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Effects of Dietary Protein and Lipid Levels on Growth and Body Composition of Juvenile Fancy Carp, *Cyprinus carpio* var. Koi

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***Abstract*—**A feeding experiment was conducted to determine the optimum dietary protein and lipid levels for juvenile fancy carp. Eight experimental diets were formulated to contain four protein levels (200, 300, 400 and 500 g kg^{-1}) with two lipid levels (70 and 140 g kg^{-1}). Triplicate groups of fish (initial weight, 12.1±0.2 g $fish^{-1}$) were hand-fed the diets to apparent satiation for 8 weeks. Fish growth performance, feed utilization and feed intake were significantly (P<0.0001) affected by dietary protein level, but not by dietary lipid level (P>0.05). Weight gain and feed efficiency ratio tended to increase as dietary protein level increased up to 400 and 500 g kg^{-1}, respectively. Daily feed intake of fish decreased with increasing dietary protein level and that of fish fed diet contained 500 g kg^{-1} protein was significantly lower than other fish groups. The protein efficiency ratio of fish fed 400 and 500 g kg^{-1} protein was lower than that of fish fed 200 and 300 g kg^{-1} protein. Moisture, crude protein and crude lipid contents of muscle and liver were significantly affected by dietary protein, but not by dietary lipid level (P>0.05). The increase in dietary lipid level resulted in an increase in linoleic acid in liver and muscle paralleled with a decrease in n-3 highly unsaturated fatty acids content in muscle of fish. In considering these results, it was concluded that the diet containing 400 g kg^{-1} protein with 70 g kg^{-1} lipid level is optimal for growth and efficient feed utilization of juvenile fancy carp.

***Keywords*—**Fancy carp, Dietary protein, Dietary lipid, Fatty acid.

I. Introduction

ORNAMENTAL fishes are attractive and colorful fishes of peaceful nature which are farmed for the purpose of enjoying their beauty. Keeping ornamental fish as pets is a hobby that has experienced considerably over the last decade [1]. At present, ornamental sector is one of the most economic and profitable areas of fish farming activities. The global wholesale value of live ornamental fish in 2000 was estimated by the Food and Agriculture Organization of the United Nations (FAO) to be US$ 900 million with a retail value of US$3 billion [2]. Among ornamental fishes, fancy carp (*Cyprinus carpio* var. Koi) are highly valued by hobbyists and the commercial pet trade. Fancy carp has been developed from common carp that are members of the largest family of freshwater fish, *Cyprinidae*. These fish are characterized by wide diversity of colors and colors combinations such as red and white, tricolor, gold and others that build up a large repertoire of phenotypes for which it is appreciated as a pet. Southeast Asia is the center of origin for this family of fish; however many species have been introduced around the world and have readily adapted to these new environments [3]–[5].

Successful cultivation of fishes depends on the suitable diet with required essential nutrients. Information on the nutritional requirements of many ornamental fish is still relatively unknown in comparison with those of food fish. The dietary nutrient requirements of ornamental fish, firstly under commercial farming conditions are concentrated on maximum growth rate, and afterward in a public or home aquaria environment shifts to other variables such as coloration and gonad maturation, rather than fast growth [1], [6], [7].

Dietary protein plays a major role in fish growth performance and feed cost. Improper dietary protein can lead to lower fish performance, increased production cost and the deterioration in water quality resulting from wasted feed [8]. Therefore, determination of dietary protein requirement of fish is essential in developing a nutrient-balanced and cost-effective fish diet. The protein requirement for some ornamental fish such as juvenile omnivorous fish guppy (30−40%), goldfish (29%), tin foil barb (41.7%), carnivorous fish discus (44.9−50.1%) and herbivorous fish redheaded cichlid (40.8%) species are in accordance with requirements reported for food fishes [9]. Generally, fish use protein as energy source when non-protein energy sources (carbohydrates and lipid) are not present in sufficient quantities in the diet. Therefore to improve protein utilization for fish growth, dietary protein could be partially replaced with lipid. The protein sparing effect lipids has been reported in several fish species [10], [11].

The objective of this study was to investigate the influence of dietary protein and lipid levels on growth, feed utilization, body composition and fatty acid composition of juvenile red- and white-colored fancy carp, *Cyprinus carpio* var. koi.

II. Material and Methods

A. Fish and Feeding Trial

Juvenile red- and white-colored fancy carp were obtained from Chung Cheong Buk-Do Inland Fisheries Research Institute (Chungju, Korea). The fish were acclimated to laboratory conditions for 2 weeks prior to the start of the

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feeding trial. Juvenile fish (initial weight, 12.1±0.2 g fish^{-1}) were allocated randomly into 24 plastic tanks, with 20 fish per tank (100 L water volume) for the feeding trial after being collectively weighted. Three replicate groups of fish were hand-fed to apparent satiation three times a day (09:00, 13:00, and 17:00 for 6 days per week) for 8 weeks. Water temperature was 27±1.2°C, and the photoperiod was left under natural conditions during the feeding trail. Records were kept of daily feed consumption, mortalities and feeding behavior of each tank.

TABLE I
INGREDIENTS AND PROXIMATE COMPOSITION OF EXPERIMENTAL DIETS

Protein levels (g kg^{-1})	200		300		400		500	
Lipid levels (g kg^{-1})	70	140	70	140	70	140	70	140
Ingredients (g kg^{-1})								
Fish meal	140	140	320	320	500	500	680	680
Wheat flour	530	340	430	240	330	140	230	40
Corn gluten meal	40	70	30	60	20	50	10	40
α-potato starch		90		90		90		90
Brewer yeast	30	30	30	30	30	30	30	30
Fish oil	30	30	20	20	10	10		
Soybean oil + Linseed oil	20	90	20	90	20	90	20	90
Cellulose	180	180	120	120	60	60		
Vitamin premix[1]	12	12	12	12	12	12	12	12
Mineral premix[2]	10	10	10	10	10	10	10	10
Vitamin C (50%)	5	5	5	5	5	5	5	5
Choline salt (50%)	3	3	3	3	3	3	3	3
Proximate composition (g kg^{-1} on dry matter basis)								
Crude protein	209	202	315	310	411	416	515	516
Crude lipid	67	146	67	139	66	132	66	134
Crude fiber[3]	186	186	126	126	66	66	6	6
Ash	50	46	87	88	133	127	166	164
Carbohydrate [4]	488	420	405	337	324	259	247	180
Energy (kJ g^{-1})[5]	15.9	17.7	17.0	18.5	17.8	19.4	18.9	20.4
Protein/energy (mg kJ^{-1})	13.1	11.4	18.6	16.8	23.1	21.5	27.3	25.3
Non-protein energy/protein (kJ g^{-1})	52.7	64.1	30.5	36.3	19.9	23.1	13.3	16.2

[1] Vitamin premix contained the following amount which were diluted in cellulose (g kg^{-1} mix): DL-α-tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid (98%), 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003.
[2] Mineral premix contained the following ingredients (g kg^{-1} mix): $MgSO_4 \cdot 7H_2O$, 80.0; $NaH_2PO_4 \cdot 2H_2O$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $ZnSO_4 \cdot 7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 1.0.
[3] Calculated based on the crude fiber content of ingredients.
[4] Calculated, 1000 - (crude protein + crude lipid + crude fiber + ash).[5] Calculated based on 23.4 MJ kg^{-1} protein, 39.2 MJ kg^{-1} lipid and 17.2 MJ kg^{-1} carbohydrate

B. Experimental Diets

Fish meal as the primary protein source, fish oil and mixture of soybean and linseed oil as lipid sources, and wheat flour as carbohydrate source were used to formulate eight experimental diets containing four levels of protein (200, 300, 400 and 500 g kg^{-1}) with two levels of lipid (70 and 140 g kg^{-1}). Ingredient and proximate composition of the experimental diets are presented in Table I. The experimental diets were pelletized by a laboratory pellet machine after 40 g water was mixed with 100 g of ingredients and dried overnight at room temperature. All diets were stored at −30°C until used. Fatty acid compositions of the experimental diets are summarized in Table II.

TABLE II
FATTY ACID COMPOSITION (% OF THE TOTAL FATTY ACIDS) OF THE EXPERIMENTAL DIETS

Protein (g kg^{-1})	200		300		400		500	
Lipid (g kg^{-1})	70	140	70	140	70	140	70	140
C12:0	0.5	0.1	0.1	0.2	0.3	0.1	0.2	0.1
C14:0	2.4	1.5	2.4	1.7	2.4	1.6	2.0	1.7
C14:1	0.3	0.2	0.4	0.3	0.4	0.3	0.5	0.4
C16:0	16.3	13.0	17.1	13.5	17.9	14.1	18.7	15.2
C16:1	3.0	1.7	2.3	1.6	2.1	1.5	1.8	1.5
C18:0	4.0	3.3	4.7	4.2	6.0	5.5	7.8	6.2
C18:1n-9	16.3	17.2	15.0	15.9	14.1	15.2	13.5	15.1
C18:2n-6	28.6	29.9	21.8	23.4	14.8	18.6	10.1	14.4
C18:3n-6	0.4	1.0	0.4	1.0	0.2	0.8	0.2	0.2
C18:3n-3	8.2	18.0	7.3	16.3	5.4	14.3	3.9	12.6
C20:0	0.7	1.2	0.6	1.5	0.1	1.3	0.1	0.1
C21:0	1.3	0.9	1.0	0.8	0.8	0.6	0.8	0.6
C20:1	0.5	0.3	0.4	0.3	0.4	0.3	0.3	0.3
C20:3n-3	1.5	0.8	1.8	1.2	2.0	1.4	2.2	1.7
C20:4n-6	0.3	0.1	0.3	0.2	0.4	0.3	0.4	0.3
C20:5n-3	4.4	2.9	5.3	3.8	6.2	4.5	6.3	5.1
C22:5n-3	0.8	0.5	1.5	1.1	2.1	1.5	2.4	1.9
C22:6n-3	8.7	5.9	14.1	10.6	20.4	14.5	22.9	17.8

C. Sample Collection and Analytical Methods

At the end of the feeding trial, all the fish in each tank were collectively weighed after anesthetizing with tricaine methanesulfonate (MS222, Sigma, St. Louis, MO, USA) at a

concentration of 100 ppm, and after starvation for 24 h. Ten fish from each tank at the end of the feeding trials were pooled and were used for the analyses of chemical composition. The crude protein content was determined using the Auto Kjeldahl System (Buchi, Flawil, Switzerland), the crude lipid content was determined by the ether-extraction method, using a Soxhlet extractor (VELP Scientifica, Italy), the moisture content was determined with a dry oven (105°C for 6 h), and the ash content was determined using a muffle furnace (600°C for 4 h). Lipid for fatty acid analyses was extracted by a mixture of chloroform and methanol (2:l v/v) according to the method of Folch et al. [12], and fatty acid methyl esters were prepared by transesterification with 14% BF_3-MeOH (Sigma, St. Louis, MO, USA). Fatty acid methyl esters were analyzed using a gas chromatography (PerkinElmer, Clarus 600, Shelton, CT, USA) with a flame ionization detector, equipped with SP^{TM}-2560 capillary column (100 m × 0.25 mm i. d. film thickness 0.20 μm; Supelco, Bellefonte, PA, USA). Injector and detector temperatures were both 240°C. The column temperature was programmed from 140 to 240°C at a rate of 5°C min^{-1}. Helium was used as the carrier gas. Fatty acids were identified by comparison with retention times of the standard fatty acid methyl esters (PUFA 37 component FAME Mix; Supelco).

TABLE III
WEIGHT GAIN, SPECIFIC GROWTH RATE, CONDITION FACTOR, HEPATOSOMATIC AND VISCERASOMATIC INDEX OF FANCY CARP FED DIETS CONTAINING VARIOUS LEVELS OF PROTEIN AND LIPID[1]

Protein levels (g kg^{-1})	Lipid levels (g kg^{-1})	WG[2]	SGR[3]	CF[4]	HSI[5]	VSI[6]
200	70	178 ± 4.1^{a}	1.8 ± 0.03^{a}	2.0 ± 0.01	3.0 ± 0.45	3.6 ± 0.39
	140	182 ± 5.6^{a}	1.9 ± 0.04^{a}	2.1 ± 0.07	3.1 ± 0.43	3.5 ± 1.21
300	70	226 ± 18.7^{b}	2.1 ± 0.10^{b}	2.1 ± 0.06	3.7 ± 0.58	3.4 ± 0.28
	140	254 ± 14.0^{b}	2.3 ± 0.07^{b}	2.1 ± 0.03	3.1 ± 0.49	4.9 ± 0.06
400	70	312 ± 12.5^{cd}	2.5 ± 0.06^{c}	2.1 ± 0.03	3.9 ± 0.15	3.2 ± 0.52
	140	303 ± 15.3^{c}	2.5 ± 0.07^{c}	2.1 ± 0.01	3.4 ± 0.10	3.4 ± 0.15
500	70	349 ± 10.4^{d}	2.7 ± 0.04^{c}	2.0 ± 0.13	2.7 ± 0.28	3.6 ± 0.63
	140	339 ± 11.5^{cd}	2.6 ± 0.05^{c}	2.1 ± 0.09	3.1 ± 0.14	3.5 ± 0.48
Two-way ANOVA: P-values						
Dietary protein		0.0001	0.0001	0.7	0.2	0.5
Dietary lipid		0.7	0.6	0.2	0.6	0.4
Interaction		0.4	0.4	0.6	0.5	0.4

[1]Values (mean ± SE of three replications) in the same column not sharing a common superscript are significantly different ($P < 0.05$).
[2]Weight gain = (final fish wt. - initial fish wt.) × 100 / initial fish wt.
[3]Specific growth rate = [ln (final fish weight) – ln (initial fish weight)] × 100/days reared.
[4]Condition factor = [fish weight (g) / fish length (cm)^3]×100.
[5]Hepatosomatic index = (liver weight / body weight) × 100.
[6]Visceralsomatic index = (viscera weight / body weight) × 100.

TABLE IV
FEED EFFICIENCY RATIO, DAILY FEED INTAKE, PROTEIN EFFICIENCY RATIO AND DAILY PROTEIN INTAKE OF FANCY CARP FED DIETS CONTAINING VARIOUS LEVELS OF PROTEIN AND LIPID[1]

Protein levels (g kg^{-1})	Lipid levels (g kg^{-1})	FER[2]	DFI[3]	PER[4]	DPI[5]
200	70	41.0 ± 0.98^{a}	4.1 ± 0.05^{b}	2.0 ± 0.05^{c}	0.9 ± 0.01^{a}
	140	37.7 ± 1.63^{a}	4.5 ± 0.12^{c}	1.9 ± 0.08^{c}	0.9 ± 0.03^{a}
300	70	43.7 ± 3.67^{ab}	4.3 ± 0.20^{bc}	1.4 ± 0.12^{ab}	1.4 ± 1.10^{b}
	140	48.3 ± 2.28^{bc}	4.1 ± 0.10^{b}	1.6 ± 0.08^{b}	1.3 ± 0.03^{b}
400	70	52.8 ± 1.80^{c}	4.1 ± 0.08^{b}	1.3 ± 0.04^{a}	1.7 ± 0.03^{c}
	140	52.8 ± 2.89^{c}	4.1 ± 0.14^{b}	1.3 ± 0.07^{a}	1.7 ± 0.06^{c}
500	70	60.8 ± 0.88^{d}	3.7 ± 0.03^{a}	1.2 ± 0.02^{a}	1.9 ± 0.02^{d}
	140	61.1 ± 1.37^{d}	3.7 ± 0.04^{a}	1.2 ± 0.03^{a}	1.9 ± 0.02^{d}
Two-way ANOVA : P-values					
Dietary protein		0.0001	0.0001	0.0001	0.0001
Dietary lipid		0.8	0.7	0.7	0.6
Interaction		0.4	0.05	0.3	0.3

[1]Values (mean ± SE of three replications) in the same column not sharing a common superscript are significantly different ($P<0.05$).
[2]Feed efficiency ratio = wet weight gain × 100 / feed intake.
[3]Daily feed intake = feed intake × 100 / [(initial fish wt. + final fish wt. + dead fish wt.) × days reared / 2].
[4]Protein efficiency ratio = (wet weight gain / protein intake).
[5]Daily protein intake = protein intake × 100 / [(initial fish wt. + final fish wt. + dead fish wt.) × days reared / 2].

D.Statistical Analysis

The data were subjected to the one-way and two-way analysis of variance (ANOVA) to test the effect of dietary protein and lipid levels on fish performance. When significant ($P<0.05$) differences were found, the Duncan's multiple range test [13] was used to rank the groups. All statistical analyses

were carried out by using the SPSS program Version 20.0 (SPSS Inc., Chicago, IL, USA).

TABLE V
PROXIMATE COMPOSITION (G KG^{-1}) OF THE MUSCLE OF FANCY CARP FED DIETS CONTAINING VARIOUS LEVELS OF PROTEIN AND LIPID[1]

Protein levels (g kg^{-1})	Lipid levels (g kg^{-1})	Moisture	Crude protein	Crude lipid	Ash
200	70	761±5.7^{a}	211±5.8^{ab}	30±6.1^{b}	11±0.1
	140	768±1.8^{ab}	207±0.8^{a}	22±4.6^{ab}	11±0.2
300	70	775±1.1^{bc}	209±3.0^{a}	21±3.5^{ab}	11±0.3
	140	770±1.7^{ab}	207±6.7^{a}	21±2.4^{ab}	11±0.2
400	70	783±3.8^{c}	227±6.2^{b}	15±3.5^{a}	11±0.4
	140	794±1.2^{d}	217±4.5^{ab}	15±3.4^{a}	11±0.1
500	70	779±4.5^{bc}	216±2.9^{ab}	11±2.2^{a}	12±0.2
	140	774±2.0^{bc}	220±7.2^{ab}	17±2.3^{a}	11±0.3
Two-way ANOVA: P-values					
Dietary protein		0.0001	0.03	0.02	0.2
Dietary lipid		0.4	0.5	0.9	0.7
Interaction		0.05	0.6	0.3	0.4

[1]Values (mean ± SE of three replications) in the same column not sharing a common superscript are significantly different (P<0.05).

TABLE VI
PROXIMATE COMPOSITION (G KG^{-1}) OF THE LIVER OF FANCY CARP FED DIETS CONTAINING VARIOUS LEVELS OF PROTEIN AND LIPID[1]

Protein levels (g kg^{-1})	Lipid levels (g kg^{-1})	Moisture	Crude protein	Crude lipid
200	70	545±15.3^{a}	121±2.0^{a}	319±4.7^{f}
	140	551±14.9^{a}	125±2.0^{a}	312±8.7^{ef}
300	70	578±9.6^{abc}	124±1.2^{a}	280±9.2^{cde}
	140	567±7.8^{ab}	125±1.2^{a}	287±9.2^{def}
400	70	587±12.1^{bc}	137±2.4^{b}	251±13.9^{bcd}
	140	604±7.5^{c}	133±0.9^{b}	227±15.3^{ab}
500	70	587±5.9^{bc}	139±1.5^{b}	245±4.2^{abc}
	140	612±5.2^{c}	136±4.0^{b}	212±18.6^{a}
Two-way ANOVA: P-values				
Dietary protein		0.001	0.0001	0.0001
Dietary lipid		0.2	0.9	0.1
Interaction		0.3	0.2	0.3

[1]Values (mean ± SE of three replications) in the same column not sharing a common superscript are significantly different (P <0.05).

III. RESULTS

The results of growth performance and organosomatic indices of juvenile fancy carp fed the experimental diets for 8 weeks are presented in Table III.

Weight gain and SGR of fish were significantly affected by dietary protein level (P<0.0001), but not by dietary lipid level (P>0.05). Weight gain and SGR were improved as dietary protein levels increased up to 400 g kg^{-1}. However, no significant differences were identified in morphological parameters, such as condition factor, hepatosomatic index and viscerasomatic index among groups (P>0.05).

Feed efficiency ratio, daily feed intake and protein efficiency ratio (Table IV) were significantly affected by dietary protein level, but not by dietary lipid level (P>0.05). Feed efficiency ratio of fish increased with increasing dietary protein level up to 500 g kg^{-1}. Daily feed intake decreased as dietary protein level increased and the lowest feed intake was observed in fish fed 500 g kg^{-1} protein. The protein efficiency ratio of fish decreased with increasing dietary protein level, and in fish fed the 400 and 500 g kg^{-1} protein diet was lower than that of fish fed 200 g kg^{-1} and 300 g kg^{-1} levels. Daily protein intake was gradually increased as dietary protein content increased.

The proximate composition of muscle and liver of fish fed the experimental diets are given in Tables V and VI. The moisture, crude protein and crude lipid contents of muscle and liver were significantly affected by dietary protein, but not dietary lipid level. The moisture content of liver and muscle tended to increase with increasing dietary protein up to 400 g kg^{-1} level. Crude protein content of liver in fish fed the diets containing 400 and 500 g kg^{-1} protein levels was significantly (P<0.0001) higher than those of fish fed 200 and 300 g kg^{-1} protein diets at both lipid levels. Crude lipid content of muscle and liver tended to decrease with increasing dietary protein level.

The major fatty acid composition of the liver and muscle of fish are presented in Tables VII and VIII. The fatty acid compositions of tissues were reflected that of the experimental diets. The percentage of linoleic acid in liver and muscle of fish were significantly increased as dietary lipid increased and dietary protein decreased. Docosahexaenoic acid (DHA; 22:6n-3) content in liver was not affected by dietary lipid levels, but affected by dietary protein. Eicosapentaenoic acid (EPA; 20:5n-3) and DHA contents in muscle were affected by dietary lipid level, the percentages were decreased with increasing dietary lipid level at the same protein level.

TABLE VII
MAJOR FATTY ACID COMPOSITION (% OF TOTAL FATTY ACIDS) OF THE LIVER OF FANCY CARP FED DIETS CONTAINING VARIOUS LEVELS OF PROTEIN AND LIPID[1]

Fatty acids	Protein levels (g kg^{-1})								Two-way ANOVA: P-values		
	200		300		400		500				
	Lipid levels (g kg^{-1})										
	70	140	70	140	70	140	70	140	Protein	Lipid	Interaction
C14:0	1.5±0.03^{b}	1.2±0.03^{a}	1.4±0.06^{b}	1.2±0.07^{a}	1.4±0.03^{b}	1.3±0.09^{ab}	1.5±0.06^{b}	1.4±0.15^{b}	0.2	0.002	0.2
C16:0	18.5±0.28^{c}	15.5±0.50^{ab}	15.3±1.33^{ab}	14.5±0.61^{a}	15.3±0.95^{ab}	16.1±0.95^{ab}	17.0±0.27^{bc}	16.3±0.10^{abc}	0.05	0.1	0.1
C16:1	8.8±0.20^{bc}	7.1±0.33^{ab}	10.0±0.86^{c}	6.3±0.34^{a}	9.6±1.03^{c}	6.9±0.52^{a}	7.6±0.23^{ab}	6.5±0.24^{a}	0.2	0.001	0.1
C18:0	4.9±0.15	4.4±0.23	4.2±0.54	4.1±0.17	4.2±0.44	4.9±0.35	5.0±0.32	4.8±0.09	0.2	0.8	0.3
C18:1n-9	47.9±0.52^{abc}	44.2±1.11^{a}	51.2±0.83^{cd}	45.0±1.82^{a}	53.1±1.33^{d}	49.5±0.62^{bcd}	51.4±1.51^{cd}	46.6±0.98^{ab}	0.003	0.001	0.7
C18:2n-6	8.0±0.32^{cd}	12.8±0.82^{f}	6.8±0.32^{bc}	12.8±0.37^{f}	5.4±0.30^{ab}	9.0±0.69^{de}	4.7±0.61^{a}	9.6±0.41^{e}	0.001	0.001	0.2
C18:3n-6	0.1±0.01^{a}	0.4±0.06^{b}	0.2±0.01^{a}	0.4±0.01^{b}	0.1±0.03^{a}	0.2±0.06^{a}	0.2±0.03^{a}	0.1±0.01^{a}	0.001	0.001	0.001
C18:3n-3	2.3±0.06^{ab}	6.0±0.67^{cd}	2.2±0.06^{ab}	6.8±0.10^{d}	1.9±0.23^{a}	3.5±0.64^{b}	2.2±0.37^{ab}	5.2±0.68^{c}	0.01	0.001	0.02
C20:0	0.2±0.01^{b}	0.6±0.03^{d}	0.2±0.01^{b}	0.6±0.01^{d}	0.1±0.03^{a}	0.3±0.06^{c}	0.2±0.03^{ab}	0.1±0.01^{a}	0.001	0.001	0.001
C21:0	2.6±0.03^{ab}	2.4±0.06^{a}	2.4±0.15^{a}	2.4±0.09^{a}	2.5±0.18^{a}	2.9±0.09^{b}	2.7±0.09^{ab}	2.7±0.19^{ab}	0.02	0.6	0.1
C22:0	0.2±0.01	0.3±0.03	0.2±0.01	0.3±0.01	0.2±0.01	0.3±0.06	0.2±0.01	0.3±0.03	0.1	0.8	0.001
C20:3n-6	0.2±0.01	0.3±0.01	0.2±0.01	0.1±0.01	0.3±0.03	0.2±0.12	0.2±0.03	0.1±0.06	0.1	0.3	0.1
C22:1n-9	0.2±0.01^{a}	0.3±0.01^{b}	0.2±0.01^{a}	0.4±0.03^{b}	0.2±0.01^{a}	0.3±0.06^{b}	0.2±0.01^{a}	0.3±0.03^{b}	0.8	0.001	0.8
C20:4n-6	0.3±0.01^{ab}	0.3±0.09^{ab}	0.3±0.03^{ab}	0.5±0.10^{b}	0.4±0.06^{b}	0.2±0.07^{a}	0.5±0.09^{b}	0.5±0.03^{b}	0.05	0.9	0.1
C20:5n-3	0.6±0.03^{ab}	0.6±0.10^{ab}	0.6±0.03^{ab}	0.7±0.03^{ab}	0.6±0.17^{ab}	0.3±0.06^{a}	0.8±0.18^{b}	0.6±0.13^{ab}	0.1	0.2	0.3
C22:5n-3	0.2±0.03^{ab}	0.2±0.06^{ab}	0.2±0.03^{ab}	0.2±0.01^{ab}	0.1±0.10^{a}	0.1±0.03^{a}	0.3±0.07^{b}	0.2±0.03^{ab}	0.01	0.5	0.6
C22:6n-3	1.2±0.03^{ab}	1.4±0.19^{ab}	1.5±0.18^{ab}	1.8±0.10^{bc}	1.7±0.55^{abc}	0.7±0.23^{a}	2.6±0.52^{c}	2.2±0.17^{bc}	0.004	0.2	0.2

[1]Values (mean ± SE of three replications) in the same row not sharing a common superscript are significantly different (P<0.05).

TABLE VIII
MAJOR FATTY ACID COMPOSITION (% OF TOTAL FATTY ACIDS) OF MUSCLE OF FANCY CARP FED DIETS CONTAINING VARIOUS LEVELS OF PROTEIN AND LIPID[1]

Fatty acids	Protein levels (g kg^{-1})								Two-way ANOVA: P-values		
	200		300		400		500				
	Lipid levels (g kg^{-1})										
	70	140	70	140	70	140	70	140	Protein	Lipid	Interaction
C14:0	1.5±0.20^{b}	0.4±0.40^{a}	0.9±0.43^{ab}	1.1±0.03^{ab}	1.3±0.10^{b}	1.1±0.01^{ab}	1.5±0.07^{b}	1.2±0.07^{b}	0.3	0.004	0.1
C16:0	19.7±0.57^{c}	17.0±0.21^{a}	20.0±0.26^{c}	18.5±0.10^{b}	20.0±0.28^{c}	18.5±0.06^{b}	20.8±0.52^{c}	20.1±0.55^{c}	0.001	0.001	0.1
C16:1	4.6±0.12	3.6±0.06	5.0±0.35	3.3±0.10	4.9±0.09	3.1±0.10	4.8±0.20	3.4±0.19	0.9	0.001	0.1
C18:0	5.7±0.12^{c}	5.0±0.06^{a}	5.2±0.12^{ab}	5.5±0.06^{bc}	5.0±0.18^{a}	5.5±0.03^{bc}	5.1±0.10^{a}	5.3±0.09^{ab}	0.3	0.4	0.001
C18:1n-9	25.3±1.15^{ab}	25.8±0.44^{abc}	27.3±0.84^{bc}	24.6±0.32^{a}	27.7±0.15^{c}	24.9±0.58^{ab}	26.7±0.75^{abc}	24.9±0.84^{ab}	0.6	0.006	0.1
C18:2n-6	10.2±0.12^{c}	15.9±0.58^{g}	8.9±0.10^{b}	14.4±0.15^{f}	7.4±0.40^{a}	13.1±0.12^{e}	6.8±0.24^{a}	11.2±0.09^{d}	0.001	0.001	0.1
C18:3n-6	0.4±0.23^{ab}	0.6±0.01^{b}	0.2±0.03^{a}	0.6±0.03^{b}	0.2±0.03^{a}	0.5±0.03^{b}	0.2±0.03^{a}	0.2±0.01^{a}	0.03	0.01	0.2
C18:3n-3	3.3±0.03^{a}	8.9±0.32^{d}	3.2±0.13^{a}	8.2±0.15^{c}	2.9±0.22^{a}	8.2±0.07^{c}	3.2±0.24^{a}	7.0±0.22^{b}	0.002	0.001	0.002
C20:0	0.4±0.01^{a}	0.8±0.01^{b}	0.5±0.01^{a}	0.8±0.01^{b}	0.3±0.15^{a}	0.8±0.01^{b}	0.3±0.17^{a}	0.3±0.03^{a}	0.09	0.001	0.3
C21:0	2.2±0.06^{c}	1.7±0.01^{ab}	1.9±0.03^{b}	1.6±0.03^{a}	2.0±0.01^{b}	1.6±0.01^{a}	1.9±0.01^{b}	1.7±0.09^{ab}	0.001	0.001	0.01
C22:0	0.8±0.03^{b}	0.7±0.06^{a}	0.7±0.03^{a}	0.7±0.01^{a}	0.6±0.01^{a}	0.6±0.01^{a}	0.6±0.06^{a}	0.6±0.01^{a}	0.001	0.3	0.1
C22:1n-9	0.2±0.17	0.3±0.01	0.2±0.03	0.4±0.03	0.2±0.03	0.4±0.03	0.2±0.01	0.4±0.03	0.9	0.001	0.9
C20:3n-3	2.1±0.22^{d}	1.4±0.09^{abc}	1.7±0.06^{bc}	1.2±0.01^{a}	1.7±0.07^{bc}	1.3±0.03^{ab}	1.8±0.12^{c}	1.4±0.07^{abc}	0.1	0.001	0.2
C20:5n-3	4.3±0.07^{b}	3.0±0.17^{a}	4.1±0.12^{b}	3.0±0.03^{a}	4.0±0.12^{b}	3.0±0.12^{a}	4.0±0.18^{b}	3.1±0.24^{a}	0.7	0.001	0.7
C22:5n-3	1.3±0.07^{b}	1.1±0.07^{a}	1.4±0.03^{b}	1.1±0.03^{a}	1.4±0.03^{b}	1.1±0.06^{a}	1.4±0.03^{b}	1.1±0.06^{a}	0.8	0.001	0.6
C22:6n-3	12.9±0.23^{bc}	10.2±0.85^{a}	14.5±0.87^{cd}	11.1±0.26^{ab}	16.0±0.72^{d}	12.4±0.76^{bc}	15.7±0.73^{d}	13.3±0.87^{bc}	0.002	0.001	0.8

[1]Values (mean ± SE of three replications) in the same row not sharing a common superscript are significantly different (P<0.05).

IV. DISCUSSION

In the present study, weight gain of fish improved with dietary protein level up to 400 g kg^{-1} diet and no further improvement was observed at higher protein level, indicating that 400 g kg^{-1} protein diet satisfied the requirement for growth of juvenile fancy carp. There are numerous reports for the optimal dietary protein requirements of literature are very wide (25−50% of dry matter). Some of these results include: 31−38% for young *Cyprinus carpio* [9]; 35% for fingerling *Puntius gonionotus* [14]; 41−43% for fry of grass carp [15]; 47% for fry *Catla catla* [16]. Such differences originate mainly because of differences in the methodological approach, culture conditions, growth rates, and dietary protein quality [17], [18].

Results of the present study have shown that survival, growth performance and feed utilization of juvenile fancy carp were not affected by dietary lipid levels, suggesting that 70 g kg^{-1}

dietary lipid may meet sufficient lipid requirement for juvenile fancy carp. Optimal dietary lipid levels have been suggested to be below 12% in the practical diets of cyprinids. The recommended dietary lipid level was 6.5% for grass carp [19] and 7–8% for Indian major carp [20]. In agreement to the present study, no protein sparing effect of dietary lipid have been observed in other fish species such as white sea bream *Diplodus sargu* [21], Malaysian mahseer, *Tor tambroides* [22], Nile tilapia [23] and grass carp [24]. In this regard, they are different with salmonids which have high ability to utilize the high dietary lipid (above 30%) as source of metabolic energy for substantially protein spare [25]. The omnivorous fish such as common carp seem to be able to digest carbohydrates more efficiently than lipids while carnivorous species such as most marine fish can use higher dietary lipid for optimal growth.

Optimum protein to energy (P/E) ratio for proper growth of several fish species has been investigated and the estimated ratios range from 19 to 27 mg kJ^{-1} [9]. In the present study the maximum weight gain was obtained from the diets containing 400 and 500 g kg^{-1} protein corresponding to P/E ratios of 23–27 mg kJ^{-1} which is lower than reported values for Asian sea bass, 31 mg kJ^{-1} [26], mutton snapper, 28−30 mg kJ^{-1} [27], and similar to that of sunshine bass, 24−27 mg kJ^{-1} [28]. It has been suggested that feed intake is regulated by the dietary available energy [29] probably because the fish eat to satisfy their energy requirements. In this study, daily feed intake tended to decrease with dietary energy level increment. The highs daily feed intake was observed in fish fed diet containing 200 g kg^{-1} protein and 140 g kg^{-1} lipid, corresponding to the lowest P/E ratio 11.4 mg kJ^{-1}. This finding suggested that feed intake in juvenile fancy carp is directly related to dietary protein and non-protein energy level. It has earlier been reported that feed intake affected by dietary protein level in channel catfish and grass carp [30], [31]. Feed intake could be increased to compensate the amino acid or energy when inadequate amount of protein or energy in the diet are provided to fish.

The proximate composition of muscle and liver of experimental fish were significantly affected by dietary protein, but not dietary lipid level in this study. Similarly, the results of other studies showed that carcass lipid of channel catfish [32] and muscle lipid content of European sea bass [33] were not affected by dietary lipid levels. In contrast, a positive correlation between body lipid content and dietary lipid level was reported in grass carp [31], [24]. The highest crude lipid content in muscle and liver were observed in fish fed the lowest dietary protein level in this experiment. Similar result has been noted in silver perch [34]. The higher lipid deposition in groups fed low protein diets can be explained by an overconsumption of non-protein nutrient per unit weight gain when protein is not sufficient in diet [35].

It has been suggested that common carp requires both n-6 and n-3 fatty acids and that a supply of 1% of each fatty acids is essential for optimum growth and feed efficiency [36]. In this experiment, no fatty acid deficiency symptoms were apparent among fishes suggesting dietary essential fatty acid requirement was satisfied for fancy carp. The fatty acid composition of tissues exhibited differences in some cases, in response to changes in dietary lipid and protein levels. The increase in dietary lipid level resulted in an increase in linoleic acid in liver and muscle paralleled with a decrease in n-3 highly unsaturated fatty acids (HUFA) content in muscle of experimental fish. Similar report, high linoleic acid and low n-3 HUFA, were recorded in the whole body of juvenile flounder fed high energy diet mainly resulting from supplementation of soybean oil into the high energy diet [37]. Also, reference [38] showed that diets with the same fatty acid composition but different lipid level can induce modifications in fatty acid composition in intestinal brush border membranes of juvenile white seabass, and the higher linoleic acid and lower n-3 HUFA content has been reported in the fish with increasing dietary lipid level. The author suggested that an increase in dietary lipid level by triglyceride incorporation resulted in a modification in tissue fatty acid composition

V. Conclusion

Considering the results of the present study, it can be concluded that the diet containing 40% protein with 7% lipid level is optimal for growth and efficient feed utilization of juvenile red- and white-colored fancy carp *Cyprinus carpio* var. Koi.

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