

## EFFECTS OF DIETARY VITAMIN B<sub>6</sub> LEVELS ON LIPID CONCENTRATION AND FATTY ACID COMPOSITION IN GROWING CHICKS

B. K. An, K. Tanaka<sup>1</sup> and S. Ohtani

Science of Biological Production, The United Graduate School of Agricultural Sciences and Department of Animal Science and Technology, Faculty of Agriculture, Gifu University, Japan

### Summary

This experiment was designed to evaluate the effect of various dietary vitamin B<sub>6</sub> levels on conversion from linoleic acid to arachidonic acid in various tissues in growing chicks. Growing chicks were fed the purified diet containing 7% safflower oil with different levels of vitamin B<sub>6</sub> (0, 4, 8, 40, 80 mg per kg diet) for 14 days. Feed intake and weight gain in chicks fed the vitamin B<sub>6</sub>-free diet were markedly depressed. Esterified and free cholesterol concentrations in serum were significantly higher, while the serum triglyceride concentration was significantly lower in chicks fed the vitamin B<sub>6</sub>-free diet compared to that fed diets with vitamin B<sub>6</sub>. The liver triglyceride content was also lower in chicks fed the vitamin B<sub>6</sub>-free diet. The liver and serum cholesterol ester fractions in chicks fed the vitamin B<sub>6</sub>-free diet showed higher rate of C<sub>18:2n6</sub> and lower rates of C<sub>18:3n6</sub>, C<sub>20:3n6</sub> and C<sub>20:4n6</sub> as compared with vitamin B<sub>6</sub> fed groups. In serum phospholipid fraction of chicks fed the vitamin B<sub>6</sub>-free diet, rates of C<sub>20:3n6</sub> and C<sub>20:4n6</sub> were markedly lower. As dietary vitamin B<sub>6</sub> level was increased, the rate of C<sub>20:4n6</sub> was slightly increased, although it was statistically not significant. The fatty acid compositions of adipose tissue showed almost the same pattern as those in liver and serum. This result suggests that the desaturation of C<sub>18:2n6</sub> to C<sub>18:3n6</sub>, elongation to C<sub>20:3n6</sub> or both steps might be impaired by vitamin B<sub>6</sub> deficiency in growing chicks.

(Key Words : Dietary Vitamin B<sub>6</sub> Level, Lipid Concentration, Fatty Acid Composition, Growing Chicks)

### Introduction

It has been known that vitamin B<sub>6</sub> plays an important role in amino acid synthesis and metabolism as a co-factor of aminotransferase and decarboxylase. Birch and Gyorgy (1936) reported that essential fatty acid rich oils had a special effect on dermatitis caused by pyridoxine deficiency in rats. Subsequent studies have showed that the level of longer chain unsaturated fatty acids in tissue lipid was decreased by pyridoxine deficiency (Medes et al., 1947; Schwartzman and Strauss, 1949; Witten and Holman, 1952; Delorme and Lupien, 1976). Cunnane et al. (1985) have suggested that vitamin B<sub>6</sub> is involved in metabolism of linoleic acid to arachidonic acid by finding the accumulation of C<sub>18:2n6</sub> and C<sub>18:3n6</sub> and decreasing of C<sub>20:4n6</sub> level in various tissues of rats fed pyridoxine

deficient diet. Sato (1970) also demonstrated that pyridoxine plays a role in essential fatty acid metabolism, especially in conversion of C<sub>18:3n6</sub> to C<sub>20:4n6</sub>, according to the investigation of different kinds and conditions of dietary fats. In contrary, it has been also reported that pyridoxine deficiency does not affect polyunsaturated fatty acid metabolism, but rather influence the oxidation rate of C<sub>18:2n6</sub> and C<sub>20:4n6</sub> (Kirschman and Coniglio, 1961; Dussault and Lepage, 1975). Thus, this study was conducted to determine whether vitamin B<sub>6</sub> is concerned in desaturation and elongation of C<sub>18:2n6</sub> and to investigate the effect of a larger amount doses of vitamin B<sub>6</sub> on fatty acid composition of various lipid fractions in growing chicks.

### Materials and Methods

#### Animal and diet

Day-old male White Leghorn chicks were used. Until 3 weeks of age, the birds were housed in battery type electric breeder and fed a commercial diet. At 4 weeks of age, all chicks were randomly assigned into 5 groups such

<sup>1</sup>Address reprint requests to Dr. K. Tanaka, Department of Animal Science and Technology, Faculty of Agriculture, Gifu University, Yanagido 1-1, Gifu 501-11, Japan.

Received October 13, 1994

Accepted July 7, 1995

that the average body weights were similar for each group and fed purified diets containing 7% safflower oil (containing more than 75% linoleic acid) and different levels of vitamin B<sub>6</sub> as shown in table 1. Room

temperature of 25 ± 3°C and photoperiod of 14 hrs. were maintained throughout experimental period. Feed and water were provided ad libitum.

TABLE 1. COMPOSITION OF EXPERIMENTAL DIETS

	Experimental diet				
	0	4	8	40	80
	.....(%).....				
Sucrose	37.8	37.8	37.8	37.8	37.8
Casein mixture <sup>1</sup>	20.4	20.4	20.4	20.4	20.4
Safflower oil	7.0	7.0	7.0	7.0	7.0
Cellulose	26.63	26.6296	26.6292	26.626	26.622
Mineral mixture <sup>2</sup>	6.0	6.0	6.0	6.0	6.0
Vitamin B mixture <sup>3</sup>	1.0	1.0	1.0	1.0	1.0
Vitamin AD mixture <sup>4</sup>	1.0	1.0	1.0	1.0	1.0
Choline-HCl	0.17	0.17	0.17	0.17	0.17
Pyridoxine-HCl	0	0.0004	0.0008	0.004	0.008
Total	100	100	100	100	100
Crude protein (%)	18	18	18	18	18
M. E. (kcal/100 g)	288.7	288.7	288.7	288.7	288.7

<sup>1</sup> Casein mixture was vitamin-free and premixed (g/kg diet) with L-arginine, 11.02; DL-methionine, 3.47; glycine, 4.08.

<sup>2</sup> Mineral mixture supplied (g/kg diet) : NaCl, 8.47; K<sub>2</sub>HPO<sub>4</sub>, 16.28; CaHPO<sub>4</sub> 2H<sub>2</sub>O, 13.10; CaCO<sub>3</sub>, 15.14; MgSO<sub>4</sub> 7H<sub>2</sub>O, 5.17; Fe SO<sub>4</sub> 7H<sub>2</sub>O, 1.38; MnSO<sub>4</sub> 4H<sub>2</sub>O, 0.40; KI, 0.04; ZnSO<sub>4</sub>, 0.013; CuSO<sub>4</sub> 5H<sub>2</sub>O; 0.015.

<sup>3</sup> Vitamin B mixture was vitamin B<sub>6</sub>-free and supplied (mg/kg diet) : thiamine-HCl, 8; riboflavin, 12; niacin, 100; folic acid, 5; Ca-pantothenate, 40; DL-tocopherol, 20; p-aminobenzoic acid, 80; menadione, 5; biotin, 0.6; cyanocobalamin, 0.05. These were premixed with lactose.

<sup>4</sup> Vitamin A D mixture supplied (IU/kg diet) : vitamin A, 55,000; vitamin D, 20,000. These were premixed with lactose.

### General procedure

After 14 days of feeding experimental diets, all chicks were weighted individually. Thereafter, blood samples were taken from the wing vein of all chicks. At necropsy, the liver, abdominal fat and thigh muscle were quickly removed. These samples including serum were stored at -20°C until analysis of contents and fatty acid composition of various lipid fractions. The various lipid fractions were separated by thin layer chromatography on silica gel chromatorod using hexane: diethyl ether: formic acid (85:15:0.14, v/v) as developing solvents, and quantitated by IATRO SCAN (TH-10 TLC/FID analyzer, Iatron Ltd.) with hydrogen as gas flow (Vandamme et al., 1978).

The total lipids of the liver, serum, abdominal fat and thigh muscle were extracted by Folch et al. (1957) and then were separated to various lipid fractions by thin layer chromatography on previously activated silica gel plates

using hexane: diethyl ether: acetic acid (70:30:1, v/v) as developing solvents. Each lipid fraction was methylated using sodium methylate as the agent. The fatty acid compositions of triglyceride, cholesterol ester and phospholipid fractions were measured by gas-liquid chromatography (GL-14A type, Shimadzu, Ltd.) using 0.25  $\phi$  × 30 m capillary column (FFS ULBON HR-SS-10, Shinwa, Ltd.). The initial column temperature was set at 150°C and increased to 220°C at 4°C/min. The injector and detector were set at 240°C. Helium was used as the carrier gas. The commercial fatty acid methyl esters were used for identification of each fatty acid. The peaks were presumed by comparison between carbon atom numbers and retention time as there was no standard material.

### Statistical method

All the data were statistically analyzed using the one-way layout design of the analysis of variance. Significant

differences among treatments were determined using Duncan's multiple range test (Duncan, 1952).

### Results

Table 2 shows initial and final body weight, body weight gain, liver weight and feed intake of chicks fed experimental diets. Feed intake of chicks fed the vitamin

B<sub>6</sub>-free diet was markedly depressed. Therefore, the final body weight and weight gain of vitamin B<sub>6</sub>-free diet group were significantly lower as compared with those of vitamin B<sub>6</sub> supplemented groups. There was not different from feed intake and weight gain among vitamin B<sub>6</sub> supplemented groups. The liver weight (g/100 g body weight) was significantly lower in chicks fed the vitamin B<sub>6</sub>-free diet than in those fed diets with vitamin B<sub>6</sub>.

TABLE 2. EFFECTS OF DIETARY VITAMIN B<sub>6</sub> LEVELS ON INITIAL AND FINAL BODY WEIGHT, BODY WEIGHT GAIN, LIVER WEIGHT/100 G BODY WEIGHT AND FEED INTAKE

	Vitamin B <sub>6</sub> level (mg/kg diet)				
	0	4	8	40	80
Initial body weight (g)	338.57 ± 9.45	337.86 ± 6.99	338.57 ± 13.14	337.86 ± 5.67	337.86 ± 8.09
Final body weight (g)	412.86 ± 21.77 <sup>1)</sup>	589.29 ± 20.50 <sup>b</sup>	572.14 ± 24.81 <sup>b</sup>	590.00 ± 42.52 <sup>b</sup>	581.43 ± 18.19 <sup>b</sup>
Body weight gain (g)	74.29 ± 16.69 <sup>a</sup>	250.00 ± 18.17 <sup>b</sup>	233.57 ± 29.68 <sup>b</sup>	252.14 ± 38.82 <sup>b</sup>	242.86 ± 22.15 <sup>b</sup>
Liver weight (g/100 BW)	2.19 ± 0.50	2.28 ± 0.21	2.35 ± 0.17	2.31 ± 0.18	2.39 ± 0.12
Feed intake (g/bird)	390.2	585.8	570.3	569.2	575.4

<sup>1)</sup> Means ± SD. Values with different superscripts differ significantly ( $p < 0.05$ ).

TABLE 3. EFFECTS OF DIETARY VITAMIN B<sub>6</sub> LEVELS ON THE CONCENTRATIONS OF VARIOUS LIPID FRACTIONS IN SERUM AND LIVER

	Vitamin B <sub>6</sub> level (mg/kg diet)				
	0	4	8	40	80
Serum (mg/100ml)					
Cholesterol ester	263.51 ± 29.27 <sup>b1)</sup>	186.11 ± 9.84 <sup>a</sup>	188.34 ± 11.19 <sup>a</sup>	186.20 ± 11.24 <sup>a</sup>	192.31 ± 11.53 <sup>a</sup>
Triglyceride	14.57 ± 4.26 <sup>a</sup>	24.00 ± 4.42 <sup>b</sup>	39.80 ± 8.64 <sup>c</sup>	37.67 ± 5.54 <sup>c</sup>	39.99 ± 2.75 <sup>c</sup>
Free cholesterol	37.77 ± 3.41 <sup>b</sup>	30.50 ± 1.46 <sup>a</sup>	31.16 ± 2.26 <sup>a</sup>	30.61 ± 0.83 <sup>a</sup>	32.45 ± 2.39 <sup>a</sup>
Phospholipid	301.94 ± 22.80 <sup>a</sup>	312.52 ± 29.75 <sup>ab</sup>	312.13 ± 16.71 <sup>ab</sup>	334.31 ± 26.80 <sup>ab</sup>	343.64 ± 14.08 <sup>b</sup>
Liver (mg/g)					
Triglyceride	1.80 ± 0.49 <sup>1)</sup>	3.31 ± 0.94 <sup>ab</sup>	3.88 ± 2.27 <sup>b</sup>	3.96 ± 0.62 <sup>b</sup>	3.69 ± 0.94 <sup>ab</sup>
Free cholesterol	4.42 ± 0.20	4.27 ± 0.25	4.17 ± 0.14	4.17 ± 0.23	4.14 ± 0.19
Phospholipid	66.78 ± 4.01	62.39 ± 2.62	61.41 ± 3.85	63.76 ± 6.86	60.05 ± 5.85

<sup>1)</sup> Means ± SD. Values with different superscripts differ significantly ( $p < 0.05$ ).

Contents of various lipid fractions in serum and liver of chicks fed experimental diets are shown in table 3. The serum triglyceride concentration of chicks fed the vitamin B<sub>6</sub>-free diet was significantly lower as compared with that of vitamin B<sub>6</sub> supplemented groups, and up to 8 mg of vitamin B<sub>6</sub>/kg diet fed chicks showed significantly higher value than of 4 mg fed chicks. In contrary, the esterified and free cholesterol concentrations in serum were significantly higher in chicks fed the vitamin B<sub>6</sub>-free diet than in those fed vitamin B<sub>6</sub> supplemented diets. The serum phospholipid concentration tended to elevate with

increasing of vitamin B<sub>6</sub> supplemented levels. The liver triglyceride content was significantly lower in chicks fed vitamin B<sub>6</sub>-free diet compared to those fed vitamin B<sub>6</sub> supplemented diets. The liver free cholesterol and phospholipid contents appeared higher values in chicks fed vitamin B<sub>6</sub>-free diet than in those fed vitamin B<sub>6</sub> supplemented diets. There was no difference from various lipid contents in liver among groups fed vitamin B<sub>6</sub>.

The fatty acid composition of various tissue lipid fractions are shown in table 4 to 6. In chicks fed the vitamin B<sub>6</sub>-free diet, some important differences were

observed the fatty acid composition in various tissue lipid fractions. These were the significant decrease of C<sub>18:3n6</sub>, C<sub>20:3n6</sub> and C<sub>20:4n6</sub> and increase of C<sub>18:2n6</sub>. The cholesterol ester fraction in liver of chicks fed the vitamin B<sub>6</sub>-free diet contained less C<sub>18:0</sub>, C<sub>18:3n6</sub>, C<sub>20:3n6</sub> and C<sub>20:4n6</sub>, but more C<sub>18:1n9</sub> and C<sub>8:2n6</sub> compared with those of vitamin B<sub>6</sub> fed groups. The rates of C<sub>18:0</sub>, C<sub>18:3n6</sub> and C<sub>20:3n6</sub> in liver triglyceride fraction of chicks fed the vitamin B<sub>6</sub>-free diet were lower values compared with those fed vitamin B<sub>6</sub> supplemented diets, whereas the rates of C<sub>18:1n9</sub> and C<sub>18:2n6</sub> showed higher values. Chicks fed vitamin B<sub>6</sub>-free diet had less C<sub>18:0</sub>, C<sub>20:3n6</sub> and C<sub>20:4n6</sub> in the liver phospholipid fraction compared to vitamin B<sub>6</sub> fed groups. The C<sub>18:2n6</sub>

fatty acid accounts for above 50% of total fatty acid in serum cholesterol ester fraction. Chicks fed the vitamin B<sub>6</sub>-free diet had more C<sub>18:2n6</sub>, but less C<sub>18:3n6</sub>, C<sub>20:3n6</sub> and C<sub>20:4n6</sub> in serum cholesterol ester fraction as compared with other groups. In serum triglyceride fraction, rates of C<sub>18:1n9</sub>, C<sub>18:3n6</sub> and C<sub>20:3n6</sub> were increased in the vitamin B<sub>6</sub>-free diet group, but C<sub>18:0</sub> and C<sub>20:4n6</sub> were decreased. But there was no significant difference from fatty acid compositions with increasing dietary vitamin B<sub>6</sub> level. The fatty acid compositions in adipose tissue, but not in thigh muscle, were appeared almost the same pattern as those in each lipid fraction in liver and serum.

TABLE 4. EFFECTS OF DIETARY VITAMIN B<sub>6</sub> LEVELS ON THE FATTY ACID COMPOSITION OF VARIOUS LIPID FRACTIONS IN LIVER

Fatty acid <sup>1)</sup>	Vitamin B <sub>6</sub> level (mg/kg diet)				
	0	4	8	40	80
..... (%) <sup>2)</sup> .....					
Cholesterol ester					
18:0	14.65 ± 2.53 <sup>ab3)</sup>	19.56 ± 1.03 <sup>c</sup>	17.53 ± 0.78 <sup>b</sup>	16.47 ± 0.76 <sup>ab</sup>	20.13 ± 1.10 <sup>c</sup>
18:1 (n-9)	26.02 ± 2.01 <sup>b</sup>	18.14 ± 1.13 <sup>a</sup>	20.25 ± 2.61 <sup>a</sup>	20.32 ± 0.54 <sup>a</sup>	18.96 ± 1.27 <sup>a</sup>
18:2 (n-6)	27.39 ± 1.67 <sup>b</sup>	21.79 ± 1.16 <sup>a</sup>	23.01 ± 1.97 <sup>a</sup>	24.15 ± 2.79 <sup>a</sup>	21.95 ± 1.39 <sup>a</sup>
18:3 (n-6)	0.11 ± 0.03 <sup>a</sup>	0.22 ± 0.03 <sup>b</sup>	0.22 ± 0.03 <sup>b</sup>	0.21 ± 0.03 <sup>b</sup>	0.20 ± 0.03 <sup>b</sup>
18:3 (n-3)	0.12 ± 0.04	Trace	Trace	0.10 ± 0.04	Trace
20:3 (n-6)	0.97 ± 0.18 <sup>a</sup>	1.57 ± 0.36 <sup>b</sup>	1.45 ± 0.09 <sup>b</sup>	1.59 ± 0.12 <sup>b</sup>	1.59 ± 0.18 <sup>b</sup>
20:4 (n-6)	2.10 ± 0.28 <sup>a</sup>	3.16 ± 0.22 <sup>b</sup>	2.92 ± 0.29 <sup>b</sup>	3.18 ± 0.40 <sup>b</sup>	2.88 ± 0.32 <sup>b</sup>
Triglyceride					
18:0	12.99 ± 1.56 <sup>a</sup>	17.92 ± 4.17 <sup>b</sup>	17.90 ± 3.68 <sup>b</sup>	19.39 ± 2.23 <sup>b</sup>	20.22 ± 3.41 <sup>b</sup>
18:1 (n-9)	25.43 ± 0.99 <sup>b</sup>	20.06 ± 2.97 <sup>a</sup>	20.78 ± 2.87 <sup>a</sup>	19.56 ± 3.33 <sup>a</sup>	18.62 ± 3.67 <sup>a</sup>
18:2 (n-6)	31.85 ± 1.56 <sup>b</sup>	24.07 ± 5.14 <sup>a</sup>	25.21 ± 2.45 <sup>a</sup>	23.86 ± 1.20 <sup>a</sup>	22.90 ± 6.49 <sup>a</sup>
18:3 (n-6)	0.11 ± 0.01 <sup>a</sup>	0.25 ± 0.04 <sup>b</sup>	0.22 ± 0.04 <sup>b</sup>	0.26 ± 0.06 <sup>b</sup>	0.23 ± 0.03 <sup>b</sup>
18:3 (n-3)	0.13 ± 0.02 <sup>b</sup>	0.06 ± 0.03 <sup>a</sup>	0.07 ± 0.02 <sup>a</sup>	0.07 ± 0.01 <sup>a</sup>	0.06 ± 0.02 <sup>a</sup>
20:3 (n-6)	0.50 ± 0.07 <sup>a</sup>	0.66 ± 0.10 <sup>bc</sup>	0.60 ± 0.09 <sup>ab</sup>	0.60 ± 0.12 <sup>ab</sup>	0.77 ± 0.11 <sup>c</sup>
20:4 (n-6)	2.75 ± 1.16	2.68 ± 0.67	2.38 ± 0.58	2.49 ± 0.42	2.51 ± 0.86
Phospholipid					
18:0	20.69 ± 1.94 <sup>a</sup>	27.13 ± 8.75 <sup>b</sup>	29.02 ± 2.90 <sup>b</sup>	25.12 ± 4.45 <sup>b</sup>	30.71 ± 6.38 <sup>b</sup>
18:1 (n-9)	13.09 ± 3.92 <sup>b</sup>	11.28 ± 6.23 <sup>b</sup>	5.28 ± 1.59 <sup>a</sup>	9.08 ± 1.76 <sup>ab</sup>	8.95 ± 2.91 <sup>ab</sup>
18:2 (n-6)	21.44 ± 4.51	20.15 ± 4.32	17.34 ± 3.85	18.46 ± 1.93	19.52 ± 1.58
20:3 (n-6)	Trace	1.77 ± 0.44 <sup>b</sup>	1.75 ± 0.24 <sup>b</sup>	1.33 ± 0.28 <sup>a</sup>	1.23 ± 0.03 <sup>a</sup>
20:4 (n-6)	5.62 ± 2.09 <sup>a</sup>	13.02 ± 6.48 <sup>b</sup>	12.61 ± 4.32 <sup>b</sup>	12.07 ± 0.93 <sup>b</sup>	16.06 ± 4.18 <sup>b</sup>

<sup>1)</sup> Number of carbon atoms : number and (position) of double bonds.

<sup>2)</sup> Values expressed as % of total fatty acids.

<sup>3)</sup> Means ± SD. Values with different superscripts differ significantly (p < 0.05).

## Discussion

At 3 days of experimental period, chicks fed the

vitamin B<sub>6</sub>-free diet became excitable and pecked out one another. After 7 days of deprivation of vitamin B<sub>6</sub>, chicks were poor plumage and squatted frequently. As deprivation

TABLE 5. EFFECTS OF DIETARY VITAMIN B<sub>6</sub> LEVELS ON THE FATTY ACID COMPOSITION OF VARIOUS LIPID FRACTIONS IN SERUM

Fatty acid <sup>1)</sup>	Vitamin B <sub>6</sub> level (mg/kg diet)				
	0	4	8	40	80
..... (%) <sup>2)</sup> .....					
Cholesterol ester					
18:0	3.45 ± 0.23 <sup>3)</sup>	4.95 ± 0.07 <sup>d</sup>	4.40 ± 0.25 <sup>b</sup>	4.62 ± 0.27 <sup>bc</sup>	4.78 ± 0.15 <sup>cd</sup>
18:1 (n-9)	9.10 ± 0.41	8.70 ± 0.90	7.89 ± 0.69	8.08 ± 1.00	8.19 ± 1.71
18:2 (n-6)	63.85 ± 1.63 <sup>c</sup>	55.01 ± 2.22 <sup>a</sup>	59.57 ± 1.07 <sup>b</sup>	58.92 ± 0.88 <sup>b</sup>	59.00 ± 2.50 <sup>b</sup>
18:3 (n-6)	0.33 ± 0.02 <sup>a</sup>	0.80 ± 0.20 <sup>c</sup>	0.64 ± 0.04 <sup>b</sup>	0.67 ± 0.07 <sup>bc</sup>	0.60 ± 0.03 <sup>b</sup>
18:3 (n-3)	Trace	Trace	0.08 ± 0.00	0.09 ± 0.01	0.08 ± 0.01
20:3 (n-6)	0.87 ± 0.16 <sup>a</sup>	1.10 ± 0.07 <sup>b</sup>	1.14 ± 0.10 <sup>b</sup>	1.09 ± 0.16 <sup>b</sup>	1.03 ± 0.17 <sup>ab</sup>
20:4 (n-6)	5.27 ± 0.47 <sup>a</sup>	7.79 ± 1.15 <sup>b</sup>	8.18 ± 0.51 <sup>b</sup>	7.91 ± 0.76 <sup>b</sup>	8.32 ± 0.66 <sup>b</sup>
Triglyceride					
18:0	9.84 ± 1.07 <sup>a</sup>	12.42 ± 1.09 <sup>b</sup>	12.30 ± 0.72 <sup>b</sup>	14.15 ± 0.52 <sup>b</sup>	12.63 ± 2.29 <sup>b</sup>
18:1 (n-9)	25.26 ± 2.57 <sup>c</sup>	19.86 ± 0.72 <sup>b</sup>	19.37 ± 2.60 <sup>ab</sup>	16.41 ± 1.80 <sup>a</sup>	17.04 ± 1.97 <sup>ab</sup>
18:2 (n-6)	32.43 ± 2.80	32.61 ± 3.66	33.62 ± 4.71	31.64 ± 2.90	36.36 ± 3.44
18:3 (n-6)	0.87 ± 0.73 <sup>b</sup>	0.47 ± 0.16 <sup>ab</sup>	0.16 ± 0.03 <sup>a</sup>	0.17 ± 0.02 <sup>a</sup>	0.15 ± 0.01 <sup>a</sup>
18:3 (n-3)	Trace	Trace	0.12 ± 0.02 <sup>ac</sup>	0.10 ± 0.02 <sup>a</sup>	0.13 ± 0.02 <sup>bc</sup>
20:3 (n-6)	0.90 ± 0.46 <sup>b</sup>	0.34 ± 0.05 <sup>a</sup>	0.34 ± 0.04 <sup>a</sup>	0.35 ± 0.04 <sup>a</sup>	0.37 ± 0.04 <sup>a</sup>
20:4 (n-6)	1.88 ± 1.23	2.70 ± 0.69	2.47 ± 0.85	2.41 ± 0.35	2.43 ± 0.56
Phosphlipid					
18:0	9.04 ± 0.70 <sup>a</sup>	10.65 ± 0.97 <sup>ab</sup>	13.95 ± 3.53 <sup>bc</sup>	17.77 ± 3.84 <sup>c</sup>	16.98 ± 2.49 <sup>c</sup>
18:1 (n-9)	28.40 ± 3.36 <sup>b</sup>	33.40 ± 2.83 <sup>c</sup>	14.06 ± 1.76 <sup>a</sup>	13.70 ± 2.66 <sup>a</sup>	14.11 ± 2.31 <sup>a</sup>
18:2 (n-6)	18.40 ± 7.68	20.68 ± 5.83	27.80 ± 7.84	27.58 ± 5.29	27.64 ± 5.29
20:3 (n-6)	Trace	Trace	0.60 ± 0.15 <sup>a</sup>	Trace	1.12 ± 0.36 <sup>b</sup>
20:4 (n-6)	Trace	Trace	6.92 ± 1.45	9.81 ± 2.91	7.64 ± 3.35

<sup>1)</sup> Number of carbon atoms : number and (position) of double bonds.

<sup>2)</sup> Values expressed as % of total fatty acid.

<sup>3)</sup> Means ± SD Values with different superscripts differ significantly ( $p < 0.05$ ).

continues, chicks had little appetite and nervous disorder became severely. The depilation and inflammation were observed around the eyes and beaks. These were the characteristic signs of vitamin B<sub>6</sub> deficiency that has been observed in chicks (Gries and Scott, 1972; McDowell, 1989). Chicks fed the vitamin B<sub>6</sub>-free diet caused marked decreases in feed intake and body weight gain. The reduction of feed intake and growth retardation resulting from vitamin B<sub>6</sub> deficiency had been also reported in other experiments using chicks and rats (Daghir and Balloun, 1962; Cunnane et al., 1985). There was no difference in weight gain among dietary vitamin B<sub>6</sub> levels.

A decreased liver triglyceride content of chicks fed the vitamin B<sub>6</sub>-free diet was conflicting with the result of the experiment using rats (Gomikawa and Okada, 1978; Abe and Kishino, 1982). Most probably a decline of hepatic fatty acid synthesis resulting from severe reduction of feed intake in vitamin B<sub>6</sub> deficient chicks might contribute to

this decrease of the triglyceride content, as suggested previously (Yeh and Leveille, 1970; Tanaka et al., 1975).

The concentrations of free and esterified cholesterol in serum were significantly higher in chicks fed the vitamin B<sub>6</sub>-free diet. This result agreed with Dam et al. (1958) and Daghir and Balloun (1962) who have suggested that serum cholesterol was elevated in vitamin B<sub>6</sub> deficient chicks. Lupien et al. (1969) have observed that the rate of incorporation of acetate - <sup>14</sup>C into liver cholesterol in pyridoxine deficient rats increased rapidly.

Chicks fed the vitamin B<sub>6</sub>-free diet appeared the increase in C<sub>18:2n6</sub> in each lipid fraction of liver and serum, while the rate of C<sub>20:4n6</sub> was decreased. These results are in agreement with those of Witten and Holman (1952) and Sato (1970) who have suggested that the accumulation of C<sub>18:2n6</sub> in vitamin B<sub>6</sub> deficiency may be caused by decrease in C<sub>18:2n6</sub> metabolism to C<sub>20:4n6</sub>. Furthermore, Hill et al. (1982) have shown that the ratio of C<sub>18:2n6</sub>/C<sub>20:4n6</sub> is

TABLE 6. EFFECTS OF DIETARY VITAMIN B<sub>6</sub> LEVELS ON THE FATTY ACID COMPOSITION IN THIGH MUSCLE AND ADIPOSE TISSUE

Fatty acid <sup>1)</sup>	Vitamin B <sub>6</sub> level (mg/kg diet)				
	0	4	8	40	80
..... (%) <sup>2)</sup> .....					
Thigh muscle					
18:0	11.00 ± 1.06 <sup>b3)</sup>	7.66 ± 0.60 <sup>a</sup>	7.49 ± 1.07 <sup>a</sup>	8.36 ± 0.98 <sup>a</sup>	7.63 ± 0.89 <sup>a</sup>
18:1 (n-9)	31.27 ± 1.86	27.79 ± 1.97	30.28 ± 2.57	28.26 ± 2.26	29.13 ± 3.69
18:2 (n-6)	32.33 ± 1.73 <sup>a</sup>	37.61 ± 1.49 <sup>b</sup>	36.74 ± 1.40 <sup>b</sup>	37.57 ± 1.08 <sup>b</sup>	36.94 ± 1.42 <sup>b</sup>
18:3 (n-6)	0.13 ± 0.02 <sup>a</sup>	0.17 ± 0.03 <sup>b</sup>	0.19 ± 0.00 <sup>b</sup>	0.18 ± 0.03 <sup>b</sup>	0.19 ± 0.02 <sup>b</sup>
18:3 (n-3)	0.21 ± 0.03 <sup>a</sup>	0.24 ± 0.04 <sup>ab</sup>	0.27 ± 0.04 <sup>b</sup>	0.25 ± 0.02 <sup>ab</sup>	0.26 ± 0.01 <sup>b</sup>
20:3 (n-6)	0.20 ± 0.03 <sup>ab</sup>	0.20 ± 0.04 <sup>ab</sup>	0.14 ± 0.02 <sup>a</sup>	0.23 ± 0.09 <sup>b</sup>	0.19 ± 0.06 <sup>ab</sup>
20:4 (n-6)	1.27 ± 0.43 <sup>ab</sup>	1.72 ± 0.45 <sup>ab</sup>	0.97 ± 0.39 <sup>a</sup>	2.29 ± 1.31 <sup>b</sup>	1.62 ± 0.82 <sup>ab</sup>
Adipose tissue					
18:0	8.95 ± 0.91 <sup>b</sup>	6.69 ± 0.92 <sup>a</sup>	7.67 ± 0.46 <sup>ab</sup>	7.69 ± 0.47 <sup>ab</sup>	7.65 ± 1.62 <sup>ab</sup>
18:1 (n-9)	36.33 ± 2.21 <sup>b</sup>	28.45 ± 1.70 <sup>a</sup>	29.34 ± 1.42 <sup>a</sup>	26.94 ± 0.66 <sup>a</sup>	27.32 ± 3.84 <sup>a</sup>
18:2 (n-6)	33.30 ± 1.95 <sup>a</sup>	42.21 ± 1.61 <sup>c</sup>	38.79 ± 2.22 <sup>b</sup>	44.19 ± 0.88 <sup>c</sup>	42.22 ± 2.86 <sup>c</sup>
18:3 (n-6)	0.14 ± 0.03 <sup>a</sup>	0.18 ± 0.03 <sup>b</sup>	0.18 ± 0.00 <sup>b</sup>	0.20 ± 0.02 <sup>b</sup>	0.19 ± 0.00 <sup>b</sup>
18:3 (n-3)	0.23 ± 0.01 <sup>a</sup>	0.24 ± 0.01 <sup>a</sup>	0.28 ± 0.02 <sup>bd</sup>	0.26 ± 0.01 <sup>bc</sup>	0.26 ± 0.01 <sup>b</sup>
20:3 (n-6)	0.06 ± 0.01 <sup>a</sup>	0.10 ± 0.02 <sup>b</sup>	0.09 ± 0.01 <sup>b</sup>	0.09 ± 0.02 <sup>b</sup>	0.09 ± 0.01 <sup>b</sup>
20:4 (n-6)	0.14 ± 0.01 <sup>a</sup>	0.13 ± 0.02 <sup>a</sup>	0.13 ± 0.02 <sup>a</sup>	0.16 ± 0.03 <sup>ab</sup>	0.17 ± 0.03 <sup>b</sup>

<sup>1)</sup> Number of carbon atoms : number and (position) of double bonds.

<sup>2)</sup> Values expressed as % of total fatty acid.

<sup>3)</sup> Means ± SD. Values with different superscripts differ significantly ( $p < 0.05$ ).

usually a valid indicator of C<sub>18:2n6</sub> desaturation. Since, in the present experiment, C<sub>18:2n6</sub> was higher in tissues of chicks fed the vitamin B<sub>6</sub>-free diet, it appeared that C<sub>18:2n6</sub> desaturation was influenced by vitamin B<sub>6</sub>. Whereas, it has been reported that vitamin B<sub>6</sub> deficiency does not affect C<sub>18:2n6</sub> metabolism, but rather may influence the rate of C<sub>18:2n6</sub> and C<sub>20:4n6</sub> oxidation (Goswami and Coniglio, 1966). The mechanisms were suggested as responsibility for decreasing of C<sub>20:4n6</sub> in tissue lipid in vitamin B<sub>6</sub> deficiency may due to acceleration of C<sub>20:4n6</sub> oxidation, if not, to inhibition of desaturative and elongative reaction from C<sub>18:2n6</sub>. In present experiment, the decrease of C<sub>18:3n6</sub>, C<sub>20:3n6</sub> and C<sub>20:4n6</sub> in vitamin B<sub>6</sub> deficiency chicks coupled to an increase of C<sub>18:2n6</sub> could possibly be caused by inhibiting of conversion from C<sub>18:2n6</sub> to C<sub>20:4n6</sub>, not be due to acceleration of C<sub>20:4n6</sub> oxidation. But the addition of high levels of vitamin B<sub>6</sub> (up to 8 mg/kg diet) did not significantly affected fatty acid composition in various tissue lipid fractions.

Cunnane et al. (1984) have showed that the desaturation of C<sub>18:2n6</sub> and elongation of C<sub>18:3n6</sub> were depressed in vitamin B<sub>6</sub> deficiency rat, but there was no evidence on depression of n3 fatty acid metabolism. Since safflower oil used in this study contains little C<sub>18:3n3</sub> and in-

corporation rate of C<sub>18:3n3</sub> into tissue lipid was less than 1%, it is obscure whether vitamin B<sub>6</sub> plays a role in C<sub>18:3n3</sub> metabolism or not. But it is generally accepted that desaturation and elongation steps of n3 and n6 essential fatty acids share with a same enzyme system (Kinsella et al., 1990). Further studies are required to clarify the role of vitamin B<sub>6</sub> on metabolism of n-3 metabolite.

#### Literature Cited

- Abe, M. and Y. Kishino. 1982. Pathogenesis of fatty liver in rats fed a high protein diet without pyridoxine. *J. Nutr.* 112:205-210.
- Birch, T. W. and P. Gyorgy. 1936. A study of the chemical nature of vitamin B<sub>6</sub> and methods for its preparation in a concentrated state. *Biochem. J.* 30:304-315.
- Cunnane, S. C., M. S. Manku and D. F. Horrobin. 1984. Accumulation of linoleic and gamma-linolenic acids in tissue lipid of pyridoxine-deficient rats. *J. Nutrition* 114:1754-1761.
- Cunnane, S. C., M. S. Manku and D. F. Horrobin. 1985. Effect of vitamin B-6 deficiency on essential fatty acid metabolism. *Vitamin B-6: Its role in health and*

- disease, page 447-451. Alan R. Liss, Inc.
- Daghir, N. J. and S. L. Balloun. 1962. Vitamin B<sub>6</sub> and cholesterol metabolism in the chick. *Poultry Sci.* 41:1868-1879.
- Dam, H., G. Kristensen, G. K. Nielsen and E. Sondergaard. 1958. Effect of pyridoxine deficiency on cholesterol and polyenoic fatty acids in chicks. *Acta. Physiol. Scand.* 44:67-79.
- Delorme, C. B. and P. J. Lupien. 1976. The effect of vitamin B-6 deficiency on the fatty acid composition of the major phospholipids in the rat. *J. Nutr.* 106:169-180.
- Duncan, D. B. 1952. Multiple range and multiple F test. *Biometric.* 11:1-42.
- Dussault, P. E. and M. Lepage. 1975. Effects of pyridoxine deficiency on the composition of plasma and liver fatty acids in rats fed low and high fat diets. *J. Nutr.* 105:1371-1376.
- Folch, J., M. Lees and G. H. Sloane-Stanley. 1957. A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226:497-509.
- Gomikawa, S. and M. Okada. 1978. Metabolism of fatty acids and the levels of ketone bodies in the livers of pyridoxine-deficient rats. *J. Nutr. Sci. Vitaminol.* 24:25-34.
- Goswami, A. and J. G. Coniglio. 1966. Effect of pyridoxine deficiency on the metabolism of linoleic acid in the rat. *J. Nutr.* 89:210-216.
- Gries, C. L. and M. L. Scott. 1972. The pathology of pyridoxine deficiency in chicks. *J. Nutr.* 102:1259-1268.
- Hill, E. G., S. B. Johnson, L. D. Lawson, M. M. Mofouz and R. T. Holman. 1982. Perturbation of the metabolism of essential fatty acids by dietary partially hydrogenated vegetable oil. *Proc. Natl. Acad. Sci. USA* 79:953-957.
- Kinsella, J. E., K. S. Broughton and J. W. Whelan. 1990. Dietary unsaturated fatty acid: interactions and possible needs in relation to eicosanoid synthesis. *J. Nutr. Biochem.* 1:123-141.
- Kirschman, J. C. and J. G. Coniglio. 1961. The role of pyridoxine in the metabolism of polyunsaturated fatty acid in rats. *J. Biol. Chem.* 236:2200-2204.
- Lupien, P. J., C. M. Hinse and M. Avery. 1969. Cholesterol metabolism and vitamin B<sub>6</sub>. 1. Hepatic cholesterogenesis and pyridoxine deficiency. *Can. J. Biochem.* 47:631-635.
- McDowell, L. R. 1989. Vitamins in animal nutrition. pp. 246-253. Academic Press, Inc., San Diego, CA.
- Medes, G., D. C. Keller and A. Kurkjian. 1947. Essential fatty acid metabolism. 1. Essential fatty acid content of rats on fat-free and pyridoxine-free diets. *Arch. Biochem.* 15:19-29.
- Sato, Y. 1970. A possible role of pyridoxine in lipid metabolism. *Nagoya J. Med. Sci.* 33:105-130.
- Schwartzman, G. and L. Strauss. 1949. Vitamin B<sub>6</sub> deficiency in the Syrian hamster. *J. Nutr.* 38:131-154.
- Tanaka, K., T. Sen and K. Shigeno. 1975. The effect of fasting and refeeding on lipids of serum and liver in the meat-type chicken. *Jap. J. Zootech. Sci.* 46:396-402.
- Vandamme, D., V. Blanton and H. Peeters. 1978. Screening of plasma lipids by thin-layer chromatography with flame ionization detection on chromarods. *J. Chromatogr.* 145:151-154.
- Witten, P. W. and R. T. Holman. 1952. Polyenoic fatty acid metabolism. 4. Effect of pyridoxine on essential fatty acid conversions. *Arch. Biochem. Biophys.* 41:266-273.
- Yeh, Y. -Y. and G. A. Leveille. 1970. Hepatic fatty acid synthesis and plasma free fatty acid levels in chickens subjected to short periods of food restriction and refeeding. *J. Nutr.* 100:1389-1398.