# EFFECTS OF DIETARY PROTEINS ON THE ACTIVITIES OF LIPOGENIC ENZYMES IN THE LIVER OF GROWING CHICKS

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## Summary

In Experiment 1, when fasted chicks were fed diets containing various sources of protein for 3 days, the activities of lipogenic enzymes (acetyl-CoA carboxylase, fatty acid synthetase, citrate cleavage enzyme and malic enzyme) in the liver of growing chicks were significantly lower in the soybean protein or gluten diet than in the casein or fish protein diet. Triglyceride contents of the liver and plasma of chicks fed the casein or fish protein diet were significantly lower than that of those fed soybean protein or gluten diet. In Experiment 2, the effects of dietary amino acid mixture simulating casein or protein on the activities of hepatic lipogenic enzyms were examined. The activities of acetyl-CoA carboxylase and fatty acid synthetase in the liver of chicks fed the casein diet were significantly higher than that of those fed the soybean protein diet or two diets of amino acid mixtures. Furthermore, there were no significant differences between the two diets of amino acid mixture based on casein or soybean protein. However, the activities of malic enzyme and citrate cleavage enzyme tended to be lower in the soybean-type amino acid diet than in the casein-type amino acid diet. Thus, some effects can be ascribed to the protein itself and some to the amino acid composition of the protein sources.

(**Key Words**: Growing Chick, Dietary Protein, Lipogenic Enzymes, Triglyceride)

## Introduction

The role of dietary composition in the regulation of fatty acid synthesis have been the subject of numerous investigations. Both the composition and level of carbohydrate, fat and protein have been demonstrated to alter the rate of fatty acid synthesis and the activities of the associated enzymes (Romsos and Leveille, 1970). On the other hand, the role of dietary protein in the regulation of hepatic fatty acid synthesis has been studied much less than either dietary fat or carbohydrate. Yeh and Leveille (1969) and Tanaka et al. (1979, 1983) reported that the hepatic lipogenesis and the activities of the associated enzymes in chickens were decreased by increased dietary protein.

Plant protein, in particular soybean protein, generally has shown hypocholesterolemic effects when compared with animal protein such as casein (Nagata et al., 1981a,b). Some of the publications also reported serum and liver triglyceride levels in addition to cholesterol levels, however, the effects of dietary plant protein on the triglyceride levels were inconsistent (Cohn et al., 1984; Goldberg et al., 1982; Lefevre and Schneeman 1984; Iritani et al., 1985). Furthermore, most of these publications were being carried out on rats. Therefore, in the present study, we have investigated the effects of the source of dietary protein on the activities of lipogenic enzymes in the liver of growing chicks.

## Materials and Methods

### Animals and Diets

Day-old male White Leghorn chicks obtained from a local hatchery were used in this study. They were raised on wire floors. Feed and water were made available at all times. In Experiment 1, at five weeks of age, all chicks were weighed individually and divided into four groups of six birds each. Thereafter, chicks were fasted for 3 days and refed the diet containing one of several protein sources for 3 days. Chicks were maintained on an automatic lighting schedule from

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Received July 9, 1991
Accepted December 23, 1991

06:00 to 20:00 h at 25℃. The dietary proteins were casein (Wako Chemicals, Osaka, Japan, 90.9% protein), fish protein (obtained from Japan Chemical Feed Co. LTD., Hakodate, Japan, 85.2% protein), gluten(Wako Chemicals, Osaka, Osaka, Japan, 90.6% protein) and soybean protein (Fujipro R, Fuji Oil Co. LTD., 90.3%) in Experiment 1. Composition of experimental diets were shown in table 1.

In Experiment 2, dietary protein (casein or soybean protein) were replaced with amino acids to simulate casein or soybean protein. Eight birds were assigned to each treatment.

**General procedure**

At the end of Experiment 1 and 2, all the chicks were weighed individually. Thereafter, using a heparinized syringe, blood samples were taken

TABLE 1. COMPOSITION OF EXPERIMENTAL DIETS

| *Ingredients* | Dietary protein | | | |
|---|---|---|---|---|
| | Casein | Fish protein | Soybean protein | Gluten |
| | | (%) | | |
| Casein | 19.8 | – | – | – |
| Fish protein | – | 21.1 | – | – |
| Soybean protein | – | – | 19.9 | – |
| Gluten | – | | – | 19.9 |
| Corn starch | 59.2 | 59.2 | 59.2 | 59.2 |
| Cellulose | 15.0 | 13.7 | 14.9 | 14.9 |
| Mineral mixture[1] | 5.3 | 5.3 | 5.3 | 5.3 |
| Vitamin A, D mixture[2] | 0.2 | 0.2 | 0.2 | 0.2 |
| Vitamin B mixture[3] | 0.2 | 0.2 | 0.2 | 0.2 |
| Choline chloride | 0.3 | 0.3 | 0.3 | 0.3 |
| Crude protein (%) | 18.0 | 18.0 | 18.0 | 18.0 |
| Metabolizable energy (kcal/kg) | 2978.5 | 2975.3 | 2968.7 | 2923.0 |

[1] 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% $FeSO_4 \cdot 7H_2O$, 0.665% $MnSO_4 \cdot 4H_2O$, 0.067% KI, 0.021% $ZnSO_4$, 0.025% $CuSO_4 \cdot 5H_2O$.

[2] Vitamin A 5000, Vitamin D 1000 (IU or ICU/g mixture).

[3] 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, cyanocabalamin 0.5, lactose 91893.5 (mg/100g mixture).

from the wing vein of each chick for determination of triglyceride and non-esterified fatty acids (NEFA) concentrations. From each blood sample, plasma was later extracted and then stored at −30℃ until analysis. At necropsy, the liver and abdominal fat were immediately removed and weighed. Livers were placed in an ice-cold saline to determine triglyceride contents, and the activities of lipogenic enzymes.

**Preparation of liver homogenates**

Livers were homogenized in 0.25 M sucrose solution containing 1 mM ethylenediaminetetraacetate-2Na (EDTA-2Na), after which the homogenates were centrifuged (Model RS-18, Tomy Seiko) at 600 x g at 4℃ for 15 min. The supernatants were recentrifuged (Model 65P, RP 40-705 rotor, Hitachi koki) at 105,000 x g at 4℃ for 60 min and the resulting clear supernatants (cytosolic fraction) was used for assaying lipogenic enzymes.

**Enzyme assay**

Acetyl-CoA carboxylase (EC 6.2.1.3) was assayed by $H^{14}CO_3^-$-fixation method (Qureshi et al., 1980). Fatty acid synthetase was assayed by 1-$^{14}$C-acetyl-CoA incorporation method (Hsu et al., 1965). Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) was determined by following the rate of glucose-6-phosphate dependent reduction of NADP (Löhr and Waller, 1974); malic enzyme (EC 1.1.1.40) by following the rate of malate-

dependent NADP reduction (Ochoa, 1955). Citrate cleavage enzyme (EC 4.1.3.8) was determined as described in Takeda et al. (1965). The protein content of solutions used for enzyme assay was determined by the method of Lowry et al. (1951). Enzyme activities are expressed as nanomole of substrate converted to product per minute per mg protein at 25℃ or 38℃. Triglyceride contents in the liver and the plasma were carried out according to the procedures as reported by Hsu et al. (1988).

**Statistical analyses**

All data were statistically analyzed using the one-way layout design of the analysis of variance (Yoshida, 1975). Significant differences between treatments were determined by Duncan's multiple range test (Duncan, 1955).

## Results and Discussion

In Experiment 1, body weight gains, feed intakes, and weights of the liver and abdominal fat of chicks fed diets containing different protein sources for 3 days are shown in table 2. Body weight gains, feed intake and the abdominal fat weight did not significantly differ among the protein sources. The liver weight was smaller in chicks fed the soybean protein or gluten diet than in those given the casein or fish protein diet. Table 3 shows the activities of hepatic lipogenic enzymes of chicks fed diets containing different protein sources for 3 days. The activities of lipogenic enzymes, except glucose-6-phosphate dehydrogenase, in the liver of chicks were significantly lower in the soybean protein and gluten diet than in the casein and fish protein diet. Malic enzyme and glucose-6-phosphate dehydrogenase are involved in the generation of NADPH for fatty acid synthesis under a variety of conditions. However, it seems that the chicks differs

TABLE 2. EFFECT OF DIETARY PROTEIN ON BODY WEIGHT GAINS, FEED INTAKES, AND WEIGHTS OF THE LIVER AND ABDOMINAL FAT OF GROWING CHICKS (EXPERIMENT 1)

| Dietary protein | Body weight gain | Feed intake | Liver weight | Abdominal fat weight |
|---|---|---|---|---|
| | (g/3 days) | (g/3 days/bird) | (%) | (%) |
| Casein | 53.3 ± 6.1[1] | 109 | 5.0 + 0.3[c] | 0.16 ± 0.01 |
| Fish protein | 54.0 ± 7.3 | 109 | 4.5 ± 0.2[bc] | 0.11 ± 0.02 |
| Soybean protein | 42.4 ± 6.5 | 105 | 4.0 ± 0.3[b] | 0.09 ± 0.01 |
| Gluten | 36.2 + 8.0 | 107 | 3.0 ± 0.1[a] | 0.11 + 0.03 |

[1] Mean ± SE for six chicks.
[a,b,c] Mean values in the same vertical column having different superscript letters are significantly different from one another ($p < 0.05$).

TABLE 3. EFFECT OF DIETARY PROTEIN ON ACTIVITIES OF LIPOGENIC ENZYMES IN THE LIVER OF GROWING CHICKS (EXPERIMENT 1)

| Dietary protein | Acetyl-CoA carboxylase | Fatty acid synthetase | Citrate cleavage enzyme | Malic enzyme | Glucose-6-phosphate dehydrogenase |
|---|---|---|---|---|---|
| | (nmole/min/mg protein) | | | | |
| Casein | 2.18 ± 0.17[b][1] | 4.73 ± 0.32[b] | 99.9 ± 5.8[b] | 167.3 ± 11.1[b] | 5.71 ± 0.36 |
| Fish protein | 2.23 + 0.12[b] | 4.14 ± 0.47[ab] | 87.2 ± 4.9[b] | 146.1 ± 16.4[b] | 6.70 ± 1.01 |
| Soybean protein | 1.39 ± 0.08[a] | 3.61 ± 0.27[a] | 61.1 ± 6.5[a] | 99.0 ± 8.4[a] | 5.67 ± 0.32 |
| Gluten | 1.14 ± 0.13[a] | 3.46 ± 0.21[a] | 47.8 ± 3.4[a] | 105.3 ± 8.5[a] | 5.48 ± 0.49 |

[1] Mean± SE for six chicks.
[a,b] Mean values in the same vertical column having different superscript letters are significantly different from one another ($p < 0.05$).

from the rat in terms of the pathways available for the production of NADPH. Usually the pentose-pathway dehydrogenases were not active in the liver of chicks, at least not active enough to supply any appreciable amount of NADPH. Also, Romsos and Leveille (1974) have pointed out that the relative importance of the pentose-pathway dehydrogenases for the generation of NADPH in the liver of chicks might be less significance than these dehydrogenases in tissues of the rat or pig, due to the fact that, in contrast to those in the rat, the pentose-pathway dehydrogenases in the liver exhibited low activities and were not responsive to dietary manipulations (Madappally et al., 1971; Goodridge, 1968). The results presented in Experiment 1 clearly demonstrated a relationship between protein sources and activities of enzymes involved in hepatic lipogenesis of chicks. However, G6PDH showed the lower activity in comparison with malic enzyme in the liver and did not affect by dietary protein. Similar results have been obtained by Herzberg and Rogerson (1984) and Iritani et al. (1986) in the study used rats.

Triglyceride contents of the liver and plasma of chicks fed the casein and fish protein diets were significantly lower than that of those fed the soybean protein and gluten diets. Whereas plasma NEFA concentration was opposite to plasma triglyceride concentration. Yeh et al. (1970) and Yeh and Leveille (1971) have pointed out that increased NEFA concentration in the serum increased hepatic long-chain acyl-CoA levels and depressed hepatic fatty acid synthesis, presumably by inhibiting acetyl-CoA carboxylase and fatty acid synthetase activities (Tubbs and Garland, 1964). The mechanism involved in dietary protein, however, appears more complex.

*In Experiment 2, the effects of dietary amino* acid mixtures simulating casein and soybean protein on hepatic lipogenic enzyme activities were

TABLE 4. EFFECT OF DIETARY PROTEIN ON CONTENTS OF HEPATIC TRIGLYCERIDE AND PLASMA TRIGLYCERIDE AND NON ESTERIFIED FATTY ACIDS (NEFA) OF GROWING CHICKS (EXPERIMENT 1)

| Dietary protein | Liver | Plasma | |
|---|---|---|---|
| | Triglyceride | Triglyceride | NEFA |
| | (mg/g) | (mg/100 ml) | |
| Casein | $7.62 \pm 0.67^{b}$ [1] | $66.3 \pm 5.1^{b}$ | $4.14 \pm 0.48^{a}$ |
| Fish protein | $7.04 \pm 0.51^{b}$ | $57.3 \pm 4.6^{b}$ | $4.81 \pm 0.48^{a}$ |
| Soybean protein | $3.32 \pm 0.21^{a}$ | $25.6 \pm 3.9^{a}$ | $7.14 \pm 0.97^{b}$ |
| Gluten | $3.09 \pm 0.25^{a}$ | $22.7 \pm 2.7^{a}$ | $5.68 \pm 0.62^{ab}$ |

[1] Mean ± SE for six chicks.
[a,b] Mean values in the same vertical column having different superscript letters are significantly different from one another ($p < 0.05$).

TABLE 5. EFFECT OF DIETARY AMINO ACIDS SIMULATING CASEIN AND SOYBEAN PROTEIN ON BODY WEIGHT GAINS, FEED INTAKES, AND WEIGHTS OF THE LIVER AND ABDOMINAL FAT OF GROWING CHICKS (EXPERIMENT 2)

| Dietary protein | Body weight gain | Feed intake | Liver weight | Abdominal fat weight |
|---|---|---|---|---|
| | (g/3 days) | (g/3 days/bird) | (%) | (%) |
| Casein | $51.5 \pm 5.0^{b}$ [1] | 103 | $3.83 \pm 0.28$ | $0.34 \pm 0.05$ |
| Soybean protein | $39.2 \pm 5.7^{a}$ | 96 | $3.91 \pm 0.36$ | $0.24 \pm 0.05$ |
| Casein amino acids | $30.4 \pm 3.1^{a}$ | 95 | $3.56 \pm 0.19$ | $0.26 \pm 0.04$ |
| Soybean protein amino acids | $31.0 \pm 3.3^{a}$ | 92 | $3.20 \pm 0.33$ | $0.37 \pm 0.06$ |

[1] Mean± SE for eight chicks.
[a,b] Mean values in the same vertical column having different superscript letters are significantly different from one another ($p < 0.01$).

examined. Body weight gains and feed intake of chicks fed the casein diet tended to be greater, although not statistically significant, than that of those fed the other diets (table 5). When dietary protein was replaced with the amino acid mixture simulating either casein or soybean protein, the activities of acetyl-CoA carboxylase and fatty acid synthetase in the liver were not different between the two groups fed amino acid mixtures, although the activities of malic enzyme and citrate cleavage enzyme tended to be lower in the soybean-type amino acid diet than in the casein-type amino acid diet (table 6). Furthermore, the activities of acetyl-CoA carboxylase and fatty acid synthetase in the liver were significantly lower in the casein-type amino acid diet than in the casein diet. Similarly, although triglyceride contents in the liver and plasma were higher in chicks fed the casein diet than in those fed the soybean protein, there were no significant differences between the two diets of amino acid mixture based on casein or soybean protein (table 7). Thus, it is suggested that some mechanisms ascribed to the protein itself as well as the amino acid composition, are involved in the effects.

TABLE 6. EFFECT OF DIETARY AMINO ACIDS SIMULATING CASEIN AND SOYBEAN PROTEIN ON ACTIVITIES OF LIPOGENIC ENZYMES IN THE LIVER OF GROWING CHICKS (EXPERIMENT 2)

| Dietary protein | Acetyl-CoA carboxylase | Fatty acid synthetase | Citrate cleavage enzyme | Malic enzyme | Glucose-6-phosphate dehydrogenase |
|---|---|---|---|---|---|
| | (nmole/min/mg protein) | | | | |
| Casein | 2.43 ± 0.16[b 1)] | 4.34 ± 0.40[b] | 130.7 ± 16.7[c] | 145.6 ± 11.3[b] | 5.21 ± 0.50 |
| Soybean protein | 1.47 ± 0.06[a] | 3.11 ± 0.36[a] | 82.9 ± 11.6[b] | 93.4 ± 12.9[a] | 6.10 ± 0.62 |
| Casein amino acids | 1.41 ± 0.05[a] | 2.75 ± 0.17[a] | 79.5 ± 8.4[ab] | 124.2 ± 15.6[ab] | 6.33 ± 0.46 |
| Soybean protein amino acids | 1.42 ± 0.09[a] | 2.40 ± 0.31[a] | 62.0 ± 5.6[a] | 96.9 ± 7.6[a] | 6.56 ± 0.55 |

[1)] Mean ± SE for eight chicks.
[a,b,c] Mean values in the same vertical column having different superscript letters are significantly different from one another ($p < 0.05$).

TABLE 7. EFFECT OF DIETARY AMINO ACIDS SIMULATING CASEIN AND SOYBEAN PROTEIN ON CONTENTS OF HEPATIC TRIGLYCERIDE AND PLASMA TRIGLYCERIDE AND NEFA OF GROWING CHICKS (EXPERIMENT 2)

| Dietary protein | Liver | Plasma | |
|---|---|---|---|
| | Triglyceride | Triglyceride | NEFA |
| | (mg/g) | (mg/100 ml) | |
| Casein | 6.91 ± 0.91[b 1)] | 54.1 ± 8.0[b] | 4.01 ± 0.33 |
| Soybean protein | 2.89 ± 0.21[a] | 35.3 ± 6.1[a] | 5.31 ± 0.30 |
| Casein amino acids | 3.22 ± 0.40[a] | 29.0 ± 3.7[a] | 6.83 ± 0.56 |
| Soybean protein amino acids | 2.19 ± 0.22[a] | 32.8 ± 3.0[a] | 5.87 ± 0.61 |

[1)] Mean ± SE for eight chicks.
[a,b] Mean values in the same vertical column having different superscript letters are significantly different from one another ($p < 0.05$).

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