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Whole-body amino acid pattern of F_4 human growth hormone gene-transgenic red common carp (*Cyprinus carpio*) fed diets with different protein levels

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Abstract

F_4 generation of human growth hormone (*hGH*) gene-transgenic red common carp, and the non-transgenic controls were fed for 8 weeks on purified diets with 20%, 30% or 40% protein. Analysis of whole-body amino acids showed that the proportions of lysine, leucine, phenylalanine, valine and alanine, as percentages of body protein, increased significantly, while those of arginine, glutamic acid and tyrosine decreased, with increases in dietary protein level in at least one strain of fish. Proportions of the other amino acids were unaffected by the diets. The proportions of lysine and arginine were significantly higher, while those of leucine and alanine were lower in the transgenics than in the controls in at least one diet group. Proportions of the other amino acids were unaffected by strain. The results suggest that the whole-body amino acid profile of transgenic carp, when expressed as proportions of body protein, was in general, similar to that of the non-transgenic controls.

Keywords: Transgenic fish; Amino acid composition; Growth hormone; *Cyprinus carpio*

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1. Introduction

Transgenic fish have been produced in a variety of species using different foreign gene constructs (Zhu, 1992; Hackett, 1993; Devlin et al., 1994). Many investigators have reported that the growth hormone (GH)-transgenic fish exhibited higher growth rates than the controls (Zhu et al., 1986, 1989; Chourrout et al., 1986; Dunham et al., 1987; Rokkones et al., 1989; Zhang et al., 1990; Du et al., 1992; Devlin et al., 1994). However, information on body composition of transgenic fish is limited (Chatakondi et al., 1995; Cui et al., 1996b; Fu et al., 1998), and there has been only one report on the body amino acid composition of transgenic fish, which suggested that the contents of 14 of the 18 muscle amino acids were higher in the transgenic carp containing rainbow trout GH gene than in the controls (Chatakondi et al., 1995). In a previous paper, we reported the growth, feed utilization and proximate body composition of F_4 generation of human growth hormone (hGH) transgenic carp fed diets with different protein levels in an 8-week growth trial (Fu et al., 1998). The study showed that transgenic carp had higher growth rates, protein retention, and contents of body dry matter and protein than the controls. The purpose of the present study was to compare whole-body amino acid pattern between the F_4 *hGH*-transgenic and the non-transgenic red common carp.

2. Materials and method

Details of fish, experimental systems and procedures were described in Fu et al. (1998). P_0 transgenic fish were produced by microinjection of the *hGH* gene capped with a mouse metallothionein promoter into the fertilized eggs of common carp (*Cyprinus carpio* — red variety). The F_4 transgenic carp were produced in 1996 by breeding the F_3 generation following spawning induction. A batch of non-transgenic control carp (red variety) was also produced on the same day. The polymerase chain reaction (PCR) method based on the procedure of Li et al. (1996) was used to detect the transgene-positive individuals. Results of PCR on 90 transgenic carp revealed that 100% of the tested fish were positive. At the age of 4 weeks, 300 transgenic and 300 control carps were transferred to the laboratory and fed an equal mixture of the three experimental diets. The growth experiment was initiated when the fish were approximately 8 weeks old and weighed 1.75 g.

Three isoenergetic purified diets, with crude protein levels of 20%, 30% and 40%, and carbohydrate levels of 50%, 40% and 30%, respectively, were formulated (see Table 1), and proximate composition and gross energy of the diets were presented in Fu et al. (1998). For amino acid analysis, triplicate samples of each diet were hydrolyzed with 6 N HCl for 24 h at 100 °C in tubes sealed after nitrogen flushing. After dilution and filtration, a small aliquot was derivitized with phenyisothiocyanate. The derivitized amino acids were analyzed by reverse phase liquid chromatography using a Pico-tag Amino Acid Analysis System (including Waters 510 pump, 717 autosampler, 996 photodiode array detector, 470 scanning fluorescence detector and 2010 work station; Millipore, Milford, MA, USA). Tryptophan was not analyzed in this study. The amino acid compositions of the diets are presented in Table 1.

Table 1
Formulation of the experimental diets

Ingredients (%)	Dietary protein (%)		
	20	30	40
Casein	16.0	24.0	32.0
Gelatin	4.0	6.0	8.0
Cod liver oil	3.0	3.0	3.0
Soybean oil	3.0	3.0	3.0
Dextrin	50.0	40.0	30.0
Others[a]	24	24	24
Amino acid composition (% dry weight)			
Essential			
Lys	1.5	2.2	3.0
Met	0.7	1.1	1.5
Thr	0.8	1.2	1.6
Leu	1.8	2.6	3.6
Ile	0.7	1.1	1.3
His	0.6	0.9	1.0
Arg	0.9	1.4	1.9
Phe	0.6	0.8	1.2
Val	1.2	1.8	2.4
Non-Essential			
Cys	0.1	0.1	0.1
Ser	1.2	1.7	2.3
Gly	1.5	2.1	2.9
Pro	2.7	4.0	5.5
Ala	1.0	1.4	1.9
Glu	3.7	5.5	7.3
Asp	0.9	1.4	1.9
Tyr	0.8	1.2	1.7

[a]Others: 1% vitamin premix and 4% mineral premix (Fu et al., 1998), 16% cellulose, 2% carboxymethylcellulose and 1% chromic oxide.

The growth experiment was conducted in 18 circular fiberglass tanks (diameter 70 cm, water volume 90 l), which were part of a recirculation system. Each tank had a flat bottom with a central drain. The water temperature varied from 26°C to 29°C (mean 27.5°C, SE 0.9 °C). Photoperiod was controlled by artificial lighting with the light period between 07:00 and 19:00.

Thirty individuals of either transgenic or control carp were weighed and put into each tank, and three tanks were assigned randomly to each combination of strain and diet. The growth experiment lasted for 8 weeks and the fish were fed to satiation twice a day at 08:00 and 16:00. At the end of the experiment, the fish were not fed for 1 day and weighed. Twenty fish from each tank were killed and used for the analysis of body composition. Amino acid analysis of fish body was as described for the diets. All analyses were carried out in triplicates.

The effects of diet and fish strain were analyzed by two-way analysis of variance (ANOVA). Multiple comparisons were made based on least-square means (Cui et al., 1996a). Differences were regarded as significant when $P < 0.05$.

3. Results and discussion

In general, the amino acid profiles of body protein in both the transgenic carp and the controls (Tables 2 and 3) were similar to those reported for common carp or other cyprinids (Schwarz and Kirchgessner, 1988; Gatlin, 1987). Of the essential amino acids, regardless of strain, the proportions of lysine, leucine, phenylalanine and valine as percentages of body protein, increased significantly, while that of arginine decreased, with increases in dietary protein level. Proportions of other essential amino acids were unaffected by the diets (Table 2). Of the non-essential amino acids, the proportion of glutamic acid decreased with increases in dietary protein level in both the transgenics and controls. The proportion of alanine in the transgenics increased with increases in dietary protein level, and the proportion of tyrosine in the controls decreased with increases in dietary protein level. Proportions of the other non-essential amino acids were unaffected by the diets (Table 3).

Few papers have reported the effect of dietary protein levels on the body amino acid composition in fish. Schwarz and Kirchgessner (1988) reported that dietary protein level had no obvious influence on the body amino acid pattern in common carp. The results were somewhat different from those of the present study. Several differences in experimental conditions may partly account for the different results between the two studies. In Schwarz and Kirchgessner's (1988) study, fish were housed at a mean water temperature of 24°C and received a fixed feeding rate. The initial weight of the fish was 170 g. In the present study, fish were fed to satiation twice a day at a mean water

Table 2
Whole body essential amino acid pattern (% protein) of control and transgenic carp fed diets with different protein levels (mean ± SE, $n = 3$)[a]

	Dietary protein (%)						ANOVA (P-value)			
	20		30		40		effect			
	Control	Transgenics	Control	Transgenics	Control	Transgenics	Overall	Diet	Strain	Diet × strain
Lys	7.4 ± 0.0^{Xa}	7.7 ± 0.0^{Ya}	7.5 ± 0.1^{Xa}	7.9 ± 0.1^{Yb}	8.0 ± 0.1^{Xb}	8.4 ± 0.1^{Yc}	0.0000	0.0000	0.0002	0.6655
Met	2.9 ± 0.1	3.1 ± 0.1	2.9 ± 0.0	3.1 ± 0.2	3.2 ± 0.1	3.3 ± 0.1	0.3325	0.2278	0.1382	0.9948
Thr	4.3 ± 0.2	4.4 ± 0.2	4.5 ± 0.5	4.4 ± 0.2	4.8 ± 0.1	4.5 ± 0.1	0.8706	0.5784	0.7885	0.6864
Leu	7.5 ± 0.1^{Xa}	6.7 ± 0.1^{Ya}	7.7 ± 0.3^{a}	7.5 ± 0.2^{b}	8.9 ± 0.5^{Xb}	8.0 ± 0.3^{Yb}	0.0039	0.0013	0.0106	0.4395
Ile	4.3 ± 0.3	4.4 ± 0.1	4.6 ± 0.2	4.2 ± 0.1	5.1 ± 0.3	4.4 ± 0.1	0.1699	0.1513	0.0767	0.2823
His	2.8 ± 0.1	2.9 ± 0.0	2.6 ± 0.1	2.9 ± 0.1	2.7 ± 0.0	2.8 ± 0.0	0.2477	0.1789	0.0609	0.8048
Arg	7.9 ± 0.2^{a}	7.9 ± 0.0	7.5 ± 0.2^{a}	7.8 ± 0.2	6.9 ± 0.1^{Xb}	7.5 ± 0.1^{Y}	0.0100	0.0048	0.0238	0.0719
Phe	4.0 ± 0.2^{a}	4.1 ± 0.2^{a}	4.6 ± 0.5^{a}	4.9 ± 0.2^{b}	3.2 ± 0.0^{b}	3.6 ± 0.1^{a}	0.0055	0.0008	0.2482	0.8781
Val	4.8 ± 0.1^{a}	4.9 ± 0.0^{ab}	5.1 ± 0.1^{b}	4.7 ± 0.1^{a}	5.2 ± 0.1^{b}	5.1 ± 0.1^{b}	0.0256	0.0266	0.1587	0.0434
Total essential amino acids										
	45.8 ± 0.2^{a}	46.0 ± 0.3^{a}	47.1 ± 0.8^{b}	47.4 ± 0.2^{b}	47.2 ± 0.7^{b}	47.4 ± 0.1^{b}	0.0431	0.0083	0.4790	0.9737

[a]Letters after each value indicate results of pair-wise comparisons. Different upper case letters (XY) indicate significant differences between fish strain within diet; different lower case letters (abc) indicate significant differences between diets within strain as ranked by least square means.

Table 3
Whole body non-essential amino acid pattern (% protein) of control and transgenic carp fed diets with different protein levels (mean ± SE, $n = 3$)[a]

	Dietary protein (%)						ANOVA (P-value)			
	20		30		40		effect			
	Control	Transgenics	Control	Transgenics	Control	Transgenics	Overall	Diet	Strain	Diet × strain
Cys	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.1469	0.1569	0.1097	0.5119
Ser	4.6 ± 0.1	4.5 ± 0.0	4.4 ± 0.1	4.3 ± 0.0	4.5 ± 0.2	4.6 ± 0.1	0.1446	0.0606	0.4787	0.5104
Asp	6.4 ± 0.1	7.2 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	7.1 ± 0.2	6.8 ± 0.2	0.0874	0.7431	0.3249	0.0242
Glu	17.1 ± 0.1[a]	17.2 ± 0.1[a]	16.2 ± 0.1[b]	16.5 ± 0.3[a]	15.5 ± 0.2[b]	15.8 ± 0.3[b]	0.0022	0.0002	0.2142	0.8918
Pro	5.5 ± 0.2	5.2 ± 0.1	5.6 ± 0.2	5.5 ± 0.2	5.4 ± 0.3	5.7 ± 0.1	0.5550	0.5291	0.7762	0.3343
Gly	8.9 ± 0.1	9.0 ± 0.1	8.7 ± 0.4	8.6 ± 0.1	8.8 ± 0.1	8.4 ± 0.1	0.1815	0.1295	0.3013	0.3595
Ala	7.3 ± 0.1[X]	6.7 ± 0.1[Ya]	7.6 ± 0.2[X]	6.9 ± 0.2[Ya]	7.6 ± 0.1	7.5 ± 0.0[b]	0.0022	0.0061	0.0016	0.0942
Tyr	4.0 ± 0.2[a]	4.0 ± 0.1	3.4 ± 0.2[b]	3.6 ± 0.1	3.6 ± 0.1[ab]	3.6 ± 0.1	0.1188	0.0270	0.7421	0.4933
Total non-essential amino acids										
	54.2 ± 0.1[a]	54.0 ± 0.4[a]	53.1 ± 0.9[ab]	52.6 ± 0.2[b]	52.8 ± 0.7[b]	52.6 ± 0.1[b]	0.0581	0.0129	0.4052	0.9039

[a]Letters after each value indicate results of pair-wise comparisons. Different upper case letters (XY) indicate significant differences between fish strain within diet; different lower case letters (ab) indicate significant differences between diets within strain as ranked by least square means.

temperature of 27.5°C. The initial weight of the fish was only 1.75 g. Formulations of the experimental diets were also different.

Of the essential amino acids, the proportion of lysine was significantly higher in transgenics than in controls regardless of diets. The proportion of leucine was significantly lower in the transgenics than in the controls fed with 20% and 40% protein diets, but was unaffected by strain in fish fed with the 30% protein diet. The proportion of arginine was significantly higher in the transgenics than in the controls fed with 40% protein diet, but was unaffected by strain in fish fed with diets that have lower protein levels. Proportions of the other essential amino acids and total essential amino acids were unaffected by strain (Table 2).

Of the non-essential amino acids, the proportion of alanine was significantly lower in the transgenics than in the controls fed with 20% and 30% protein diets. Proportions of the other non-essential amino acids and total non-essential amino acids were unaffected by strain (Table 3).

Chatakondi et al. (1995) reported that the contents of 14 of the 18 muscle amino acids were higher in the transgenic carp containing rainbow trout GH gene than in the controls. In the present study, only 4 of the 17 body amino acids analyzed were significantly different between the transgenics and the controls. Amino acid composition was expressed as grams per 100 g of muscle in Chatakondi et al. (1995), and as grams per 100 g of protein in the present study. As the body protein content was significantly higher in the transgenics than in the controls in the present study, the contents of most amino acids, when expressed as grams per unit body weight, would be expected to be higher in the transgenics than in the controls.

In conclusion, results of the present study suggested that the whole-body amino acid profile of the transgenic carp, when expressed as proportions of body protein, was in general, similar to that of the non-transgenic controls.

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References

Chatakondi, N., Lovell, R.T., Duncan, P.L., Hayat, M., Chen, T.T., Powers, D.A., Weete, J.D., Cummins, K., Dunham, R.A., 1995. Body composition of transgenic common carp, *Cyprinus carpio*, containing rainbow trout growth hormone gene. Aquaculture 138, 99–109.

Chourrout, D., Guyomard, R., Houdebine, L.-M., 1986. High efficiency gene transfer in rainbow trout (*Salmo gairdneri* Rich.) by microinjection into egg cytoplasm. Aquaculture 51, 143–150.

Cui, Y., Hung, S.S.O., Zhu, X., 1996a. Effect of ration and body size on the energy budget of juvenile white sturgeon. J. Fish Biol. 49, 863–876.

Cui, Z., Zhu, Z., Cui, Y., Li, G., Xu, K., 1996b. Food consumption and energy budget in *MThGH*-transgenic F_2 red carp (*Cyprinus carpio* L. red var.). Chin. Sci. Bull. 41, 591–596.

Devlin, R.H., Yesaki, T.Y., Biagy, C.A., Donaldson, E.M., Swanson, P., Chan, W.K., 1994. Extraordinary salmon growth salmon growth. Nature 371, 209–210.

Du, S.J., Gong, Z., Fletcher, G.L., Shears, M.A., King, M.J., Idler, D.R., Hew, C.L., 1992. Growth enhancement in transgenic Atlantic salmon by the use of an 'all fish' chimeric growth hormone gene construct. Biotechnology 10, 176–181.

Dunham, R.A., Eash, J., Askins, J., Townes, T.M., 1987. Transfer of metallothionein-human growth hormone fusion gene into channel catfish. Trans. Am. Fish. Soc. 116, 87–91.

Fu, C., Cui, Y., Hung, S.S.O., Zhu, Z., 1998. Growth and feed utilisation by F_4 human growth hormone transgenic carp fed diets with different protein levels. J. Fish Biol. 53, 115–129.

Gatlin, D.M. III, 1987. Whole-body amino acid composition and comparative aspects of amino acid nutrition of the goldfish, golden shiner and fathead minnow. Aquaculture 60, 223–229.

Hackett, P.B., 1993. The molecular biology of transgenic fish. In: Hochachka, P., Mommsen, R. (Eds.), Biochemistry and Molecular Biology of Fishes vol. 2 Elsevier, Amsterdam, pp. 207–240.

Rokkones, E., Alestrom, P., Skjervold, H., Gautvik, K.M., 1989. Microinjection and expression of a mouse metallothionein human growth hormone fusion gene in fertilized salmonid eggs. J. Comp. Physiol. 158, 751–758.

Schwarz, F.J., Kirchgessner, M., 1988. Amino acid composition of carp (*Cyprinus carpio* L.) with varying protein and energy supplies. Aquaculture 72, 307–317.

Zhang, P., Hayat, M., Joyce, C., Gonzalez-Villasenor, L.I., Lin, R.A., Dunham, R.A., Chen, T.T., Powers, D.A., 1990. Gene transfer, expression and inheritance of pRCV-rainbow trout-GHcDNA in the common carp *Cyprinus carpio*. Mol. Reprod. Dev. 25, 13–25.

Zhu, Z., 1992. Generation of fast growing transgenic fish: methods and mechanisms. In: Hew, C.L., Fletcher, G.L. (Eds.), Transgenic Fish. World Scientific Press, Singapore, pp. 92–119.

Zhu, Z., Xu, K., Li, G., Xie, Y., He, L., 1986. Biological effects of human growth hormone gene microinjected into the fertilized eggs of loach *Misgurnus anguillicaudatus*. KeXue TongBao 31, 988–990, (Science Bulletin, Academia Sinica).

Zhu, Z., Xu, K., Xie, Y., Li, G., He, L., 1989. A model of transgenic fish. Sci. China, Ser. B 2, 147–155.