飼料蛋白質レベルの変化はニワトリの血漿インスリン様増殖因子-I濃度を変化させる

誌名	日本家禽学会誌
ISSN	00290254
巻/号	361
掲載ページ	p. 25-30
発行年月	1999年1月

Dietary Protein Levels Alter Plasma Insulin-Like Growth Factor-I Concentration of Chicks

Kazumi KITA* and Jun-ichi OKUMURA

Laboratory of Animal Nutrition, School of Agricultural Sciences, Nagoya University, Nagoya 464-8601

The plasma concentration of glucose, free fatty acid, triacylglycerol, cholesterol, albumin, protein, insulin-like growth factor-I (IGF-I) was measured in chicks (7-d-old) given diets with varying dietary protein levels (0, 10, 20, 40 and 60% protein) *ad libitum* for 10 days. Plasma glucose concentration decreased with rising dietary protein levels from 10 to 40%. When dietary protein levels increased from 0 to 60%, plasma cholesterol concentration decreased significantly. Plasma IGF-I concentration increased significantly with elevating dietary protein levels up to 20%, and above the level, it decreased gradually. The response of body weight change in chicks to the alteration in nutritional condition such as dietary protein levels over 10 days would be partially regulated by the change in plasma IGF-I concentration.

*(Jpn. Poult. Sci., **36** : 25-30, 1999)*

Key words : dietary protein, insulin-like growth factor-I, blood glucose, chickens

Introduction

The insulin-like growth factors-I (IGF-I) found in chickens has been characterized and shown to be 70 amino acid polypeptides (BALLARD *et al.* 1990). Recently some findings pointing to an important role for IGF-I in the control of growth and metabolism in chickens, as in the case of mammals have been reported. During the early stage of growth in chicks after hatching, plasma IGF-I concentration increases rapidly accompanied with aging, reaches a peak before sexual maturity, and then declines (HUYBRECHTS *et al.* 1985 ; JOHNSON *et al.* 1990 ; MCGUINNES and COGBURN, 1990). Plasma IGF-I levels are also responsive to the change in nutritional conditions and are reduced in chickens fed low protein diets (ROSEBROUGH *et al.* 1988, 1996, 1998, ROSEBROUGH and MCMURTRY, 1993, KITA *et al.*, 1996 b, 1998). However, the influence of dietary protein levels excess the requirement on plasma IGF-I concentration has not been clarified. Therefore, in the present study, we examined the influence of dietary protein levels varying widely from protein deprivation to the dietary protein levels three times the requirement on plasma IGF-I concentration.

Materials and Methods

Two hundreds single-comb White Leghorn male chicks from a local hatchery

Received May 28, 1998 Accpted October 19, 1998

* Correspondence and reprint requests should be addressed to Dr. Kazumi KITA, Laboratory of Animal Nutrition, School of Agricultural Sciences, Nagoya University, Nagoya 464-8601, Japan. Tel : 052-789-4078. Fax : 052-789-4077. E-mail : kitak@.agr.nagoya-u.ac.jp

(Hattori Yokei Ltd, Nagoya, Japan) were fed a commercial chick mash diet (Pre-Chick™, crude protein 215 g/kg, metabolizable energy 12.1 kJ/g ; Marubeni Siryou Ltd, Tokyo, Japan) from hatching until 7 days of age in electrically heated brooders. At this age, 30 birds of uniform body weight (average initial body weight, 81.5±0.52 g) were selected and divided evenly into 5 experimental groups of 6 birds each. The birds were placed in individual stainless steel metabolism cages in a temperature-controlled (29±1℃) room. Continuous illumination was provided. According to SCOTT *et al.* (1982) and NRC (1984), the dietary protein requirement for male Leghorn-type chicks are 21.5% and 18.0%, respectively. In the present study, 20% dietary protein was set as the dietary protein requirement. At 7 days of age, the birds were allowed free access to experimental diets with 0, 10, 20, 40 and 60% dietary protein. Calculated metabolizable energy of all diets was set at 12.6 kJ/g. The composition of the diets is shown in Table 1. Ten days after placement of the experimental diets, the birds were anesthetized and blood was collected by heart puncture. After centrifuge at 3,000×g and 4℃ for 20 minutes, plasma was collected for measuring plasma concentration of glucose, free fatty acid, triacylglycerol, cholesterol, albumin, protein and IGF-I. Plasma samples were stored at −20℃ until analyzed. After blood sampling, liver and right breast muscle (*M. pectoralis major*) were removed and weighed.

Plasma IGF-I concentration was measured by radioimmunoassay described in detail previously (KITA *et al.*, 1997, 1998). Plasma concentration of glucose, free fatty acid, triacylglycerol, cholesterol, albumin and protein was measured by using commercial kits (glucose : Glucose C II-Test Wako ; free fatty acid : NEFA C-Test Wako ; triacylglycerol : Triglyceride G-Test Wako ; cholesterol : Cholesterol E-Test Wako ; albumin and protein : A/G B-Test Wako ; Wako Pure Chemical Co. Ltd., Osaka).

Statistical analysis of data was performed by one-way ANOVA followed by

Table 1. Composition of experimental diets

Protein level (%)	0	10	20	40	60
			g/kg diet		
Isolated soybean protein[1)]	0	119.5	239.0	478.0	717.0
L-Methionine	0	1.5	2.9	5.8	8.7
L-Threonine	0	0.6	1.2	2.4	3.6
Glycine	0	2.1	4.2	8.4	12.6
Corn starch	752.7	629.0	505.4	258.1	10.8
Cellulose			154.3		
Corn oil			30.0		
Mineral mixture[2)]			58.5		
Vitamin mixture[2)]			2.0		
Choline chloride			1.5		
Inositol			1.0		
Metabolizable energy (kJ/g)	12.6	12.6	12.6	12.5	12.4

[1)] : Crude protein content was 840 g/kg.

[2)] : The composition of vitamin mixture and mineral mixture was reported previously (Kita *et al.*, 1996 a).

DUNCAN's multiple range test (DUNCAN, 1955) using the General Linear Model Procedures (GLM ; SAS/STAT Version 6, SAS Institute, Cary, NC, U.S.A.). Differences between means were considered to be significant at $P<0.05$. Regression equation was also calculated using GLM.

Results

Body weight change, food intake, liver and muscle weights, and plasma concentration of glucose, free fatty acid, triacylglycerol, cholesterol, albumin, protein and IGF-I of chicks fed diets with various dietary protein levels are shown in Table 2. During the experimental period, body weight increased in all treatments except for chicks fed a protein-free diet. Body weight increased with the increment of dietary protein from 0 to 20%, and above the protein requirement (20%), it decreased significantly with increasing dietary protein levels from 40 to 60%. Food intake of chicks fed the protein-free diet was significantly lower than those of the 10, 20 and 40% dietary protein groups and was also reduced in chicks fed high protein diets containing 60% protein. Liver weight of chicks fed the protein-free diet was significantly lower than those of other treatments. The differences in muscle weight due to varying dietary protein levels was proportional to body weight.

Plasma glucose concentration decreased with rising dietary protein levels from 10 to 40%. When dietary protein levels increased form 0 to 60%, plasma cholesterol concentration decreased significantly. Plasma IGF-I concentration increased significantly with elevating dietary protein levels up to 20%, and above the level, it decreased gradually. Varying dietary protein levels did not affect plasma concentration of free fatty acid, triacylglycerol, albumin and protein.

Table 2. Influence of varying dietary protein levels on body weight change, food intake, liver weight, muscle (*M. pectoralis major*) weight and plasma concentration of glucose, free fatty acid, triacylglycerol, cholesterol, albumin, protein and insulin-like growth factor-I (IGF-I) of chicks

	Dietary protein levels (%)					Pooled
	0	10	20	40	60	SEM
Body weight change (g/10 days)	-24^{c}	45^{b}	80^{a}	71^{a}	54^{b}	4.2
Food intake (g/10 days)	58^{c}	168^{a}	172^{a}	158^{a}	136^{b}	6.9
Liver weight (g)	2.1^{b}	4.5^{a}	5.3^{a}	5.2^{a}	5.3^{a}	0.29
Muscle weight (g)	1.0^{c}	4.0^{b}	5.4^{a}	5.4^{a}	4.4^{b}	0.26
Plasma concentration						
Glucose (mg/d*l*)	368^{a}	366^{a}	316^{ab}	295^{b}	268^{b}	19
Free fatty acid (mEq/*l*)	0.50	0.36	0.31	0.26	0.33	0.042
Triacylglycerol (mg/d*l*)	107	96	65	51	47	21
Cholesterol (mg/d*l*)	322^{a}	195^{b}	143^{c}	108^{cd}	101^{d}	12
Albumin (g/d*l*)	1.3	1.3	1.5	1.5	1.6	0.082
Protein (g/d*l*)	1.4	1.6	1.9	1.9	1.8	0.18
IGF-I (ng/m*l*)	6.4^{c}	13.3^{b}	21.7^{a}	17.9^{ab}	14.3^{b}	1.9

$^{a, b, c, d)}$: Means not sharing with a common superscript in the same row are significantly different at $P<0.05$.

Discussion

Plasma IGF-I concentration increased with the level of dietary protein up to the requirement of 20%, whereas above that level plasma IGF-I decreased gradually with increasing dietary protein levels. ROSEBROUGH *et al.* (1988, 1996, 1998) and our previous findings (KITA *et al.*, 1996 b) reported that plasma IGF-I concentration increased with rising dietary protein levels from approximate half of the requirement to the dietary protein requirement, as is in good agreement to the data obtained in the present study. The results in this study also showed that plasma IGF-I concentration of chicks deprived dietary protein was lower than that in the low dietary protein group (10% protein). Furthermore, it was clarified that dietary protein levels more than twice the requirement decreased plasma IGF-I concentration. As shown in Table 2, food intake was also influenced by dietary protein levels. The variation of food intake is accompanied by the change in protein and energy intakes, which has the potency to fluctuate plasma IGF-I levels (ROSEBROUGH and MCMURTRY, 1993). In the present study, the statistical analysis indicated that the effect of varying dietary protein levels was significant ($P=0.015$), but not food intake ($P=0.436$). Therefore, it was concluded that the change in plasma IGF-I concentration observed in this study was brought about by the change in the dietary protein levels.

When chicks fed diets with different dietary protein levels from 14 to 28%, there was no significant difference in plasma glucose and triglyceride concentrations (ROSEBROUGH *et al.*, 1988). As shown in Table 2, plasma triglyceride concentration was stable to the change in dietary protein levels, which is good agreement in ROSEBROUGH *et al.* (1988). In the present study, however, glucose concentration decreased with rising dietary protein levels from 10 to 40%. This discrepancy suggests that plasma glucose levels changes significantly when dietary protein levels varies very widely. Varying dietary protein levels did not affect plasma concentrations of free fatty acid and albumin (Table 2). Previously, we reported that hepatic albumin content in chicks fed a high protein diet (CP 40%) was significantly higher than that of chicks in the 20% CP group. These results suggest that hepatic albumin level is more sensitive to the change in dietary protein levels compared to plasma albumin. ROSEBROUGH *et al.* (1996) reported that plasma free fatty acid concentration of chicks did not change when dietary protein levels varied from 12 to 21%, as is consistent with the result observed in the present study.

One of the most important factors influencing whole-body and tissue protein metabolism in animals is nutrient intake. In avian species, varying dietary protein levels significantly alter whole-body protein synthesis in chicks, and the change in whole-body protein synthesis contributed to body weight change (MURAMATSU *et al.*, 1987, KITA *et al.*, 1989, 1993 a). As shown in Table 2, body weight change increased with elevating dietary protein levels up to 20% and the above level body weight gain decreased gradually. Plasma components were also measured in the present study and the significant positive correlation was observed between body weight change and plasma IGF-I concentration ($r=0.69$, $P<0.001$). This correlation suggests that in

chicks fed diets with widely varying dietary protein levels, body weight change would be regulated, at least in part, by the alteration in plasma IGF-I concentration.

We conclude that the response of body weight change in chicks to the alteration in nutritional condition such as dietary protein levels over 10 days would be partially regulated by the change in plasma IGF-I concentration.

References

BALLARD, F.J., JOHNSON, R.J., OWENS, P.C., FRANCIS, G.L., UPTON, F.Z., MCMURTRY, J.P. and WALLACE, J.C. (1990) Chicken insulin-like growth factor-I : Amino acid sequence, radioimmunoassay, and plasma levels between strains and during growth. General and Comparative Endocrinology, **79** : 459-468.

DUNCAN, D.B. (1955) Multiple range and multiple F tests. Biometrics **11** : 1-42.

HUYBRECHTS, L.M., KING, D.B., LAUTERIO, T.J. MARSH, J. and SCANES, C.G. (1985) Plasma concentration of somatomedin-C in hypophysectomized, dwarf and intact growing domestic fowl as determined by heterologous radioimmunoassay. Journal of Endocrinology, **104** : 233-239.

JOHNSON, R.J., MCMURTRY, J.P. and BALLARD, F.J. (1990) Ontogeny and secretory pattern of plasma insulin-like growth factor-I concentrations in meat-type chickens. Journal of Endocrinology, **124** : 81-87.

KITA, K., IWATA, J., SHIBATA, T., CONLON, M.A. and OKUMURA, J. (1997) Influence of fasting on plasma insulin-like growth factor-I concentration in chickens at early stages of growth. Japanese Poultry Science **34** : 410-413.

KITA, K., KANOKWAN, H., SHIBATA, T., CONLON, M.A., SASAKI, K., SAITO, N. and OKUMURA, J. (1998) Influence of refeeding on hepatic insulin-like growth factor-I (IGF-I) gene expression and plasma IGF-I concentration in fasted chicks. British Poultry Science **39** : in press.

KITA, K., MATSUNAMI, S. and OKUMURA, J. (1996 a) The relationship of protein synthesis to mRNA in the liver of chicks under various nutritional conditions. Journal of Nutrition, **126** : 1610-1617.

KITA, K., MURAMATSU, T. and OKUMURA, J. (1989) Influence of excess protein intake on whole-body protein synthesis in chicks. Nutrition Report International, **39** : 1091-1097.

KITA, K., MURAMATSU, T. and OKUMURA, J. (1993 a) Effect of protein and energy intakes on whole-body protein turnover and its contribution to heat production in chicks. British Journal of Nutrition, **69** : 681-688.

KITA, K. and OKUMURA, J. (1993 b) Protein synthesis in the liver and breast muscle of chicks fed on a high protein diet. Br. Poult. Sci. **34** : 553-558.

KITA, K., TOMAS, F.M., OWENS, P.C., KNOWLES, S.E., FORBES, B.E., UPTON, Z., HUGHES, R. and BALLARD, F.J. (1996 b) Influence of nutrition on hepatic IGF-I mRNA levels and plasma concentrations of IGF-I and IGF-II in meat-type chickens. Journal of Endocrinology, **149** : 181-190.

MCGUINNES, M.C. and COGBURN, L.A. (1990) Measurement of developmental changes in plasma insulin-like growth factor-I levels of broiler chickens by radioreceptor assay and radioimmunoassay. General and Comparative Endocrinology, **79** : 446-458.

MURAMATSU, T., KITA, K., TASAKI, I. and OKUMURA, J. (1987) Influence of dietary protein intake on whole-body protein turnover in chicks. British Poultry Science **28** : 471-482.

National Research Council (1984) Nutrient Requirements of Poultry, 8th Rev. Ed. National Academy Press. Washington, D.C., U.S.A.

ROSEBROUGH, R.W. and MCMURTRY, J.P. (1993) Protein and energy relationship in the broiler chicken. 11. Effects of protein quantity and quality on metabolism. British Journal of Nutrition, **70** : 667-678.

ROSEBROUGH, R.W., MCMURTRY, J.P., MITCHELL, A.D., and STEELE, N.C. (1988). Chicken hepatic metabolism *in vitro*. Protein and energy relations in the broiler chicken —VI. Effect of dietary protein and energy restriction on in vitro carbohydrate and lipid metabolism and metabolic hormone profiles. Comparative Biochemistry and Physiology, **90** : 311-316.

ROSEBROUGH, R.W., MCMURTRY, J.P. and VASILATOS-YOUNKEN, R. (1998). Further studies on carry-over effects of dietary crude protein and triiodothyronine (T 3) in broiler chickens. British Journal of Nutrition, **79** : 89-95.

ROSEBROUGH, R.W., MITCHELL, A.D. and McMurtry, J.P. (1996) Dietary crude protein changes rapidly alter metabolism and plasma insulin-like growth factor I concentrations in broiler chickens. Journal of Nutrition, **126** : 2888-2898.

SCOTT, M.L., NESHEIM, M.C. and YOUNG, R.J. (1982) Nutrition of the Chicken. 3rd Ed. M.L. Scott & associates, Ithaca, NY., U.S.A.

飼料蛋白質レベルの変化はニワトリの血漿インスリン様増殖因子-Ⅰ濃度を変化させる

喜多一美・奥村純市

名古屋大学農学部，名古屋市千種区不老町 464-8601

飼料蛋白質レベルの異なる飼料（CP 0, 10, 20, 40 および 60%）をニワトリヒナに 10 日間自由摂取させた後，血漿中のグルコース，遊離脂肪酸，トリグリセライド，コレステロール，アルブミン，蛋白質およびインスリン様増殖因子-Ⅰ（IGF-Ⅰ）濃度を測定した。血漿中のグルコース濃度は，飼料蛋白質レベルが 10% から 40% まで増加するのにともなって減少した。飼料蛋白質レベルが 0% から 60% まで増加すると血漿中のコレステロールは飼料蛋白質レベルの増加にともなって減少した。血漿中の IGF-Ⅰ濃度は，飼料蛋白質レベル 20% までは飼料蛋白質レベルの増加にともなって上昇したが，飼料蛋白質レベルが 20% を越えると血漿中の IGF-Ⅰは徐々に減少した。飼料蛋白質レベルの変化にともなう体重変化は，血漿中の IGF-Ⅰ濃度の変化によってその一部が制御されている可能性が示唆された。

（家禽会誌，***36*** : *25-30, 1999*）

キーワード：飼料蛋白質，インスリン様増殖因子-Ⅰ，血糖，ニワトリ