Effect of Dietary Protein Level on Alanine Aminotransferase Activity in Liver and Kindney of Growing Chicks

Keiichi TANAKA, Norio TOKITA, Shigeru OHTANI and Kakichi SHIGENO

Faculty of Agriculture, Gifu University, Kakamigahara-shi, Gifu 504

The influence of nutritional or physiological factors on gluconeogenesis has not been extensively investigated in the chicken. In almost all the published studies, interest has been centered on factors affecting the post-embryonic developmental changes in the activities of liver enzymes associated with gluconeogenesis in the chicken¹⁻⁴). On the other hand, compared with mammals, blood glucose level in the chicken is markedly higher and is maintained at a high level (it only drops by 20%) even after prolonged periods of fasting⁵). It is possible that the chicken may have some mechanisms to maintain their blood glucose level, that is, it is probably capable of producing glucose from non-carbohydrate precursors such as gluconeogenic amino acids. In fact, it has been observed that chicks fasted or fed high-protein diet had elevated glucose produced from non-carbohydrates *e. g.* alanine^{6,7}). The enzymes initially involved in the process are the aminotransferases which convert such amino acids into their α -keto acids, *i. e.* pyruvate and oxaloacetate. Similary, KREBS⁸) showed that the sites of regulation of gluconeogenesis exist at the initial steps in the degradation of gluconeogenic amino acids. Most of the studies available on the influences of diets and the mechanism by which enzymatic adaptations occur, have been carried out mainly on mammals. This being the case, we therefore investigated the effects of dietary protein level on activities of alanine amionotransferase in the liver and kidney of growing chicks.

Materials and Methods

Animals and diets: Day-old male White Leghorn chicks obtained from a local hatchry (Goto Poultry Farm, Gifu) were used in this study. They were housed in electrically-heated pens with raised wire floors and fed commercial chick starter *ad libitum* for the first 7 days. At this time, all the chicks were individually wing-banded and assigned to the various treatments with five chicks each. The composition of experimental diets is shown in Table 1. They were formuatted isocalorically by holding the carbohydrate content and at the same time inversely varying the protein and carbohydrate. Feeding was done from the 8th to the 15th day, at which time the birds were killed in the fed state. Feed and water were made available at all times.

Tissue preparation and enzyme assay: At the termination of the experiment, the chicks were weighed and then killed by decapitation. Liver and kidney were rapidly removed, weighed and placed immediately in an ice-cold 0.25 M sucrose solution containing 1 mM EDTA. After blotting dry, a portion of the tissue was weighed and homogenized in 20

Table 1. Composition of experimental diets

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4
Casein mixture ¹⁾	8.9	17.6 ^(%)	17.6	17.6
Casein	—	—	15.8	31.6
Sucrose	76.1	67.3	49.2	31.3
Corn oil	1.8	1.8	1.8	1.8
Cellulose	7.0	7.1	9.4	11.5
Mineral mixture ²⁾	5.0	5.0	5.0	5.0
Vitamin AD mixture ³⁾	0.5	0.5	0.5	0.5
Vitamin B mixture ⁴⁾	0.5	0.5	0.5	0.5
Choline chloride	0.2	0.2	0.2	0.2
Crude protein (%) ⁵⁾	7.9	15.6	29.6	43.5
Metabolizable energy (kcal/100 g) ⁶⁾	326	326	326	326
Percentage of energy (ME base)				
Protein	10	20	40	60
Carbohydrate	85	75	55	35
Fat	5	5	5	5

1) 90.0% casein, 5.4% L-arginine, 1.7% DL-methionine, 2.0% glycine.

2) 14.1% NaCl, 27.1% $K_2HPO_4 \cdot 3H_2O$, 21.8% $CaHPO_4 \cdot 2H_2O$, 25.2% $CaCO_3$, 8.6% $MgSO_4 \cdot 7H_2O$, 2.3% $FeSO_4 \cdot 7H_2O$, 0.666% $MnSO_4 \cdot 4H_2O$, 0.067% KI, 0.021% $ZnSO_4$, 0.025% $CuSO_4 \cdot 5H_2O$.

3) Vitamin A 5000, vitamin D₃ 1000 (IU or ICU/g mixture).

4) Thiamine-HCl 1200, riboflavin 120, niacin 1000, pyridoxine-HCl 80, Ca-pantothenate 400, inositol 4000, folic acid 50, DL- α -tocopherol 200, β -aminobenzoic acid 1000, biotin 6, menadione 50, cyanocobalamin 0.5, lactose 91893.5 (mg/100 g mixture).

5) Calculated value.

6) Calculated value. Casein mixture 3.9, casein 4.1, corn oil and Sucrose 3.7 (kcal/g).

volumes of the same medium. Homogenates were centrifuged at $600 \times g$ for 10 minutes. One aliquot of supernatant was used to measure the activities of total tissue alanine aminotransferase (AAT) and phosphoenolpyruvate carboxykinase (PEPCK), the second aliquot was recentrifuged at $105,000 \times g$ for 60 minutes. Supernatant from this high-speed centrifugation was used to measure the activities of AAT and PEPCK in soluble fraction. The activities of these enzymes in particulate fraction were assumed to represent the difference between the activities of particulate and soluble fraction. All operations were performed at 4°C. Samples for total enzyme activities were rapidly frozen, and stored for no more than one day. They were then thawed and sonicated in ice-cold water immediately before assay. Enzymes activities in soluble fraction was measured the same day the chicks were killed.

The activities of AAT and PEPCK were determined according to previously described methods by BERGMAYER and BERN⁹⁾ and JOMAIN-BAUM *et al.*¹⁰⁾. Assays were carried out at 25°C and enzyme activities were expressed as μ moles NADH oxidized/min/g wet tissue. Protein was determined by the method of LOWRY *et al.*¹¹⁾ using bovine serum albumin as the standard. Enzyme activity was also expressed on the basis of the protein. Since concentrations of protein in the organs changed only minimally in the various treatments, the

date on them are not presented. Serum was separated by centrifugation from the blood collected at killing time. It was used in the determinations of the non-esterified fatty acids (NEFA) and glucose level in serum which were carried out in accordance with the simplified procedures suggested in the mini-manual enclosed with the NEFA-Test Kit and the Glucose C-Test Kit from a commercial chemical manufacture-distributor (Wako Junyaku Kogyo K.K.).

Statistical analysis: TUKEY's procedure⁽²⁾ was used to test treatment mean when significant ($P < 0.05$) treatment F values were found.

Results and Discussion

The influence of dietary protein level on body weight gain, liver and kidney weights is shown in Table 2.

Increasing the level of dietary protein significantly increased body weight gain although there was no significant difference between 10% and 20% energy from protein.

Liver weight, expressed as percentage of body weight, was not affected by dietary protein level. Kidney weight per 100g body weight in chicks fed diet containing 10% energy from protein was significantly lower than in those fed other experimental diets.

The influence of dietary protein level on concentrations of glucose and NEFA in serum is shown in Table 3.

The concentration of NEFA in serum was significantly increased when the percentage of energy from dietary protein was increased from 10% to 20%, however, when the per-

Table 2. Effect of percentage of energy from protein on energy intakes, body weight gain, liver and kidney weights of growing chicks

	Percentage of energy from protein (ME base)				P ¹⁾
	10	20	40	60	
Energy intakes (kcal/chick/day)	48.9	48.2	44.4	45.7	
Body weight gain (g)	62.2 ± 3.6 ^{2)a}	65.5 ± 1.5 ^a	73.2 ± 3.6 ^b	89.0 ± 3.3 ^c	P < 0.01
Liver weight (g/100 g body weight)	2.4 ± 0.3	3.0 ± 0.5	2.8 ± 0.2	2.5 ± 0.1	N.S.
Kidney weight (g/100 g body weight)	0.7 ± 0.1 ^a	1.0 ± 0.1 ^b	1.0 ± 0.1 ^b	1.1 ± 0.1 ^b	P < 0.01

1) Probability of a significant treatment effect. N.S.=Not significant.

2) Mean ± S.D. for five chicks.

a, b, c Mean values in the same horizontal column having different superscript letters are significantly different from one another ($P < 0.01$).

Table 3. Effect of percentage of energy from protein on concentrations of glucose and NEFA in serum of growing chicks

	Percentage of energy from protein (ME base)				P ¹⁾
	10	20	40	60	
Glucose (mg/100 ml)	223.2 ± 6.4 ^{2)a}	243.2 ± 8.8 ^b	215.4 ± 8.8 ^a	141.2 ± 8.8 ^c	P < 0.01
NEFA (μ Eq/l)	364.0 ± 37.3 ^a	441.0 ± 37.2 ^b	296.0 ± 44.9 ^c	309.0 ± 27.3 ^c	P < 0.01

1) Probability of a significant treatment effect.

2) Mean ± S.D. for five chicks.

a, b, c, Mean values in the same horizontal column having different superscript letters are significantly different from one another ($P < 0.05$).

Table 4. Effect of percentage of energy from protein on AAT and PEPCK activities in liver and kidney of growing chicks

	Percentage of energy from protein (ME base)				P ¹⁾
	10	20	40	60	
AAT activity	 μ moles/min/g wet tissue.....				
Liver					
Particulate fraction	0.64 $\pm$ 0.11 ^{2)a}	1.51 $\pm$ 0.23 ^b	2.60 $\pm$ 0.41 ^c	5.16 $\pm$ 0.55 ^d	P < 0.01
Soluble fraction	0.33 $\pm$ 0.03 ^a	0.38 $\pm$ 0.08 ^a	0.52 $\pm$ 0.08 ^a	1.29 $\pm$ 0.28 ^b	P < 0.01
Kidney					
Particulate fraction	3.04 $\pm$ 0.26 ^a	4.72 $\pm$ 0.34 ^b	9.39 $\pm$ 0.37 ^c	19.77 $\pm$ 0.38 ^d	P < 0.01
Soluble fraction	0.57 $\pm$ 0.05 ^a	1.07 $\pm$ 0.15 ^b	1.47 $\pm$ 0.17 ^c	2.19 $\pm$ 0.03 ^d	P < 0.01
PEPCK activity*					
Liver					
Particulate fraction	1.43 $\pm$ 0.61	1.21 $\pm$ 0.44	1.23 $\pm$ 0.22	1.35 $\pm$ 0.31	N. S.
Kidney					
Particulate fraction	1.13 $\pm$ 0.55	1.16 $\pm$ 0.43	1.11 $\pm$ 0.41	1.11 $\pm$ 0.34	N. S.

1) Probability of a significant treatment effect. N. S. = Not significant.

2) Mean $\pm$ S. D. for five chicks.

a, b, c, d, Mean values in the same horizontal column having different superscript letters are significantly different from one another ($p < 0.05$).

* Most of PEPCK activity in liver and kidney of chicks was located in the particulate fraction. Consequently only particulate fraction was shown in the table.

centage of energy from dietary protein was increased from 20% to 40% or 60%, the concentration significantly decreased. Although glucose concentration in serum in chicks fed diet containing 20% energy from protein was significantly higher than in those fed diets containing 10% and 40% energy from protein, increasing it to 60% cause the glucose concentration to decrease significantly.

Table 4 shows the influence of dietary protein level on the AAT and PEPCK activities in liver and kidney of growing chicks.

AAT activity, expressed per g tissue, was higher in kidney than in liver, and was higher in particulate fraction than in soluble fraction. Approximately 85% to 90% of activity in kidney was associated with the particulate fraction and only 10% to 15% with the soluble fraction. Increased protein in the diet resulted in a significant increase in AAT activity in both fractions of kidney, although the magnitude of change in the particulate fraction was greater than that observed in the soluble fraction. In liver, approximately 65% to 80% of activity was associated with the particulate fraction and 20% to 35% with the soluble fraction. As observed in kidney, increased protein in the diet resulted in a significant increase in AAT activity in the particulate fraction of liver. On the other hand, AAT activity in the soluble fraction of liver tended to increase, although not significantly, when the percentage of energy from dietary protein increased from 10% to 40%. However, chicks fed diet containing 60% energy from protein had significantly higher AAT activity in the soluble fraction of liver than those fed diets containing from 10% to 40% energy from protein.

The cellular distribution of AAT presented in this paper markedly differ from those of SARKER'S^{13,14)}, SWICK *et al.*^{15,16)} and DeROSA and SWICK'S¹⁷⁾ experiments with rats. They have demonstrated that in rats, most of AAT activity in liver was located in the cytosol fraction

Figure 1. Regression lines relating AAT activity in liver and kidney of chicks to the percentage of energy from protein in diets. Each point represents the mean for five chicks. Equations for liver particulate and soluble fractions were $Y = 0.087 X - 0.351$ ($r = 0.982$, $p < 0.05$) and $Y = 0.018 X - 0.026$ ($r = 0.833$), respectively. Equations for kidney particulate and soluble fractions were $Y = 0.337 X - 1.494$ ($r = 0.971$, $p < 0.05$) and $Y = 0.031 X + 0.242$ ($r = 0.970$, $p < 0.05$), respectively.

ent experiment, increased protein, at the expense of carbohydrate in the diet, resulted in a linear increase in AAT activity in the particulate (mitochondrial) fraction of liver and kidney, although the enzyme activity in kidney was greater than that observed in liver (Fig. 1). On the other hand, the activity of liver and kidney PEPCK, which is the most important enzyme in the regulation of gluconeogenesis, was not affected by the dietary protein level. Furthermore, in the present experiment, glucose concentration in serum was decreased when chicks fed diet containing 60% energy from protein, although, increasing from 10% to 40% energy from the dietary protein did not decrease glucose level in serum. Therefore, from the results presented in this paper, no definite conclusions can be drawn as to whether a close parallelism between the increased rate of gluconeogenesis and increased activity of AAT in liver and kidney of chicks exists or not.

Summary

The influence of increasing dietary protein level, at the expense carbohydrate, on alanine aminotransferase (AAT) activity in liver and kidney of growing chicks was investigated.

and also, that there was little activity of AAT in kidney. DeROSA and SWICK¹⁷⁾ proposed that the main function of the mitochondrial AAT is to form pyruvate from alanine while the cytosolic enzyme may mainly be involved in the reverse reaction. In the present experiment, AAT and PEPCK necessary for the conversion of alanine to pyruvate, to oxaloacetate and to phosphoenolpyruvate, are mostly present in the particulate (mitochondrial) fraction in liver and kidney of chicks, unlike in liver of rats¹³⁻¹⁵⁾ and guinea-pig¹³⁾. This restricts the conversion of oxaloacetate to phosphoenolpyruvate in the cytosol fraction, although permitting this reaction to take place in the mitochondrial fraction, phosphoenolpyruvate, after being formed in the mitochondrial fraction, can be transported to the cytosol fraction and be utilized for glucose synthesis. It is assumed, in fact, that phosphoenolpyruvate can also pass through the mitochondrial membrane¹⁸⁾. The reaction catalyzed by the AAT is the initial step in the reaction sequences in gluconeogenesis for alanine^{8,19)}, and AAT plays an important role in the regulation of gluconeogenesis from alanine. In the present

Approximately 85% to 90% of AAT activity in kidney was associated with the particulate fraction and only 10% to 15% with the soluble fraction. In liver, approximately 65% to 80% of AAT activity was associated with the particulate fraction and 20% to 35% with the soluble fraction.

Increasing dietary protein, at the expense of carbohydrate, resulted in a linear increase in AAT activity in the particulate fraction of liver and kidney, although the magnitude of change in kidney was greater than that observed in liver. AAT activity in the soluble fraction of kidney was significantly increased when the dietary protein level increased. On the other hand, AAT activity in the soluble fraction of liver tended to increase, although insignificantly, when the percentage of energy from dietary protein increased from 10% to 40%. However, chicks fed diet containing 60% energy from protein had significantly higher AAT activity in the soluble fraction of liver than those fed diets containing from 10% to 40% energy from protein.

Activity of phosphoenolpyruvate carboxykinase in the particulate fraction of liver and kidney was not affected by the dietary protein level.

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飼料中蛋白質含量が成長中ヒナの肝臓および腎臓におけるアラニン・アミノトランスフェラーゼ活性におよぼす影響について

田中桂一・時田昇臣・大谷 滋・重野嘉吉

岐阜大学農学部・各務原市 〒504

飼料中の蛋白質含量が成長中のヒナの肝臓および腎臓における alanine aminotransferase (AAT) 活性を検討した。

腎臓における AAT 活性の 85%~90% は particulate 画分中に、残りの 10%~15% が soluble 画分中に分布していた。肝臓においては、AAT 活性の 65%~80% は particulate 画分中に、そして 20~35% は particulate 画分中に分布している。

飼料中の蛋白質含量が増加すると肝臓および腎臓の particulate 画分中の AAT 活性は直線的に増加し、そしてその増加の程度は肝臓に比較し腎臓の方が大きかった。腎臓での soluble 画分中の AAT 活性は、飼料中

の蛋白質含量が増加するのに伴い、統計的に有意な増加を示した。一方肝臓での soluble 画分中の AAT 活性は、飼料中の全エネルギーのうち蛋白質の占める割合が 10% から 40% までの増加では、統計的に有意なものではなかったが増加する傾向にあった。しかし飼料中の全エネルギーのうち蛋白質の占める割合が 60% に増加した時の活性は、飼料中の蛋白質によるエネルギーの割合が 10% から 40% の時の活性に比べ、統計的に有意に高い値を示した。

肝臓および腎臓の particulate 画分中の phosphoenolpyruvate carboxykinase 活性は飼料中の蛋白質含量による影響はみられなかった。

(家禽会誌, 18, 164~170, 1981)