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Is dietary iron requirement of broiler breeder hens at the late stage of production cycle influenced by phytase supplementation?

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The purpose of this study was to investigate the effects of phytase on iron requirement of broiler breeder hens at the late stage of production cycle. Ninety-six Cobb (500) broiler breeder hens were weighed and individually placed in galvanized wire cages at 59 weeks of age. In order to depletion of hens iron reserved, after placement, hens were offered a semi-purified iron-deficient (37 mg iron/kg) diet for three weeks. At 62 weeks of age, hens were randomly allocated to eight dietary treatments in a factorial arrangement with four replicates of three hens in each. Factors included two levels of phytase (0 and 600 U/kg) and four levels of iron (37, 52, 67 and 82 mg/kg). Hens were fed the experimental diets from 62 to 68 weeks of age. Iron in the diet had significant effects on egg weight ($P < 0.05$). Increasing dietary iron content significantly increased the concentration of iron in the bone marrow, liver, spleen ($P < 0.05$), egg yolk and blood serum ($P < 0.01$). Added phytase enhanced the iron concentration of the egg yolk and serum ($P < 0.01$). It seems that phytase can release iron from inositol in old broiler breeder hens and increased chicken iron reserve. Two slope, broken-line analysis of hen house egg production regressed on the dietary added iron indicated that 65 mg/kg supplemental iron without phytase and 58 mg/kg supplemental iron with phytase were required for the optimal egg production of broiler hens.

Keywords: iron; phytase; requirement; broiler breeder hens; nonlinear models

1. Introduction

Iron is an essential mineral for animal growth and development because it takes part in an array of biochemical processes vital in maintaining normal cellular function. Biochemical processes involved include electron transport (cytochromes and iron–sulphur proteins), handing of molecular oxygen (peroxidase and catalase), oxygen transport and storage (haemoglobin and myoglobin) porphyrin metabolism, collagen synthesis, lymphocyte and granulocyte function and neurotransmitter anabolism and catabolism (McDowell 2003). Also, iron is especially essential during development, as it is required for proper myelination and also is a cofactor for enzymes in neurotransmitter synthesis (Pollitt & Leibel 1976; Reichmann et al. 1995). Few studies were performed on the iron requirements of poultry (Chio et al. 1976; Morck & Austic 1981; Aoyagi & Baker 1995). But none of the researches have been conducted on effect of phytase on iron requirement of poultry. On the other hands, in spite of the broiler breeder hens' importance in meat production cycle, no investigation of the iron requirement of them has been reported. Only one study has been done on the effect of iron on broiler breeder hens that investigated the effect of iron on performance and iron content of broiler breeder hens' egg (Bess et al. 2012). They suggested that egg production in hens fed no supplemented iron diets showed a significant reduction in comparison with hens fed supplemented iron diets. Cao et al. (1996) stated that the kidney, liver and bone marrow iron concentrations increased linearly by increasing dietary iron. Ramadan et al. (2010) investigated 0, 100 and 200 mg/kg iron in laying hens and suggested that egg mass for layers fed by supplemental iron either at the level of 100 mg or at the level of 200 mg/kg diet in comparison with diet without iron was improved by 15% and 4.9%, respectively. Vahl and Van 'T Klooster (1987) recommended 100 mg/kg of iron for broiler. There are many factors that can affect iron absorption; proteins, calcium, polyphenols and Phytic acid (inositol hexaphosphate) can all reduce iron bioavailability (Hurrell 1997). Phytate can account for up to 5% of the weight in cereal grains and legumes (Maga 1982). It accounts for 0.62–1.35% of dry weight in wheat, 0.84–1.01% of dry weight in oats and 0.97–1.08% of dry weight in barley (Maga 1982). More than 80% of the total phosphorous in corn is in the form of phytate phosphorous (De Boland et al. 1975). There appears to be a dose–response relationship between the level of phytate in a food and iron absorption (Hallberg & Hulthén 2000). It is poorly digestible for humans and monogastric animals due to a lack of effective endogenous phytase (Bitar & Reinhold 1972). Phytate acts as an anti-nutritional factor, exerting its

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effects via a reduction in the solubility, and so availability of phosphorus (P) and to a lesser extent, calcium, zinc and iron (Hallberg et al. 1987, Hurrell et al. 2003). Several studies have shown that supplementing animal diets with phytase improves mineral bioavailability (Simons et al. 1990; Lei et al. 1993; Stahl et al. 1999; Augspurger & Baker 2004; Shelton & Southern 2006). Compared to broiler chicks, phytase inclusion in diets for laying hens has been the subject of less research. Exogenous phytase enzymes are known to release not only phosphorus bound by the phytase molecule but also other nutrients including calcium and amino acids (Cosgrove 1966; Selle & Ravindran 2007). Recently, it has been reported that phytase enzymes may also affect iron metabolism. Liebert et al. (2005) contend that the benefits of phytase supplementation of layer diets are 'still under discussion'. These studies were conducted to determine the effect of iron deficiency in the broiler breeder hens and to determine the amount of dietary iron required with phytase and without phytase for maximal performance.

2. Materials and methods

2.1. Animals and sampling

Ninety-six Cobb 500 broiler breeder hens were weighed and individually placed in galvanized wire cages (45 cm height × 38 cm depth × 30 cm width) at 59 weeks of age. Soft plastic wires were placed on the cage floors to inhibit bird's foot damages. In order to depletion of hens iron reserved, until 62 weeks of age, hens were offered a semi-purified iron deficient (37 mg iron/kg) diet that contained 13.7% of crude protein and 2680 kcal/kg of AMEn. The composition of the basal diets is shown in Table 1. After the depletion period, hens were randomly allocated to eight dietary treatments in a factorial arrangement of two levels of phytase (0 and 600 U/kg) and four levels of iron (37, 52, 67 and 82 mg/kg) with four replicates of three hens in each. Dietary treatments were made with the addition of $FeSO_4.4H_2O$ to the basal diet. The composition of the experimental diets is shown in Table 2. Hens received a constant schedule of 16 h light (beginning at 6 am). The consumed water contained approximately 41.68 µg iron/l that was measured by polarography (Model VA 797 Metrohm). In this experiment, plastic trough drinker and individual feeder were used. Birds did not have any access to the feed of each other. Egg production and egg weight were recorded daily, and the egg mass was calculated at the end of experiment (egg weight × egg production). To evaluate blood serum iron concentration, first bleeding was carried out before initiation and next was done at the end of experiment. Blood samples were collected in heparin-coated tubes from the brachial vein. Blood samples were immediately centrifuged at 3500× g for 14 min to collect blood serum. Serum samples were stored at –20°C pending for iron concentration assays. Blood serum iron was determined by calorimetric method using an automated spectrophotometric analyzer (enzyme-linked immunosorbent assay plate reader model no. 259293) instrument. Egg component, Haugh units, shell-quality test and shell-breaking strengths measurements were done at the end of experiment. Shell-breaking strengths and shell thickness were measured by using an egg shell force gauge (model no. 55R1123, Instron Corp., Canton, MA) and Karl Deutsch D-56 (wuppertal echometer 1061), respectively. At 68 weeks of age, two hens per replicate were slaughtered by a neck cutter, and carcass characteristics were measured. The liver, heart and spleen were extracted from the carcass then weighed and frozen immediately for further analysis. The right tibia was pooled and then frozen for analysis of bone marrow iron content. The iron content of liver, heart, spleen, bone marrow and egg yolk was determined by

Table 1. Composition of basal diets.

Ingredients	Amount
Corn starch	50.67
Soybean meal	30.60
Cellulose	7.45
Oil corn	1.51
Phosphoric acid	0.95
Potassium sulphate	0.25
Carbonate calcium	7.66
NaCl	0.34
Vitamin premix[a]	0.03
Mineral premix[b]	0.13
DL-Met	0.33
L-Thr	0.08
Calculated nutrients	%
AMEn (kcal/kg)	2680
Crude protein	13.7
Calcium	3
Available phosphor	0.35
Sodium	0.15
Digestible Lys[c]	0.75
Digestible Met	0.5
Digestible M + C	0.66
Digestible Thr	0.55
Digestible Arg	0.88
Iron (mg/kg)	37

[a]Provides per kg of diet: vitamin A (from vitamin A acetate) 12000 IU; cholecalciferol 3000 IU; vitamin E (from dl-alpha-tocopheryl acetate) 50 IU; menadione (from menadione sodium bisulphate) 6 mg; vitamin B1 (from thiamin mono nitrate) 2.5 mg; riboflavin 10 mg; niacin 40 mg; pantothenic acid 25 mg; pyridoxine (from pyridoxine HCl) 6 mg; folic acid 4 mg; cobalamin (from) 0.035 mg; d-biotin 0.066 mg; antioxidant 0.5 mg.
[b]Provides per kg of diet: Mn (form manganese oxide) 120 mg; Zn (form zinc oxide) 110 mg; Cu (from copper sulphate) 10 mg; Se (from sodium selenite) 0.3 mg; choline (form choline chloride) 250 mg; iodine (from calcium iodate) 0.2 mg.
[c]Calculated amino acid composition is reported on a standardized ileal digestible amino acid basis (Parsons 1996).

Table 2. Composition of experimental diets.[a]

Iron (mg/kg)	37	52	64	82	37	52	64	82
Phytase (U/kg)	0	0	0	0	600	600	600	600
Ingredients (g/kg)								
Phytase[b]	0	0	0	0	0.24	0.24	0.24	0.24
$FeSO_4.4H_2O$[c]	0	0.048	0.096	0.1446	0	0.048	0.096	0.1446
Cellulose	74.50	74.45	74.40	74.35	74.26	74.21	74.16	74.11

[a]All diets were identical to the basal diet except for phytase and iron content.
[b]Quantum® is an *Escherichia coli* 6-phytase. AB Agri Ltd Woodstock Court, Blenheim Road, Marlborough Business Park, UK.
[c]$FeSO_4.4H_2O$ content 22.4% iron.

atomic absorption spectrophotometry instrument (Shelton & Southern 2006).

2.2. *Statistical analyses*

The experimental design was a completely randomized design using individual broiler breeder hens as experimental units. The obtained results from experiments were analysed by general linear model (GLM) procedure of SAS Institute (2002). Significant differences among treatment means were determined using Duncan's multiple range tests. In this experiment, the broken-line (Robbins et al. 2006), logistic and quadratic models (Pesti et al. 2009) were used to determine iron requirement.

3. Results

The effects of phytase and iron on performance of hens are shown in Table 3. Iron in the diet had significant effects on egg weight ($P < 0.05$). No significant effect of dietary added phytase of phytase and iron were observed on egg production, egg mass and body weight. Also, no significant interactions were observed between supplemented iron and phytase for egg production, egg weight, egg mass and body weight. The effects of phytase and iron levels on carcass characteristics of hens are shown in Table 4. Analysis showed that there was no significant effect of iron on the liver, heart and spleen weight. Significant differences were observed in heart weight ($P < 0.01$) and spleen weight ($P < 0.05$) of phytase-supplemented hens, but there was no significant effect on the liver weight. Lack of significant interactions were observed between iron levels and phytase supplementation for heart, spleen and liver weight. The effects of iron and phytase on egg quality of broiler breeder hens are presented in Table 5. Haugh unit, albumen height, shell thickness, egg shell-breaking strength, shell and yolk percentage were not significantly affected by dietary of iron. Also the phytase supplementation showed no significant differences on Haugh unit, albumen height, egg shell-breaking strength, shell and yolk percentage. Significant differences were observed in shell thickness ($P < 0.05$) and egg shell-breaking strength ($P < 0.01$) of phytase supplementation. No significant interactions were observed between iron and phytase supplementation for Haugh unit, albumen height, egg shell-breaking strength and shell and yolk fractional weight. But interaction between dietary added iron and phytase on shell thickness was significant ($P < 0.05$; Figure 1). The

Table 3. Effect of dietary iron and phytase levels on performance of broiler breeder hens.[a,b]

Treatments						
Phytase (U/kg)	Iron (ppm)	Body weight (g)	Egg production (%)	Egg mass (g)	Egg weight (g)	Egg production (hen house)
	37	4506.9	24.64	16.39	67.58[b]	10.40
	52	4490.6	32.57	20.86	68.28[b]	13.71
	67	4432.0	38.40	26.37	71.28[a]	13.91
	82	4519.6	29.64	20.21	69.74[a,b]	12.42
SEM		58.09	5.908	4.16	0.902	2.32
0		4537.8	31.82	20.76	68.43	13.15
600		4436.7	30.81	21.14	70.01	12.07
SEM		38.50	4.17	2.94	0.64	1.65
P-value						
Fe		0.7356	0.4337	0.4201	0.0373	0.6983
Phytase		0.088	0.8661	0.9280	0.0923	0.6444
Fe × phytase		0.2465	0.7489	0.6946	0.2820	0.5420

[a]Means within the same column without common letters differ significantly ($P < 0.05$).
Values are means of four replicates.
SEM, standard error of the mean.

Table 4. Effect of dietary iron and phytase levels on carcass characteristics of broiler breeder hens.[a,b]

Treatments							
Phytase (U/kg)	Iron (ppm)	Liver (%)	Heart weight (g)	Heart (%)	Spleen weight (g)	Spleen (%)	Fat weight (g)
	37	1.27	21.67	0.47	2.94	0.06	122.67
	52	1.29	21.49	0.47	2.79	0.06	133.95
	67	1.38	20.00	0.48	3.11	0.07	99.87
	82	1.24	21.68	0.46	2.78	0.06	101.91
SEM		0.049	0.825	0.022	0.228	0.0048	13.27
0		1.25	23.14[a]	0.50[a]	2.67[b]	0.06[b]	110.32
600		1.33	20.28[b]	0.43[b]	3.15[a]	0.07[a]	118.87
SEM		0.035	0.583	0.016	0.16	0.0034	9.38
P-value							
Fe		0.2338	0.9699	0.9116	0.7130	0.6638	0.2281
Phytase		0.1396	0.0021	0.0065	0.0472	0.0164	0.5254
Fe × Phytase		0.3418	0.9671	0.4649	0.5125	0.6638	0.1568

[a]Means within the same column without common letters differ significantly. ($P < 0.05$).
[b]Values are means of four replicates.
SEM, standard error of the mean.

effects of iron and phytase on concentration of iron in tissue of broiler breeder hens are presented in Table 6. Based on the results by increasing dietary iron levels, the concentration of iron in the bone marrow, liver, spleen ($P < 0.05$), egg yolk and blood serum increased ($P < 0.01$). Dietary iron linearly increased iron concentrations in spleen, egg yolk, liver and bone marrow. The hens received the highest amount of iron and stored more iron in their organs. Hens fed with phytase supplementation showed high iron concentration in the egg yolk and blood serum ($P < 0.01$). According to result of this study, the phytase levels showed no significant differences on iron concentration in the bone marrow, spleen and liver. No significant interaction between iron levels and phytase supplementation for concentration of iron in the bone marrow, liver, spleen and blood serum of hens was observed. But interaction between dietary added iron and phytase on egg yolk iron content was significant ($P < 0.05$). Highest and lowest amount of egg yolk iron storage was observed at 37 mg/kg dietary iron, 0 phytase and 82 mg/kg dietary iron level and 600 unit supplemented phytase, respectively (Figure 2). Table 7 summarized the statistics, estimated requirement values and appropriate models used for estimation. According to the results of the broken-line models, estimated iron requirement without phytase for hen house egg production, egg production percentage, egg mass, iron content of the liver, blood serum, spleen and egg yolk was 65, 65, 64, 54, 68, 63 and 68 mg/kg, respectively (Figures 3–8). Based on the quadratic

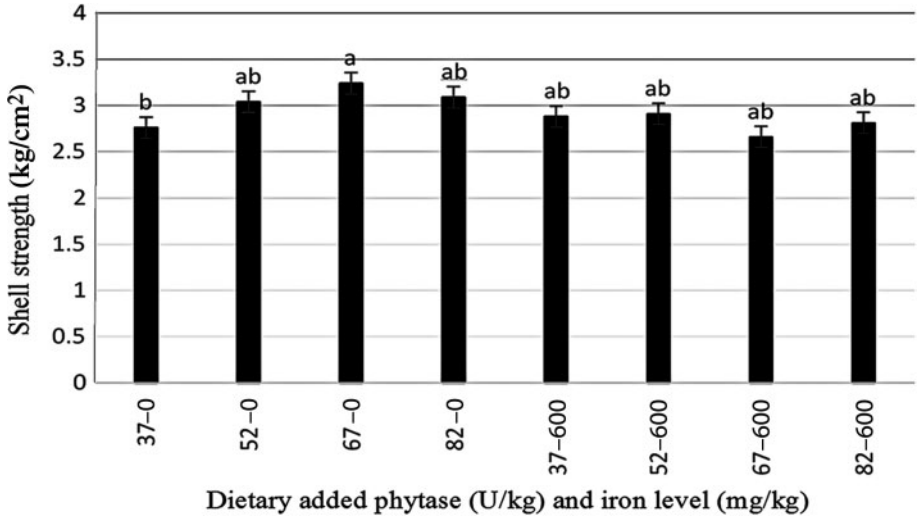

Figure 1. Interaction between dietary added iron and phytase on shell strength.

Table 5. Effect of dietary iron and phytase levels on egg quality of broiler breeder hens.[a,b]

Treatments							
Phytase (U/kg)	Iron (ppm)	Haugh unit	Albumen height (cm)	Shell strength (kg/cm^2)	Shell thickness (mm)	Yolk (%)	Shell (%)
	37	87.97	8.14	2.82	0.28	34.03	11.48
	52	88.25	8.31	2.98	0.29	34.05	11.61
	67	90.52	8.76	2.95	0.29	32.46	11.01
	82	90.55	8.52	2.95	0.29	32.87	11.50
SEM		3.15	0.59	0.079	0.005	0.584	0.214
0		91.25	8.79	3.04[a]	0.29[a]	33.21	11.48
600		87.39	8.07	2.82[b]	0.28[b]	33.48	11.32
SEM		2.23	0.422	0.056	0.0036	0.437	0.151
P-value							
Fe		0.8904	0.8711	0.5614	0.6764	0.0759	0.7199
Phytase		0.2606	0.2651	0.0087	0.0473	0.4531	0.3742
Fe × phytase		0.8782	0.8132	0.0410	0.1212	0.5965	0.1807

[a]Means within the same column without common letters differ significantly ($P < 0.05$).
[b]Values are means of four replicates.
SEM, standard error of the mean.

model, estimated iron requirement without phytase for egg weight was 66 mg/kg (Figure 9). The logistic model that was fitted to iron content of spleen proposed that iron requirement without phytase was equal to 53 mg/kg (Figure 10). Two slope, broken-line analysis of hen house egg production regressed on the supplemental iron indicated that 58 mg/kg supplemental iron with phytase was required for the optimal egg production of broiler breeder (Figure 11).

4. Discussion

The results of this study showed that, increasing the amount of iron in the diet increases egg weight. The highest egg weight was observed in hens fed supplemented diet with 67 mg iron/kg. Morck and Austic (1981) and Park et al. (2004) reported that the iron can influence egg weight. Ramadan et al. (2010) agreed with results of this experiment and stated that egg weight increases by dietary iron in the layer hens but had no effects on the egg quality. Ovotransferrin is one of the iron-containing compounds in the egg white albumin which forms 12% of the amount of the egg protein. Increased egg weight in the present study may be due to increasing the amount of ovotransferrin in the albumen of hens that received 67 mg iron/kg of iron. These results are in disagreement with the published data by Bess et al. (2012). The choice of a model to evaluate data should depend on objectives of the experiment (Baker 1986). Pesti et al. (2009) suggested that the broken-line models

Table 6. Effect of dietary iron and phytase levels on iron content in tissues of broiler breeder hens.[a,b]

Treatments		Iron content (ppm)				
Phytase (U/kg)	Iron (mpp)	Bone marrow	Spleen	Liver	Egg yolks	Serum
	37	508[b]	762[b]	734[b]	212[c]	496[b]
	52	502[b]	834[a,b]	822a[b]	215[bc]	471[b]
	67	538[ab]	882[a]	841[ab]	229[b]	641[a]
	82	620[a]	893[a]	872[a]	243[a]	619[a]
SEM		28.27	31.00	29.07	4.68	35.37
0		520	842	798	216[b]	499[b]
600		563	843	837	233[a]	614[a]
SEM		19.96	23.68	22.20	3.31	26.03
P-value						
Fe		0.023	0.0298	0.0255	0.0018	0.0046
Phytase		0.129	0.974	0.1895	0.0002	0.0064
Fe × Phytase		0.446	0.532	0.287	0.0344	0.702

[a]Means within the same column without common letters differ significantly ($P < 0.05$).
[b]Values are means of four replicates.
SEM, standard error of the mean.

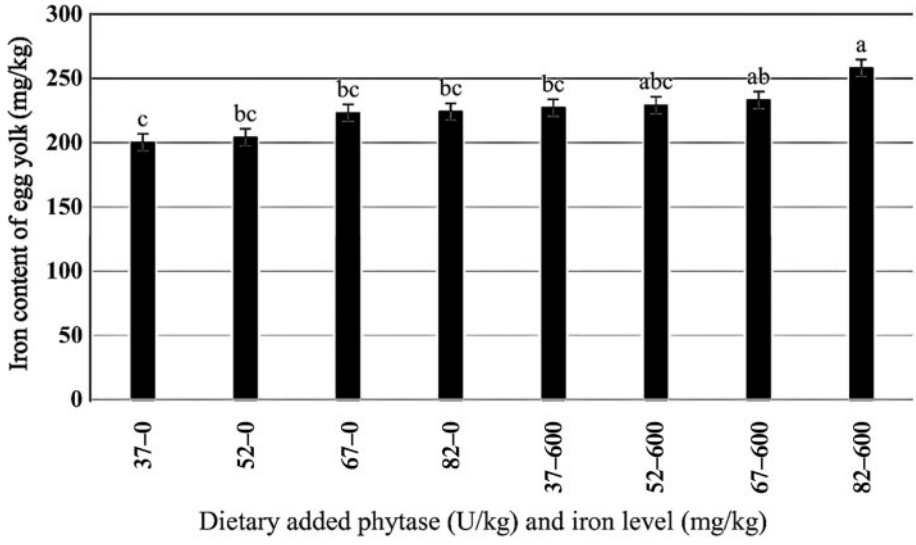

Figure 2. Interaction between dietary added iron and phytase on egg yolk iron content.

more closely represent theoretical ideas of the nature of nutritional responses than polynomial models. The ranges of optimal iron requirement of broiler breeder hens at late stage of production cycle are 54–72 g/kg of diet (Figure 1–4). The model: $Y = 17.18–0.24 \times (65–x)–0.393 \times (x–65)$, where $(65–x)$ is defined as zero at values of $x > 65$, and $(x–65)$ is defined as zero when $x < 65$, fitted to the data from groups without supplementation of phytase. The model indicated that the breakpoint occurred at 65 mg/kg diet (Figure 6). In White Leghorn hens, the iron requirement was 35–45 ppm for maintenance of hematocrit and 55 ppm for maximum of hatchability (Morck & Austic 1981). These researchers determined the iron requirement of White Leghorn hens by diet that contained levels of 15–65 mg iron/kg. Davis et al. (1962) and McNaughton and Day (1979) suggested that the iron requirements of broiler were 79–80 mg iron/kg. Aoyagi and Baker (1995) showed that the weight gain, hematocrit, haemoglobin and iron content in blood serum increases when graded levels 0, 5, 10, 20, 30, 40

Table 7. The estimated requirement of broiler breeder hens.

Requirement (mg/kg)	*P*-value	R^2	Equation	Model	Response
65	0.584	0.15	$Y = 17.18 - 0.24 \times (Z1) - 0.3933 \times (Z2)$[a]	Broken-line	Egg production (H.H)
65	0.5751	0.16	$Y = 41.78 - 0.59 \times (Z1) - 1.01 \times (Z2)$	Broken-line	Egg production (%)
64	0.49	0.17	$Y = 23.4 - 0.49 \times (Z1) - 0.74 \times (Z2)$	Broken-line	Egg mass (g)
66	0.065	0.33	$Y = 44.6 + 0.8x - 0.006x^{2}$[b]	Quadratic equation	Egg weight (g)
54	0.013	0.64	$Y = 844.8 - 10.62 \times (Z1) - 0.2778 \times (Z2)$	Broken-line	Iron of liver (mg/kg)
68	0.0038	0.67	$Y = 635.6 - 8.2 \times (Z1)$[c]	Broken-line	Iron content of serum (mg/kg)
63	0.09	0.4	$Y = 954.3 - 7.9 \times (Z1) - 3 \times (Z2)$	Broken-line	Iron content of spleen (mg/kg)
53	0.0001	0.99	$Y = a=(1 + ((a - b)=b) \times e^{[(-cx)]}$[d]	Logistic	
68	0.0043	0.59	$Y = 229.5 - 1.068 \times (Z1)$	Broken-line	Iron content of egg yolk (mg/kg)

[a]Y = L + U (R − x) + (x − R), where Y = performance parameter (e.g., egg production, egg weight), L = the ordinate of the breakpoint in the curve; R = the abscissa of the breakpoint in the curve (requirement estimate), $(R–x) = Z1$ is defined as zero at values of $x > R$, and $(x–R) = Z2$ is defined as zero when $x < R$. We define parameters for the breakpoint x value (R), an asymptote for the first segment (L) and slopes for the two line segments (U, V).

[a]$Y = a + bx + cx^2X$ = value required to achieve maximal Y.

[c]Y = L + U (R − XLR), where Y = performance parameter (e.g., egg production, egg weight), L = the ordinate of the breakpoint in the curve; R = the abscissa of the breakpoint in the curve (requirement estimate); X_{LR}= value of x less than R and $(R–x) = Z1$ is defined as zero when $x > R$; and U = slope of the line for X less.

[d]$Y = a=(1 + ((a - b)=b) \times e^{[(-cx)]}$, where Y = performance (e.g., egg production, egg weight); $a–c$ = are constants; x = dietary nutrient concentration; and e = base of natural logarithms.

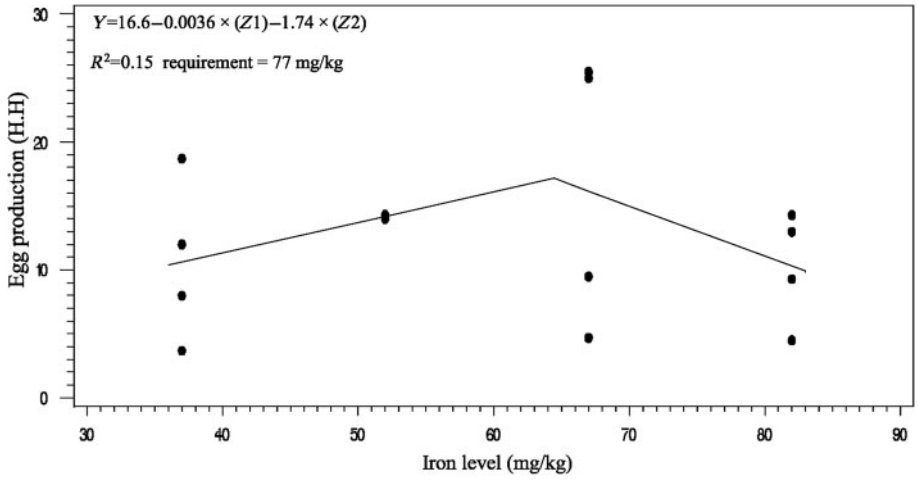

Figure 3. Hen house egg production response to consumption iron based on two-slope broken-line model.

and 50 mg/kg of iron from analytical grade $FeSO_4.7H_2O$ were added to iron-deficient basal diet containing 46 mg iron/kg. Also suggested that the maximum response in hematocrit, haemoglobin, weight gain, feed intake and iron content in blood serum occurred between 30 and 40 mg/kg supplemental iron. Cobb 500 and Ross 308 breeder management guide recommended 50–55 mg/kg of iron of diet for broiler breeder hens. The model: $Y = 13.68–0.285 \times (58–x) + 0.036 \times (x–58)$, where $(58–x)$ is defined as zero at values of $x > 58$ and $(x–58)$ is defined as zero when $x < 58$, fitted to the data from groups with supplementation of phytase. The model indicated that the breakpoint occurred at 58 mg/kg diet (Figure 6). Based on these data, the iron equivalency of 600 U/kg phytase is 7 mg/kg. Significant differences were observed in shell thickness and egg shell-breaking strength of phytase supplementation. Decrease in shell-breaking strengths and shell thickness was observed due to addition of phytase to the diet. Also it can be related to greater egg weight in the hens' received phytase which is in disagreement with reports by Lim et al. (2003) and Casartelli et al. (2005) who reported that phytase does not affect the shell-breaking strengths and shell thickness. Figure 1 indicated that hens received diet without phytase had more shell thickness, furthermore, addition of phytase to the basal diet decreased shell thickness. Iron is necessary for making haemoglobin which is essential for transferring oxygen in blood from the lungs to the tissues. Life of animal can be endangered by reducing available oxygen of tissues (hypoxia). Heart increases flow of blood to the lungs for oxygenation to prevent hypoxia but increasing of blood flow causes heart hypertrophy. Aoyagi and Baker (1995) reported the heart hypertrophy in chicks fed by diets severely deficient in iron. The heart weight was not affected by iron levels in our study that may be due to the high age of hens. In this research, the high spleen weight and the low heart weight were observed in hens fed diet supplemented with phytase. Phytase can influence on the carcass traits because of high impact of it on the availability of minerals, fat, protein, amino acids and starch. Mohamed Arabi (2013) stated that phytase did

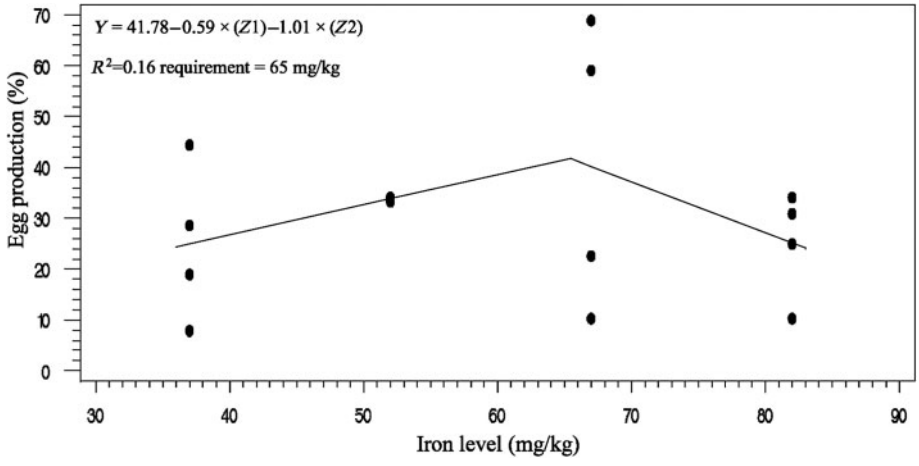

Figure 4. Egg production percentage response to consumption iron based on two-slope broken-line model.

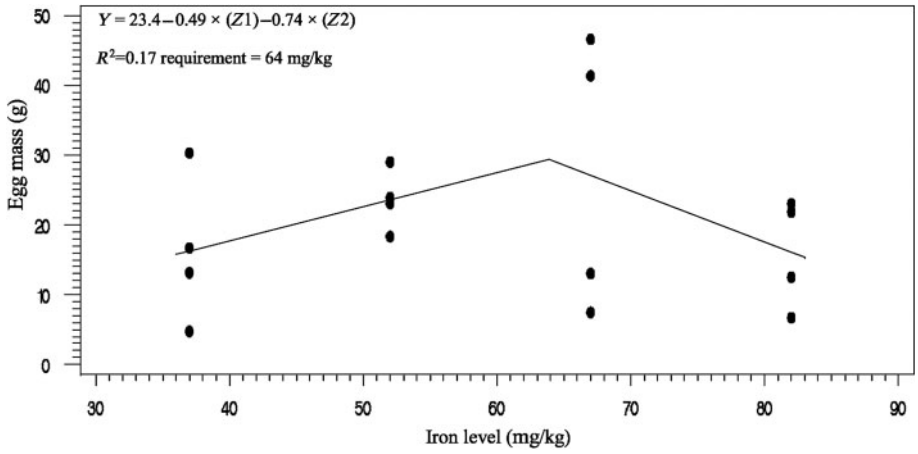

Figure 5. Egg mass response to consumption iron based on two-slope broken-line model.

not have significant effect on heart and liver percentage. Concentrations of mineral tissue are commonly used to evaluate the mineral status of animals and humans. In this experiment, we found linear responses in the concentrations of iron in liver, spleen and bone marrow by increasing levels of iron. Our findings are similar to (Aoyagi & Baker 1995; Cao et al. 1996; Yu et al. 2000) results that observed that the iron concentration of tissues enhances with increasing of dietary iron. Ma et al. (2012) suggested that adding 120 and 160 mg iron/kg from iron–glycine and 160 mg iron/kg from $FeSO_4$ increases iron concentration in serum, liver, breast muscle, tibia and faeces at 21 and 42 days of broiler age. The poor iron status will lead to embryonic malformations and delayed development and also it can cause death in post-hatch. One of embryonic sign in mortality of post-hatch is heart hypertrophy (Tako & Glahn 2011). In this experiment, significant effect of dietary iron on iron content of egg yolk was observed, which agree with (Morck & Austic 1981; Park et al. 2004; Bess et al. 2012) the findings. Park et al. (2004) found that iron content of eggs increased by 5% and 18% by the addition of either $FeSO_4$ and Fe-methionine, respectively. Hens fed diets with phytase enhanced the iron concentration of the egg yolk and blood serum. This result showed that phytase can release iron from inositol in old broiler breeder hens and increases chicken iron reserve. The result showed that 600 U/kg of phytase supplementation led to an increase in iron concentration of 6% in egg yolk and 18.7% in blood serum. Ao et al. (2007) stated that adding phytase to diet increase zinc concentration in blood plasma and bone marrow of broiler. Statistical methods used to determine the iron requirement can be effective on the amount of iron requirements estimation.

5. Conclusion

The present study showed that phytase increased iron bioavailability, and the supplements of iron provided as organic iron in the semi-purified diet required for hen

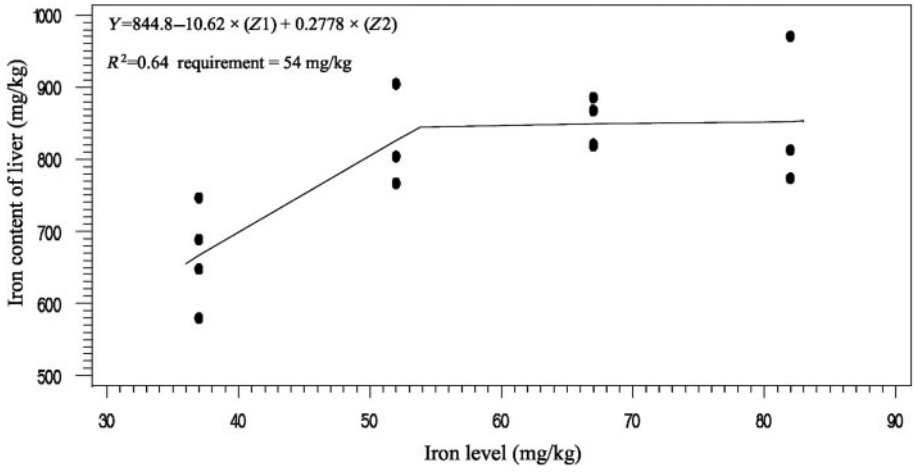

Figure 6. Iron content of liver response to consumption iron based on two-slope broken-line model.

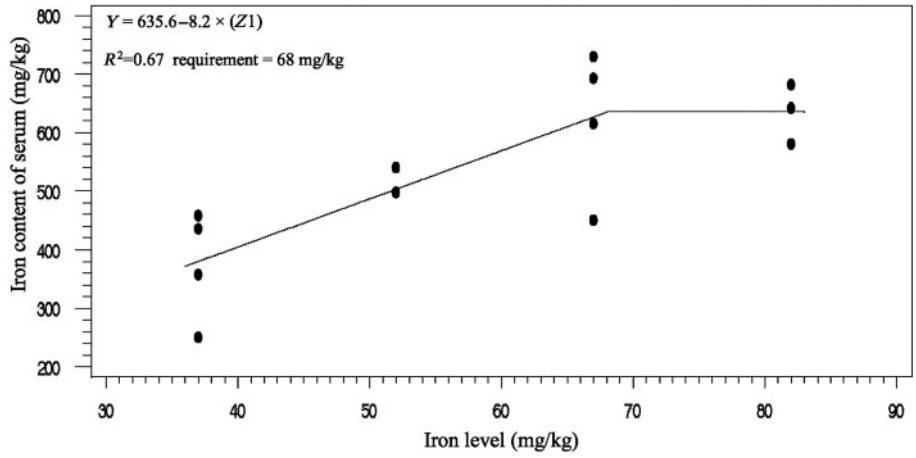

Figure 7. Iron content of serum response to consumption iron based on one-slope broken-line model.

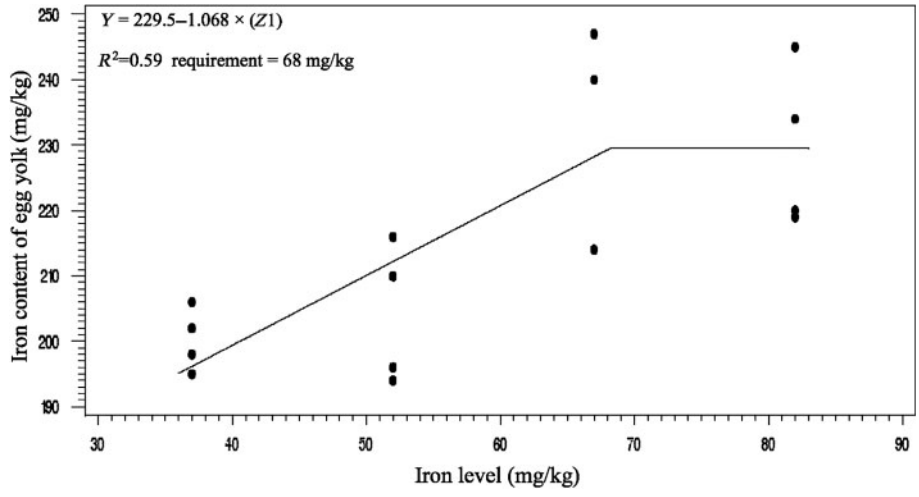

Figure 8. Iron content of egg yolk response to consumption iron based on one-slope broken-line model.

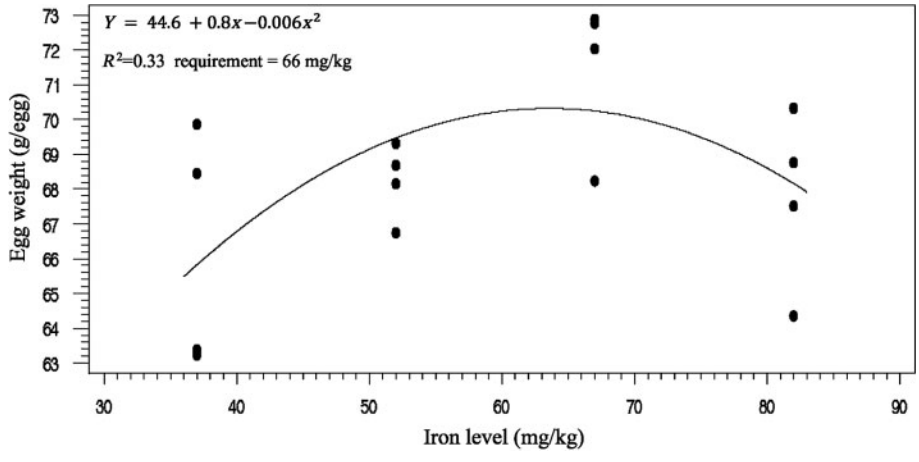

Figure 9. Egg weight response to consumption iron based on quadratic model.

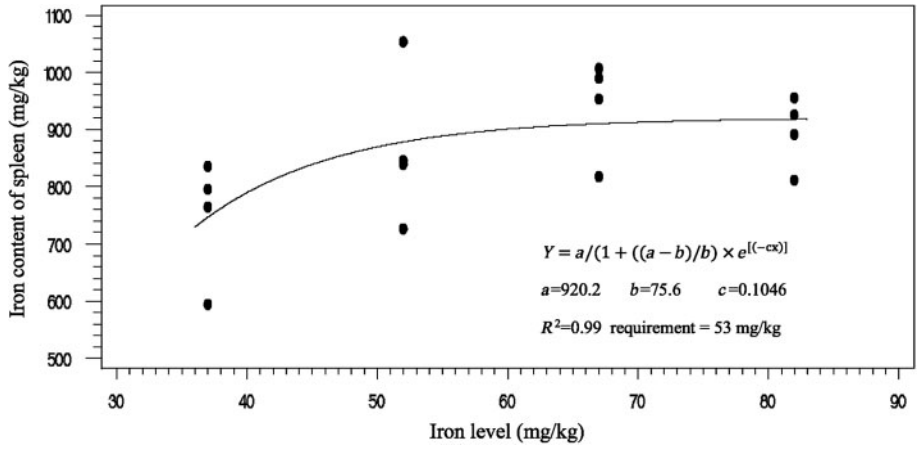

Figure 10. Iron content of spleen response to consumption iron based on logistic model.

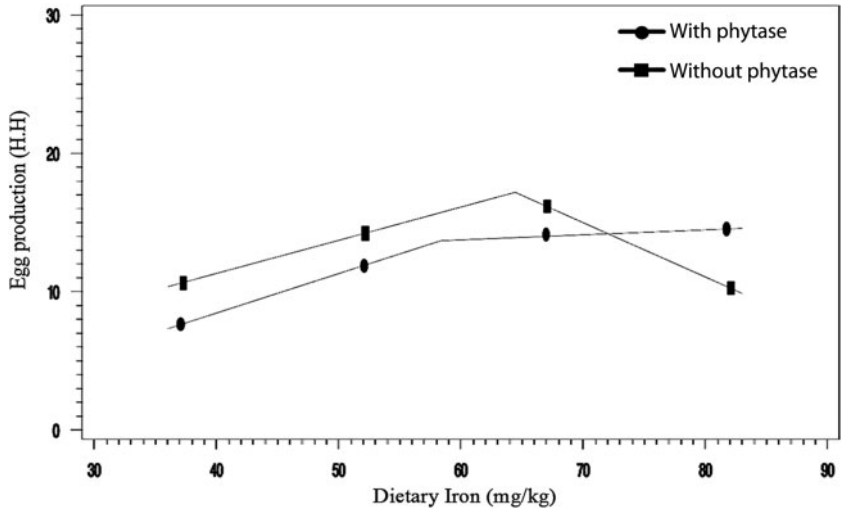

Figure 11. Two slope broken-line analysis plot of hen house egg production (egg numbers during 6 weeks per hens) as a function of supplemental iron with or without phytase.

house egg production of broiler breeder hens at late stage of production cycle were 65 and 58 mg/kg without and with phytase supplementation, respectively. Based on our data on hen house egg production, about 1.16 mg of iron was released per 100 U/kg of phytase, which means that 7 mg iron/kg will be released if 600 U/kg of phytase is used.

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