg Cu	
45 mg Zn	
20 mg Cu + 45 mg Zn	
0.0	200
20 mg Cu	
45 mg Zn	
20 mg Cu + 45 mg Zn	

The productive traits were calculated as egg/hen/period for each replicate during the three interval periods (from 24-36 Wks of age). Egg mass was calculated by multiplying egg number by egg weight average. Feed conversion was calculated as kg feed consumption divided by Kg egg mass (kg feed\kg egg).

Egg quality was measured at the end of each interval period, in which 18 eggs were randomly chosen from each treatment (6 eggs from each replicate). Eggs were individually broken out, shape index, yolk index values were measured according to Sauter *et al.* (1951) in addition to shell, yolk, albumen percentages were calculated according to Paganelli *et al.* (1974) Also Haugh Units according to Williams (1997).

The concentrations of Fe, (mg/100 gm egg) were determined in 6 chosen randomly eggs for each treatment in the principal center lab (Cairo University-Faculty of Agriculture), samples were digested by the Advanced Microwave Digestion System and measured by ICP spectrometer (ICAP 6000 series; Thermo Scientific).

At the end of the study, blood samples were collected from 6 birds chosen randomly from each treatment and then hemoglobin (g/100 ml) was determined by hemoglobinmeter and hematocrit (%) by heparinized microhematocrite tubs. Remaining blood was centrifuged at 3000 Xrpm for 10 min and plasma separated then stored at -20°C for analysis. Creatinin (mg/100 ml) Alanine Transaminase (ALT) and Aspartate Transaminase (AST) concentration were calorimetrically determined. The manufacturer recommendations of commercial kits were used for all determinations.

Data were subjected to a factorial arrangement (3 x 4), statistical analysis using General Linear Model of SAS (SAS Institute, 2004). Means were separated by Duncan Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Productive traits: Impact of supplemental Iron (Fe), Copper (Cu) and Zinc (Zn) and their interaction on feed intake, feed conversion, egg number, egg weight and

Table 3: Productive performance of Mandarah laying hens as affected by iron supplementation during the experimental period (24-36 wks of age)

	Iron supplementation (mg/kg diet)			
Items	0.0 iron	100	200	Significance
Feed intake (kg/hen/period)				
First period 24-28	2.87±0.04[a]	2.84±0.03[a]	2.64±0.04[b]	**
Second period 28-32	2.70±0.02	2.71±0.01	2.64±0.05	NS
Third period 32-36	2.57±0.05	2.61±0.04	2.58±0.05	NS
Overall mean	2.72±0.03[a]	2.72±0.02[a]	2.62±0.03[b]	**
Feed conversion (kg feed/kg egg mass)				
First period 24-28	3.50±0.19	3.18±0.15	3.39±0.15	NS
Second period 28-32	3.72±0.26[a]	3.11±0.13[b]	3.60±0.17[ab]	*
Third period 32-36	3.74±0.26[a]	3.08±0.11[b]	3.03±0.12[b]	**
Overall mean	3.63±0.21[a]	3.11±0.10[b]	3.30±0.07[ab]	*
Egg number (hen/period)				
First period 24-28	17.70±0.73	19.28±0.74	17.25±0.79	Ns
Second period 28-32	15.55±0.90[b]	18.32±0.69[a]	16.00±0.62[b]	**
Third period 32-36	14.65±0.96[b]	17.48±0.62[a]	17.87±0.48[a]	**
Overall mean	15.96±0.80[b]	18.36±0.58[a]	17.03±0.44[ab]	*
Egg weight (g)				
First period 24-28	46.77±0.28	46.48±0.46	45.91±0.22	NS
Second period 28-32	47.59±0.45[ab]	47.95±0.43[a]	46.43±0.31[b]	*
Third period 32-36	48.25±0.37	48.55±0.35	47.87±0.36	NS
Overall mean	47.53±0.34	47.66±0.39	46.74±0.23	NS
Egg mass (kg/hen)				
First period 24-28	0.828±0.04[b]	0.897±0.03[a]	0.792±0.04[b]	*
Second period 28-32	0.739±0.04[b]	0.879±0.03[a]	0.742±0.04[b]	**
Third period 32-36	0.707±0.05[b]	0.849±0.03[a]	0.856±0.03[a]	**
Overall mean	0.759±0.04[b]	0.875±0.03[a]	0.795±0.02[b]	*

[a-ab]Means in the same row with different letters are significant. NS: Not Significant, *: $p \leq 0.05$, **: $p \leq 0.01$

Table 4: Productive performance of Mandarah laying hens as affected by copper or/and zinc supplementation during the experimental period (24-36 wks of age)

	Cu and Zn supplementation (mg/kg diet)				
Items	0.0	Cu	Zn	Cu+Zn	Significance
Feed intake kg/hen/period					
First period 24-28	2.90±0.05[a]	2.76±0.04[b]	2.75±0.04[b]	2.73±0.07[b]	**
Second period 28-32	2.73±0.02[a]	2.71±0.03[ab]	2.70±0.05[ab]	2.61±0.04[b]	*
Third period 32-36	2.75±0.02[a]	2.48±0.04[b]	2.51±0.03[b]	2.60±0.05[b]	**
Overall mean	2.79±0.03[a]	2.65±0.02[b]	2.65±0.03[b]	2.65±0.04[b]	**
Feed conversion (kg feed/kg egg mass)					
First period 24-28	3.43±0.25[ab]	3.60±0.14[a]	3.38±0.17[ab]	2.98±0.11[b]	*
Second period 28-32	3.21±0.28	3.70±0.26	3.69±0.25	3.30±0.13	NS
Third period 32-36	3.60±0.28[a]	3.39±0.26[ab]	3.01±0.17[b]	3.11±0.15[b]	*
Overall mean	3.40±0.25[ab]	3.54±0.18[a]	3.33±0.16[b]	3.12±0.10[c]	*
Egg number (hen/period)					
First period 24-28	18.53±1.08[ab]	16.58±0.86[b]	17.56±0.81[ab]	19.64±0.54[a]	*
Second period 28-32	18.13±1.07[a]	15.67±0.97[b]	15.73±0.92[b]	16.96±0.60[ab]	*
Third period 32-36	16.24±0.91	15.76±1.27	17.24±0.83	17.42±0.72	NS
Overall mean	17.63±0.94	16.00±0.86	16.83±0.66	18.00±0.54	NS
Egg weight (g)					
First period 24-28	45.69±0.47	46.46±0.39	46.73±0.41	46.66±0.25	NS
Second period 28-32	47.11±0.43	47.88±0.59	47.54±0.47	46.76±0.48	NS
Third period 32-36	47.44±0.42	48.52±0.46	48.79±0.39	48.13±0.30	NS
Overall mean	46.75±0.38	47.62±0.44	47.69±0.39	47.18±0.32	NS
Egg mass (kg/hen)					
First period 24-28	0.844±0.05[b]	0.770±0.04[c]	0.822±0.04[b]	0.916±0.03[a]	*
Second period 28-32	0.854±0.05	0.750±0.05	0.750±0.05	0.793±0.03	NS
Third period 32-36	0.770±0.04	0.764±0.06	0.842±0.04	0.839±0.04	NS
Overall mean	0.824±0.04	0.762±0.04	0.804±0.04	0.849±0.03	NS

[a-c]Means in the same row with different letters are significant. NS: Not Significant, *: $p \leq 0.05$, **: $p \leq 0.01$

Table 5: Feed intake and feed conversion of Mandarah laying hens as affected by the interaction between iron copper and zinc supplementation during the experimental period (24-36 wks of age)

Iron levels mg/kg	Cu and Zn supplementation mg/kg diet	Feed intake (kg/hen/period)				Feed conversion (kg feed/kg egg mass)			
		24-28 wks	28-32 wks	32-36 wks	Overall mean	24-28 wks	28-32 wks	32-36 wks	Overall mean
0.0	0.0	3.04±0.14^{a}	2.72±0.04^{a}	2.78±0.04^{a}	2.85±0.07^{a}	3.55±0.62^{ab}	3.50±0.83^{abc}	4.16±0.72^{a}	3.71±0.72^{ab}
	20 mg Cu	2.77±0.03^{bc}	2.73±0.06^{a}	2.42±0.06^{e}	2.64±0.02^{bcd}	3.67±0.35^{ab}	4.45±0.46^{a}	4.40±0.18^{a}	4.13±0.26^{a}
	45 mg Zn	2.86±0.04^{ab}	2.70±0.01^{a}	2.55±0.08^{ebcd}	2.70±0.03^{bc}	3.57±0.24^{ab}	3.80±0.32^{abc}	3.48±0.28^{ab}	3.61±0.27^{abc}
	20 mg Cu + 45 mg Zn	2.83±0.02^{bc}	2.66±0.04^{a}	2.54±0.08^{ebcd}	2.68±0.04^{bc}	3.15±0.30^{ab}	3.11±0.13^{c}	2.90±0.23^{b}	3.05±0.22^{bc}
100	0.0	2.86±0.04^{ab}	2.76±0.04^{a}	2.70±0.04^{abc}	2.77±0.01^{ab}	3.47±0.48^{ab}	2.96±0.16^{c}	3.19±0.17^{b}	3.19±0.25^{abc}
	20 mg Cu	2.89±0.04^{ab}	2.70±0.02^{a}	2.53±0.1^{ebcd}	2.71±0.04^{bc}	3.30±0.19^{ab}	3.00±0.20^{c}	3.01±0.18^{b}	3.11±0.17^{bc}
	45 mg Zn	2.73±0.02^{bc}	2.70±0.02^{a}	2.52±0.05^{ecd}	2.65±0.02^{bcd}	2.88±0.19^{ab}	2.96±0.23^{c}	2.78±0.19^{b}	2.87±0.18^{c}
	20 mg Cu + 45 mg Zn	2.86±0.1^{ab}	2.69±0.03^{a}	2.68±0.03^{abcd}	2.74±0.04^{ab}	3.04±0.12^{ab}	3.48±0.35^{abc}	3.33±0.31^{b}	3.27±0.20^{abc}
200	0.0	2.80±0.02^{bc}	2.70±0.04^{a}	2.76±0.03^{ab}	2.75±0.03^{ab}	3.26±0.26^{ab}	3.17±0.15^{bc}	3.45±0.22^{ab}	3.29±0.18^{abc}
	20 mg Cu	2.62±0.04^{dc}	2.69±0.06^{a}	2.50±0.06^{edc}	2.60±0.03^{cd}	3.84±0.13^{a}	3.64±0.13^{abc}	2.77±0.01^{b}	3.37±0.03^{abc}
	45 mg Zn	2.64±0.06^{dc}	2.70±0.16^{a}	2.46±0.01^{ed}	2.60±0.06^{cd}	3.68±0.23^{ab}	4.29±0.41^{ab}	2.77±0.25^{b}	3.51±0.15^{abc}
	20 mg Cu + 45 mg Zn	2.50±0.09^{d}	2.49±0.03^{b}	2.60±0.14^{abcde}	2.53±0.07^{d}	2.756±0.06^{b}	3.31±0.09^{bc}	3.11±0.24^{b}	3.04±0.09^{bc}
Sig.		**	*	*	**	*	*	*	*

$^{a-c}$Means in the same raw with different letters are significant ($p<0.05$). *: ($p<0.05$), **: ($p<0.01$), Sig.: Significance

egg mass of Mandarah laying hens are presented in Tables 3, 4, 5 and 6. Concerning the effect of supplemental iron (Table 3), it was clearly noted that, feed intake was significantly decreased by increasing the level of add iron during the first period (24-28 wks of age), while during the second and third periods (28-32 and 32-36 wks of age, respectively) there were no significant differences were observed. In this respect, Cao *et al*. (1996) stated that feed intake decreased significantly ($p\leq 0.001$) with iron supplementation in broiler chicks diets.

Respecting feed conversion, significant effect was detected between layers fed diet without Fe supplementation and those received supplemental Fe (100 or 200 mg Fe/kg diet) during the second and third period. Layers fed 200 mg Fe/kg diet exhibited the best feed conversion during the third period.

Regarding to egg mass, it is well recognized that, egg mass resulted from multiplying egg number by egg weight. Results obtained showed that, layers received 100 mg Fe/kg diet gave the highest values ($p\leq 0.01$) of egg mass during the first and second periods, as compared to the other treatments. Notable, during the third period, data illustrated in Table 3 showed no significant differences between layers fed diets supplemented with 100 or 200 mg Fe/kg diet.

The overall mean revealed that, feed conversion for layers fed supplemental iron either at the level of 100 mg or 200 mg/kg diet was improved by 14% and 8.84%, respectively. Also, egg mass was improved by 15% and 4.9%, respectively for layers fed the two mentioned iron levels comparable to diet without iron.

As regards to supplemented 20 mg Copper (Cu)/kg or/with 45 mg zinc (Zn)/kg in layer's diets, the overall mean (Table 4) of feed conversion and egg mass values showed that, the combination between Cu and Zn in layers diet gave the best values. These improvements were 8.2% for feed conversion and 3% for egg mass as compared to those fed diet without Cu or Zn.

Regarding the interaction between iron, Copper and zinc supplementation it was noted that (Table 5), the overall mean of feed intake was significantly ($p\leq 0.05$) decreased for layers fed diets supplemented with Cu or/with Zn whatever under the level 100 or 200 mg Fe/kg diet comparable to the control diet without minerals supplementation. While the overall mean of feed conversion indicated that, layer fed diet supplemented with iron at the level of 100 mg with Zn gave the best feed conversion followed by those fed 200 mg Fe/kg diet with Zn + Cu the improvements were 22.37 % and 18%, respectively.

As for the overall mean of egg mass (Table 6), it was observed that, the highest value was detected for layers fed supplemental iron at the level of 100 mg/kg diet with Zn, the increment was 20% as compared to those fed the control diet.

Results obtained for egg production agree with those reported by Balat *et al*. (1995) for Mandarah layer hens and Abou El-Ella *et al*. (1997) for Gimmezah layer as local strains.

Effect of supplemental Fe, Cu and Zn in layer's diets on Fe concentration in egg (Yolk and White): Egg iron concentration as affected by iron, copper, zinc supplementation and their interaction is shown in Table 7. It was clearly noted that increasing iron level from 0.0 to 100 and 200 mg\kg diet significantly ($p\leq 0.01$) increase egg iron content by 33.97 and 57.89% respectively compared to 0.0 iron supplementation. As regard to the effect of supplemental Cu or\with Zn, hens fed diets fortified with Cu + Zn combination showed the highest value of egg iron content. Concerning the interaction between Fe, Cu and Zn the best value of iron concentration in egg was observed for hens fed diets supplemented with Fe at the level of 200 mg/kg diet in combination with Cu and Zn, being 3.85 mg/100 gm egg, followed by those fed diets supplemented with Fe at the level of 100 mg/kg diet in combination with the same mentioned elements, being 3.59 mg/100 gm egg.

Table 6: Egg weight, egg number and egg mass of Mandarah laying hens as affected by the interaction between iron, Copper and zinc supplementation during the experimental period (24-36 wks of age)

Iron levels mg/kg	Cu and Zn supplementation mg/kg diet	Egg weight (g) 24-28 wks	28-32 wks	32-36 wks	Overall mean
0.0	0.0	46.73±0.35	47.18±0.77^{ab}	47.68±0.56^{ab}	47.20±0.34^{ab}
	20 mg Cu	46.95±0.99	48.19±1.72^{ab}	48.56±1.44^{ab}	47.90±1.38^{ab}
	45 mg Zn	46.38±0.13	47.19±0.15^{ab}	48.21±0.43^{ab}	47.26±0.09^{ab}
	20 mg Cu + 45 mg Zn	47.02±0.67	47.79±0.68^{ab}	48.53±0.52^{ab}	47.78±0.6^{ab}
100	0.0	45.32±1.28	47.65±1.11^{ab}	48.06±1.08^{ab}	47.01±1.13^{ab}
	20 mg Cu	46.57±0.47	48.53±0.59^{ab}	48.50±0.50^{ab}	47.86±0.29^{ab}
	45 mg Zn	47.67±1.12	48.96±0.94^{a}	49.42±0.86^{a}	48.68±0.98^{a}
	20 mg Cu + 45 mg Zn	46.35±0.30	46.68±0.09^{ab}	48.23±0.09^{ab}	47.08±0.08^{ab}
200	0.0	45.03±0.29	46.50±0.26^{ab}	46.59±0.31^{b}	46.04±0.09^{b}
	20 mg Cu	45.87±0.47	46.93±0.59^{ab}	48.51±0.50^{ab}	47.11±0.29^{ab}
	45 mg Zn	46.15±0.17	46.47±0.28^{ab}	48.75±0.71^{ab}	47.12±0.38^{ab}
	20 mg Cu + 45 mg Zn	46.60±0.34	45.82±1.15^{b}	47.63±0.77^{ab}	46.68±0.72^{ab}
Significance		NS	*	*	*

Iron levels mg/kg	Cu and Zn supplementation mg/kg diet	Egg number (egg/hen/period) 24-28 wks	28-32 wks	32-36 wks	Overall mean
0.0	0.0	18.33±2.15^{ab}	16.47±2.74^{abc}	14.00±1.72^{bc}	16.27±2.16^{ab}
	20 mg Cu	16.07±1.39^{ab}	12.73±0.68^{c}	11.33±0.57^{c}	13.37±0.53^{b}
	45 mg Zn	17.27±0.84^{ab}	15.07±1.29^{bc}	15.20±1.46^{abc}	15.83±1.07^{ab}
	20 mg Cu + 45 mg Zn	19.13±1.38^{ab}	17.93±0.87^{ab}	18.07±1.77^{ab}	18.37±1.34^{a}
100	0.0	18.20±2.65^{ab}	19.60 ±1.45^{a}	17.60±1.31^{ab}	18.47±1.73^{a}
	20 mg Cu	18.80±1.63^{ab}	18.53±1.48^{ab}	17.33±1.92^{ab}	18.23±1.57^{a}
	45 mg Zn	19.87±0.93^{a}	18.60±1.22^{ab}	18.33±0.75^{ab}	18.93±0.84^{a}
	20 mg Cu + 45 mg Zn	20.27±0.47^{a}	16.53±1.54^{abc}	16.67±1.30^{ab}	17.80±0.99^{a}
200	0.0	19.07±1.46^{ab}	18.33±1.30^{ab}	17.13±1.16^{ab}	18.17±1.23^{a}
	20 mg Cu	14.87±0.57^{b}	15.73±0.35^{abc}	18.60±0.53^{a}	16.40±0.21^{ab}
	45 mg Zn	15.53±1.25^{ab}	13.53±0.44^{c}	18.20±1.39^{ab}	15.73±0.30^{ab}
	20 mg Cu + 45 mg Zn	19.53±1.05^{ab}	16.40±0.72^{abc}	17.53±0.96^{ab}	17.83±0.79^{a}
Significance		*	*	*	*

Iron levels mg/kg	Cu and Zn supplementation mg/kg diet	Egg mass (kg/hen) 24-28 wks	28-32 wks	32-36 wks	Overall mean
0.0	0.0	0.856±0.10^{bac}	0.777±0.14^{abc}	0.667±0.09^{bc}	0.768±0.11^{ab}
	20 mg Cu	0.754±0.08^{bac}	0.613±0.05^{c}	0.550±0.03^{c}	0.640±0.04^{b}
	45 mg Zn	0.801±0.04^{bac}	0.711±0.06^{bc}	0.733±0.06^{abc}	0.748±0.05^{ab}
	20 mg Cu + 45 mg Zn	0.899±0.08^{bac}	0.856±0.05^{ab}	0.877±0.09^{ab}	0.878±0.07^{a}
100	0.0	0.825±0.12^{bac}	0.934±0.05^{a}	0.846±0.05^{ab}	0.868±0.07^{a}
	20 mg Cu	0.876±0.05^{bac}	0.899±0.06^{ab}	0.841±0.08^{ab}	0.872±0.06^{a}
	45 mg Zn	0.947±0.07^{a}	0.911±0.07^{ab}	0.906±0.04^{a}	0.922±0.05^{a}
	20 mg Cu + 45 mg Zn	0.940±0.02^{ab}	0.772±0.07^{abc}	0.804±0.06^{ab}	0.838±0.04^{a}
200	0.0	0.859±0.07^{abc}	0.852±0.06^{ab}	0.798±0.05^{ab}	0.836±0.06^{a}
	20 mg Cu	0.682±0.03^{c}	0.738±0.02^{abc}	0.902±0.02^{a}	0.772±0.01^{ab}
	45 mg Zn	0.717±0.05^{bc}	0.629±0.02^{c}	0.887±0.08^{a}	0.741±0.02^{ab}
	20 mg Cu + 45 mg Zn	0.910±0.04^{abc}	0.751±0.02^{abc}	0.835±0.04^{ab}	0.832±0.03^{a}
Significance		*	*	*	*

$^{a-c}$Means in the same raw with different letters are significant (p<0.05). *: (p<0.05)

The previously increment were 79 and 67%, respectively when compared to the control diet. This may be due to the minerals synergistically effect which improve the utilization of Fe. It is well known that there are strong interactions among divalent minerals such as Fe, Zn and Cu (Paik *et al.*, 2009). This may be scientifically logic since, the combination of 120 mg Fe/kg diet with 80 mg of Zn/kg and 25 mg of Cu/kg increased Fe concentration in the Yolk and White by 36.7 and 34.9% respectively (Skrivan *et al.*, 2005). In addition Yang *et al.* (2004) indicated that Fe contents were increased in egg Yolk of hens fed diets supplemented with Fe, Zn, I and Se. In these respect Mabe *et al.* (2003) found an increase in Zn, Mn and Cu concentration in egg Yolk when they supplemented the laying hen diets with the same minerals.

It is worthy to note that, in laying hens, there are two specific proteins transmissive of iron (F II) in their blood plasma. They are transferrin and phosvitin. At onset of eggs, phosvitin increases in blood plasma. It may be attributed to the estrogen hormone change in blood, which stimulate phosvitin constituting in bird's liver, also promote iron metabolism, so phosvitin increase till the pike of egg production reaching the outmost level, where the binding capacity of ferrus (Fe II) with phosvitin was maximize producing ferrus-phosvitin compound which

Table 7: Egg iron content (mg/100 g egg) as affected by iron, copper and zinc supplementation and their interaction

Iron effect

0.0 iron supplementation	100 mg iron/kg diet	200 mg iron/kg diet	Sig.
2.09±0.07[c]	2.80±0.15[b]	3.30±0.18[a]	**

Effect of Cu and Zn

0.0 Cu or Zn	Cu (20 mg/kg diet)	Zn (45 mg/kg diet)	Cu 20 + Zn 45 mg/kg diet	
2.29±0.04[c]	2.60±0.19[b]	2.68±0.23[b]	3.36±0.25[a]	**

Interaction effect

0.0 iron				100 mg iron/kg diet				200 mg iron/kg diet				
(Control)	Cu	Zn	Cu + Zn	0.0	Cu	Zn	Cu + Zn	0.0	Cu	Zn	Cu + Zn	
2.15± 0.03[g]	1.96± 0.08[h]	1.85± 0.06[h]	2.41± 0.03[ef]	2.31± 0.03[f]	2.55± 0.07[e]	2.76± 0.06[d]	3.59± 0.05[b]	2.40± 0.06[ef]	3.28± 0.05[c]	3.44± 0.05[b]	3.85± 0.04[a]	**

[a-h]Means in the same row with different letters are significant. **: p≤0.01

directly go to egg Yolk and deposit in it (El-Hossiny and Abo-El alla, 1990). On the contrary supplemented Cu or Zn individually to hen's diets without iron supplementation reduced the concentration of iron in egg than the control diet. This results are in coincide with that of Skrivan *et al.* (2005) who found that, the concentration of iron in egg Yolk was reduced when hen's diets supplemented with Cu 25mg or Zn 80mg/kg diet individually as compared with those fed the control diet.

Notable, supplemented Cu or Zn individually in combination with iron in each of them at the levels of 100 or 200 mg Fe/kg diet, raised iron concentration in egg by 18.60 and 52.56% for Cu at the two mentioned levels of iron respectively while the increment were 28.37 and 60.00% for Zn with the previously two levels of iron respectively when compared to the control diet.

Adding 100 or 200 mg Fe/kg diet without Cu or Zn increased the concentration of iron in egg by 7.44 and 11.63%, respectively as compared to the control diet. In this respect, Skrivan *et al.* (2005) reported that, when layers diets supplemented with 120 mg iron/kg diet, the concentration of iron increased by 6.3% and 2.2% in egg Yolk and White respectively. Moreover Bertchini *et al.* (2000) and Youjin *et al.* (2004) showed that the content of egg Yolk iron (mg/100 g) increased significantly when iron supplementation increased in diet at the level of 80 ppm.

Blood parameters: Some blood parameters of Mandarah laying hens as affected by Fe, Cu and Zn supplementation and their interaction are shown in Table 8. Concerning the effect of supplemental iron only, it is clearly noted that, blood hemoglobin increased by increasing supplemental iron. These results agree with Biehl *et al.* (1997) who indicated that added 80 mg Fe/kg diet improved hemoglobin and hemotocrit. In this respect, El-Hossiny and Abo-El alla (1990) showed that iron is an essential constituent of blood hemoglobin.

Regarding to Cu or/and Zn supplementation in laying hen diets, results obtained showed that, supplemental Cu + Zn increased blood hemoglobin (p≤0.05). While no significant variations were found due to supplemented Cu or Zn alone.

Respecting the interaction between supplemental Fe and Cu or/and Zn, inspection of obtained data revealed that, supplemented iron without Cu or Zn in layer hens diets lead to advanced blood hemoglobin values than the control, while supplemented laying hen diets with Cu + Zn in combination of iron either at the level of 100 or 200 mg/kg diet gave the highest values (p≤0.01) of blood hemoglobin, being 9.71 and 9.45 g/100 ml. Also, supplemented Cu in combination of iron at the level of 200 mg/kg diet gave a preferable value (p≤0.01) of blood hemoglobin, being 9.39 g/100 ml.

This highest values of hemoglobin produce an effect upon the concentration of iron in egg, since layer hen diets supplemented with iron either at the level of 100 or 200 mg/kg diet in combination of Cu + Zn gave the best values for egg iron contents (Table 7). Concerning this, El-Hossiny and Abo-El alla (1990) reported that, copper in the presence of iron, has a marked catalytic effect on the synthesis of hemoglobin, therefore it is essential to normal health and well-being. The same trend was obtained for blood hematocrit.

These results agree with those reported by McNaughton and Day (1979) who found that feeding 21 day old chicks with 80 ppm of dietary Fe and 8 ppm of dietary Cu increased the hemoglobin levels and Packed Cell Volumes (PCV) %.

As for creatinin and liver function (ALT and AST), analysis of variance showed no significant response to layer hens fed different dietary treatments. Yang *et al.* (2004) stated that, there were no obvious difference in the activity of ALT or AST in plasma between layers fed 360 mg supplemental iron/kg diet in combination with 420 mg zinc/kg diet and those fed the control diet.

Egg quality traits: Egg shape index, shell, yolk and albumen percentages, yolk index and Haugh units are

Table 8: Some blood parameters of Mandarah laying hens as affected by iron, copper and zinc supplementation and their interaction

Treatments	Blood parameters				
	Hemoglobin (mg/100 ml)	Hematocrit (%)	Creatinine (mg/100 ml)	ALT (Unit/ml)	AST (Unit/ml)
Iron levels (mg/kg)					
0.0	8.14±0.22^{b}	30.12±0.028^{b}	2.86±0.02	40.70±0.22	121.52±0.73
100	8.74±0.19^{a}	32.00±0.031^{a}	2.84±0.05	40.94±0.15	121.32±1.12
200	9.02±0.10^{a}	33.43±0.032^{a}	2.84±0.03	41.00±0.21	121.33±1.32
Significance	*	*	NS	NS	NS
Cu and Zn (mg/kg diet)					
0.0	8.30±0.11^{b}	30.78±0.031^{b}	2.80±0.03	40.82±0.19	119.55±0.80
20 mg Cu	8.55±0.19^{b}	31.74±0.37^{ab}	2.88±0.04	40.64±0.22	121.47±0.83
45 mg Zn	8.30±0.24^{b}	31.75±0.29^{ab}	2.84±0.03	40.97±0.39	121.09±1.80
20 mg Cu + 45 mg Zn	9.38±0.15^{a}	33.13±0.33^{a}	2.87±0.05	41.07±0.27	123.46±1.12
Significance	*	*	NS	NS	NS

Interaction effect

Iron levels mg/kg	Cu and Zn supplementation mg/kg diet	Hemoglobin (mg/100 ml)	Hematocrit (%)	Creatinine (mg/100 ml)	ALT (Unit/ml)	AST (Unit/ml)
0.0	0.0	7.81±0.15^{d}	29.90±0.31^{c}	2.80±0.07	40.50±0.33	120.06±0.93
	20 mg Cu	7.90±0.12^{d}	30.02±0.29^{c}	2.90±0.03	40.30±0.31	122.01±0.72
	45 mg Zn	7.88±0.22^{d}	30.03±0.32^{c}	2.85±0.05	41.00±0.29	120.09±0.77
	20 mg Cu + 45 mg Zn	8.98±0.18^{b}	30.51±0.33^{bc}	2.90±0.03	41.01±0.23	124.00±1.13
100	0.0	8.58±0.13^{c}	30.84±0.25^{bc}	2.79±0.05	40.90±0.22	119.83±1.24
	20 mg Cu	8.36±0.12^{c}	31.04±0.31^{bc}	2.91±0.04	40.80±0.31	120.40±0.93
	45 mg Zn	8.31±0.23^{c}	31.99±0.29^{b}	2.82±0.03	41.03±0.36	123.00±0.72
	20 mg Cu + 45 mg Zn	9.71±0.19^{a}	34.14±0.27^{a}	2.86±0.05	41.06±0.25	122.08±0.81
200	0.0	8.51±0.21^{c}	31.60±0.21^{b}	2.81±0.02	41.08±0.33	118.83±1.12
	20 mg Cu	9.39±0.23^{a}	34.14±0.33^{a}	2.85±0.02	40.83±0.22	122.00±0.81
	45 mg Zn	8.71±0.19^{bc}	33.23±0.29^{a}	2.85±0.03	40.89±0.29	120.19±0.81
	20 mg Cu + 45 mg Zn	9.45±0.13^{a}	34.74±0.24^{a}	2.86±0.05	41.22±0.27	124.30±0.99
Significance		*	*	NS	NS	NS

$^{a-c}$Means in the same column with different letters are significant. NS: Not Significant, *: $p \leq 0.05$

presented in Table 9. It was clearly noted that, the mentioned egg quality parameters showed no significant response due to iron supplementation in layer's diets either at the level of 100 or 200 mg Fe/kg diet. On the contrary there were significant effect ($p \leq 0.05$) for shell and yolk percentages, Also yolk index and Haugh units referable to supplemented layer diets by Cu or/and Zn. Respecting Haugh units, it is well known that, Haugh units define as the height of albumen in relative to egg weight, so it is used as a measure for the interior egg quality (Kamer and Samy, 1984). Once more, inspection of obtained data revealed that, the best value of Haugh units was detected for layers fed diets supplemented with Cu + Zn being 78.58. The increment was 6.97%. When compared with those fed the control diet.

Concerning the interaction between supplemented Fe and Cu or/and Zn it was observed that, no significant effect between dietary treatments applied for shape index and albumen percentages. However, layers fed diets supplemented with iron at the level of 100 and 200 mg/kg diet in combination with Cu + Zn gave the best values ($p \leq 0.01$) of Haugh units, being 80.56 and 80.02, respectively.

It was observed that, layers fed diets supplemented with iron at the level of 200mg/kg diet in combination with Cu gave the preferable value ($p \leq 0.05$) for Yolk index. While those fed diets supplemented with Zn without supplemented iron gave favorite value ($p \leq 0.01$) of shell percentage.

In agreement with obtained egg quality results Abo El-Ella *et al.* (1997), Tollba *et al.* (2007) and Aly *et al.* (2010) reached to same results for the local strains of Gimmezah, Dandarawi and Mandarah laying hens, respectively. Notable, the results of Bertchini *et al.* (2000) found that there were no significant effects on the external and internal egg quality when diet supplemented with 20, 40, 60 and 80 ppm Fe/kg. From the other hand Guo *et al.* (2002) supported the results of this experiment in regarding to Zinc supplementation.

Economical efficiency: From the economical point of view, the profitability of supplemented Mandarah laying hens diets by Fe, Cu or/and Zn depend on the price of these minerals and the layers production performance, so we used inorganic compounds because they are common ingredients of minerals supplements for farm animals due to their low price.

In this study iron addition with Cu or/and Zn improved the economical efficiency comparable to the control. The lowest feed cost/kg egg was observed for layers fed

Table 9: Some egg quality of Mandarah laying hens as affected by iron, copper and zinc supplementation and their interaction

Treatments	Items Shape index	Shell percentage	Yolk percentage	Albumen percentage	Yolk index	Haugh units
Iron levels (mg/kg)						
0.0	76.16±0.41	13.21±0.17	32.67±0.32	54.13±0.30	40.96±0.26	75.47±0.59
100	75.44±0.34	13.35±0.17	32.80±0.23	53.85±0.27	40.50±0.29	74.44±1.23
200	76.24±0.31	13.41±0.15	32.81±0.28	53.77±0.32	41.04±0.41	76.92±0.96
Significance	Ns	Ns	Ns	Ns	Ns	Ns
Cu and Zn (mg/kg diet)						
0.0	75.85±0.30	12.92±0.19^{c}	33.53±0.34^{a}	53.55±0.40	41.42±0.29^{a}	73.46±0.80^{b}
20 mg Cu	75.88±0.51	12.81±0.15^{c}	32.81±0.37^{ab}	54.38±0.41	41.47±0.50^{a}	76.70±0.92^{a}
45 mg Zn	76.03±0.41	13.99±0.15^{a}	32.44±0.35^{b}	53.57±0.36	40.49±0.42^{b}	73.71±1.46^{b}
20 mg Cu + 45 mg Zn	76.02±0.46	13.57±0.11^{b}	32.26±0.22^{b}	54.17±0.25	39.96±0.29^{b}	78.58±0.98^{a}
Significance	Ns	*	*	Ns	*	*

Interaction effect

Iron levels mg/kg	Cu and Zn supplementation/kg diet	Shape index	Shell percentage	Yolk percentage	Albumen percentage	Yolk index	Haugh units
0.0	0.0	76.09±0.36	12.62±0.11^{c}	33.74±0.65^{ab}	53.64±0.68	40.76±0.5^{bcde}	74.92±1.04^{ebcd}
	20 mg Cu	75.44±1.11	12.31±0.21^{c}	33.01±0.53^{abc}	54.68±0.67	42.02±0.4^{ab}	76.55±0.94^{abcd}
	45 mg Zn	77.15±0.52	14.14±0.11^{a}	31.92±0.63^{c}	53.95±0.57	40.95±0.52^{bcde}	75.27±1.1^{bcd}
	20 mg Cu + 45 mg Zn	75.96±1.09	13.76±0.13^{ab}	32.01±0.57^{bc}	54.23±0.55	40.14±0.42^{de}	75.15±1.66^{bcd}
100	0.0	75.97±0.65	13.52±0.36^{ab}	33.01±0.51^{abc}	53.48±0.51	41.27±0.56^{abcd}	73.01±1.73^{de}
	20 mg Cu	74.90±0.77	12.66±0.17^{c}	33.13±0.63^{abc}	54.22±0.76	39.66±0.76^{de}	74.24±1.5^{cde}
	45 mg Zn	74.37±0.40	13.85±0.40^{ab}	32.66±0.41^{abc}	53.48±0.54	40.69±0.56^{bcde}	69.94±2.9^{e}
	20 mg Cu + 45 mg Zn	76.52±0.64	13.36±0.25^{b}	32.42±0.23^{abc}	54.23±0.38	40.37±0.33^{cde}	80.56±1.34^{a}
200	0.0	75.49±0.58	12.61±0.36^{c}	33.85±0.63^{a}	53.54±0.97	42.24±0.18^{ab}	72.45±1.34^{de}
	20 mg Cu	77.31±0.44	13.47±0.10^{ab}	32.30±0.8^{abc}	54.23±0.80	42.74±0.85^{a}	79.30±1.71^{abc}
	45 mg Zn	76.58±0.62	13.99±0.18^{ab}	32.74±0.17^{abc}	53.27±0.20	39.83±0.3^{de}	75.91±1.76^{abcd}
	20 mg Cu + 45 mg Zn	75.57±0.66	13.59±0.20^{ab}	32.36±0.32^{abc}	54.06±0.43	39.36±0.69^{e}	80.02±1.32^{ab}
Sig.		Ns	**	*	Ns	*	**

$^{a-c}$Means in the same row with different letters are significant. Ns: Not significant, *: $p \leq 0.05$, **: $p \leq 0.01$, Sig.: Significance

Table 10: Input-output analysis and economic efficiency of different dietary treatments

Iron levels mg/kg	Cu and Zn supplementation/kg diet	Feed price (LE/kg)	Total feed intake/hen (kg)	Total feed cost/hen (LE)	Total egg mass/hen (kg)	Feed cost/kg egg (LE)	Feed cost/kg egg (%)
0.0	0.0	1.59	8.54	13.58	2.306	5.89	100.00
	20 mg Cu	1.59	7.92	12.59	1.922	6.55	111.04
	45 mg Zn	1.60	8.11	12.98	2.240	5.78	98.13
	20 mg Cu + 45 mg Zn	1.60	8.03	12.85	2.637	4.87	82.68
100	0.0	1.60	8.32	13.31	2.597	5.12	86.93
	20 mg Cu	1.60	8.12	12.99	2.616	4.96	84.21
	45 mg Zn	1.61	7.95	12.80	2.766	4.63	78.61
	20 mg Cu + 45 mg Zn	1.61	8.23	13.25	2.515	5.27	89.47
200	0.0	1.61	8.32	13.31	2.509	5.30	89.98
	20 mg Cu	1.62	7.81	12.65	2.322	5.45	92.53
	45 mg Zn	1.62	7.80	12.63	2.234	5.66	96.09
	20 mg Cu + 45 mg Zn	1.62	7.59	12.29	2.494	4.93	83.66

diets supplemented with 100 mg Fe/kg diet in combination with 45 mg Zinc/kg diet, it was being 4.63LE and the relative economical efficiency was 78.61% Therefore, the previously mentioned dietary treatment was more economic than the other treatments as shown in Table 10.

Notable, most of dietary treatments gave good economical efficiency and increased iron content in eggs which is the main point of this study irrespective of the profitability.

Conclusion: Feeding Mandarah laying hens diets supplemented with 200 mg iron/kg diet in combination with 20 mg Copper + 45 mg Zinc/kg diet gave the best concentration of egg iron, being 3.85 mg/100 g egg without any adverse effect on the performance of laying hens. In addition, iron supplementation improved feed conversion, egg mass and economical efficiency.

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