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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

EFFECTS OF DIETARY PROTEIN AND LIPID LEVELS ON GROWTH, FEED UTILIZATION AND BODY COMPOSITION OF JUVENILE GRASS CARP (*Ctenopharyngodon idella*)

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Abstract: A growth trial was conducted for 90 days to evaluate the effects of dietary protein and lipid levels on growth performance, feed utilization, and whole body chemical composition of juvenile grass carp. Six practical diets containing two dietary digestible protein levels (P1: 33% and P2: 37%) and three lipid levels (L1: 4%, L2: 6% and L3: 8%) were prepared. Fish were fed to satiation three times a day. There were significant differences between the weight gain (387-594%), specific growth rate (1.7-2.1%), feed intake (46.1-51.4 g fish^{-1}), feed conversion ratio (FCR, 1.2-1.7), protein efficiency ratio (PER, 1.6-2.4), hepatosomatic index (3-3.2%), digestible protein (87.4-93.5%), lipid (91.1-97.6%) and energy (87.6-93.5%) values and the whole body fat content (16.6-18.6%) for fish fed on the experimental diets. Growth-performance, FCR, PER, feed intake, digestible protein, lipid and energy values were found higher for P1L2 diet group compared to all other experimental diets ($P<0.05$). These results suggest that P1L2 diet appears to be more adequate for a better growth of these fish. Protein utilization was enhanced by a sparing effect of dietary lipid at 6%. Under the light of this study results, it is concluded that when growth and nutrient utilization are considered, even dietary levels of 33% digestible protein, 6% lipid and 10.7 kJ g^{-1} digestible energy could be assumed to be suitable levels in formulating practical diets for juvenile grass carp.

Keywords: Diet, Digestibility, Grass carp, Growth, Lipid, Protein

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Özet:

Yemdeki Protein ve Lipid Düzeylerinin Yavru Ot Sazanı (*Ctenopharyngodon idella*)'nın Büyüme, Yem Değerlendirme ve Vücut Kompozisyonuna Etkileri

Protein ve lipit düzeyleri farklı olan yemlerin yavru ot sazanının büyüme performansı, yem değerlendirmesi ve et kalitesine etkisini belirlemek amacıyla 90 günlük bir büyüme denemesi yürütülmüştür. Sindirilebilir protein düzeyi %33 (P1) ve %37 (P2) olan ve sırasıyla %4 (L1), %6 (L2) ve %8 (L3) oranlarında lipit içeren 6 adet deneme yemi hazırlanmış ve balıklar günde üç defa olmak üzere doyuncaya kadar beslenmiştir. Deneme yemleriyle beslenen balıkların ağırlık artışı (% 387-594), spesifik büyüme oranı (% 1.7-2.1), yem tüketimi (46.1-51.4 g balık^{-1}), yem dönüşüm oranı (1.2-1.7), protein etkinlik oranı (1,6-2,4), hepatosomatik indeks (%3-3.2), yemlerdeki protein (%87.4-93.5), lipit (%91.1-97ç6) ve enerjinin (%87.6-93.5) sindirilme oranları, tüm vücut etindeki yağ miktarları (%16.6-18.6) arasındaki farklılıklar önemli bulunmuş, bu parametreler bakımından en yüksek değerler P1L2 yemiyle beslenen balıklardan elde edilmiştir ($P<0.05$). Bu sonuçlar, P1L2 yeminin balıkların büyümesi için daha verimli olduğunu göstermektedir. Yemde %6 oranında lipid bulunması proteinin değerlendirilme oranını arttırmıştır. Bu çalışmada elde edilen verilerin ışığı altında, yavru ot sazanının yeminde %33 oranında sindirilebilir protein, %6 lipid ve 10.7 kJ g^{-1} sindirilebilir enerji bulunması gerektiği sonucuna varılmıştır.

Anahtar Kelimeler: Yem, Sindirilebilirlik, Ot sazanı, Büyüme, Lipit, Protein

Introduction

It was well known that fish utilize protein preferentially to lipid or carbohydrate as an energy source. Therefore, from the nutritional, environmental and economical points of view, it is important to improve protein utilization for tissue synthesis rather than for energy purpose. Within certain limits, increasing dietary lipid levels improve diet utilization (Sargent et al., 1999).

Dietary lipid was also reported to bring protein sparing effect, replacing protein, which may otherwise be used to provide energy (NRC, 1992; Vargara et al., 1996), to reduce organic matter and nitrogen losses (Du et al., 2005). But, some authors have observed no protein sparing effect of lipid in some fish species (Danielssen and Hjertnes, 1993). In salmon aquaculture today, high fat diet has normally been used. However, excessive dietary lipid negatively affected growth performance and reduced feed consumptions and utilization of other nutrients, which led to fat deposition on viscera or the carcass (Shiau and Huang, 1990). Excessive lipid in the diet not only suppresses fatty acid synthesis, but also reduces the ability of fish to digest and assimilate lipids (Sargent et al., 1999). Therefore, a proper ratio of digestible protein to digestible lipid is necessary to achieve high growth rate and feed conversion efficiency (Takeuchi et al., 1978).

Grass carp (*Ctenopharyngodon idella*) is a typical herbivorous finfish without stomach. In its natural environment, it consumes water plants. It has a long history in aquaculture and is one of the most important species cultured in inland water bodies in China. Although studies were initiated as far back as the late 1970s on the nutrition of grass carp (Dabrowski, 1977; Law, 1986; Cai et al., 2005), there is no adequate knowledge on the suitable protein and lipid levels in juvenile diet of grass carp. Only protein (Dabrowski, 1977) and lipid (Du et al., 2005) and energy (Carter and Brafield, 1991; Cui et al., 1992) have been studied. It is necessary to determine the dietary protein and lipid requirements of grass carp to achive optimum growth rates in aquaculture. Since the protein content in finfish diets usually constitutes the largest single cost factor in feeds, the objective is to minimize protein while optimizing growth and survival.

In the present study, the effects of dietary protein and lipid levels on the growth performance, feed utilization, body composition and digestibility of dietary nutrient and energy in juvenile grass carp were investigated. Responses to these parameters of the fish were used to derive an optimum dietary protein and lipid levels.

Materials and Methods

This study was conducted at the Fish Nutrition Unit of Fisheries Faculty of Firat University, Elazig, Turkey. Each treatment had three replicates, 30 juvenile grass carp per replicate, with mean initial fish weights of 7.1 ± 0.1 g. Each treatment combination was randomly assigned to three conic glass aquaria (50 L). Fish were exposed to natural daily light regime. During the experimental periods, the aquaria continuously supplied with 250 mL min^{-1} of freshwater with 26 ± 2 ^{o}C temperatures, 7.0 ±0.4 mg L^{-1} dissolved oxygen and 7.2 ± 0.2 pH.

Feed ingredients were obtained from a local supplier (Oz Ugur-Feed Manufacture Company, Elazig, Turkey). Chromic oxide (marker) was donated by Merck Products Ltd. Vitamin and mineral premixes were obtained from Roche Products Ltd. All test ingredients were grounded until achiving a diameter of 500 µm. Diets were mechanically mixed with distilled water, and pelleted using a hand mincer fitted with a 3-mm die. The resultant moist pellets were then oven dried at 70 °C for approximately 12-h and then allowed to cool to ambient temperature in the oven and stored in polythene bags at 4 ^{o}C until used (Cai et al., 2005).

In the present experiment, a feeding trial was performed, at juvenile stage, to identify suitable protein and lipid levels and optimum protein to energy ratio to be used in formulating dry diets for this species. In the study, six different treatments were conducted. Triplicate groups were fed to satiation one of six practical diets (P1L1, P1L2, P1L3, P2L1, P2L2 and P2L3) with increasing levels of digestible protein (P1: 33% and P2: 37%) and three lipid levels (L1: 4%, L2: 6% and L3: 8%) within each protein level (Table 1).

All fish were fed three times at 4-h intervals from 09:00 to 18:00-h during 90 days. On termination of the experiment, fishes were weighed individually and mean weight for each aquaria was calculated. During acclimatization period, fish were fed with experimental diets for 7 days prior to the beginning of fecal collection. After that first fecal collection was carried out. One hour after the feed was administered; any feed and feces present in the aquaria were removed. Fresh feces produced by the fish after this period and before the second feed was given, were siphoned out. At least two siphonings were done from each aquaria during each period to minimise nutrient leaching from the feces. Fecal samples collected by siphoning were filtered onto filter paper and dried at 105 ^{o}C for 12-h. The faecal samples from each treatment replicate were separately analysed for the determination of nutrients and energy digestibility (Windell et al., 1978).

Whole body composition was determined after homogenization of individual fish. The dry matter, protein, fat, fiber and ash in all samples were determined by standard methods (AOAC, 2000). The chromic oxide content of the experimental diets and fecal samples was determined by the acid digestion method of Furukawa and Tsukahara (1966). These samples were analysed for dry matter at 65 ^{o}C for 24-h in a vacuum oven. Crude protein (%N x 6.25) was determined by the micro-Kjeldahl method using an Auto Kjeldahl System and lipid by ether extraction. Nitrogen-free extract (NFE) was calculated by subtracting the percentages calculated for crude protein, fat, ash and fiber from 100. Gross energy content was determined in an Adiabatic Calorimetric Bomb.

Apparent digestibility coefficients for control and test diets were calculated according to the following equation described by Cho and Slinger (1979).

$$ADC = 100 \times [1 - (F/D) \times (Di/Fi)]$$

where ADC = % apparent digestibility coefficient; F = % nutrient or energy of feces; D = % nutrient or energy of diet; Di = % marker in diet; Fi = % marker in feces.

The trial consisted of six treatments and three replications arranged in a completely randomized design. The data were expressed as mean ± SE of three replicate groups. The data of different treatments were subjected to ANOVA. When significant ($P<0.05$) difference was found, a Duncan's multiple range test was used to estimate the difference. Correlation analysis was performed to compare differences between dietary lipid level and whole body fat content of fish. All statistical analyses were made using the SPSS 10.1 computer program for Windows (SPSS Inc. Chicago, Illinois, USA).

Table 1. Formulation and proximate composition of the experimental diets

Components (% as fed)	P1L1	P1L2	P1L3	P2L1	P2L2	P2L3
Anchovy meal	20	20	20	25	25	25
Soybean meal	43.9	43.9	43.9	47.9	47.9	47.9
Wheat flour	5	5	5	5	5	5
Wheat bran	26.5	24.5	22.5	17.5	15.5	13.5
Soybean oil	2	4	6	2	4	6
Antioxidant[1]	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin premix[2]	1	1	1	1	1	1
Mineral premix[3]	1	1	1	1	1	1
Chromic oxide	0.5	0.5	0.5	0.5	0.5	0.5
Proximate composition (% in dry matter)						
Dry matter	92	92.5	93	92.1	92.6	93.1
Crude protein	35.1	35	34.9	40	40.1	40
Digestible protein[5]	33	33	33	37	37	37
Crude lipid	4.1	6	8	4.2	6.1	8.1
Crude fiber	3.8	3.7	3.6	3.4	3.3	3.2
Ash	4.1	4	3.9	4.5	4.4	4.3
NFE[4]	52.9	51.3	49.6	47.9	46.1	44.4
Digestible energy[5] (kJ g^{-1})	10.5	10.7	10.9	11.5	11.7	11.9

[1] Antioxidant: butylated hydroxytoluene, 12.5 mg g^{-1}
[2] Vitamin premix contains (IU or mg g^{-1} of premix): retinol, 1600 IU; calciferol, 800 IU; choline chloride, 200; ascorbic acid, 80, tocopherol, 20; pantothenic acid, 20; niacin, 12; riboflavin, 6; menadione, 4; thiamine, 4; pyridoxine, 4; folic acid, 2; biotin, 0.4; cobalamin, 0.008
[3] Mineral premix contains (mg g^{-1} of premix): zinc 200, iron 88, manganese 50, iodine 10, copper 6, selenium 0.6, cobalt 0.02
[4] NFE: nitrogen-free extract = 100 – (crude protein % + lipid % + fiber % + ash %)
[5] Digestible protein and digestible energy were calculated from NRC (1992)

Results and Discussion

All the experimental diets were well accepted by juvenile grass carp, which actively fed on them, confirming the voracious feeding nature of this species. During the experimental period, a 100% survival rate was observed in all treatments; no external pathological signs were observed during the experiment.

The specific growth rates (SGR) determined in all treatments showed mean values higher than 1.7% of body weight per day. The grass carp fed the P1L1 diet group had the lowest weight gain (387%) and SGR (1.7%), which was significantly lower than those fed other experimental diets. But, when the lipid levels of diet containing 33% digestible protein was increased, the weight gain and SGR significantly increased. The feed conversion ratio (FCR) ranged from 1.7 to 1.2. Thus, in the P1L2 diet (containing 33% digestible protein, 6% lipid and 10.7 kJ g^{-1} digestible energy) group, FCR was near to 1 and it was higher than those fed other diets ($P<0.05$). The protein efficiency ratio (PER) followed the same general pattern as FCR (Table 2).

No significant differences were found in whole body protein, fiber, ash or gross energy contents of fish fed diets with different protein and lipid levels (Table 3). On the other hand, body fat level increased with the increase from 4% to 8% in dietary lipid levels ($P<0.05$). Furthermore, the results showed a positive correlation ($R = 0.926$, $P<0.01$) between dietary lipid level and body fat content.

In addition, the P1L2 diet group had the highest ADC values for protein, lipid and energy, and the P2L3 diet group the lowest ($P<0.05$). But, there were no significant differences between the digestibility values of dry matter, fiber, ash and NFE (carbohydrate) in the experimental diets (Table 4).

Dietary crude (digestible) protein content, which supported optimal growth performance of the juvenile grass carp, under experimental conditions in this study, varied from 35% (33%) to 40% (37%). In the previous studies, crude protein content in juvenile diet for grass carp has been suggested to be about 35-43% (Dabrowski, 1977; Du et al., 2005).

The SGR of fish fed the test diets in experiment showed high mean values (> 1.7%). Increasing dietary lipid from 4% to 6% allowed digestible protein level to be decreased from 37% (P2L1 diet) to 33% (P1L2 diet) without negative affects on the SGR and FCR. Likewise, when comparing diets contained 33% digestible protein, the increase in dietary lipid led to an improvement in growth performance and feed utilization, suggesting that protein may be utilized for growth rather than for energy and, therefore, energy from lipid spares protein in juvenile grass carp. This fact has been demonstrated in a number of freshwater fish and marine carnivorous teleosts (Vergara et al., 1996).

A significant improvement in growth performance due to the sparing effect of lipid on dietary digestible protein has been reported for the gilthead seabream, *Sparus aurata* (Vergara et al., 1996). This conclusion is supported by the PERs found for grass carp in the present study. Thus, in this work, a repeated trend was observed towards a higher PER as dietary lipid increased. This fact was evident when comparing, once again, diets with the same protein contents but different energy levels. This phenomenon has been observed in rainbow trout, *Oncorhynchus mykiss* (Takuechi et al., 1978). However, some reports showed no effect of dietary lipid on body weight gain in juvenile turbot, *Scophthalmus maximus* (Danielssen and Hjertnes, 1993) and grass carp, *C. idella* (Du et al., 2005). Peres and Oliva-Teles (1999) believed this lack of protein sparing effect by dietary lipid may be related to the high protein level of the diet and according to Dias et al. (1998), the beneficial effects of an increase of the lipid level from 10 to 18% in sea bass (*Dicentrarchus labrax*) diets were significant only with a low protein diet, but not with a high protein diet. Similarly in the present study, the dietary protein content was relatively low, when lipid level was below 6%, the protein utilization increased with the lipid level. This suggests, in low protein diets, the protein sparing effect by lipid is possible within a high upper limit.

The results of whole body composition showed positive correlation between dietary lipid level and body fat content, as reported in most other fish species investigated to date (Vergara et al., 1996). The body lipid content of grass carp increased with increasing dietary lipid levels, indicating that this fish could deposit lipid in the muscle. These results agreed with the report of gilthead sea bream (Vergara et al., 1996) and grass carp (Du et al., 2005). Thus in many fish species, the increase of dietary lipid levels should be evaluated carefully for it may lead to increased fat deposition in fish.

Digestible protein, lipid and energy values decreased significantly with an increase in dietary lipid level from 4% to 8% (except P1L2 diet), which is probably one of the causes of the lowered growth performance described above. Hernandez et al., (2001) reported that sharp snout seabream (*Diplodus puntazzo*) showed no decrease in protein digestibility and an increase of lipid digestibility with an increase in dietary lipid level. The digestibilities of protein and lipid for a diet with high lipid might differ among fish species. The present study shows that protein digestibility values (93 -93.5%) in diet P1L1, P1L2 and P2L1 for juvenile grass carp was found higher than the value (90%) reported by Law (1986) who fed commercial feed containing 38% of protein to grass carp. But, the digestible lipid (91.1-97.6%) and energy (87.6-93.5%) values for all diets used in this study are lower than the values (lipid: 100% and energy: 98%) determined by Law (1986).

Conclusions

In conclusion, the maximum growth performance, feed intake, FCR, PER, digestible protein, lipid and energy values were obtained from the P1L2 diet group. These results suggest that P1L2 diet appears to be more adequate for growth of fish. However, a high-lipid diet (P2L3 diet) containing 37% digestible protein negatively affected growth performance and protein digestibility in juvenile grass carp. Thus in low protein diets, the protein sparing affect by lipid is possible within a high upper limit. Dietary levels of 33% digestible protein, 6% lipid and 10.7 kJ g^{-1} digestible energy could be assumed to be adequate levels in formulating practical diets for juvenile grass carp.

Table 2. Growth and feed utilization of juvenile grass carp (*Ctenopharyngodon idella*) fed the diets with different protein and lipid levels at 26 ± 2 °C of water temperature

Parameters [1]	Dietary group					
	P1L1	P1L2	P1L3	P2L1	P2L2	P2L3
Final body weight (g)	34.6 ± 1.69^{d}	49.3 ± 2.01^{a}	40.4 ± 1.78^{b}	45.3 ± 1.98^{a}	41.5 ± 1.85^{b}	37.2 ± 1.72^{c}
Final total length (cm)	13.1 ± 0.51^{b}	13.9 ± 0.78^{a}	13.4 ± 0.58^{ab}	13.7 ± 0.71^{a}	13.5 ± 0.61^{ab}	13.2 ± 0.55^{b}
Feed intake (g/fish)	46.1 ± 0.80^{d}	51.4 ± 0.96^{a}	49 ± 0.84^{abc}	49.6 ± 0.94^{ab}	47.8 ± 0.88^{bc}	47 ± 0.81^{cd}
WG[2] (%)	387 ± 15.9^{e}	594 ± 19.1^{a}	469 ± 16.8^{c}	537 ± 18.8^{b}	484 ± 17.5^{c}	424 ± 16.2^{d}
SGR[3] (% day^{-1})	1.73 ± 0.07^{c}	2.13 ± 0.13^{a}	1.93 ± 0.07^{b}	2.07 ± 0.12^{a}	1.93 ± 0.08^{b}	1.87 ± 0.07^{b}
FCR[4]	1.7 ± 0.06^{a}	1.2 ± 0.02^{f}	1.5 ± 0.04^{c}	1.3 ± 0.03^{e}	1.4 ± 0.05^{d}	1.6 ± 0.05^{b}
PER[5]	1.7 ± 0.09^{e}	2.4 ± 0.11^{a}	2.0 ± 0.10^{b}	1.9 ± 0.10^{c}	1.8 ± 0.09^{d}	1.6 ± 0.08^{f}
Hepatosomatic index (%)[6]	3.0 ± 0.12^{b}	3.0± 0.19^{b}	3.1± 0.15^{ab}	3.0 ± 0.14^{b}	3.1 ± 0.21^{ab}	3.2± 0.18^{a}

[1] Mean ± SE of three replicates and values within the same row with different superscripts are significantly ($P<0.05$). Initial body weight of fish was 7.1 ± 0.1 g

[2] WG: weight gain (%) = (final body weight - initial body weight) x 100 / initial body weight

[3] SGR: spesific growth rate (% day^{-1}) = (ln final body weight - ln initial body weight) x 100 / 90 days

[4] FCR: feed conversion ratio = (feed intake) / (body weight gain)

[5] PER: protein efficiency ratio = (body weight gain) / (protein intake)

[6] Hepatosomatic index (%) = (individual liver weight) x 100 / (body weight)

Table 3. Body composition of juvenile grass carp (*Ctenopharyngodon idella*) fed the diets with different protein and lipid levels at 26 ± 2 °C of water temperature for 90 days

Components (% in dry matter)[1]	Initial	Dietary group					
		P1L1	P1L2	P1L3	P2L1	P2L2	P2L3
Moisture	77.0 ± 0.67	78.1 ± 0.92	78.3 ± 0.99	78.6 ± 1.27	78.2 ± 1.34	78.4 ± 0.98	78.8 ± 0.95
Crude protein	67.9 ± 0.67	70.5 ± 0.56	72.4 ± 0.52	71.3 ± 0.70	72.6 ± 0.58	71.8 ± 0.55	71.0 ± 0.50
Crude fat	15.5 ± 0.36	16.6 ± 0.18^{c}	18.2 ± 0.24^{ab}	18.6 ± 0.21^{a}	17.3 ± 0.25^{bc}	17.9± 0.25^{a}	18.5 ± 0.34^{a}
Crude fiber	0.8 ± 0.07	1.0 ± 0.04	1.02 ± 0.05	1.0 ± 0.07	1.03 ± 0.12	1.0 ± 0.09	1.01 ± 0.08
Ash	1.5 ± 0.14	1.78 ± 0.12	1.77 ± 0.14	1.73 ± 0.19	1.79 ± 0.22	1.7 ± 0.17	1.68 ± 0.15
NFE[2]	14.3 ± 0.41	10.1 ± 0.20^{a}	6.6 ± 0.18^{d}	7.4 ± 0.14^{c}	7.3 ± 0.13^{c}	7.6 ± 0.17^{bc}	7.8 ± 0.19^{b}
Gross energy (kJ g^{-1})	23.8 ± 0.22	24.0 ± 0.12	24.2 ± 0.15	24.4 ± 0.18	24.3 ± 0.20	24.6 ± 0.16	24.8 ± 0.13

[1] Mean ± SE (N = 12) and values within the same row with different superscripts are significantly (P<0.05)
[2] NFE: nitrogen-free extract

Table 4. Nutrient and energy digestibility values of the experimental diets for juvenile grass carp (*Ctenopharyngodon idella*) at 26 ± 2 °C of water temperature

Digestibility values (%)[1]	Dietary group					
	P1L1	P1L2	P1L3	P2L1	P2L2	P2L3
Dry matter	80.1 ± 0.12	80.5 ± 0.10	79.7 ± 0.15	80.0 ± 0.10	79.8 ± 0.19	79.4 ± 0.11
Protein	93.0 ± 0.09^{ab}	93.5 ± 0.15^{a}	88.7 ± 0.12^{bc}	93.2 ± 0.07^{ab}	88.8 ± 0.16^{bc}	87.4 ± 0.14^{c}
Lipid	97.1 ± 0.13^{a}	97.6 ± 0.19^{a}	94.3 ± 0.16^{ab}	97.5 ± 0.11^{a}	94.9 ± 0.20^{ab}	91.1 ± 0.17^{b}
Fiber	89.9 ± 0.14	90.4 ± 0.20	89.4 ± 0.17	89.9 ± 0.12	89.6 ± 0.16	89.3 ± 0.10
Ash	53.2 ± 0.07	53.7 ± 0.06	52.6 ± 0.09	53.1 ± 0.05	52.9 ± 0.09	52.5 ± 0.12
NFE (nitrogen-free extract)	67.2 ± 0.04	67.5 ± 0.12	66.8 ± 0.08	67.1 ± 0.05	66.9 ± 0.07	66.3 ± 0.09
Energy	93.0 ± 0.10^{a}	93.5 ± 0.18^{a}	91.1 ± 0.13^{a}	93.2 ± 0.08^{a}	91.3 ± 0.11^{a}	87.6 ± 0.09^{b}

[1] Mean ± SE of three replicates and values within the same row with different superscripts are significantly (P<0.05)

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