

Effect of Dietary Energy to Protein Ratios on Growth Performance and Feed Efficiency of Juvenile Grass Carp (*Ctenopharyngodon idella*)

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Abstract: A feeding trial of three dietary protein (DP) (25, 35 and 45%) and four dietary lipid (DL) levels (3, 6, 9 and 12%) with different dietary energy to protein (E/P) ratios (from 5.93 to 13.84 kcal/g protein) was conducted to investigate the proper dietary E/P ratio for juvenile grass carp. After acclimation of 2 weeks by a lipid-free diet, fish were reared for 10 weeks in a water recirculation system at $22.2 \pm 2.8^\circ\text{C}$ under natural light-dark cycle. The highest growth and feed efficiency were obtained with the diet of 45% DP and 3% DL (E/P ratio of 5.93 kcal/g protein), followed closely by the group with 35% DP and 3% DL (E/P ratio of 7.62 kcal/g protein), without any significant difference between them. At the same DP level, growth and feed efficiency decreased with increasing DL level. However, at the same DL level, growth and feed efficiency enhanced with increasing DP level. But at the 9% and 12% DL level (energy of 321 and 348 kcal/100g diet), there were no significant differences between groups in terms of growth indices, irrespective of the DP level. Except intraperitoneal fat ratio, condition factor, viscera ratio and hepatosomatic index, all studied parameters showed increasing trends with enhancing dietary E/P ratio. Lipid contents of whole body and liver aroused with the increase of DL level at the same DP level. Taking into account the cost of feeding, the diet containing 35% DP and 3% DL (E/P ratio of 7.62 kcal/g protein, GE of 267 kcal/100g diet) is recognized as an optimal diet for juvenile grass carp in the present study. However, some future investigations will need to address the effects of temperature on optimal E/P ratio in grass carp.

Keywords: Grass carp; energy to protein ratio; growth; feed efficiency.

INTRODUCTION

In fish nutrition, dietary protein is the most important factor affecting growth performance of fish and feed cost [1], but excessive amount of dietary protein could cause the increase of ammonia excretion. Dietary protein utilization may be improved by partially replacing dietary protein with lipid and/or carbohydrate to benefit from protein-sparing criterion [2]. However, excessive energy in the diet can lead to increase body lipid deposition and to reduce growth [3]. Therefore, dietary energy to protein (E/P) ratio is an important index in fish feed formulation. The optimal dietary E/P ratio (kcal/g protein) has been determined in some aquatic animal species including channel catfish, 11.5 [4]; Nile tilapia, 9.1 [5]; blue tilapia, 9.3 [6]; hybrid striped bass, 8 [7]; African catfish, 6.9 [8] and Asian seabass, 7.8 [9].

As a typical herbivorous and stomachless finfish, grass carp is one of the most popular fish species for aquaculture in China throughout the year. Although protein and energy requirement of grass carp have been already studied [10-17], the data of dietary E/P ratio is not complete, especially in the cold seasons. Because grass carp normally grows slowly in lower temperatures, a study to demonstrate the optimum dietary E/P ratio would help to improve the growth of grass

carp in low temperatures. The objective of the present study was to determine the effect of dietary E/P ratio on growth, protein efficiency ratio and body composition of juvenile grass carp during the cold seasons.

MATERIALS AND METHODOLOGY

Diet Preparation

12 purified diets with different E/P ratios were prepared as described by [18], and were stored at -30°C until use. The formulation of diets was shown in Table 1. The available energy was calculated using physiological fuel value of 4.0, 4.0 and 9.0 kcal·g⁻¹ (16.7, 16.7 and 37.7 KJ·g⁻¹) for protein, carbohydrate and lipid respectively [19,20].

Supply and Maintenance of Fish

Juvenile grass carp were obtained from our own fish farm and maintained in 36 circular fiberglass tanks (120L) in a half-indoor room under natural light-dark cycle. The fish were acclimated to experimental conditions and feed for 2 weeks. During the acclimating period, fish were fed with lipid-free experimental diet (GE 2312 kcal·kg⁻¹) at 1% body weight per day (BW/d). After acclimation period, healthy fish with similar body weight were selected for the experiment, with 20 fish per tank. The fish were weighed and the differences in tanks were no more than 5%. The initial weight of fish was $3.30 \pm 0.03\text{g}$. Fish were fed four times a day (9:00AM, 12:00AM, 3:00PM, 6:00PM). The feeding rate was 2.0% BW/d. Fish were weighed every two weeks

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Table 1. Composition of Experimental Diets (% dry Weight)

Ingredients	Energy to Protein (E/P) Ratios (kcal/g protein)											
	5.93	6.53	7.13	7.73	7.62	8.39	9.16	9.93	10.63	11.70	12.77	13.84
Casein	40	40	40	40	31.2	31.2	31.2	31.2	22.4	22.4	22.4	22.4
Gelatin	10	10	10	10	17.8	17.8	17.8	17.8	5.6	5.6	5.6	5.6
Corn starch	15	15	15	15	25	25	25	25	35	35	35	35
Fish oil ¹	1.5	3	4.5	6	1.5	3	4.5	6	1.5	3	4.5	6
Corn oil	1.5	3	4.5	6	1.5	3	4.5	6	1.5	3	4.5	6
Cellulose	21.50	18.50	15.50	12.50	22.50	19.50	16.50	13.50	23.50	20.50	17.50	14.50
Vitamin mix ¹	1	1	1	1	1	1	1	1	1	1	1	1
Mineral mix ²	8	8	8	8	8	8	8	8	8	8	8	8
Ascorbic phosphate ester	1	1	1	1	1	1	1	1	1	1	1	1
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Proximate Composition (calculated / analyzed)												
Crude protein	45/44.81	45/44.98	45/44.90	45/45.19	35/34.65	35/35.03	35/34.63	35/35.49	25/25.38	25/25.30	25/25.48	25/26.07
Crude lipid	3/2.85	6/5.74	9/8.58	12/11.39	3/2.74	6/5.24	9/8.63	12/11.47	3/2.46	6/5.33	9/8.42	12/11.71
Estimated available energy (kcal/100g)	267	294	321	348	267.4	294.4	321.4	348.4	267.8	294.8	321.8	348.8
Moisture	8.79	8.66	8.59	8.48	9.15	8.75	10.01	8.94	8.20	7.09	6.95	9.23
Ash	5.60	5.49	5.63	5.67	5.52	5.56	5.62	5.73	5.65	5.61	5.66	5.62

¹Vitamin mix contained (mg.100g-1 of diet): thiamine HNO₃, 5; riboflavin, 5; vitamin A, 2500IU; vitamin E, 40; vitamin D₃, 2400IU; menadione, 4; pyridoxine HCl, 4; cyanocobalamin, 0.01; biotin, 0.6; calcium pantothenate, 10; folic acid, 1.5; niacin, 20; inositol, 200; and cellulose was used as a carrier.

²Mineral mix: (g.100g-1 diet): calcium biphosphate, 0.98; calcium lactate, 3.79; sodium chloride, 0.26; potassium sulfate, 1.31; potassium chloride, 0.53; ferrous sulfate, 0.09; ferric citrate, 0.31; magnesium sulfate, 0.35; zinc sulfate, 0.004; manganese sulfate, 0.003; cupric sulfate, 0.002; cobalt chloride, 0.003; potassium iodide, 0.0002; and cellulose 4.2.

and the daily rations were adjusted accordingly. Wasted feed and feces were removed from tanks with a rubber pipe every morning and were dried for feed intake calculation. The experiment lasted for 70 days (from October to December in Guangzhou, South China). During the experiment, the water temperature, dissolved oxygen, pH and ammonia were $22.2 \pm 2.8^\circ\text{C}$, $7.96 \pm 0.39\text{mg/L}$, 7.46 ± 0.23 and $0.14 \pm 0.05\text{mg/L}$, respectively.

Sample Collection and Chemical Analysis

At the end of the experiment, fish were weighed and weight gain (WG), specific growth rate (SGR) and feed efficiency ratio (FER) were calculated using the equals showed in footnotes in Table 2. After the final weighing, three fish were randomly sampled from each tank to determine the whole body chemical composition. Another five fish were randomly taken, killed, and body weight, body length, vis-

cera, liver and intraperitoneal fat were measured, respectively. The moisture was analyzed by drying at 105°C for 24h. Protein, lipid and ash of diets and samples were analyzed by the Kjeldhal method with Tecator Kjelte (1030-Auto-analyzer, Tecator AB, Höganäs, Sweden), Soxhlet Extraction with Tecator Soxtem (HT6, Tecator AB, Höganäs, Sweden) and combustion at 550°C for 16h, respectively.

Statistical Analysis

Possible effects of dietary energy and protein on fish performance were statistically analyzed using SPSS 9.0. The normality of data was estimated by using Percent-Percent plot method (P-P plot). Mean of each variables were analyzed using two-way ANOVA, to find the effect of dietary lipid (energy), protein and any possible interactions between lipid and protein levels. The significant ($P < 0.05$) differences

Table 2. Growth Performance and Feed Efficiency of Grass Carp Fed Diets with Different E/P Ratios¹

DP (%)	DL (%) / Energy (kcal/100g diet)	E/P Ratios	Initial (g)	FI (g/fish) ²	WG ³	SGR ⁴	FER ⁵	PER ⁶
45	3 / 267	5.93	3.28	8.59 ^c	102.20 ^d	1.00 ^d	0.39 ^f	0.95 ^d
	6 / 294	6.53	3.31	7.68 ^b	73.51 ^c	0.79 ^c	0.32 ^{de}	0.77 ^{bcd}
	9 / 321	7.13	3.32	6.67 ^a	50.14 ^{ab}	0.58 ^{ab}	0.25 ^{bc}	0.60 ^{ab}
	12 / 348	7.73	3.32	6.44 ^a	41.35 ^a	0.49 ^a	0.21 ^{ab}	0.51 ^a
35	3 / 267.4	7.62	3.32	8.45 ^c	91.86 ^d	0.93 ^d	0.36 ^{ef}	1.16 ^e
	6 / 294.4	8.39	3.31	7.24 ^b	61.94 ^{bc}	0.69 ^{bc}	0.28 ^{cd}	0.89 ^d
	9 / 321.4	9.16	3.30	6.41 ^a	41.05 ^a	0.49 ^a	0.21 ^{ab}	0.66 ^{abc}
	12 / 348.4	9.93	3.31	6.36 ^a	35.45 ^a	0.43 ^a	0.18 ^a	0.56 ^a
25	3 / 267.8	10.63	3.30	7.65 ^b	73.00 ^c	0.78 ^c	0.32 ^{de}	1.36 ^f
	6 / 294.8	11.70	3.32	6.64 ^a	46.79 ^{ab}	0.54 ^{ab}	0.23 ^{abc}	0.98 ^{de}
	9 / 321.8	12.77	3.29	6.31 ^a	37.46 ^a	0.45 ^a	0.19 ^{ab}	0.82 ^{cd}
	12 / 348.8	13.84	3.32	6.39 ^a	39.13 ^a	0.47 ^a	0.20 ^{ab}	0.85 ^{cd}
ANOVA P				<0.001	<0.001	<0.001	<0.001	<0.001
DP level				<0.001	<0.001	<0.002	<0.003	<0.001
DL (Energy) Level				<0.001	<0.001	<0.001	<0.001	<0.001
DP × DL (Energy)				<0.1	<0.2	<0.3	<0.5	<0.6
Pooled SEM				0.51	5.31	0.048	0.019	0.065

¹Mean of three replicates. Numbers with different superscripts within each column are significantly different (P<0.05).

²Feed intake (FI)

³Weight gain (WG)=(final weight – initial weight)×100/(initial weight)

⁴Specific growth rate (SGR)=(ln final weight – ln initial weight)×100/days

⁵Feed efficiency ratio (FER)=(Fish weight gain)/(feed intake)

⁶Protein efficiency ratio (PER)=(Fish weight gain)×100/(protein intake)

of each variable were detected by the one-way ANOVA test, Duncan's multiple range test was used to rank the groups.

RESULTS

After a 10-week feeding trial, no fish died. Feed intake (FI), weight gain (WG), specific growth rate (SGR), feed efficiency ratio (FER) and protein efficiency ratio (PER) are presented in Table 2 and Fig (1). All growth and feed efficiencies indices were significantly affected by the DL and DP levels. The highest FI, growth and FER were obtained with the diet containing 45% DP and 3% DL (E/P ratio of 5.93 kcal/g protein), followed closely by the group of 35% DP and 3% DL (E/P ratio of 7.62 kcal/g protein). At the same DP level, FI, WG, SGR, FER and PER decreased with the increasing DL level. However at the same DL level, FI, WG, SGR and FER increased as the DP levels increased. At the 9% and 12% DL levels (energy of 321 and 348 kcal/100g diet), the FI, WG, SGR and FER didn't show any significant differences, irrespective of the DP level. At the same DL level, PER decreased with the increase of DP level and the highest PER was obtained by the group with 25% DP and 3%DL (E/P ratio of 10.63kcal/g protein).

Data related to condition factor (CF), viscera percentage (VP), hepatosomatic index (HSI) and intraperitoneal fat ratio (IPF ratio) are presented in Table 3. CF was significantly affected by DP level (P<0.05), but not by DL level. With the increase of E/P ratio, CF showed an increasing trend. VP and HSI were affected significantly both by DP and DL (energy) level, and they both showed an increasing trend with the arising dietary E/P ratio. IPF ratio was not affected by either DP or DL levels.

The whole body and liver composition are shown in Table 4. Neither dietary DP nor DL levels had any significant effect on crude protein of whole body (P>0.05). However, lipid content of whole body and liver was affected significantly by DP and DL level and tended to enhance with the increase of DL level at the same DP level. The moisture content of whole body and liver were significantly affected only by DL level, and its trend was inversely related to that of lipid content in liver.

DISCUSSION

The main purpose of this experiment was to investigate the utilization of dietary protein and energy of grass carp under low temperature. The water temperature in the present

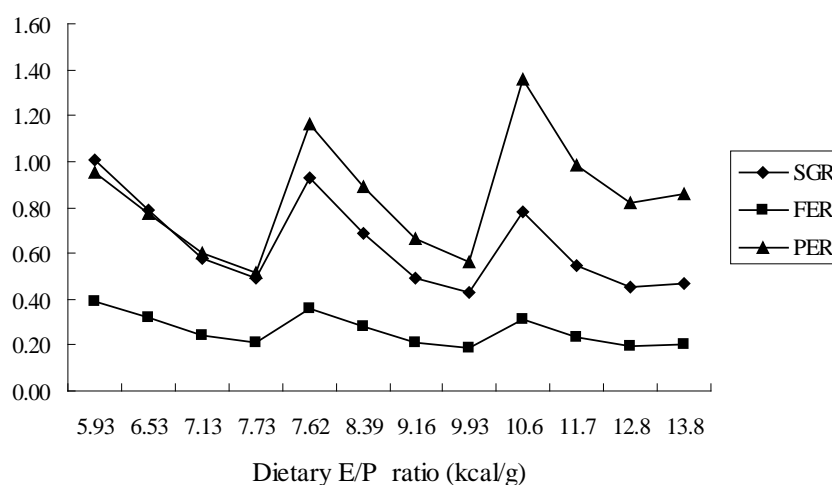


Fig. (1). Relationship of SGR, FER and PER to dietary E/P ratio.

Table 3. Biological Measurement of Grass Carp Fed Diets with Different E/P Ratios¹

DP (%)	DL (%) / Energy (kcal/100g diet)	E/P Ratios	CF ²	VP ³	HSI ⁴	IPF Ratio ⁵
45	3 / 267	5.93	2.12 ^a	9.66 ^a	3.01 ^a	2.13
	6 / 294	6.53	2.21 ^{ab}	10.17 ^{ab}	3.19 ^{ab}	2.27
	9 / 321	7.13	2.10 ^a	10.80 ^{bcd}	3.37 ^{abc}	2.22
	12 / 348	7.73	2.13 ^a	10.24 ^{abc}	3.23 ^{ab}	2.02
35	3 / 267.4	7.62	2.17 ^{ab}	10.27 ^{abc}	3.33 ^{abc}	2.08
	6 / 294.4	8.39	2.15 ^a	10.56 ^{abc}	3.49 ^{abc}	2.30
	9 / 321.4	9.16	2.20 ^{ab}	10.85 ^{bcd}	3.75 ^{bcd}	2.26
	12 / 348.4	9.93	2.18 ^{ab}	11.62 ^{de}	3.90 ^{cd}	2.61
25	3 / 267.8	10.63	2.29 ^b	11.22 ^{cde}	3.49 ^{abc}	2.77
	6 / 294.8	11.70	2.28 ^b	11.71 ^{de}	4.20 ^d	2.35
	9 / 321.8	12.77	2.30 ^b	12.18 ^e	4.31 ^d	2.81
	12 / 348.8	13.84	2.23 ^{ab}	12.13 ^e	4.30 ^d	2.50
ANOVA P			<0.02	<0.001	<0.001	<0.2
DP level			<0.001	<0.001	<0.001	\
DL (Energy) Level			<0.8	<0.004	<0.004	\
DP × DL (Energy)			<0.5	<0.6	<0.6	\
Pooled SEM			0.04	0.31	0.17	0.19

¹ Mean of three replicates. Numbers with different superscripts within each column are significantly different (P<0.05).

² Condition factor (CF)=Fish weight (g) × 100/body length³ (cm)

³ Viscera percentage (VP)= Viscera weight × 100/fish weight

⁴ Hepatosomatic index (HSI)=Liver weight × 100/fish weight

⁵ Intraperitoneal fat ratio (IPF ratio)= Intraperitoneal fat weight × 100/fish weight

study (22°C) was lower than the optimum temperature (about 27-28°C) of grass carp, and it was the main reason that the growth rate of this experiment was lower than those of summer [21]. But it was the real condition in grass carp aquaculture from October to December in south China and

results still seem helpful for practical aquaculture for this part of the year.

In the present study, feed intake and growth performance of grass carp decreased as DL level increased at the same DP

Table 4. Composition of Whole Body and Liver in Grass Carp Fed Diets with Different E/P Ratios¹

DP (%)	DL (%) / Energy (kcal/100g diet)	E/P Ratios	Whole Body			Liver	
			Moisture	Protein	Lipid	Moisture	Lipid
45	3 / 267	5.93	77.84 ^{bc}	13.88	4.00 ^a	69.87 ^f	4.30 ^{ab}
	6 / 294	6.53	76.57 ^{bc}	14.56	4.04 ^a	68.80 ^{def}	6.28 ^{cde}
	9 / 321	7.13	78.84 ^c	13.65	4.99 ^b	67.28 ^{cd}	7.53 ^e
	12 / 348	7.73	77.37 ^{bc}	14.55	5.20 ^b	65.12 ^b	11.03 ^f
35	3 / 267.4	7.62	78.16 ^{bc}	13.77	4.01 ^a	69.63 ^f	3.39 ^a
	6 / 294.4	8.39	77.20 ^{bc}	14.11	6.36 ^{cd}	69.27 ^{ef}	4.41 ^{abc}
	9 / 321.4	9.16	76.52 ^{bc}	13.94	6.60 ^d	67.32 ^{cd}	6.01 ^{bcd}
	12 / 348.4	9.93	75.32 ^{ab}	14.41	7.07 ^d	63.28 ^a	10.94 ^f
25	3 / 267.8	10.63	77.30 ^{bc}	13.10	5.74 ^{bc}	68.97 ^{def}	5.13 ^{abcd}
	6 / 294.8	11.70	77.62 ^{bc}	12.99	5.22 ^b	67.70 ^{cde}	6.02 ^{bcd}
	9 / 321.8	12.77	76.64 ^{bc}	13.32	5.48 ^b	67.00 ^c	6.97 ^{de}
	12 / 348.8	13.84	73.81 ^a	14.62	6.76 ^d	63.96 ^{ab}	9.97 ^f
ANOVA P			<0.04	<0.1	<0.001	<0.001	<0.001
DP level			<0.2	\	<0.001	<0.2	<0.04
DL (Energy) Level			<0.03	\	<0.001	<0.001	<0.001
DP × DL (Energy)			<0.2	\	<0.001	<0.4	<0.3
Pooled SEM			0.86	0.41	0.26	0.55	0.59

¹ Mean of three replicates. Numbers with different superscripts within each column are significantly different (P<0.05).

level, and the best growth was obtained in diet containing the lowest GE. It suggests that grass carp has low capacity to utilize dietary lipid as an energy source which is in agreement with the previous studies [18, 21]. Lovell [1] demonstrated when fish was fed a diet containing excess energy, growth might be reduced due to reduced feed consumption. In the present study, the uneaten feed was also found at the bottom of groups fed diet containing DL level exceeding 9% (GE exceeding 321 kcal/100g diet) which was also represented by the data of decreased feed intake in Table 2. It is assumed that fish, as well as homeothermic animals [22] can adjust their feed intake to satisfy their digestible energy requirements [23, 24]. Higher water temperature increases the demand for energy for maintenance and activity of fish [25]. In contrast, Ng *et al.* [26] demonstrated that low water temperature decreased the digestibility of dietary lipids, especially saturated fatty acids, whereas increase the content of triglyceride in the fecal lipids of rainbow trout. Taking into account the above information, low water temperature of the present study might reduce the energy requirement of fish and high dietary lipid level (or energy level) may not be suitable for grass carp in cold reason. On the other hand, since the food of grass carp in natural environment is water plant

with low energy, the relative low capacity to utilize dietary lipid in grass carp is conceivable.

Protein-sparing effect of dietary lipid was not obviously found in the present study. When DL level was 3% or 6%, growth performance of groups with higher DP and lower DL levels were always better than that of groups with lower DP and higher DL levels. Meanwhile, when DL level was 9% or 12%, the growth rate of all treatments were not significantly different, but are much lower than the groups with lower DL level.

Our summer experiments [21] showed that grass carp was sensitive to DL level, and high DL content would induce excess lipids accumulation of liver. This result was also consistent with the results from the present experiment in cold season. With the increase of dietary lipid level, the lipid content in liver increased rapidly, even faster than the increase of our summer data. It is suggested that in the cold season, the energy utilization of grass carp is lower than that in warm season and it also implies that hepatic symptom induced by high fat diet would be more easily happened in cold season.

Improved growth and feed efficiency ratio with increasing dietary protein level is well known in many species [27].

In the present study, WG, SGR and FER were also improved with the increasing DP level at the DL levels below 12%. Our previous work (unpublished data) found the optimum protein requirement of juvenile grass carp is 38% when water temperature is 25.5°C. In the present study, 35% DP level was similar with the optimum protein requirement attained from our previous work, and the higher protein level (45%) might have no additional growth response. Millikin [28] obtained that PER of striped bass increased as both dietary protein and lipid increased from 37 to 57% and 7 to 17%, respectively. However, in the present study, PER of grass carp decreased as both dietary protein and lipid increased from 25 to 45% and 3 to 12%, respectively. This result also differs from yellowtail [29], red drum [3] and partly differs from Atlantic cod [30], in which PER had negative and positive correlation with dietary protein and lipid level, respectively. Considering the different feeding habit of fish, it is suggested that herbivorous grass carp has a lower capacity to utilize excess protein and lipids than other carnivorous or omnivorous fish.

Dietary E/P is an important criterion in fish feed formulation. Optimum dietary E/P ratios for some fish species were investigated and the estimated ratios range from 8.9 to 12.3 kcal/g protein [27]. In the present study, the maximum growth and feed efficiency ratio was obtained in the group with 45% DP, 3% DL and E/P ratio of 5.93 kcal/g protein and the group with 35% DP, 3% DL and E/P ratio of 7.62 kcal/g protein. These E/P ratios are lower than the optimum E/P ratios of several species investigated [27], and it confirms again that grass carp has lower dietary energy requirement. According to Table 1 and the Fig. (1), it is obvious that the growth of fish didn't correlate with E/P ratio linearly, which indicate that actual amount of dietary protein and energy must be carefully considered when optimum E/P ratio is estimated. This is also similar to the observation on rockfish [31].

The results of this study indicate that at cold season, juvenile grass carp grows well by the diets containing 45% protein, 3% lipid, E/P ratio of 5.93 kcal/g protein and GE of 267 kcal/100g diet or 35% protein, 3% lipid, E/P ratio of 7.62 kcal/g protein and GE of 267 kcal/100g diet. Taking into account the cost of feeding, the latter diet is recognized as an optimal one for juvenile grass carp in the present study.

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Received: November 17, 2008

Revised: January 05, 2009

Accepted: January 12, 2009

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