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Effects of dietary protein and lipid levels on growth, feed utilization and meat quality of clown knifefish (*Chitala chitala*)

Tran Thi Thanh Hien, Lam My Lan, and Tran Le Cam Tu
College of Aquaculture and Fisheries, Can Tho University, Viet Nam
**Correspondence: Tran Thi Thanh Hien (email: ttthien@ctu.edu.vn)*

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ABSTRACT

Effects of dietary protein and lipid levels on growth, feed utilization and fillet quality of clown knifefish (Chitala chitala) were implemented to determine the protein and energy requirements of this species at 400 g stage. A 4 x 3 factorial design with four dietary protein levels (25%, 30%, 35% and 40%) and three dietary lipid levels (6, 9, and 12%) corresponded to 3 energies levels 18, 19 and 20 MJ/kg. Clown knifefish (407 ± 5.0 g) was stocked at a density of 10 fish/m^2. The experiment was arranged in a completely randomized design in triplicate tanks for 8 weeks. There was a significant interaction effect (P<0.05) between protein and lipid levels on weight gain and specific growth rate with the fish growth being highest in the 35 CP-9% lipid treatment. The survival rate of fish was not affected (P>0.05) by dietary protein and lipid levels and ranged from 93.3 to 100%. The feed efficiency of fish was influenced by dietary protein or lipid content as well as the interaction between these two factors. Protein utilization efficiency increased as protein content increased, and diet contained t 35% protein and 9% lipid, enhanced chewiness and hardness of fish paste. These results showed that clown knifefish fed 32.8% dietary protein and 19 MJ energy with a P/E ratio of 18.6 had the optimal growth and the best quality of fish meat.

1. INTRODUCTION

Feed accounts for about 70% of the total production cost in aquaculture, and its protein content is the most important factor affecting both feed cost and the growth of aquatic animals (Lovell, 1989). The protein requirements of aquatic animals range from 30% to 50%, and they are influenced by their feeding preferences, which are largely determined by whether they are herbivores, omnivores, or carnivores. Increasing the protein content of the feed often improves the performance of fish, especially carnivorous fish, because the protein requirements of this group of fish are much higher than those of herbivorous fish (Hien & Tuan, 2009). However, using protein as an energy source is undesirable because it increases feed costs due to the higher cost of protein compared to lipids and carbohydrates, and it can also lead to the release of ammonia when amino acids are converted to energy (National Research Council [NRC], 2011). Dietary protein not only provides essential amino acids (EAA) for protein synthesis of fish body, but is also the main component of metabolically active substances in the body (Collins et al., 2013; Yadata et al., 2020; Wu et al., 2020), so it is the main factor affecting fish physiological responses (Lee et al., 2002; Liu et al., 2013a, 2013b). Consequently, the optimal dietary protein level in fish diets needs to be chosen carefully because fish growth can be restricted by a diet insufficient in protein (Jin et al., 2015), while excess dietary protein will increase feed cost and ammonia nitrogen excretion, posing a threat to the environment and fish health (Wu & Gatlin, 2014; Barreto-Curiel et al., 2019). Replacing some of the protein in feed with lipids and carbohydrates can improve the efficiency of protein use for growth (Garling & Wilson, 1976), and the addition of lipids as an energy source in feed is more efficient than carbohydrates because lipids are used more efficiently as an energy source in fish (NRC, 1993). The energy sharing of lipids and carbohydrates for protein as well as the ratio of protein to the energy of several fish species have been studied (Cho & Kaushik, 1990; Lee & Lee, 2005). The optimal protein to energy ratio in feed for aquatic animals varies by species and developmental stages, and normally ranges from 20 to 25 g DP/ KJ DE (Wang et al., 2006). The DE/DP ratio in Atlantic salmon, *Salmo salar* L. suggests that this species has a good lipid utilization capacity of 20 g DP/KJ DE (Einen & Roem, 2003), while the ratio of carnivores is relatively poor tolerant of dietary lipid, such as Asian sea bass (*Lates calcarifer*), cobia (*Rachycentron canadum*), snakehead (*Channa striata)* (Cattacuta & Coloso, 1997; Webb et al., 2010; Hien et al, 2020). Some feed species have low P/E ratio with high lipid content, improved protein utilization efficiency, but results in undesirable level of lipid deposition (Tibbett et al., 2005) which may have long term implications in terms of final quality or health of wildlife. Other studies found that protein requirements in fish diet decrease as the fish grow larger. The protein requirement for channel catfish (*Ictalurus punctatus*) is 35% for small fish sizes of 14-100 g, but drops to 25% for larger fish sizes of 114-500 g (Page & Andrews, 1973). Similar results have been reported for salmon, carp, and tilapia (Wilson & Halver, 1986). Research results on optimal DP:DE ratio were reported sea bream *(Sparus aurata L.*) (Lupatsch et al., 2001), snakehead (Hien et al., 2020), striped catfish (*Pangasianodon hypophthalamus)* (Glencross et al., 2011).

Clown knifefish inhabit a wide range of freshwater bodies (Menon, 1999), and feed on aquatic invertebrates, mollusks, shrimp and small fish (Rahman, 1989). The wide tolerance range for temperature, dissolved oxygen, and pH, make this species a potential candidate for aquaculture with a maximum growth up to 1–2 kg/year has been reported under polyculture system (Sarkar et al., 2006). The quality of fish at harvest greatly affects the quality of fish meat when making fish paste. Many studies have shown that feed composition affects not only the chemical composition of the fish but also affects the ratio of fillet and the chewiness of fish (NRC, 2011).

In Viet Nam, clown knifefish are commonly farmed in the Mekong Delta, where fish can reach an average weight of 0.5–0.8 kg after 8–10 months. Clown knifefish are usually weaned to formulated feed at around 25 days after hatching and protein requirements for fish at the 2–5 g stage are 45% (Hien et al., 2013), and at the 50–100 g stage are 38.6% (Lan et al., 2014). The objectives of this study was to determine the protein and energy requirements for growth, feed efficiency and quality of clown knifefish at the 400 g stage, at around which time they are harvested.

2. METHODOLOGY

2.1. Experimental fish

Clown knifefish with an average initial weight of 407 ± 5.0 g, and without visible signs of disease or deformities, were transferred from ponds at the Fish hatchery Center, Can Tho city, Viet Nam to a 100 m^2 hapa net located in a 1,000 m^2 pond of 1.5 m in depth, in order to adapt to the water conditions of the feeding experiment.

2.2. Experimental diets

The experiment was conducted in a 4 x 3 factorial design with four dietary protein levels (25, 30, 35 and 40 %), and three dietary lipid levels (6, 9, and 12%), corresponding to 3 energy levels 18, 19 and 20 MJ/kg (Table 1). Each treatment had three replicates. The experimental diets were formulated from fishmeal, soybean meal, cassava meal, fish oil premix (Table 1). All ingredients were finely ground and 30% water added, then pelleted (3 mm diameter) and dried in an oven at 45ºC for 12 h to reach moisture of about 10%. All experimental diets were stored in a freezer at -20ºC during the experiment. The chemical compositions of all dietary treatments were shown in Table 2.

Table 1. Composition of the experimental diets *(all values are percentages)*

Lipid (%)	Protein (%)	Ingredients					
		Fish meal[1]	Soybean meal[2]	Cassava meal[3]	Fish oil[4]	CMC[5]	Premix[6]
6	25	18.94	25.80	45.44	3.49	4.32	2.0
	30	22.76	31.00	36.57	3.06	4.60	2.0
	35	26.58	36.20	27.70	2.63	4.88	2.0
	40	30.40	41.40	18.84	2.2	5.16	2.0
9	25	18.95	25.80	44.37	6.5	2.38	2.0
	30	22.76	31.00	35.50	6.07	2.66	2.0
	35	26.58	36.20	26.63	5.64	2.94	2.0
	40	30.40	41.40	17.77	5.21	3.23	2.0
12	25	18.95	25.81	43.30	9,50	0.44	2.0
	30	22.77	31.01	34.43	9.07	0.73	2.0
	35	26.58	36.21	25.56	8.64	1.01	2.0
	40	30.40	41.41	16.70	8.21	1.29	2.0

*[1]Ca Mau Fishmeal, [2]soybean meal, *Premix: Bio-premix C-1 AG: vitamin A (6,000,000 IU), vitamin D3 (2,000,000 IU), vitamin E (50,000 mg), vitamin K_3 (6,000 mg), vitamin B1 (11,000 mg), vitamin B2 (7,000 mg), vitamin B6 (8,000 mg), vitamin C Stay (50,000 mg), inositol (5,000 mg), acid folic (3,000 mg), biotin (500 mg), pantothenic acid (35,000 mg), niacine (60,000 mg), Cu (34,000 mg), Fe (50,000 mg), Mn (12,000 mg), Zn (125,000 mg), I (500 mg), Co (250 mg), Se (200 mg), CMC: carboxymethyl cellulose.*

2.3. Experiment system

The experiment was carried out in a system of 36 hapas of 1m x 1m in cross section, a depth of 1.5 m, and a water volume of 1 m^3. Clown knifefish were stocked at a density of 10 fish/m^2. The experiment was arranged in a completely randomized design and lasted for 8 weeks.

Table 2. Chemical compositions of the experimental diets (% dry matter)

Treatment	25P-6L	30P-6L	35P-6L	40P-6L	25P-9L	30P-9L	35P-9L	40P-9L	25P-12L	30P-12L	35P-12L	40P-12L
Protein	24.51	30.37	36.10	41.14	24.98	28.93	35.21	39.56	25.59	30.14	35.29	40.21
Lipid	5.87	5.73	5.82	5.86	8.92	8.94	8.99	9.03	11.75	11.86	11.82	12.16
Ash	9.15	9.82	11.60	12.85	9.25	10.00	11.59	12.25	9.30	10.85	11.66	12.78
Energy (KJ g^{-1})	18.05	18.09	18.06	18.12	18.89	18.96	18.93	19.03	19.89	19.91	20.06	20.12
P/E ratio	13.82	16.79	19.98	22.70	13.22	15.26	18.60	20.79	12.87	15.14	17.59	19.99

2.4. Experimental management and sample analysis

Fish were fed twice daily (at 8:00 and 16:00) and observed during feeding. The amount of food consumed by the fish was recorded, and uneaten feed was removed after the one-hour feeding period for further feed intake calculation.

Monitoring environmental factors: Water temperature was measured daily and ranged from 28˚C to 34˚C. Water pH, dissolved oxygen (DO), NO_2^--N, TAN were measured weekly using test kits (SERA, Germany). Dissolved oxygen ranged from 5.5 to 6.4 mg/L, the contents of NO_2^--N concentrations varied from 0.04 to 0.06 mg/L, and the TAN were from 0.04 to 0.1 mg/L.

Fish samples: Fish in each hapa were totally weighed at monthly intervals to determine weight gain. At the end of the 8-week feeding period, three fish from each hapa were randomly sampled and killed using ice water for 30 minutes, and then stored at -20ºC for further analyses.

The moisture, ash, fiber, protein, fat, and carbohydrate (NFE) of feed and fish were determined using standard methods (AOAC, 2000). Energy content was measured with a Parr 6100 calorimeter machine.

2.5. Methods for fish meat quality analysis

Preparation of clown knifefish paste: fillet the fish, scrape the flesh and pound it for 15 min then shape the paste into round cylinders of 25 mm in diameter and 25 mm in height (Pons, 1996) to measure the texture. For the texture measurement of the fish balls, all the fish balls were cooled to 5°C and kept in an insulated container containing ice (Park, 2000) to ensure temperature stability.

Measure the structure of the fish balls with the Texture Analyzer (TA - XT2*i*), based on the compression force (using the P/75 probe), the deformation 60% compared to the original height of the fish ball. From the TPA (Texture Profile Analysis) curves of the structural measurement to calculate the hardness, springiness and chewiness of fish muscle.

2.6. Calculations and statistical analysis

The initial mean weight (W_i) and final mean weight (W_f) of individual fish were determined before and after the experiment, respectively. The survival rate (SR), weight gain (WG), feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), and net protein utilization (NPU).

SR (%) = (number of fish at harvest)/(number of fish at stock) × 100

DWG (g/day) = $(W_f - W_i)/t$

SGR (%/day) = $(\ln W_f - \ln W_i) \times 100/t$

FCR = dry food fed /wet weight gain)

PER = $(W_f - W_i)$/protein intake

NPU = (the amount of protein in the final fish – the amount of protein in the initial fish)/the amount of protein intake.

Where W_i is the initial weight of fish (g), W_f is the final weight of fish (g), t is the experimental period (day).

2.7. Data analysis

Treatment means were compared using one or two-way ANOVA and the Duncan test with a significance level of $P < 0.05$. For the protein and lipid requirements, after considering the interaction of the two factors, the difference of each feed was examined to select the best treatment. Data were processed using the SPSS program Version 20.0.

The optimum protein level was estimated using second-degree polynomial regression analysis ($Y = ax^2 + bx + c$) as described by Zeitoun et al. (1976).

3. RESULTS

3.1. Growth and survival

There was an interaction effect between dietary protein and lipid levels in final mean weight, weight gain and specific growth rate of fish (P<0.05). Fish growth was affected by dietary protein or lipid levels (P<0.05). Fish fed the diets containing 9% lipid had significantly higher weight gain and specific growth rates at corresponding dietary protein levels than those fed diets containing 6% and 12% lipid, except for a dietary protein level of 25%, where weight gain and specific growth rate were similar for diets containing 9% and 12% lipid (Table 3). At a dietary lipid level of 9%, weight gain and specific growth rate increased linearly with dietary protein level from 25% to 35%, but then dropped sharply at a dietary protein level of 40%. Consequently, the highest weight gain and specific growth rate were found at a dietary protein level of 35% and lipid level of 9% (Table 3). Survival rates ranged from 93.3% to 100%, and there was no significant difference (p>0.05) among treatments.

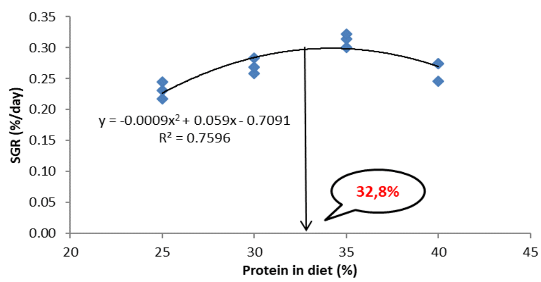

Figure 1. Relationship between specific growth rate and dietary protein content in clown knifefish (>400 g) with dietary lipid level of 9%

At 9% lipid level, when considering the correlation between protein and SGR through the equation $y= -0.0009 x^2 + 0.059 x - 0.7091$ ($r^2= 0.76$). It showed that at 32.8% protein level, the SGR of fish was 0.26%/day (Figure 1).

Table 3. Weight gain (g) and specific growth rate (%) of clown knifefish fed diets with different lipid and protein contents

Lipid (%)	Protein (%)	Wi (g)	Wf (g)	WG (g)	SGR (%/day)	Survival rate (%)
6	*25*	408±1.53	444±2.91[ab]	35.67±1.45[ab]	0.16±0.007[ab]	93.3±11.55[a]
	30	409±3.93	448±3.93[abc]	39.00±0.00[ab]	0.18±0.000[b]	96.7±5.77[a]
	35	407±5.84	448±5.51[abc]	40.67±0.67[bc]	0.19±0.006[bc]	96.7±5.77[a]
	40	407±5.33	450±5.90[abc]	42.33±6.33[bcd]	0.19±0.028[bcd]	96.7±5.77[a]
9	*25*	407±4.04	458±6.35[cd]	50.00±2.31[def]	0.23±0.006[cdf]	96.7±5.77[a]
	30	408±1.76	468±0.58[de]	59.67±1.67[f]	0.27±0.007[f]	100±0.00[a]
	35	407±2.19	478±1.45[de]	70.33±1.20[g]	0.31±0.006[g]	96.7±5.77[a]
	40	408±4.00	467±4.04[de]	59.00±2.08[ef]	0.26±0.007[e]	96.7±5.77[a]
12	*25*	407±2.19	458±3.53[cd]	51.00±5.69[de]	0.23±0.024[de]	100±0.00[a]
	30	408±2.19	461±3.76[cd]	52.33±1.86[ef]	0.24±0.007[e]	96.7±5.77[a]
	35	403±1.00	453±0.50[bc]	49.50±1.50[cde]	0.23±0.005[cde]	100±0.00[a]
	40	408±1.53	438±3.18[a]	29.67±3.33[a]	0.14±0.013[a]	93.3±5.77[a]
	25	407±1.41	453±3.31	45.89±3.14[ab]	0.21±0.014[ab]	95.6±7.26[a]
	30	408±1.40	459±3.37	50.33±3.11[bc]	0.23±0.013[bc]	97.8±4.41[a]
	35	406±2.17	460±5.47	54.00±4.98[c]	0.24±0.020[c]	97.5±4.63[a]
	40	408±1.98	451±4.82	43.67±4.76[a]	0.20±0.021[a]	95.6±5.27[a]
6		408±1.92	447±2.12[a]	39.42±1.58[a]	0.18±0.007[a]	95.8±6.69[a]
9		408±1.36	468±2.66[b]	60.00±2.22[b]	0.27±0.009[b]	96.7±4.92[a]
12		407±1.02	452±3.28[a]	45.27±3.44[a]	0.21±0.015[a]	97.3±4.67[a]
Protein		ns	0.063	0.004	0.002	ns
Lipid		ns	0.000	0.000	0.000	ns
Protein x Lipid		ns	0.007	0.000	0.000	ns

Values are represented as mean ± standard deviation. Different letters in the same column represented a significant difference (P<0.05).

3.2. Feed utilization

The feed conversion ratio (FCR) was consistently lower for diets containing 9% lipid than for diets containing 6% and 12% lipid, except at a protein level of 40%, when the FCR was not significant difference for the 9% and 6% treatments (P>0.05) (Table 4); the lowest FCR was observed in treatment 35-9 (Table 4). For diets containing 6% lipid, the FCR tended to decrease with increasing protein levels in the feed, whereas, FCR tended to increase with increasing protein level in diets containing 12% lipid (Table 4). Both the protein efficiency ratio (PER) and net protein utilization (NPU) were significantly higher in treatments containing 9% lipid compared to those with 6% and 12% lipid, except for a sharp drop in both PER and NPU at a dietary protein level of 40% in the 9% lipid treatment (Table 4). There was little or no change in either PER and NPU with increasing dietary protein level in diets containing 6% lipid, while in diets containing 12% lipid, both PER and NPU progressively decreased with increasing dietary protein levels.

Table 4. Feed conversion ratio (FCR), protein efficiency ratio (PER) and NPU

Lipid (%)	Protein (%)	FCR	PER	NPU (%)
6	25	3.81±0.11^{e}	1.07±0.03^{c}	16.10±0.43
	30	2.72±0.17^{c}	1.22±0.08^{c}	18.45±1.34
	35	2.66±0.01^{c}	1.04±0.01^{c}	15.69±0.09
	40	2.29±0.17^{bc}	1.07±0.09^{c}	16.25±1.34
9	25	2.31±0.02^{bc}	1.73±0.01^{d}	26.02±0.37
	30	2.11±0.09^{ab}	1.65±0.07^{d}	25.14±0.99
	35	1.74±0.13^{a}	1.65±0.11^{d}	25.47±1.91
	40	2.28±0.16^{bc}	1.12±0.07^{c}	17.091.07
12	25	3.18±0.20^{d}	1.24±0.08^{c}	18.54±1.12
	30	3.17±0.16^{d}	1.05±0.05^{c}	15.96±0.65
	35	3.52±0.13^{de}	0.81±0.03^{b}	12.32±0.45
	40	4.50±0.19^{f}	0.56±0.02^{a}	8.48±0.40
	25	3.10±0.23^{b}	1.35±0.10^{b}	20.22±1.53
	30	2.67±0.17^{a}	1.31±0.09^{b}	19.85±1.47
	35	2.53±0.27^{a}	1.21±0.14^{ab}	18.51±2.19
	40	3.02±0.38^{b}	0.92±0.10^{a}	13.94±1.46
6		2.87±0.18^{b}	1.10±0.03^{a}	16.62±0.53^{b}
9		2.11±0.08^{a}	1.54±0.08^{b}	23.43±1.22^{c}
12		3.60±0.19^{c}	0.92±0.09^{a}	13.96±1.29^{a}
P value				
Protein		0.001	0.000	0.130
Lipid		0.000	0.000	0.004
Protein x Lipid		0.000	0.000	0.529

Values were represented as mean ± standard deviation. Different letters in the same column represented a significant difference (P<0.05).

3.3. Proximate composition and meat quality of clown knifefish

Body protein levels were almost identical for all dietary protein levels for fish fed diets containing 6% and 9% lipid, but fish fed diets containing 12% lipid had significantly lower body protein levels, and they declined with increasing dietary protein content (Table 5). Body lipid contents were quite low (<6%) in all dietary treatments, and tended to increase with increasing dietary protein in fish fed diets containing 9% lipid, and decrease with increasing protein level in fish fed diets containing 6% and 12% lipid. Changes of protein and lipid levels in diets had no effect (p>0.05) on the moisture and mineral contents of fish (Table 5).

The chewiness of fish meat is affected by its hardness and cohesion. The influence of protein, lipid content and the interaction of protein and lipid on the hardness and chewiness were significantly different among treatments (P<0.05). Hardness and chewiness were highest in fish feeding a diet containing 35% protein-9% lipid. At the same dietary lipid level, when the protein content increased from 25 to 40%, the strain and muscle chewiness increased, the highest in the 35% protein treatments, but when the protein increased (40%) the chewiness increased, the hardness tends to decrease and the difference is not statistically significant (9% lipid); decreased and the difference was statistically significant (12% lipid). When the lipid content in the feed increased from 6 to 9%, the accumulation of lipids in the fish muscle also increased, resulting in increased chewiness and hardness.

Table 5. Proximate composition of clown knifefish

Lipid (%)	Protein (%)	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
6	25	75.1±0.51	15.01±0.02	5.27±0.08[f]	4.92±0.01
	30	75.6±0.24	15.08±0.10	3.68±0.10[bc]	4.99±0.02
	35	75.6±0.65	15.08±0.15	3.55±0.02[abc]	4.98±0.04
	40	73.9±1.12	15.10±0.08	3.27±0.10[ab]	4.97±0.01
9	25	75.5±1.03	14.99±0.08	3.08±0.09[a]	5.00±0.06
	30	75.2±0.95	15.27±0.05	4.27±0.22[de]	4.94±0.04
	35	72.6±1.11	15.40±0.13	4.76±0.07[e]	4.98±0.11
	40	74.2±0.61	15.30±0.06	4.49±0.15[de]	5.01±0.07
12	25	75.0±1.39	14.98±0.08	5.41±0.30[f]	4.96±0.02
	30	73.7±0.57	15.20±0.15	5.37±0.23[f]	4.95±0.06
	35	74.9±0.64	15.27±0.02	4.59±0.05[e]	5.01±0.06
	40	72.8±0.29	15.28±0.12	3.98±0.25[cd]	5.02±0.05
	25	75.2±0.53	14.99±0.03[a]	4.59±0.39[c]	4.96±0.02
	30	74.8±0.43	15.18±0.06[b]	4.44±0.27[ab]	4.96±0.02
	35	74.3±0.67	15.25±0.08[b]	4.26±0.21[ab]	4.99±0.04
	40	73.6±0.43	15.23±0.05[b]	3.92±0.20[a]	5.00±0.03
6		75.0±0.37	15.07±0.04	3.94±0.24[a]	4.97±0.01
9		74.4±0.53	15.24±0.06	4.15±0.20[a]	4.98±0.03
12		74.0±0.47	15.17±0.06	4.86±0.22[b]	4.98±0.02
P value					
Protein		0.134	0.016	0.00	0.726
Lipid		0.314	0.057	0.00	0.916
Protein x Lipid		0.261	0.708	0.00	0.929

Values were represented as mean ± standard deviation. Different letters in the same column represented a significant difference (P<0.05).

Table 6. Meat quality of clown knifefish fed diets containing different levels of protein and lipid

Lipid (%)	Protein (%)	Hardness (g/)	Chewiness (g/)
6	25	502±6.08	504±21.3a
	30	540±2.19	554±3.71ab
	35	551±27.7	564±3.53ab
	40	585±26.0	600±38.3b
9	25	530±4.33	630±22.2bc
	30	634±3.51	712±14.7cd
	35	642±9.29	751±22.9d
	40	605±28.5	684±42.6cd
12	25	538±16.4	553±18.3ab
	30	567±44.0	585±16.9ab
	35	625±22.5	697±9.00cd
	40	482±56.2	498±48.9a
	25	523±7.52[a]	562±21.1[a]
	30	580±19.0[ab]	617±25.1[a]
	35	604±18.7[b]	667±32.3[b]
	40	557±27.3[ab]	594±34.5[a]
6		545±12.1[a]	555±13.9[a]
9		603±14.8[b]	694±17.7[b]
12		546±23.2[a]	573±24.8[a]
P value			
Protein		0.008	0.001
Lipid		0.001	0.000
Protein x Lipid		0.053	0.000

Values are represented as mean ± standard deviation. Different letters in the same column represented a significant difference (p<0.05).

4. DISCUSSION

The protein and lipid requirements of fish vary by species and stage of development. In this study, the protein and lipid requirements of clown knifefish of around 400 g in weight were 35% protein and 9% lipid, corresponding to an energy level of 19 MJ/kg. At lipid levels of 6 and 9%, fish growth increased from protein levels of 25-35%, and increasing protein levels to 40% did not improve growth in the 6% lipid diet, and growth was greatly reduced in fish fed the 9% dietary lipids. Previous studies have shown that non-protein energy promotes protein utilization for body growth rather than energy consumption (Ward et al., 2003; Yuan et al., 2010; Jiang et al., 2015), a process known as 'protein sparing' (Lee et al., 2002). The protein-sparing effect of lipids has been well demonstrated in black sea bream *(Acanthopagrus schlegelii)* (Wang et al., 2019), cobia *(Rachycentron cannadum)* (Wang et al., 2005), Atlantic salmon *(Salmo salar)* (Mock et al., 2018). However, in our study, the growth of clown knifefish fed different levels of protein was significantly higher at a lipid level of 9% compared with their growth at corresponding dietary protein levels with 6% lipid. In the other hand, raising lipid content to 12% in the diet (corresponding to energy 20 MJ/kg feed) significantly reduced growth rates at corresponding dietary protein levels. Tu and Hien (2006) studied the energy sharing of lipids for protein in the feed of climbing perch (*Anabas testudineus*) and found no energy sharing of lipids for protein. In comparison with protein requirements of clown knifefish at different stages, the results in the present work revealed that the protein requirement of clown knifefish gradually decreased with the growth stage of fish, for instance, 45% dietary protein at the 2-5 g stage (Hien et al., 2013), 50-100 g (38.6% dietary protein) (Lan et al., 2014). The protein requirement of 400 g clown knifefish in this study was 32.8%, and similar with that found in 200 g clown knifefish (33.6%) (unpublished data). Small fish have a fast growth rate, so they need a higher protein level than large fish. For tilapia the protein requirement is 45-50% for first feeding larvae, 35-40% for fry and fingerlings (0.02-10 g), 30-35% for juveniles (10.0-25.0 g), and 28-30% for on-growing (>25.0 g) ((Lim et al., 2006), and in chanel catfish, 25% for 104-500 g fish, but 35% for 14-100 g fish (Page & Andrew, 1973). Similar results have been reported for seabass (Glencross, 2006), striped catfish (Glencross et al., 2011), mudskipper (*Pseudapocryptes elongatus*) (Be and Hien, 2020), snakehead fish (Hien et al., 2020). Corresponding to the decrease in protein requirements, the P/E ratio also decreases with fish growth. The optimal P/E ratio for aquatic animals varies from species to species, but it is generally greater than 20 g/MJ, and has been reported to range from 19 to 27 g/MJ (NRC, 1983). However, most studies on the P/E ratio in fish have been carried out on very young fish at the seed stage. In this study, the best P/E ratio for 400 g clown knifefish was 17.5, lower than that of the 3 g was 24 (Hien et al., 2013), 50-100 g (Lan et al., 2014) and was equivalent to 200 - 300 g (17.7) fish stage (Hien, 2014). The survival rate of pompano in the experiment ranged from 96.6 to 100% and was not affected by the interaction between dietary protein and lipid levels (Hien et al., 2022, unpublished data)

The feed efficiency of clown knifefish is influenced by the dietary protein and lipid content and the interaction between these two factors. Protein utilization efficiency increases as protein content increases. At a lipid level of 12%, FCR was high due to the slow growth rate, possibly because high lipid and energy levels affected the digestion and absorption of fish. According to Hien and Tuan (2009), with the same source of protein supplied to the feed, the efficiency of protein use would be high in low-protein feed because aquatic animals would make the most of the protein source in the feed to build their bodies. In the present study, the growth performance and feed utilization of clown knifefish were both significantly affected by dietary protein and lipid, and this may affect body composition. The crude lipid content of fish increased with the increasing dietary lipid. Similar results were observed in *Nibea diacanthus* (Li et al., 2017), *Epinepheus fuscoguttatus* (Shapawi et al., 2014), protein content in fish body tends to increase gradually with increasing protein content in feed which approximately paralleled the growth performance. This change is related to different growth rates (Abdel-Tawwab et al., 2010). The finding is in accordance with the study by Samantaray and Mohanty (1997) on snakehead, and by Lee and Lee (2005) on catfish bragid (*Pseudobagrus fulvidraco*). The lipid composition in the fish body tends to decrease gradually with the increase of protein content in the feed and increase with the increase of lipid content in the feed. Low energy with high lipid content, improved protein utilization efficiency, but results in undesirable level of lipid deposition (Tibbett et al., 2005) which may have long term implications in terms of final quality or health of aquatic animals. De Silva et al. (1991), with feed with the same protein level, the lipid content in tilapia hybrids increased significantly when the lipid content in the feed increased. Lipids on fingerling snakeheads also gave similar results. Lipid content of some fish species increased with increased lipid and energy content of feed (Hillestad & Johnsen, 1994; Peres & Oliva, 1999).

The results show that there is an effect of feed with different protein and lipid content on the springiness of fish muscle. Houlian et al. (1995) studying the fish protein synthesis, concluded that low-protein feed consumption in fish leads to low protein accumulation in fish muscle and low weight gain. The development of muscle fibers is poor and the muscle fibers are small hence the springiness of fish muscle is low. The highest springiness was achieved in the treatment 35-9 (protein/lipid) showing that the optimal protein content (35%) for protein accumulation in fish muscle and best growth was achieved. This result may be explained that each fish species has a specific limit of determining the appropriate protein in the feed composition for growth and protein accumulation in fish muscle, so when the protein content is excess, the fish cannot use it effectively (Siddiqui et al., 1988). According to Hultmann and Rustad (2004) when studying endogenous proteolysis affecting fish muscle structure, it is also found that the quality of fish, texture of fish muscle is influenced by protein content. Therefore, it can be concluded that the optimal protein levels could induce good weight gain, higher protein accumulation and stronger muscle based on higher hardness and chewiness.

Optimum lipid content (9%) for the accumulation of lipids in fish flesh and the best fish growth, the highest fish muscle structure. Another study in arctic salmon (*Salvelinus alpinus*) showed that as lipid accumulation in fish flesh increased, muscle coagulation also increased and therefore springiness increased (Ginés et al., 2004). Interestingly, when the lipid content in the feed increased to 12% and exceeded the lipid requirement of fish, the weight gain begins to decrease because the energy-rich food reduces the capture of prey, and the accumulation of protein in the muscle is also reduced, while the accumulation of lipids increased, so the hardness and chewiness of fish muscle tend to decrease. Ohanna et al. (2005) suggested that when the lipids in the feed increase, the growth of the fish slows down, the growth of fish muscle is reduced, the muscle becomes loose, less firm, so the muscle structure of the fish decreases. The study on the growth and flesh quality of carp (*Cyprinus carpio*) also showed that the accumulation of lipid content in fish flesh increases, the fish flesh was soft, so the muscle structure decreases (Fauconneau et al., 1995). Therefore, similar to protein, the lipid content of the feed also needs to be in the appropriate range to ensure the accumulation of protein for fish, because protein is the main component of muscle tissue, so fish can accumulate good protein to build strong muscle tissue, increasing the hardness and chewiness of fish meat.

In conclusion, our results showed that the dietary protein requirements based on the SGR and FCR of 400 g clown knifefish were 32,8% with a diet containing 9% lipid in diets, 19 MJ energy/kg, P/E ratio of 18.6 which significantly promoted the full utilization of feed, and the best quality of fish muscle.

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