Director's Digest
FATS AND PROTEINS RESEARCH FOUNDATION, INC.

DR. GARY G. PEARL D.V.M.
Director Technical Services

16551 Old Colonial Road
Bloomington, Illinois 61704
Telephone: 309-829-7744 FAX: 309-829-5147
<www.fprf.org>

#333
February 2005

Effects of Dietary Protein and Energy Levels on Growth, Feed Utilization and Body Composition of Cuneate Drum (*Nibea miichthioides*)

Jinlu Guo[a], Yan Wang[a*], Dominique P. Bureau[b], Zhenghe Cui[a]

[a]Laboratory of Aquatic Ecology and Fish Nutrition,
Shanghai Fisheries University, Shanghai 200090, China

[b]Fish Nutrition Research Laboratory,
Department of Animal and Poultry Science,
University of Guelph, Guelph, ON, N1G2W1, Canada

* Corresponding author: wangyan@shfu.edu.cn; Tel/Fax: (8621) 65710764

Running title: Growth and body composition of cuneate drum (Nibea miichthioides) fed practical diets with different dietary protein and energy levels

Abstract

Effects of dietary protein and energy levels in practical feeds on growth, feed utilization and body composition of cuneate drum, *Nibea miichthioides* were examined in a 8 week net pen experiment carried out in Shenao Bay, Shantou. One-year old fish with body weight of 19.1 g $fish^{-1}$ were fed nine formulated feeds with combination of three digestible protein (DP) (36, 38 and 40%) and three digestible energy (DE) (14, 16 and 18 MJ g^{-1}) levels. Each feed treatment was triplicated. Frozen *Sardinella* sp (RF) was used as a comparison to formulated feed. During the experiment, water temperature fluctuated in a range between 27°C and 31°C. Feed intake (FI), growth, feed conversion ratio (FCR), carcass and liver composition of cuneate drum were significantly affected by dietary protein and energy levels. At same dietary energy level, weight gain of fish increased with the increase of dietary protein level. There were not significant trend in FI, FCR, condition index (CI), hepatosomatic index (HSI), carcass and liver composition among the fish fed various dietary protein levels. At same dietary protein level, weight gain of fish increased with the increase of energy from 14 MJ g^{-1} to 16 MJ g^{-1} DE, then declined with continuously increasing energy level up to 18 MJ g^{-1} DE, while FI, FCR, and HSI decreased with the increase of dietary energy levels. Lipid content of carcass and liver was higher in the fish fed the feed containing high dietary energy than the fish fed the feed with low dietary energy. The fish exhibited the best growth when fed 40% DP and 16 MJ g^{-1} DE. Compared to the fish fed raw fish, the fish fed the formulated feed containing 40% DP and 16 MJ g^{-1} DE showed higher weight gain, lower FI, lower FCR, higher protein retention efficiency, lower CI, higher HSI, and higher lipid content of both carcass and liver.

Keywords: Cuneate drum; protein, energy;

1. Introduction

Cuneate drum (*Nibea miichthioides*), a commercially important warm water carnivorous sciaenid, have been widely cultured in near shore net pens along the coast of South and East China Sea, due to its desirable characteristics, such as good fillet quality, rapid growth and strong resistance against disease. Wild cuneate drum are predator of small finfish and shellfish (Zhu and Wu, 1985). The fish have been generally fed raw fish under commercial production conditions. Feeding raw fish as feed results in high feed cost and serious environment pollution. Using high quality formulated feed is one of solutions for the establishment of economically and ecologically sustainable cuneate drum industry.

Fish reared in intensive production systems obtain nutrients and energy for sustaining growth and metabolism mainly from the supplemental feed, in which protein is usually the most expensive single component. The determination of protein and energy requirement of fish is essential to formulating nutritionally adequate and cost-effective feed, as growth performance and carcass composition of fish closely depend upon dietary protein and energy level. Although protein and energy requirement have been well studied for many fish species, knowledge concerning sciaenids has been limited in the information for red drum (Daniels and Robinson, 1986; Williams and Robinson, 1988; Ellis and Reigh, 1991; Serrano et al., 1992;

Moon and Gatlin, 1994; McGoogan and Gatlin III, 1998, 1999; Thoman et al., 1999; Williams and Robinson, 1988), giant croaker (Lee et al., 2001) and large yellow croaker (Duan et al., 2001), and protein and energy requirement of cuneate drum have not been determined. The purpose of the present study was to examine effects of dietary protein and energy levels in practical diets on growth, feed utilization and body composition of cuneate drum.

2. Material and Methods

A factorial layout including 3 levels of dietary digestible protein (DP) and 3 levels of dietary digestible energy (DE) was established, and 9 practical feeds, L1 (36% DP×14 MJ kg^{-1} DE), L2 (36% DP ×16 MJ kg^{-1} DE), L3 (36% DP ×18 MJ kg^{-1} DE), M1 (38% DP ×14 MJ kg^{-1} DE), M2 (38% DP ×16 MJ kg^{-1} DE), M3 (38% DP ×18 MJ kg^{-1} DE), H1 (40% DP ×14 MJ kg^{-1} DE), H2 (40% DP ×16 MJ kg^{-1} DE) and H3 (40% DP ×18 MJ kg^{-1} DE) were formulated. In addition, frozen *Sardinella* sp, a quality raw fish used as feed in commercial production of cuneate drum, served as a comparison to formulated feeds. Thus 10 feed treatments, including 9 formulated feed treatment and 1 raw fish treatment, were examined in the experiment. DP and DE levels in the formulated feeds were estimated with the published apparent digestible coefficients for the feedstuffs used in the experiment (NRC, 1993; Bureau et al., 1999). Proximate composition and amino acid profile of the feedstuffs was described in Wang et al. (Wang et al, unpublished data), and formulation and chemical composition of the experimental feeds are shown in Table 1. The formulated feeds were made into slow sinking pellets using a laboratory-scale single screw extruder.

A feeding trial was carried out in net pens in Shenao Bay from August 10 to October 8, 2003. Cuneate drum fingerlings were collected from Raoping marine fish hatchery, and were transported to the experimental site. The fish were reared in commercial net pens (3 × 3 m, 2 m in depth) for one month, during which the fish were gradually weaned from minced raw fish onto formulated feed. Two weeks prior to the feeding trial, 1200 fish with similar body size were selected and acclimated in 30 experimental pens (1 × 1 m, 1.5 m in depth) at 40 fish per pen. During the acclimation, the fish were fed a mixture of the formulated feeds at 0800 and 1600 everyday.

At the start of the feeding trial, the acclimated fish were deprived of feed 24 h, and pooled, and 30 groups each of 30 fish, with initial body weight of 19.1±0.2 g per fish (mean± S.E), were batch weighed, and then randomly stocked in 30 experimental pens. Each feed treatment was triplicated. Eight sub-samples each of 3 fish were collected for the analysis of chemical composition in whole body, liver and carcass. Standard length (SL) and body weight of the sampled fish were measured for the determination of condition index (CI), and 4 sub-samples were dissected and the liver weighed for the determination of hepatosomatic index (HSI). The sampled fish, carcass and liver were stored at -20°C until analysis.

During the feeding trial, the fish were hand fed twice per day except the days wave was rough or temperatures extremely high in Shenao Bay. For feeding the fish formulated feeds, 30-40 pellets were dropped in one pen each time. The process was performed alternately and repeatedly for each of 27 pens, in which fish were fed the formulated feeds, until no fish came to water surface to accept the dropped feed. By this way most of the dropped feed were eaten by the fish. Died fish was recorded and weighed for calibrating feed intake and feed conversion ratio (FCR). Water temperature was measured daily and salinity weekly. Water

temperature fluctuated in a range of 25℃ to 32 ℃, and salinity of 31 ppt to 32 ppt in Shenao Bay throughout 8-week course of the feeding trial.

At the end of the feeding trial, the fish in each of 27 pens were batch weighed. Two groups each of 3 fish were sampled from each pen for the determination of CI, HSI and chemical composition in whole body, carcass and liver.

Moisture, crude protein, lipid and ash in the feedstuffs, the feeds, the raw fish, and in whole body, carcass and liver of the sampled fish were analyzed following the procedures described in Wang et al. (unpublished data).

Feed intake (FI), FCR, CI, HSI and protein retention efficiency was calculated as below:

$$FI\ (\%\ d^{-1}) = 100 \times I / (W_0 \times t)$$

$$FCR = I / (W_t - W_0 + W_d)$$

$$CI\ (g\ cm^{-3}) = 100 \times W / L^3$$

$$HSI\ (\%) = 100 \times W_l / W$$

Where I is consumed feed (g), W_0 is initial body weight (g), t is duration of the feeding trial (day), W_t is final body weight (g), W_d is body weight of died fish (g), W is body weight of the sampled fish (g), L is SL of the sampled fish (cm), W_l is liver weight of the sampled fish (g),

The differences in FI, weight gain, FCR, CI, HSI, content of moisture, lipid, protein and ash in carcass, and content of moisture and lipid in liver among the treatments fed formulated feeds were examined using factorial ANOVA, and comparison between the treatments were performed using HSD test. Student t test was used to examine differences in FI, weight gain, FCR, CI, HSI, content of moisture, lipid, protein and ash in carcass, and content of moisture and lipid in liver between the RF and the formulated feed treatment with the best growth performance (H2). CI, HSI, content of moisture, lipid, protein and ash in carcass, and content of moisture and lipid in liver were arcsine transformed. Relationships between CI and lipid content in carcass, and between HSI and lipid content in carcass, and between HSI and lipid content in liver were examined using multiple regressions. $P<0.05$ was regarded as significant difference.

3. Results

Survival was 100% in the RF treatment, and over 98% in the formulated feed treatments (Table 2). There was no significant difference in survival among formulated feed treatments and between the RF and the H2.

Weight gain of the fish fed the formulated feeds depended on DP and DE level (Table 2). At the same DE level, weight gain increased with the increase of DP level from 36 % to 40%. At the same dietary DP level, weight gain increased with the increase of dietary DE level from 14 MJ kg^{-1} to 16 MJ kg^{-1}, and declined with the continuous increase of dietary DE from 16 MJ kg^{-1} to 18 MJ kg^{-1}. The H2 treatment showed the highest weight gain among the formulated feed treatments (Table 3).

Dietary DP and DE levels had significant influences on FCR, while FI was only closed dependent on dietary DE (Table 2). FI and FCR usually decreased with the increase of dietary

DP and/or DE level, the trend, however, were not very clear. The H2 and H3 treatment exhibited the lowest FCR, while the L1 treatment had the highest FCR among the formulated feed treatments (Table 4).

The level of dietary DP in formulated feed significantly affected CI, and HSI was dependent on DP and DE levels (Table 2). At the same DE level, CI increased with the elevation of dietary DP level. There was not a clear trend in HSI among the formulated feed treatments (Table 4).

Moisture content in carcass is closely dependent on both dietary DP and DE level, while content of crude protein, crude lipid and ash were only affected by dietary DE level (Table 2). At the same dietary DP level, lipid content in carcass increased with the increase of dietary DE levels. The L1 treatment exhibited the highest lipid content in carcass (Table 5). Crude lipid content in liver increased with the increase of dietary DE levels (Table 2). The L1, M1 and H1 treatments showed higher moisture content in liver, and lower lipid content, than that of the remainder formulated feed treatments (L2, L3, M2, M3, H2 and H3) (Figure 1).

In the formulated feed treatments, CI did not correlated to lipid content in carcass ($P>0.05$), and HSI did not correlated to lipid content in carcass ($P>0.05$) and liver ($P>0.05$).

There were no significant differences in weight gain, CI, lipid content in carcass between the RF and H2 treatment (Table 6). The H2 treatment showed higher HSI (Table 4) and lipid content in liver (Figure 1), and lower FI (Table 3), FCR (Table 3), moisture content in carcass (Table 5) and liver (Figure 1) than that of the RF treatment.

4. Discussion

Elevation of dietary protein level usually promotes growth of fish. In the present experiment, the fish fed 40% dietary DP showed the best growth performance than that of the fish fed 36-38% dietary DP at all DE levels tested, preliminarily suggesting cuneate drum required at least 40% dietary DP for sustaining normal growth. Atlantic croaker *Micropogonias undulatus* required 45% dietary crude protein (Davis and Arnold, 1997). Red drum required 35-45% dietary crude protein when reared in brackish water (Daniels and Robinson, 1986; Serrano et al, 1992; Thoman et al., 1999; McGoogan and Gatlin III, 1999) and at least 45% dietary crude protein when reared in sea water (Thoman et al., 1999). Large yellow croaker *Pseudosciaena crocea* required 47% dietary crude protein (Duan et al., 2001), and giant croaker *Nibea japonica* 45% dietary crude protein (Lee et al., 2001). Compared with above sciaenids, cuneate drum exhibited similar dietary protein requirement. Extending the comparison to other fish species, optimal dietary protein level for cuneate drum was similar with that for small mouth bass (45% crude protein)(Anderson et al. 1981), European sea bass *Dicentrarchus labrax* (44-45% crude protein) (Ballestrazzi et al., 1994; Pérez et al., 1997) and cobia *Rachycentron canadum* (45% crude protein) (Chou et al, 2001), but lower than that for striped bass *Morone saxatilis* (47% crude protein)(Millikin, 1983), grouper *Epinephelus malabaricus*, (more than 48% crude protein) (Chen and Tsai, 1994; Shiau and Lan, 1996), mediterranean yellow tail *Seriola dumerilii* (50% crude protein) (Jover et al. 1999) and haddock *Melangogrammus aeglefinus* (50% crude protein) (Kim and Lall, 2001). Therefore, dietary protein requirement of cuneate drum is at intermediate level among that for carnivorous fish.

In the present experiment, weight gain of cuneate drum increased with the increase of

dietary DE level from 14 KJ g^{-1} to 16 KJ g^{-1}, then decreased with the continuous increase of DE at the same DP levels. Earlier studies revealed turbot (Bromly, 1980) and red drum (McGoogan and Gatlin III, 1999) showed poor growth performance when the fish was fed excess dietary lipid. Atlantic croaker showed decreased weight gain as dietary lipid elevated from 8% to 16% (Davis and Arnold, 1997), and red drum reared in sea needed 13% dietary lipid for normal growth (Thoman et al., 1999), and large yellow croaker showed declined growth when dietary lipid level was higher than 10.5% (Duan et al., 2001), and cobia fed 6-19% dietary lipid did not show any distinguished difference in weight gain (Chou et al, 2001). The dietary energy requirement was 17-18 KJ g^{-1} gross energy (GE) for yellow tail (Jover et al. 1999), and 21-23 KJ g^{-1} GE for haddock (Kim and Lall, 2001). Optimal dietary energy level for cuneate drum is higher than that of yellow tail, but lower than that of haddock. In the present experiment, the cuneate drum fed 16 KJ g^{-1} DE (equal to 18 KJ g^{-1} GE or 15-17% dietary lipid) showed the best growth performance and relatively low FCR among the 3 energy levels tested, preliminarily indicating cuneate drum could tolerate higher dietary lipid than that of Atlantic croaker (Davis and Arnold, 1997) and large yellow croaker (Duan et al., 2001), or higher dietary energy level than that of yellow tail (Jover et al. 1999).

Energy for sustaining growth and metabolism of fish comes from either protein or non-protein (lipid and carbohydrate) reserves. Dietary protein requirement of fish relates to dietary energy level, and protein in feed can be spared by properly elevating levels of dietary no-protein energy (Shiau and Lan, 1996). Properly using no-protein energy for sparing dietary protein is not only helpful to reduce feed cost, but also to reduce nitrogen waste output derived from feed, and ratio of dietary protein to energy (P/E) should be considered for formulating low pollution and cost-effective fish feed. Optimal dietary P/E varied largely between cold water fish and warm water fish. Cold water fish could utilize lipid well, and dietary P/E was generally low than 23 g MJ^{-1}, e.g. 22 g MJ^{-1} for rainbow trout (Lee and Putnam, 1980), and 18 g MJ^{-1} for Atlantic salmon *Salmo salar* (Hillestad and Johnsen, 1994). In contrast, dietary P/E for warm water fish was relatively high, e. g. 31 g MJ^{-1} for grouper (Shiau and Lan, 1996), 22 g MJ^{-1} for European sea bass (Pérez et al., 1997), 28 g MJ^{-1} for mediterranean yellow tail (Jover et al. 1999), 28 g MJ^{-1} for red drum (McGoogan and Gatlin III, 1999) and 26 g MJ^{-1} for *Labeo rohita* (Sethuramalingam and Haniffa, 2001). In the present experiment, cuneate drum fed the fed with DP/DE of 25 g MJ^{-1} (GP/GE =24 g MJ^{-1}) showed the highest weight gain and relatively low FCR, suggesting optimal of dietary P/E for cuneate drum was lower than that for grouper (Shiau and Lan, 1996), yellow tail (Jover et al. 1999) and red drum (McGoogan and Gatlin III, 1999).

Lipid content in body of the fish deprived of feed or restricted feeding decreased, while moisture content increased during starvation (Wang et al, 2000). Oppositely, content of lipid in body of the fish fed dietary energy in excess was usually abnormally high. In the present experiment, lipid content in carcass and liver of the fish increased with the increase of dietary energy level, this is insistent with the tendency reported on yellow croaker (Duan et al., 2001), Atlantic croaker (Davis and Arnold, 1997) and red drum (Daniels and Robinson, 1986). Weight gain and lipid content in carcass of the grouper fed formulated feed was higher than that of the fish fed raw fish (Milliamena, 2002). In the present experiment, cuneate drum fed the formulated feed of 40% DP and 16 KJ g^{-1} DE exhibited similar weight gain, but higher lipid content in both carcass and liver, compared to that of the fish fed raw fish, suggesting the formulated feed of 40% DP and 16 KJ g^{-1} DE adequate dietary protein and energy requirement

of cuneate drum. The higher lipid deposition in carcass and liver of the fish fed the formulated feeds may associate with relatively low P/E in the formulated feed. This hypothesis need to be further examined through a long period histology experiment.

CI and HSI were good indicator of body energy reserve for many fish species (Chellappa et al, 1995). The results of the present experiment, however, revealed CI and HSI could not be used as adequate indicator for the estimation of energy reserves in body of cuneate drum.

Acknowledgements

We thank Fats and Protein Research Foundation (FPRF) and Education Committee of Shanghai for funded on the project. We also thank Dr. Bud Harmon and Dr. Gary Pearl for valuable suggestions and Dr. Yu Yu for helps with procurement of rendered animal ingredients.

References

Anderson R J, Kienholz E W and Flickinger S A. 1981. Protein requirements of smallmouth bass and largemouth bass. *J. Nutr.* 111: 1085-1097

Ballestrazzi, R., Lanari, D., D'Agaro, E., Mion, A., 1994. The effect of dietary protein level and source on growth, body composition, total ammonia and reactive phosphate excretion of growing sea bass (*Dicentrarchus labrax*). Aquaculture, 127, 197-206.

Bromly P J. 1980. Effects of dietary protein, lipid and energy content on the growth of turbot (*Scophthalmus maximus* L.). Aquaculture, 19, 359-369.

Bureau, D. P., Harris, A. M., Cho, C. Y. ,1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 180, 345-358.

Chen, H. Y., Tsai, J. C., 1994. Optimal dietary protein level for the growth of juvenile grouper, *Epinephelus malabaricus*, fed semipurified diets. Aquaculture 119, 265-271.

Chellappa, S., Huntingford, F. A., Strang, R. H. C., Thomson, R. Y..1995. Condition factor and hepatosomatic index as estimates of energy status in male three-spined stickleback. Journal of Fish Biology, 47, 775-787.

Chou R L, Su M S. Chen H Y. 2001. Optimal dietary protein and lipid levels for juvenile cobia (*Rachycentron canadum*). *Aquaculture*. 193: 81-89

Daniels, W. H., Robinson, E. H., 1986. Protein and energy requirements of juvenile Red Drum (*Sciaenops Ocellatus*). Aquaculture 53, 243-252

Davis, D. A., Arnold, C.R. 1997. Response of Atlantic croaker fingerlings to practical diet formulations with varying protein and energy contents. Journal of World Aquaculture Society. 28, 241-248

Duan, Q., Mai, K., Zhong, H., Si, L., Wang, X., 2001. Studies on nutrition of large yellow croaker, *Pseudosciaena crocea* R 1: growth response to graded levels of dietary protein and lipid. Aquaculture Research 32, 46-52.

Ellis, S. C., Reigh, R. C., 1991. Effects of dietary lipid and carbohydrate levels on growth and body composition of juvenile red drum, *Sciaenops ocellatus*. Aquaculture 97, 383-394.

Hernández M D, Egea M A., Rueda F M, Aguado F A., Martínez F J, García B. 2001. Effects of commercial diets with different P/E ratios on sharpsnout seabream (*Diplodus puntazzo*) growth and nutrient utilization. *Aquaculture*. 195: 321-329

Hillestad M, Johnsen F. 1994. High-energy/low-protein diets for Atlantic salmon: effects on growth, nutrient retention and slaughter quality. *Aquaculture*. 124: 109-116

Hillestad M, Alliot E. 1988. Influence of water temperature on protein requirement and protein utilization in juvenile sea bass, *Dicentrarchus labrax*. *Aquaculture*. 72: 115-129

Jover M García-Gómez A, Tomás, De la Gándara F, Pérez L. 1999. Growth of Mediterranean yellowtail (*Seriola dumerilii*) fed extruded diets containing different levels of protein and lipid. *Aquaculture*. 179: 25-33

Kim J D, Lall S P. 2001. Effects of dietary protein level on growth and utilization of protein and energy by juvenile haddock (*Melangogrammus aeglefinus*). *Aquaculture*. 195: 311-319

Lee H. Y. M., Cho K. C. & Lee J. E., Y. S. G.(2001) Dietary protein requirement of juvenile giant croaker, *Nibea japonica*, Temminck & Schlegel. *Aquaculture Research* 32,112-118.

Lee D J, Putnam G B. The response of rainbow trout to varying protein and energy ratios in a test diet. J. Nutrition. 103,916-922.

McGoogan, B. B, Gatlin III, D. M., 1998. Metabolic requirement of red drum, *Sciaenops ocellatus*, for protein and energy based on weight gain and body composition. J. Nutrition 128, 123-129

McGoogan, B. B, Gatlin III, D. M., 1999. Dietary manipulations affecting growth and nitrogenous waste production of red drum, *Sciaenops ocellatus* I. Effects of dietary protein and energy levels. Aquaculture 178, 333-348

Milliamena O. M.(2002) Replacement of fish meal by animal by-product meals in a practical diet for grow-out culture of grouper *Epinephelus coioides*. *Aquaculture* 204,75-84.

Millikin M. R. 1983. Interactive effects of dietary protein and lipid on growth and protein utilization of age-0 striped bass. *Transactions of the American Fisheries Society*. 112: 185-193

Moon, H. Y. L, Gatlin III, D. M., 1994. Effects of dietary animal proteins on growth and body composition of the red drum (*Sciaenops ocellatus)*.Aquaculture 120, 327-340.

Pérez L, Gonzalez H, Jover M, Fernández-Carmona J. 1997. Growth of Europen sea bass (*Dicentrarchus labrax*) fingerlings fed extruded diets containing varying levels of protein, lipid and carbohydrate.

Seenappa D, Devaraj. 1995. Effect of different levels of protein, fat and carbohydrate on growth, feed utilization and body carcass composition of fingerlings in Catla catla (Ham.). *Aquaculture*. 129: 243-249

Serrano, J. A., Nematipour, G. R., Gatlin III, D. M., 1992. Dietary protein requirement of the red drum (*Dicentrarchus labrax*) and relative use of dietary carbohydrate and lipid. *Aquaculture*. 101,283-291.

Sethuramalingam A. Haniffa M A. Optimization of energy to protein ratio in feed for *Labeo rohita* fingerlings. Fishery Technology 2001 38(2): 115-120

Shiau S.Y. and Lan, C. W. 1996. Optimal dietary protein level and protein to energy ratio for growth of grouper (*Epinephelus malabaricus*). *Aquaculture*. 145: 259-266

Thoman, E. S., Davis, D. A., Arnold, C. R., 1999. Evaluation of growout diets with varying protein and energy levels for red drum (*Sciaenops ocellatus*). Aquaculture 176, 343-353

Williams, C. D., Robinson, E. H., 1988. Response of red drum to various dietary levels of menhaden oil. Aquaculture 70, 107-120

Zhu, Y. D., Wu, H. L. 1985. Sciaenidae. In The fishes of Fujian Province, Part II ("Fishes of Fujian Province" Editorial Subcommittee). Fujian Science and Technology Press. 121-122.

Table 1 Formulation and chemical composition of the experimental feeds in the experiment

Ingredient	L1	L2	L3	M1	M2	M3	H1	H2	H3	RF
					kg kg^{-1}					
Fish meal	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	0.300	
Rapeseed meal	0.090	0.100	0.100	0.110	0.150	0.090	0.150	0.095	0.095	
Blood meal	0.035	0.040	0.050	0.045	0.045	0.070	0.065	0.090	0.095	
Soybean meal	0.080	0.090	0.090	0.110	0.110	0.110	0.100	0.090	0.100	
Poultry meal	0.110	0.110	0.110	0.110	0.110	0.110	0.110	0.110	0.110	
Wheat flour	0.295	0.200	0.110	0.235	0.115	0.090	0.185	0.170	0.080	
CaHPO4	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	
Met	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	
Fish oil	0.050	0.120	0.200	0.050	0.130	0.190	0.050	0.105	0.180	
Vitamin premix	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	
Mineral premix	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	
DM, %	89.2	90.3	91.0	89.5	90.5	90.9	89.2	91.1	90.7	25.2
CP, %	43.1	40.4	41.9	42.9	43.1	42.5	46.0	45.2	44.9	17.8
Lipid, %	9.4	16.3	24.1	9.4	17.3	23.2	9.5	14.9	22.2	3.3
Ash, %	9.1	9.2	9.2	9.4	9.7	9.4	9.8	9.5	9.5	3.9
GE, MJ kg^{-1}	17.3	19.0	21.0	17.4	19.4	20.9	17.6	19.0	20.8	
CP/GE, g MJ^{-1}										
DDM, %	67.0	69.3	72.2	66.9	68.6	72.8	66.3	70.1	72.9	
DP, %	36.0	36.1	36.1	38.0	38.1	38.1	40.1	40.0	40.0	
DE, MJ kg^{-1}	14.2	16.0	18.1	14.2	16.0	18.1	14.2	16.0	18.0	
DP/DE, g MJ^{-1}	25.3	22.6	19.9	26.8	23.7	21.1	28.4	25.0	22.2	

Vitamin mixture (mg per kg of feed): vitamin A, 2500 I. U.; vitamin D_3, 2000 I. U.; vitamin E, 50 I. U.; vitamin K, 1.; choline, 1000; niacin, 10; riboflavin, 6; pyridoxine, 5; thiamin, 1; D-calacium pantothenate, 20; biotin, 0.14; foliacin, 1; vitamin B_{12}, 0.02; ascorbic acid, 50

Mineral mixture (mg per kg of feed): NaCl, 1200; $FeSO_4$., 13; $ZnSO_4$., 60; $MnSO_4$, 32; $CuSO_4$, 7; KI, 8.

DM, dry matter; GP, crude protein; DDM, digestible dry matter; DP, digestible protein; DE, digestible energy; L1, formulated feed of 36% digestible protein (DP) and 14 MJ kg^{-1} digestible energy (DE); L2, formulated feed of 36% DP and 16 MJ kg^{-1} DE; L3, formulated feed of 36% DP and 18 MJ kg^{-1} DE; M1, formulated feed of 38% DP and 14 MJ kg^{-1} DE; M2, formulated feed of 38% DP and 16 MJ kg^{-1} DE; M3, formulated feed of 38% DP and 18 MJ kg^{-1} DE; H1, formulated feed of 40% DP and 14 MJ kg^{-1} DE; H2, formulated feed of 40% DP and 16 MJ kg^{-1} DE; H3, formulated feedff of 40% DP and 18 MJ/kg DE; RF, raw fish (*Sardinella* sp)

Table 2 Results of ANOVA on weight gain, feed intake, feed conversion ratio, survival, condition index and hepatosomatic index, content of moisture, crude protein, crude lipid and ash in carcass, and content of of moisture and crude protein in liver among the formulated feed treatments in the experiment.

Feed treatment	DP	DE	DP*DE
Weight gain	P<0.01	P<0.01	P=0.052
FI	P>0.05	P<0.01	P>0.05
FCR	P<0.01	P<0.01	P<0.01
Survival,	P>0.05	P>0.05	P>0.05
CI	P>0.05	P<0.01	P>0.05
HSI	P<0.01	P<0.01	P<0.01
Moisture in carcass	P<0.01	P<0.01	P>0.05
Crude protein in carcass	P>0.05	P<0.01	P>0.05
Crude lipid in carcass	P>0.05	P<0.01	P>0.05
Ash in carcass	P>0.05	P<0.01	P>0.05
Moisture in liver	P>0.05	P<0.01	P>0.05
Crude lipid in liver	P<0.05	P<0.01	P>0.05

DP, digestible protein; DE, digestible energy; FI, feed intake; FCR, Feed conversion ratio; CI, condition index; HSI, hepatosomatic index

Table 3 Weight gain, feed intake, food conversion ratio and survival of cuneate drum in the experiment (Mean±S.E.).

Feed treatment	Weight gain (g)	FI (%.d^{-1})	FCR	Survival (%)
L1	73.8±3.2^{ac}	2.9±0.1^{a}	1.24±0.05^{ac}	100
L2	80.7±1.7^{cd}	2.7±0.1^{ab}	1.11±0.03^{a}	100
L3	62.2.1±3.8^{e}	2.8±0.1^{ab}	1.38±0.08^{c}	100
M1	79.8±2.0^{cd}	2.9±0.1^{a}	1.25±0.03^{ac}	99±1
M2	82.7±3.3^{ad}	2.8±0.1^{ab}	1.16±0.05^{a}	99±1
M3	69.8±5.8^{ec}	2.6±0.2^{bc}	1.14±0.09^{a}	100
H1	84.6±1.3^{ad}	2.7±0.0^{ab}	1.13±0.06^{a}	98±2
H2	97.0±0.5^{b}	2.5±0.0^{cd}	0.95±0.02^{b}	100
H3	86.0±4.7^{d}	2.3±0.0^{d}	0.92±0.01^{b}	100
RF	92.9±2.5	2.7±0.1	1.05±0.03	100

Values within the same column without superscript are not significantly different (ANOVA, $P > 0.05$). Mean values within the same column having the common superscript are not significantly different (HSD test, $P > 0.05$). H3 and RF within the same column without the same superscript before the values are not significantly different (Student t test, $P > 0.05$).

FI, food intake; FCR, food conversion ratio; L1, formulated feed of 36% digestible protein (DP) and 14 MJ kg^{-1} digestible energy (DE); L2, formulated feed of 36% DP and 16 MJ kg^{-1} DE; L3, formulated feed of 36% DP and 18 MJ kg^{-1} DE; M1, formulated feed of 38% DP and 14 MJ kg^{-1} DE; M2, formulated feed of 38% DP and 16 MJ kg^{-1} DE; M3, formulated feed of 38% DP and 18 MJ kg^{-1} DE; H1, formulated feed of 40% DP and 14 MJ kg^{-1} DE; H2, formulated feed of 40% DP and 16 MJ kg^{-1} DE; H3, formulated feed of 40% DP and 18 MJ/kg DE; RF, raw fish (*Sardinella* sp)

Table 4 Condition index and hepatosomatic index of cuneate drum in the experiment (Mean±S.E.).

Diet treatment	CI	HSI
L1	1.1±0.01 [ab]	2.4±0.33 [a]
L2	1.2±0.03 [b]	1.9±0.16 [b]
L3	1.1±0.03 [a]	2.1±0.27 [c]
M1	1.1±0.02 [ab]	2.3±0.12 [d]
M2	1.1±0.03 [ab]	2.2±0.19 [e]
M3	1.1±0.01 [ab]	1.9±0.13 [f]
H1	1.2±0.02 [b]	2.1±0.17 [g]
H2	1.1±0.01 [ab]	2.3±0.38 [h]
H3	1.1±0.04 [ab]	2.0±0.13 [i]
RF	1.0±0.03	1.3±0.08

Mean values of the formulated feed treatments within the same column having the common superscript are not significantly different (HSD test, $P > 0.05$).

CI, Condition index; HSI, hepatosomatic index; L1, formulated feed of 36% digestible protein (DP) and 14 MJ kg^{-1} digestible energy (DE); L2, formulated feed of 36% DP and 16 MJ kg^{-1} DE; L3, formulated feed of 36% DP and 18 MJ kg^{-1} DE; M1, formulated feed of 38% DP and 14 MJ kg^{-1} DE; M2, formulated feed of 38% DP and 16 MJ kg^{-1} DE; M3, formulated feed of 38% DP and 18 MJ kg^{-1} DE; H1, formulated feed of 40% DP and 14 MJ kg^{-1} DE; H2, formulated feed of 40% DP and 16 MJ kg^{-1} DE; H3, formulated feed of 40% DP and 18 MJ/kg DE; RF, raw fish (*Sardinella* sp)

Table 5 Proximate composition in carcass of cuneate drum in the experiment (Mean±S.E.).

Feed treatment	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)
Initial	76.6±0.17	16.2±0.43	1.5±0.03	4.0±0.16
L1	73.0±0.08 ^{acd}	17.7±0.17 ^{ac}	3.7±0.26 ^{ac}	4.3±0.04 ^{ab}
L2	72.4±0.11 ^{abd}	17.4±0.14 ^{ace}	4.8±0.05 ^{ace}	4.5±0.01 ^{a}
L3	71.8±0.39 ^{b}	16.7±0.66 ^{bd}	6.1±0.32 ^{bd}	4.5±0.10 ^{a}
M1	73.5±0.25 ^{ac}	17.8±0.07 ^{ace}	4.0±0.29 ^{ace}	4.2±0.07 ^{b}
M2	72.7±0.27 ^{abcd}	16.8±0.20 ^{cde}	5.0±0.01 ^{cde}	4.4±0.07 ^{ab}
M3	72.2±0.30 ^{bd}	16.1±0.15 ^{d}	5.9±0.31 ^{d}	4.5±0.04 ^{ab}
H1	73.7±0.25 ^{c}	17.5±0.04 ^{e}	4.0±0.21 ^{e}	4.3±0.05 ^{ab}
H2	72.8±0.20 ^{abcd}	17.4±0.23 ^{e}	4.8±0.19 ^{e}	4.4±0.02 ^{ab}
H3	72.7±0.10 ^{abcd}	16.3±0.13 ^{bd}	6.0±0.14 ^{bd}	4.4±0.06 ^{ab}
RF	73.7±0.17	17.2±0.30	4.1±0.21	4.2±0.05

Mean values of the formulated feed treatments within the same column having the common superscript are not significantly different (HSD test, $P > 0.05$).

L1, formulated feed of 36% digestible protein (DP) and 14 MJ kg^{-1} digestible energy (DE); L2, formulated feed of 36% DP and 16 MJ kg^{-1} DE; L3, formulated feed of 36% DP and 18 MJ kg^{-1} DE; M1, formulated feed of 38% DP and 14 MJ kg^{-1} DE; M2, formulated feed of 38% DP and 16 MJ kg^{-1} DE; M3, formulated feed of 38% DP and 18 MJ kg^{-1} DE; H1, formulated feed of 40% DP and 14 MJ kg^{-1} DE; H2, formulated feed of 40% DP and 16 MJ kg^{-1} DE; H3, formulated feed of 40% DP and 18 MJ/kg DE; RF, raw fish (*Sardinella* sp)

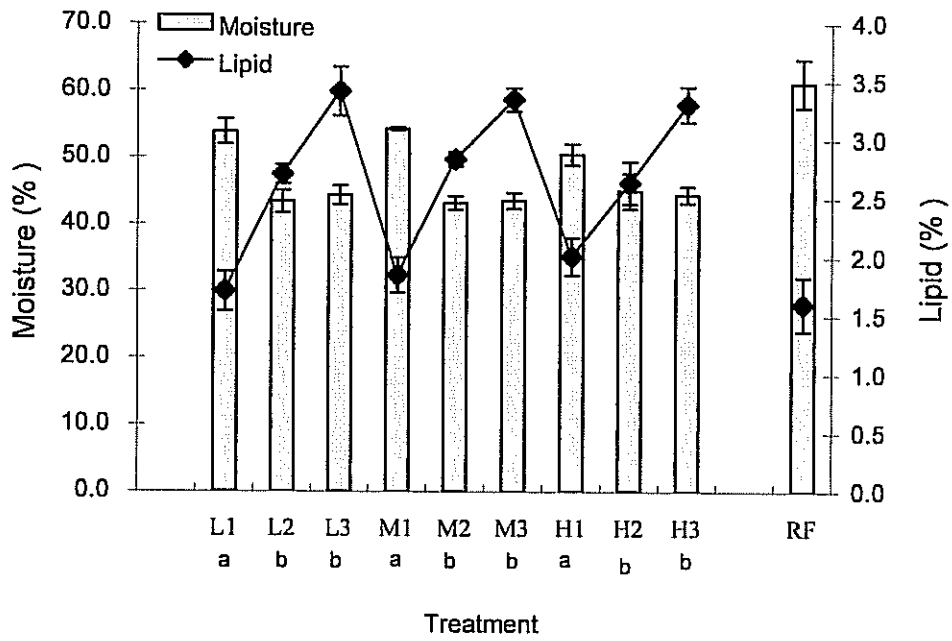
Table 2 Results of student t test on weight gain, feed intake, feed conversion ratio, survival, condition index and hepatosomatic index, content of moisture, crude protein, crude lipid and ash in carcass, and content of of moisture and crude protein in liver between H2 and RF in the experiment.

Feed treatment	df	t value	P
Weight gain	4	1.04	P>0.05
FI	4	-37.37	P<0.01
FCR	4	-26.05	P<0.01
CI	4	2.39	P>0.05
HSI	4	-7.34	P<0.01
Moisture in carcass	4	-3.57	P<0.05
Crude protein in carcass	4	2.54	P>0.05
Crude lipid in carcass	4	4.20	P<0.05
Ash in carcass	4	0.63	P>0.05
Moisture in liver	4	-3.59	P<0.05
Crude lipid in liver	4	4.37	P<0.05

H2, formulated feed of 40% DP and 16 MJ kg^{-1} DE; RF, raw fish (*Sardinella* sp); DP, digestible protein; DE, digestible energy; FI, feed intake; FCR, Feed conversion ratio; CI, condition index; HSI, hepatosomatic index

Figure legends

Figure 1 Content of moisture and lipid in liver of cuneate drum at the end of the experiment. L1, formulated feed of 36% digestible protein (DP) and 14 MJ kg^{-1} digestible energy (DE); L2, formulated feed of 36% DP and 16 MJ kg^{-1} DE; L3, formulated feed of 36% DP and 18 MJ kg^{-1} DE; M1, formulated feed of 38% DP and 14 MJ kg^{-1} DE; M2, formulated feed of 38% DP and 16 MJ kg^{-1} DE; M3, formulated feed of 38% DP and 18 MJ kg^{-1} DE; H1, formulated feed of 40% DP and 14 MJ kg^{-1} DE; H2, formulated feed of 40% DP and 16 MJ kg^{-1} DE; H3, formulated feed of 40% DP and 18 MJ/kg DE; RF, raw fish (*Sardinella* sp). Vertical bar denote standard error, and letters denote the results of HSD test (between L, M and H). Mean values of the formulated feed treatments without the same letter are not significantly different.



Guo J et al. Figure 1
Effects of dietary protein and energy levels on growth, feed utilization and body composition of cuneate drum (*Nibea miichthioides*)