Interaction between Iron and Vitamin A in Broilers**

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ABSTRACT : A 3×3 (Fe×VA) experiment with repeats was designed to study the interaction between iron and vitamin A in broilers. 504 broilers were divided into 9 groups (50% males, 50% femals), each group with 4 repeats. Iron supplemental amount was 0, 30 and 60 mg/kg; Vitamin A supplemental amount was 750, 1,500 and 2,700 IU/kg. Iron concentration in liver, serum, tibia and duodenum and vitamin A concentration in liver and serum were measured, and erythrocyte count was also observed. Results showed with the increase of dietary supplemental iron levels, vitamin A concentration in liver significantly decreased lineally (p<0.05) (0.861, 0.671, 0.639 mg/100 g at the end of 4th week; 0.900, 0.765, 0.739 mg/100 g at the end of 7th week), and vitamin A concentration in serum significantly increased lineally (p<0.05) (82.725, 97.842, 109.475 μg/100 mL at the end of 4th week; 62.288, 91.900, 95.117 μg/100 mL at the end of 7th week), meaning iron could promote the mobilization of vitamin A from liver to serum. With the increase of dietary supplemental vitamin A levels, liver iron concentration decreased and serum iron concentration increased, vitamin A could promote the mobilization of iron from liver to blood. Iron concentration in Duodenum and tibia and erythrocyte count increased significantly with higher dietary vitamin A supplementation (p<0.01), vitamin A could promote iron absorption, iron mobilization from liver to target tissues and erythropoiesis. Effects of the interaction between iron and vitamin A on vitamin A concentrations in liver and serum, iron concentration in tibia and erythrocyte count were significant (p<0.05). (*Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 4 : 558-564*)

Key Words : Broilers, Iron, Vitamin A, Interaction

INTRODUCTION

Studies with children (Mejia, 1982; Bloem, 1989; Wolde-Gebriel, 1993) and pregnant women (Suharno, 1992) have shown that vitamin A deficiency is associated with impaired iron status, while vitamin A supplementation produced an increase in blood hemoglobin concentrations (Bloem, 1989; Muhilal, 1988; Mejia, 1988; Bloem, 1990). The administration of vitamin A in addition to supplemental iron has been found to enhance the response of serum iron concentration, transferring saturation and blood hemoglobin concentration in children and pregnant women with low vitamin A and iron status (Mejia, 1988). This suggests that vitamin A improves the utilization of ingested iron and affects the iron concentrations of organs.

The mechanism by which vitamin A affects iron metabolism is still unknown. The fact that vitamin A deficiency in rats may impair erythropoiesis (Mejia, 1979; Roodenburg, 1994) but increased iron concentrations in liver (Mejia, 1979; Stabb, 1984; West, 1988) and spleen (Roodenburg, 1994; Mejia, 1979) may point to disturbed iron transport.

Many researchers studied the effects of vitamin A on iron status and the results showed vitamin A interfered with iron metabolism. There were few studies about the effects

of iron on vitamin A metabolism. The previous studies were all about the mammal. The interaction between vitamin A and iron in the poultry has not yet been studied. It was anticipated that this study would provide clues as to the interaction between vitamin A and iron in poultry.

MATERIALS AND METHODS

Experimental design and birds

Interaction between Iron and Vitamin A in Broilers was studied using a 3×3 factorial design of treatment. The experimental design was a randomized-block design. Four replicates per treatment (50% males, 50% femals). Factors were dietary iron and vitamin A. Three supplemental levels of iron added as feed grade $FeSO_4 \cdot 7H_2O$ were 0, 30, 60 g/kg diet. Three supplemental levels of vitamin A added as palmitate retinyl ester were 750, 1,500, 2,700 IU/kg diet. 504 one-day-old Avian broiler chicks from Shan Xi China Tai Co., LTD. were divided into nine groups of 56 chicks each (14 birds per replicate) in an experiment lasting 7 weeks.

Birds were fed a practical diet, the diet was formulated according to the Chinese Nutrient Requirement of Broilers (Chinese Academy of Agricultural Science, 1998. Table 1). The broilers were caged in metal cages painted with paint, with each replicate in a two story cages. Feed and water were provided for *ad libitum* consumption. All chickens were cared for in accordance with the University Council on Animal care guidelines of animal welfare. Blood was collected in heparinized orbital by orbital puncture. Blood samples were collected from one bird per replicate (4 birds

Table 1. Dietary composition and nutrient levels

Ingredients	Compositions (%)		Nutrient levels		
	0-4 week	5-7week		0-4 week	5-7 week
Corn	46.1	60.02	ME, Mcal/kg	3	3.1
Soybean oil	4.1	3.96	CP, %	22.3	20.5
Soybean meal	37.5	20.23	Met+Cys, %		
Cottonseed cake	3	5	Lys, %	0.83	0.68
Rapeseed meal	3	5	Calcium, %	1.13	0.94
Fish meal	0.1	2	Available P, %	1	0.9
Meat and Bone meal	4.8	2	Fe tested in basal diet, mg/kg	0.5	0.45
Bone meal	-	0.66	Retinol tested in basal diet, IU/kg	231	296
Stone meal	0.36	0.83		1,000	1,000
Met	0.15	0.06			
Salt	0.36	0.24			
Choline	0.13	0.13			
Trace mineral premix[1]	0.2	0.2			
Vitamin premix[2]	0.02	0.02			

[1] Mineral composition (mg/kg of diet): copper 8 mg; manganese 80 mg; zinc 70 mg; iodine 0.4 mg; selenium 0.2 mg.

[2] Vitamins in amounts per kilogram diet: D_3 400 IU; E 20IU; k_3 0.5 mg; B_1 1.5 mg; B_2 3.6 mg; B_3 10 mg; B_5 3 mg; B_6 27 mg; B_{12} 0.009 mg; B_7 0.15 mg; B_{11}, 0.55 mg.

per treatment, 50% males, 50% femals) at 28 days and 49 days of age respectively. Immediately after bleedings, the dead broilers were cut open. The liver, duodenum, and tibia were removed and stored at -20°C until analysis.

Experimental parameters measured

Determination of iron concentration : Liver was washed with saline (9 g NaCl/L). Liver (1 g) and tibia (1 g) were dried (100°C, 12 h) and ashed (500°C, 16 h), then all the ash was dissolved in 1 mL 6 M-HCl and diluted with demineralized water to 50 mL. Fe was measured by flame atomic absorption spectrometry (Modle AA-475, Varian, Springvale, Australia). Serum Fe concentrations was determined spectrophotometrically using a commercial test kit (Roche Nederlang, Mijdrecht, The Netherlands). All analyses were carried out singly. Fe in tibia was calculated as the mean of left and right tibia.

Determination of retinal concentration : Serum and Liver retinal concentration were measured by reversed phase HPLC as described by Roodernburg et al. (1996).

Determination of RCC : Blood erythrocyte count was analyzed with a blood cell counter as described by Han (1995).

Statistical analysis

All the data were analyzed statistically according to the two-way ANOVA procedure (SAS. Institute, 1989) and the treatment means were separated by Duncan's multiple range test. Statistical significance was at $p < 0.05$ for all statistical tests.

RESULTS AND DISCUSSION

The effects of iron on vitamin A metabolism

Vitamin A concentration in liver and serum : The concentration of liver vitamin A and the concentration of serum vitamin A were significantly ($p < 0.05$) affected by dietary supplemental iron levels at the end of 4th and 7th week respectively (Table 2), and with the increase of dietary iron levels, vitamin A concentration in liver significantly lineally decreased (Figure 1), and vitamin A concentration in serum significantly lineally increased (Figure 2). Munoz's study (2000) showed iron supplementation significantly increased plasma retinal, retinol binding protein, Transthyretin, this suggested iron could enhance the synthesis of vitamin A transport protein. These indicated iron promoted vitamin A mobilization from liver to blood by enhancing the synthesis of vitamin A. Rosale et al. (1999) on young rats showed that liver vitamin A concentration was significantly higher and plasma retinal concentration was significantly lower when diets lower in iron were fed ($p < 0.05$), suggesting the lower dietary iron will impair vitamin A mobilization from liver. This agreed to the result of this study.

Schultink (1997) reported that iron supplementation raised retinal of anaemic pre-school children. Schweigert (2000) reported that iron supplementation prevented the reduction in the concentration of plasma retinal in piglets, resulting in higher plasma retinal levels ($p < 0.01$). All these studies suggested that vitamin A played an important role in mobilizing iron from liver to blood.

The effects of vitamin A on iron metabolism

Table 2. Retinal concentrations in liver (mg/100 g) and serum (μg/100 ml)

Supplemental amount			At the end of 4th week		At the end of 7th weeks	
Fe (mg/kg)	VA (IU/kg)	n	Liver (mg/100 g)	Serum (μg/100 ml)	Liver (mg/100 g)	Serum (μg/100 ml)
0	750	4	0.679±0.478[bc1]	72.4±17.457	0.354±0.099[d]	55.187±6.978[d]
0	1,500	4	1.138±0.19[a]	83.375±16.469	1.128±0.004[ab]	65.575±12.263[cd]
0	2,700	4	0.766±0.057[abc]	92.4±17.429	1.218±0.042[a]	66.1±8.687[cd]
30	750	4	0.683±0.156[bc]	98.1±31.864	0.868±0.064[bc]	78.2±16.021[bc]
30	1,500	4	0.543±0.056[bc]	97.375±18.331	0.738±0.048[c]	89.025±15.825[bc]
30	2,700	4	0.787±0.101[abc]	98.05±12.696	0.689±0.143[c]	108.475±17.205[a]
60	750	4	0.402±0.025[c]	82.825±23.054	0.37±0.051[d]	97.6±12.115[b]
60	1,500	4	0.625±0.057[bc]	109.1±1.326	0.758±0.105[c]	82.975±15.906[bc]
60	2,700	4	0.89±0.034[ab]	136.5±37.798	1.089±0.419[ab]	104.775±12.354[a]
0		12	0.861[a]	82.725[b]	0.9[a]	62.288[b]
30		12	0.671[b]	97.842[ab]	0.765[b]	91.9[a]
60		12	0.639[b]	109.475[a]	0.739[b]	95.117[a]
	750	12	0.588[b]	84.442[b]	0.531[b]	76.996[b]
	1,500	12	0.768[a]	96.617[ab]	0.875[a]	79.192[b]
	2,700	12	0.814[a]	108.983[a]	0.998[a]	93.117[a]
P-value	Fe		0.0142	0.0212	0.043	0.0001
	VA		0.0147	0.037	0.0001	0.0136
	Fe*VA		0.0029	0.2228	0.0001	0.0137

[a-d] Means within a column with different superscripts are significantly different (p<0.05).
[1] Treatment Mean±SD.

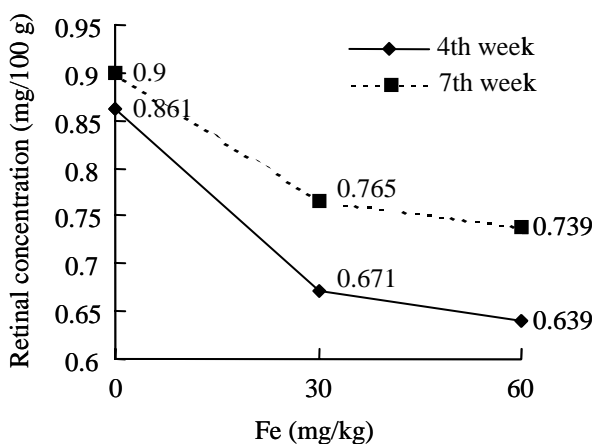**Figure 1.** Retinal concentration in liver.

Figure 2. Retinal concentration in serum

Duodenum iron concentration : The iron concentration of duodenum was significantly affected by dietary vitamin A levels at the end of 4th week (p<0.05), and with the increase of dietary vitamin A levels, the iron concentration of duodenum lineally increased (Table 3). Duodenum is the primary place to iron absorption in broilers (wang, 1996). These suggested that vitamin A could improve iron absorption. The effect was not significant (p>0.05) at the end of 7th, but duodenum iron concentration in the group with diet lower in vitamin A was lower than other groups. The effect of di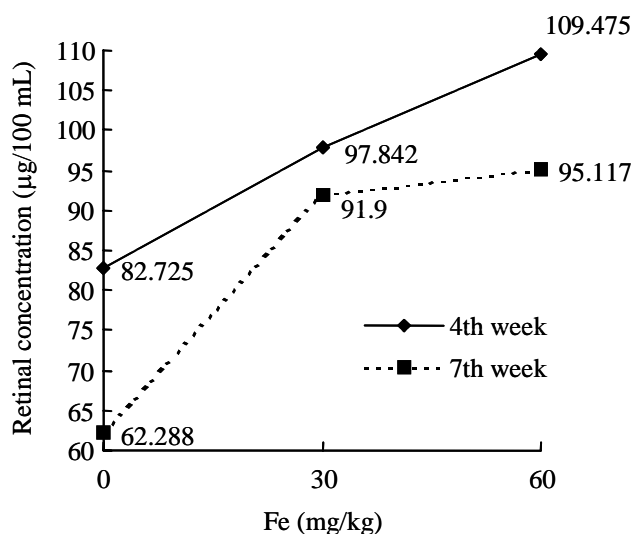etary vitamin A levels on the iron concentration of duodenum was significant at the end of 4th week (p<0.05), while was not significant at the end of 7th week (p>0.05). The reason may be that the ability of iron absorption of broilers decreases with the age increasing. The studies of Layrisse (1998) and Garcia (1998) both showed vitamin A might form a complex with iron, keeping it soluble in the intestinal lumen and preventing the inhibitory effects of phytates and polyphenols on iron absorption, so vitamin A can improve iron absorption.

Table 3. Fe Concentrations in liver, duodenum (mg/kg) and serum (mg/l)

Supplemental level			At the end of 4th week			At the end of 7th weeks		
Fe mg/kg	VA IU/kg	n	Duodenum	Liver	Serum	Duodenum	Liver	Serum
0	750	4	185.18±24.67[1]	430.78±54.79	0.48±0.06	108.58±16.25	312.65±21.26	0.73±0.17
0	1,500	4	232.31±17.11	340.93±23.08	0.4±0.06	107.71±28.98	307.81±55.2	0.8±0.02
0	2,700	4	273.64±39.55	334.22±74.32	0.61±0.13	93.37±5.1	289.96±36.1	0.73±0.19
30	750	4	195.29±59.33	442.42±94.57	0.54±0.06	196.34±5.38	337.14±18.01	0.68±0.14
30	1,500	4	181.19±9.77	431.64±31.33	0.72±0.14	156.39±31.62	304.46±38.21	0.92±0.2
30	2,700	4	201.59±53.51	363.9±75.21	0.67±0.17	194.21±19.06	309.28±27.84	0.97±0.13
60	750	4	163.16±72.17	465.65±41.49	0.68±0.14	90.46±9.79	447.51±35.42	0.87±0.01
60	1,500	4	185.30±48.22	500.01±114.2	0.65±0.17	152.02±19.08	347±34.44	0.87±0.27
60	2,700	4	188.78±77.26	456.44±118.4	0.72±0.09	113.18±32.68	341.06±52.45	0.97±0.31
0		12	230.38[a]	368.65[b]	0.5[b]	103.22[b]	303.48[b]	0.761
30		12	192.69[ab]	412.66[ab]	0.646[a]	178.98[a]	316.96[b]	0.86
60		12	179.08[b]	474.04[a]	0.687[a]	118.56[b]	378.53[a]	0.908
	750	12	171.21[b]	446.29	0.569	125.93	365.77[a]	0.765
	1,500	12	199.6[a]	424.2	0.596	140.07	319.76[b]	0.868
	2,700	12	221.34[a]	384.86	0.668	133.59	313.44[b]	0.894
P-value	Fe		0.0483	0.0088	0.0031	0.0001	0.0001	0.1971
	VA		0.0331	0.1608	0.1652	0.3019	0.0035	0.2551
	Fe*VA		0.5106	0.5427	0.1893	0.0613	0.0877	0.5521

[a-b] Means within a column with different superscripts are significantly different (p<0.05).

[1] Treatment Mean±SD.

Another reason why vitamin A can improve iron absorption maybe vitamin A enhances the growth of the cells in the epithelial intestine (Hong, 1994).

Iron concentration in liver and serum : The iron concentration in liver was significantly (p<0.05) affected by dietary vitamin A levels at the end of 7th week, and serum iron concentration was not significant (p>0.05) affected at the end of 4th or 7th week, and with the increase of dietary vitamin A levels, liver iron concentration decreased (Figure 3) and serum iron concentration increased (Figure 4) (Table 3). And low dietary vitamin A levels is disadvantageous to iron absorption. These facts above indicated the increase of liver iron concentration was caused not through the increase of iron absorption, but through the inhibition of the iron mobilization from liver due to lower dietary vitamin A levels. The mechanism how vitamin A will interfere with iron mobilization is still unknown. Chan
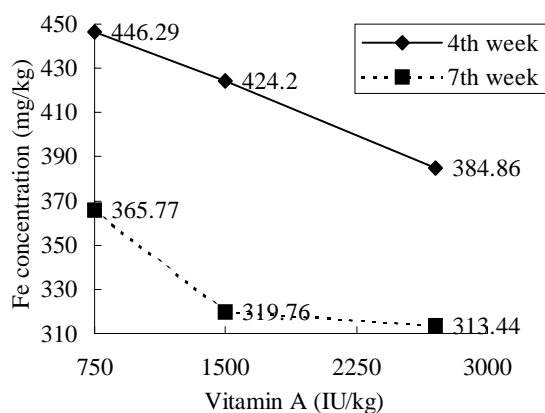
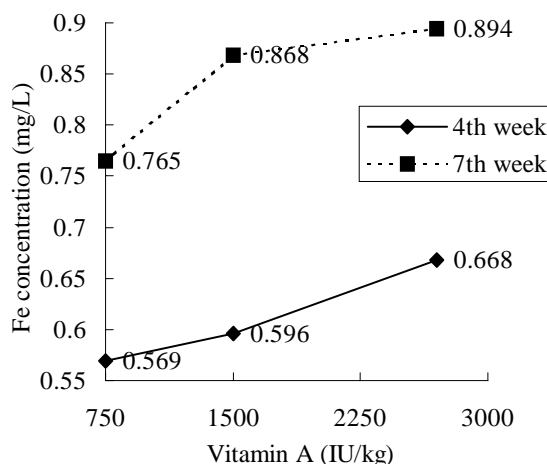

Figure 3. Fe concentration in liver

Figure 4. Fe concentration in serum

and Wolf (1987) reported vitamin A was involved in the synthesis of the glycosyl moieties of the transferring molecule; so vitamin A could affect iron mobilization from liver when transferring synthesis was impaired in vitamin A deficiency. Moreover, there was a hypothesis that lower vitamin A levels impair the iron utilization in tissues, resulting in the increase of liver iron concentration. The studies of Beynen (1992) and sijltsma (1993) both showed liver iron concentration significantly increased when rats were given diet lower in vitamin A. Suharno (1992) reported there was significantly positive correlation between plasma retinal and plasma iron in pregnant women. These studies supported the result of this study.

In this study, the effect of vitamin A on liver iron concentration at the end of 4th week and serum iron concentration at the end of 4th and 7th week were not significant, it maybe vitamin A wasn't deficient enough for boilers. Roodenburg (1996) reported that vitamin A supplementary effect in vitamin A deficiency condition was different from vitamin A supplementary effect in vitamin A adequacy condition.

From the effects of vitamin A on the concentration of iron in liver and serum, we could know vitamin A could promote iron mobilization from liver to blood in boilers.

Tibia iron concentration : The concentration of tibia iron significantly ($p<0.01$) increased with the increase of dietary vitamin A levels at the end of 7th week (Table 4), this indicated vitamin A promoted tibia iron uptake. Beynen (1992) and Sijtsma (1993) both reported femur iron concentration significantly decreased in rats given diet lower in vitamin A ($p<0.05$). Sijtsma (1993) pointed out that depressed uptake of iron by bone marrow was the primary feature of altered iron status in rats with marginal vitamin A deficiency.

RCC : Red cell count (RCC) was significantly ($p<0.01$) affected at the end of 4th and 7th week, and RCC increased significantly with the increase of dietary vitamin A levels. It suggested vitamin A could improve iron utilization in erythropoiesis in bone marrow. Roodenburg (1996) reported vitamin A promoted formation of blood red cell in rats. Li (1996) reported vitamin A improved hematopoietic microenvironment of rats by promoting BMSC (Bone Marrow Stromal Cell) to secrete hematopoietic growth factors (HGF) and enhancing its adhesive function. The effects of vitamin A on the amplification and differentiation of Blood red cell family early ancestor cell BFU-E are via changing the expression of pro-oncogene c-jun, C-fos mRNA in BMSC and further regulating the secretion of HGF.

In the effects of vitamin A on iron metabolism, tibia iron concentration significantly reduced, RCC significantly decreased and liver iron concentration significantly increased in groups given diet lower in vitamin A (Table 3). These findings suggested lower dietary vitamin A decreased the uptake of iron by bone marrow and increased the iron deposit in liver.

From the results and discussion of the effects of vitamin A on Iron Metabolism, it was concluded that the increase of

Table 4. Iron concentrations in tibia (mg/kg) and RCC (10^{10}/l)

Supplemental level			At the end of 4th week		At the end of 7th week	
Fe mg/kg	VA IU/kg	n	Tibia	RCC	Tibia	RCC
0	750	4	68.432±11.784[1]	90.75±12.5[d]	32.8±1.535[de]	258.25±19.293
0	1,500	4	84.380±14.752	139.5±32.675[cd]	38.44±2.016[de]	256.025±45.536
0	2,700	4	83.587±6.368	194.5±36.263[cd]	77.103±0.273[bc]	270±18.165
30	750	4	76.593±20.3	187±9.812[bc]	56.12±5.993[cd]	176.500±20.550
30	1,500	4	79.333±19.188	196±34.779[bc]	75.74±4.004[bc]	231.5±23.216
30	2,700	4	81.785±12.805	230.25±35.505[b]	122.28±9.871[a]	246.75±38.586
60	750	4	88.443±27.205	283±23.366[a]	45.77±12.269[de]	212.5±17.078
60	1,500	4	75.745±20.259	225.25±18.945[ab]	86.608±1.579[b]	216.5±33
60	2,700	4	78.620±7.424	244.25±18.661[b]	22.357±2.307[e]	240±8.164
0		12	78.8	141.58[c]	49.448[b]	261.5[a]
30		12	79.237	204.42[b]	84.713[a]	218.25[b]
60		12	80.936	250.83[a]	51.578[b]	223[b]
	750	12	77.823	186.92[b]	44.897[b]	215.75[b]
	1,500	12	79.819	186.92[b]	66.929[a]	234.75[ab]
	2,700	12	81.331	223[a]	73.913[a]	252.25[a]
P-value	Fe		0.9475	0.0001	0.0001	0.0008
	VA		0.3561	0.0028	0.0002	0.0091
	Fe*VA		0.8102	0.0002	0.0001	0.1576

[a-e] Means within a column with different superscripts are significantly different ($p<0.05$).
[1] Treatment Mean±SD.

dietary vitamin A levels improved iron mobilization from liver to target tissues, iron uptake, utilization in bone marrow and haematopoiesis.

The effects of interaction between vitamin A and iron on all index in the study

Interaction between Vitamin A and iron significantly affected liver vitamin A concentration at the end of 4th and 7th week ($p < 0.01$) and serum vitamin A concentration at the end of 7th week ($p < 0.05$) (Table 2). The higher level of supplemental iron (60 mg/kg) actually resulted in a similar vitamin A concentration in serum as seen in groups with higher dose of supplemental vitamin A (2,700 IU/kg) (Table 2). Interaction between vitamin A and iron significantly ($p < 0.01$) affected RCC at the end of 4th week. RCC was the highest in the group with supplemental Fe (60 mg/kg)×V_A (750 IU/kg) at the end of 4th week and Fe (0 mg/kg)×V_A (2,700 IU/kg) at the end of 7th week; significantly affected tibia iron concentration at the end of 7th week ($p < 0.01$). Tibia iron concentration was the highest in the group with supplemental Fe (60 mg/kg)×V_A (750 IU/kg) at the end of 4th week and in the group with supplemental Fe (30 mg/kg)×V_A (2,700 IU/kg) at the end of 7th week. These results suggested vitamin A and iron improve their utilization in the body of broilers each other.

IMPLICATIONS

This study indicated iron improved vitamin A mobilization from liver to blood and vitamin A improved iron mobilization from liver to target tissues, iron uptake and utilization in bone marrow, and haematopoiesis in broilers.

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