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Effects of Dietary Protein and Energy on Growth, Feed Utilization and Body Composition of Cuneate Drum"
and
Replacement of Fish Meal by rendered Animal Protein Ingredients in Feeds for Cuneate Drum
by
Yan Wang, Jin-Lu Guo, Kai Li and Dominique Bureau

Dr. Yan Wang of the Shanghai Fisheries University in China has been a grantee of FPRF aquaculture research for a number of years. Dr. Dominique Bureau also a FPRF grantee and a member of the FPRF Research Committee has served as an advisor to these projects. The attached two draft reports have been submitted for peer review publishing but also as final reports to the Fats and Proteins Research Foundation, Inc. These reports should not be duplicated for distribution outside of the foundation. The data should be used as formulation guidelines for the cuneate drum species. This species is an important commercial seafood provider in China and other Asian countries. It is a carnivore species that under aquaculture production operations generally uses between 30% and 60% fish meal as well as raw fish ingredients.

The initial study was directed at establishing the nutrient requirements for the major nutrient components for protein and energy for this species and as additional data for establishing recommendations for other carnivore species. The second study addressed the objectives of assessing the effect of various rendered animal proteins, either alone or in combination as ingredients in practical feeds on the growth, feed utilization and body composition of cuneate drum. The study indicated that MBM can be used at an inclusion rate of up to 10%. Feather meal without methionine and lysine supplementation did not perform well as a substitute for fish meal. This study did demonstrate a combination of Poultry Byproduct Meal, Meat and Bone Meal, Soy Meal, Blood Meal and Feather Meal in which amino acids were formulated similar to fish meal could be incorporated at `7.5% to replace up to 50% of fish meal.

The research has resulted in the development of a diet containing 8-10% fish meal in which 80% of fish meal were replaced with rendered protein ingredients. The current challenge is that animal by-products are not available in China to produce the diets for commercial farm validation studies.

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Effects of dietary protein and energy levels on growth, feed utilization and body composition of cuneate drum (*Nibea miichthioides*)

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Abstract

An 8-week experiment was conducted in net pens to assess the effects of dietary protein and energy levels on growth, feed utilization and body composition of cuneate drum. Cuneate drum (initial body weight 19 g fish^{-1}) were fed 9 feeds formulated to contain 3 levels of digestible protein (DP 36%, 38% and 40%) and 3 levels of digestible energy (DE 14, 16 and 18 MJ kg^{-1}). Groups of fish were fed raw fish (*Sardinella* spp.) to serve as a commercial control. Specific growth rate (SGR), final body weight (FBW), feed intake, feed conversion ratio (FCR), energy retention efficiency (ERE), and moisture and protein contents in carcass of the fish were significantly affected by DP and DE levels. Nitrogen retention efficiency (NRE) was dependent on DP level, and lipid and ash contents in carcass of the fish were affected by DE level. Specific growth rate and FBW of fish fed feeds with the same DE level increased when DP increased from 36% to 40%, whereas SGR and FBW of fish fed the feeds formulated at the same DP level increased when dietary DE increased from 14 to 16 MJ kg^{-1}. No improvement, or even a slight decline in SGR and FBW, occurred with the further increase of DE to 18 MJ kg^{-1}. For the same DP level, NRE and ERE increased with the increase in DE from 14 to 16 MJ kg^{-1}. Carcass lipid content of fish fed the feeds with the same DP level increased with increases in DE level. There were no significant differences in SGR, FBW, feed intake, FCR, and protein and lipid contents in carcass of fish fed the raw fish and feed containing 40% DP and 16 MJ kg^{-1} DE. Nitrogen retention efficiency and ERE were higher for fish fed the formulated feed containing 40% DP and 16 MJ kg^{-1} DE than fish fed the raw fish.
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Keywords: Cuneate drum; Protein; Energy; Growth; Body composition

1. Introduction

Cuneate drum is a native sciaenid species of commercial importance in China. It is widely cultured in net pens along the coast of the China Sea, due to its desir-

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able characteristics, such as good fillet quality, fast growth, and high resistance against diseases. Wild cuneate drum are predators of small finfish and shellfish (Chu and Wu, 1985), and fish reared in commercial pens are generally fed raw fish. Feeding raw fish results in high feed costs for commercial operations, as well as high waste outputs, an increasingly important concern in some regions due to the high farm densities encountered. Wider use of high quality formulated feed could help improve the economical and environmental sustainability of the Chinese cuneate drum industry, as it has been the case for other species in various countries.

Growth, feed utilization and body composition of fish closely depend upon contents of protein and energy in feed. Although dietary protein and energy requirements have been studied in many fish species, information of nutritional requirements of sciaenid fish species is still limited, with most studies having been limited to 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, 1998, 1999; Thoman et al., 1999), Atlantic croaker (Davis and Arnold, 1997), giant croaker (Lee et al., 2001), and large yellow croaker (Duan et al., 2001). Dietary protein and energy requirements for cuneate drum have not been determined. In the present study, we examined the effects of protein and energy levels in practical feeds on growth, feed utilization and body composition of cuneate drum.

2. Material and methods

2.1. Test feeds

A 3 × 3 factorial layout including 3 levels of digestible dietary protein (DP 36%, 38% and 40%) and 3 levels of digestible dietary energy (DE 14, 16 and 18 MJ kg^{-1}) was established. In addition, frozen *Sardinella* spp., a raw fish feed widely used in commercial cuneate drum farming, served as a comparison to the formulated feeds. A total of 10 feed treatments (9 formulated feeds and 1 raw fish diet) were, therefore, examined in this experiment. Contents of DP and DE in the formulated feeds were estimated using published digestible coefficients (Bureau et al., 1999). Amino acids in whole body of cuneate drum were analyzed, served as a reference to establish adequate dietary amino acid levels. Amino acids (expressed on a dry weight basis) in whole body of cuneate drum included: threonine 2.21%, valine 2.79%, cysteine 0.21%, methionine 1.77%, isoleucine 2.46%, leucine 4.28%, tyrosine 1.54%, phenylalanine 2.25%, lysine 4.56%, histidine 1.23%, arginine 3.95%. Synthetic methionine (DL-methionine) was added to the formulated feeds as it was predicted to be the first limiting amino acid. Formulation, chemical composition and energy content of the feeds are shown in Table 1, and amino acid profile in Table 2.

The formulated feeds were made into slow-sinking pellets (diameter 3 mm and length 7–10 mm) using a laboratory-scale single screw extruder. The pellets were dried at room temperature, and fish oil was quantitatively sprayed on surface of the pellets with a sprayer in a rotating stirring drum. The raw fish used was from the same batch of fish and stored in a refrigerator at −20 °C until used.

2.2. Feeding and sampling

An 8-week experiment was carried out in net pens in Shenao Bay, Nanao, China. Cuneate drum (*Nibea miichthioides*) fingerlings were collected from Raoping marine fish hatchery, and transported by boat to the experimental site. The fish were reared in net pens (3 m × 3 m × 2 m) for 4 weeks, during which the fish were gradually weaned from raw fish onto the formulated feed containing 38% DP and 16 MJ kg^{-1} DE. After the pre-acclimation period, 1200 fish, with similar body size, were moved into 30 experimental pens (1 m × 1 m × 1.5 m) at 40 fish per pen, and acclimated to the formulated feed (38% DP and 16 MJ kg^{-1} DE) for 2 weeks.

At the start of the experiment, fish were deprived of feed 24 h and pooled. Thirty groups of 30 fish each, with initial body weight of 19.1 ± 0.2 g $fish^{-1}$ (mean ± S.E., n = 30), were batch weighed and randomly distributed into 30 experimental pens. Each feed treatment had 3 replications. Eight sub-samples of 3 fish each were randomly collected from the remaining acclimated fish, and sacrificed for calculating condition factor (CF) and hepatosomatic index (HSI), and for analysis of proximate composition in whole body and carcass. Total length and body weight of the fish sampled were measured, and then liver of 4 sub-samples were dissected and weighed. Whole body

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t1.1 Table 1
t1.2 Formulation (%), chemical composition (%) and energy content (MJ kg^{-1}) of the test feeds

t1.3		Feeds									
t1.4		L1	L2	L3	M1	M2	M3	H1	H2	H3	RF
t1.5	*Feed formulations*										
t1.6	Herring meal	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	
t1.7	Rapeseed meal	9.0	10.0	10.0	11.0	15.0	9.0	15.0	9.5	9.5	
t1.8	Blood meal	3.5	4.0	5.0	4.5	4.5	7.0	6.5	9.0	9.5	
t1.9	Soybean meal	8.0	9.0	9.0	11.0	11.0	11.0	10.0	9.0	10.0	
t1.10	Poultry by product meal	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	
t1.11	Wheat flour	29.5	20.0	11.0	23.5	11.5	9.0	18.5	17.0	8.0	
t1.12	$CaHPO_4$	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	
t1.13	DL-methionine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	
t1.14	Fish oil	5.0	12.0	20.0	5.0	13.0	19.0	5.0	10.5	18.0	
t1.15	Vitamin premix[a]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
t1.16	Mineral premix[b]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
t1.17											
t1.18	*Feed nutrient and energy contents*[c,d]										
t1.19	Dry matter	89.2	90.3	91.0	89.5	90.5	90.9	89.2	91.1	90.7	25.2
t1.20	Crude protein	40.3	40.4	41.9	42.9	43.1	42.5	46.0	45.2	44.9	70.6
t1.21	Crude lipid	9.4	16.3	24.1	9.4	17.3	23.2	9.5	14.9	22.2	13.1
t1.22	Ash	9.1	9.2	9.2	9.4	9.7	9.4	9.8	9.5	9.5	15.5
t1.23	Gross energy	17.3	19.0	21.0	17.4	19.4	20.9	17.6	19.0	20.8	19.2
t1.24	DDM	67.0	69.3	72.2	66.9	68.6	72.8	66.3	70.1	72.9	
t1.25	DP	36.0	36.1	36.1	38.0	38.1	38.1	40.1	40.0	40.0	
t1.26	DE	14.2	16.0	18.1	14.2	16.0	18.1	14.2	16.0	18.0	
t1.27	DP/DE (g MJ^{-1})	25.3	22.6	19.9	26.8	23.7	21.1	28.4	25.0	22.2	

t1.28 [a] Vitamin mixture provided (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-calcium pantothenate, 20; biotin, 0.14; foliacin, 1; vitamin B_{12}, 0.02; ascorbic acid, 50.
t1.29 [b] Mineral mixture provided (mg per kg of feed): NaCl, 1200; $FeSO_4$, 13; $ZnSO_4$, 60; $MnSO_4$, 32; $CuSO_4$, 7; KI, 8.
t1.30 [c] Digestible dry matter (DDM), digestible protein (DP) and digestible energy (DE) were calculated using published digestible coefficients (Bureau et al., 1999).
t1.31 [d] Crude protein, crude lipid, ash, gross energy, DP and DE are expressed on a dry weight basis.

122 and carcass of the fish sampled were frozen at −20 °C
123 until analysis.
124 During the experiment, the fish were hand fed at
125 08:00 and 16:00 h daily except the days with rough
126 waves or water temperatures in excess of 30 °C. For

t2.1 Table 2
t2.2 Essential amino acid profile (%) of the test feeds (on a dry weight basis)

t2.3	Feeds	Thr	Val	Cys	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg
t2.4	L1	0.91	1.41	0.25	0.91	1.12	2.22	0.72	1.06	1.83	0.83	1.60
t2.5	L2	0.81	1.26	0.22	0.79	0.98	1.97	0.63	0.93	1.64	0.74	1.42
t2.6	L3	0.71	1.11	0.18	0.69	0.84	1.73	0.55	0.81	1.46	0.66	1.23
t2.7	M1	0.97	1.51	0.26	0.92	1.17	2.37	0.76	1.13	1.96	0.89	1.70
t2.8	M2	0.86	1.33	0.23	0.80	1.02	2.07	0.65	0.98	1.74	0.78	1.49
t2.9	M3	0.75	1.19	0.19	0.70	0.86	1.87	0.57	0.88	1.57	0.73	1.29
t2.10	H1	1.04	1.64	0.28	0.94	1.21	2.56	0.79	1.21	2.11	0.98	1.77
t2.11	H2	0.90	1.46	0.23	0.82	1.01	2.30	0.69	1.08	1.88	0.90	1.51
t2.12	H3	0.79	1.29	0.20	0.72	0.88	2.03	0.60	0.95	1.68	0.80	1.33

feeding fish the formulated feeds, some pellets were 127
dropped into each pen until no fish were observed to 128
come to the water surface to accept the feed. Dead fish 129
was recorded and weighed for calculating feed con- 130
version ratio (FCR). Water temperature was measured 131
daily and salinity weekly. Water temperature ranged 132
from 25 to 32 °C, and salinity from 31‰ to 32‰ 133
during the 8-week experiment. 134

At the end of the experiment, the fish were col- 135
lected from each pen and batch weighed. Two groups 136
of 3 fish each were randomly collected from each pen 137
and sacrificed for the determination of CF, HSI, and 138
proximate composition of whole body and carcass. 139

2.3. Chemical analysis 140

The cuneate drum sampled at the start and end of 141
the experiment and the raw fish sampled during the 142

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experiment were autoclaved at 120 °C for 20 min, homogenized, and dried at 105 °C for 24 h. Samples of the formulated feeds, raw fish and cuneate drum were ground into fine power with a laboratory grinder prior to chemical analysis. Contents of moisture, crude protein (Kjeldahl method) and lipid (ether extract) of the feeds and fish were analyzed following the AOAC procedures (AOAC, 1975), and ash was determined following combustion at 550 °C for 6 h. Gross energy was measured using a bomb calorimeter (Parr 1281, USA), and amino acids with an automatic amino acid analyzer (Hitachi 835-80, Japan).

2.4. Calculation and statistical analysis

Feed intake, specific growth rate (SGR), FCR, nitrogen retention efficiency (NRE), energy retention efficiency (ERE), CF and HSI were calculated as below:

$$\text{Feed intake } (\%\text{day}^{-1}) = 100 \times I/[(W_0 + W_t)/2 \times t]$$

$$\text{SGR } (\%\text{day}^{-1}) = [\text{Ln}(W_t/N_t) - \text{Ln}(W_0/N_0)]/t$$

$$\text{FCR } (\text{dry feed gain}^{-1}) = I/(W_t - W_0 + W_d)$$

$$\text{NRE } (\%) = 100 \times (W_t \times C_{Nt} - W_0 \times C_{N0} + W_d \times C_{N0})/(I \times C_{Nf})$$

$$\text{ERE } (\%) = 100 \times (W_t \times C_{Et} - W_0 \times C_{E0} + W_d \times C_{E0})/(I \times C_{Ef})$$

$$\text{CF } (\text{g cm}^{-3}) = 100 \times W_s/L_s^3$$

$$\text{HSI } (\%) = 100 \times W_l/W_s$$

where I (g) is total amount of the consumed feed on a dry weight basis, W_t (g) is total final body weight and W_0 (g) total initial body weight, t (day) is duration of the experiment, N_t is number of fish at the end of the experiment and N_0 at the start of the experiment, W_d (g) is total body weight of the dead fish, C_{Nt} (%) is nitrogen content in whole fish body at the end of the experiment and C_{N0} (%) at the start of the experiment, C_{Et} (kJ g^{-1}) is energy content in whole fish body at the end of the experiment and C_{E0} (kJ g^{-1}) at the start of the experiment, C_{Nf} (%) is nitrogen content in the feeds and C_{Ef} (kJ g^{-1}) energy content, W_s (g $fish^{-1}$) is body weight of the fish dissected at the end of the experiment and L_s (cm) total length, W_l (g) is liver weight of the fish dissected at the end of the experiment. Survival, SGR, final body weight (FBW), feed intake, FCR, NRE, ERE, CF, HSI, and contents of components (moisture, crude protein, crude lipid and ash) in carcass, among fish fed the formulated feeds, were examined using the variance of analysis for factorial layout, and mean comparison between the treatments were performed using Tukey HSD test. Survival, SGR, feed intake, NRE, ERE, CF, HSI, and components in carcass were arcsine transformed prior to the variance of analysis. Differences in above variables between fish fed the raw fish and formulated feed containing 40% DP and 16 MJ kg^{-1} DE were examined using Student's t-test. Correlation between HSI and carcass lipid content was examined. $P<0.05$ was regarded significantly different.

3. Results

Survival was above 98% for all treatments. There was no significant difference in survival among fish fed the formulated feeds, or between fish fed the raw fish and formulated feed containing 40% DP and 16 MJ kg^{-1} DE.

Table 3
Summary of analysis of variance in variables among fish fed the formulated feeds

Sources of variance	*df*	Significances				
		Feed conversion ratio, energy retention efficiency	Specific growth rate, final body weight, feed intake, moisture and crude protein contents in carcass	Protein retention efficiency	Lipid and ash contents in carcass	Condition factor	Hepatosomatic index
DP	2	$P<0.01$	$P<0.01$	$P<0.05$	NS	NS	NS
DE	2	$P<0.01$	$P<0.01$	NS	$P<0.01$	$P=0.046$	NS
DP*DE	4	$P<0.05$	NS	NS	NS	NS	NS

DP=dietary digestible protein; DE=dietary digestible energy.

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Table 4
Final body weight (g fish^{-1}), specific growth rate (% day^{-1}), feed intake (% day^{-1}) and feed conversion ratio of cuneate drum in the experiment (Mean ± S.E., $n=3$)

Feeds	Final body weight	Specific growth rate	Feed intake	Feed conversion ratio
L1	92.4 ± 3.3^{bc}	2.86 ± 0.06^{bcd}	2.9 ± 0.1^{a}	1.24 ± 0.05^{ac}
L2	99.2 ± 1.5^{ac}	2.99 ± 0.05^{ad}	2.7 ± 0.1^{ab}	1.11 ± 0.03^{bc}
L3	81.1 ± 3.9^{b}	2.60 ± 0.07^{b}	2.8 ± 0.1^{ab}	1.38 ± 0.08^{a}
M1	99.7 ± 2.5^{ac}	2.88 ± 0.05^{cd}	2.9 ± 0.1^{a}	1.25 ± 0.03^{ac}
M2	102.0 ± 4.0^{ac}	2.98 ± 0.01^{ad}	2.8 ± 0.1^{ab}	1.16 ± 0.05^{abc}
M3	89.6 ± 6.4^{bc}	2.69 ± 0.08^{bc}	2.6 ± 0.2^{ab}	1.14 ± 0.09^{abc}
H1	103.7 ± 1.2^{ac}	3.02 ± 0.05^{ad}	2.7 ± 0.0^{ab}	1.13 ± 0.06^{abc}
H2	115.8 ± 0.6^{a}	3.24 ± 0.02^{a}	2.5 ± 0.0^{b}	0.95 ± 0.02^{b}
H3	104.9 ± 4.9^{ac}	3.06 ± 0.07^{ad}	2.3 ± 0.0^{b}	0.92 ± 0.01^{b}
RF	111.7 ± 2.9	3.18 ± 0.04	2.7 ± 0.1	1.05 ± 0.03

The superscripts present results of Tukey HSD test among fish fed the formulated feeds or Student's t-test between fish fed the raw fish (RF) and feed containing 40% DP and 16 MJ kg^{-1} DE (H2). The values within the same column with different superscripts are significantly different at $P<0.05$.

Feed intake was expressed on a dry feed basis.

Dietary DP and DE levels both significantly affected SGR and FBW of fish fed the formulated feeds (Table 3). Specific growth rate and FBW of fish fed the feeds formulated to contain the same DE content increased with the increase of DP from 36% to 40%. For fish fed the feeds formulated at the same DP level, SGR and FBW increased with the increase of DE from 14 to 16 MJ kg^{-1}. Further increase of dietary DE to 18 MJ kg^{-1} resulted in no improvement or even a slight decline in SGR and FBW. Fish fed the feed containing 40% DP and 16 MJ kg^{-1} DE exhibited the highest SGR and FBW among fish fed the formulated feeds (Table 4).

Feed intake and FCR were dependent on both DP and DE levels (Table 3). Feed intake tended to decrease with the increase of DP, and FCR tended to decrease with the increase of DP and DE. However, these tendencies were not statistically significant. Fish fed the feeds containing DP of 40% and DE of 16 and 18 MJ kg^{-1} exhibited better FCR ($P<0.05$), whereas fish fed the feed containing 36% DP and 18 MJ kg^{-1} DE had the highest FCR ($P<0.05$, Table 4).

Nitrogen retention efficiency was dependent on DP level, while ERE was affected by DP and DE levels (Table 3). Nitrogen retention efficiency and ERE increased with the increase of DE from 14 to 16 MJ

Table 5
Nitrogen retention efficiency (%) and energy retention efficiency (%) of cuneate drum in the experiment (Mean ± S.E., $n=3$)

Feeds	Nitrogen retention efficiency	Energy retention efficiency
L1	32.11 ± 1.50^{ab}	29.56 ± 1.15^{bc}
L2	35.03 ± 1.43^{ab}	32.79 ± 1.54^{ac}
L3	27.84 ± 1.26^{b}	26.03 ± 1.05^{b}
M1	30.62 ± 0.81^{ab}	29.58 ± 0.50^{bc}
M2	31.84 ± 1.75^{ab}	30.00 ± 1.40^{bcd}
M3	31.20 ± 2.63^{ab}	29.66 ± 1.45^{bc}
H1	32.07 ± 1.90^{ab}	31.78 ± 0.97^{acd}
H2	36.32 ± 0.33^{aA}	37.16 ± 0.41^{aA}
H3	36.00 ± 0.74^{a}	35.46 ± 0.66^{ad}
RF	22.60 ± 0.94^{B}	29.05 ± 0.88^{B}

The superscripts present results of Tukey HSD test among fish fed the formulated feeds or Student's t-test between fish fed the raw fish (RF) and feed containing 40% DP and 16 MJ kg^{-1} DE (H2). The values within the same column with different superscripts are significantly different at $P<0.05$.

Table 6
Condition factor (g cm^{-3}) and hepatosomatic index (%) of cuneate drum at the end of the experiment (Mean ± S.E., $n=3$)

Feeds	Condition factor	Hepatosomatic index
L1	1.1 ± 0.01	2.4 ± 0.33
L2	1.2 ± 0.03	1.9 ± 0.16
L3	1.1 ± 0.03	2.1 ± 0.27
M1	1.1 ± 0.02	2.3 ± 0.12
M2	1.1 ± 0.03	2.2 ± 0.19
M3	1.1 ± 0.01	1.9 ± 0.13
H1	1.2 ± 0.02	2.1 ± 0.17
H2	1.1 ± 0.01	2.3 ± 0.38^{A}
H3	1.1 ± 0.04	2.0 ± 0.13
RF	1.0 ± 0.03	1.3 ± 0.08^{B}

The superscripts present results of Tukey HSD test among fish fed the formulated feeds or Student's t-test between fish fed the raw fish (RF) and feed containing 40% DP and 16 MJ kg^{-1} DE (H2). The values within the same column with different superscripts are significantly different at $P<0.05$.

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kg^{-1} at the same DP level. Fish fed the feeds containing DP of 40% and DE of 16 and 18 MJ kg^{-1} exhibited higher NRE ($P<0.05$) and ERE ($P<0.05$) relative to fish fed the feeds containing 36% DP and 18 MJ kg^{-1} DE (Table 5). Levels of DP and DE in the formulated feeds did not significantly affect CF and HSI ($P<0.05$, Tables 3 and 6).

At the end of the experiment, moisture and protein contents in carcass of fish were affected by DP and DE levels, while crude lipid and ash contents in carcass were affected by DE level (Table 3). Lipid content in carcass of fish fed the feeds with the same DP level increased with increase in DE. Fish fed the feed containing 36% DP and 18 MJ kg^{-1} DE exhibited the highest lipid content ($P<0.05$, Table 7). There were no correlation between HSI and lipid content in carcass for fish fed the formulated feeds.

There were no significant differences in SGR, FBW, feed intake, FCR (Table 4), CF (Table 6), and protein and lipid contents (Table 7) between fish fed the raw fish and feed containing 40% DP and 16 MJ kg^{-1} DE. Fish fed the feed containing 40% DP and 16 MJ kg^{-1} DE showed higher NRE ($P<0.05$), ERE ($P<0.05$, Table 5), HSI ($P<0.05$, Table 6) and carcass ash content ($P<0.05$, Table 7) than those of fish fed the raw fish.

t7.1 Table 7
t7.2 Carcass composition (%) of cuneate drum in the experiment (mean ± S.E., $n=3$)

t7.3 Feeds	Moisture	Crude protein	Crude lipid	Ash
t7.4 Initial	76.6±0.17	16.2±0.43	1.5±0.03	4.0±0.16
t7.5 L1	73.0 ± 0.08^{ac}	17.7 ± 0.17^{a}	3.7 ± 0.26^{b}	4.3 ± 0.04^{ab}
t7.6 L2	72.4 ± 0.11^{bcd}	17.4 ± 0.14^{ad}	4.8 ± 0.05^{cd}	4.5 ± 0.01^{a}
t7.7 L3	71.8 ± 0.39^{b}	16.7 ± 0.66^{bcd}	6.1 ± 0.32^{a}	4.5 ± 0.10^{a}
t7.8 M1	73.5 ± 0.25^{ad}	17.8 ± 0.07^{acd}	4.0 ± 0.29^{bc}	4.2 ± 0.07^{b}
t7.9 M2	72.7 ± 0.27^{abc}	16.8 ± 0.20^{cd}	5.0 ± 0.01^{ac}	4.4 ± 0.07^{ab}
t7.10 M3	72.2 ± 0.30^{bc}	16.1 ± 0.15^{bc}	5.9 ± 0.31^{acd}	4.5 ± 0.04^{ab}
t7.11 H1	73.7 ± 0.25^{a}	17.5 ± 0.04^{cd}	4.0 ± 0.21^{bc}	4.3 ± 0.05^{ab}
t7.12 H2	72.8 ± 0.20^{abcA}	17.4 ± 0.23^{cd}	4.8 ± 0.19^{ce}	4.4 ± 0.02^{abA}
t7.13 H3	72.7 ± 0.10^{abc}	16.3 ± 0.13^{b}	6.0 ± 0.14^{ae}	4.4 ± 0.06^{ab}
t7.14 RF	73.7 ± 0.17^{B}	17.2 ± 0.30	4.1 ± 0.21	4.2 ± 0.05^{B}

The superscripts present results of Tukey HSD test among fish fed the formulated feeds or Student's *t*-test between fish fed the raw fish (RF) and feed containing 40% DP and 16 MJ kg^{-1} DE (H2). The values within the same column with different superscripts are significantly different at $P<0.05$.

t7.15

t7.16 Contents of crude protein, crude lipid and ash are expressed on a wet weight basis.

4. Discussion

In the present study, fish fed the feeds containing 40% DP, at the same dietary DE level, showed higher SGR and FBW than those of fish fed the feeds containing dietary DP of 36% and 38%, suggesting cuneate drum requires dietary DP of at least 40% to sustain its fast growth. Previous studies indicated Atlantic croaker required dietary crude protein (CP) of 45% (Davis and Arnold, 1997), and red drum of 35% to 45% CP (Daniels and Robinson, 1986; Serrano et al., 1992; McGoogan and Gatlin, 1999; Thoman et al., 1999), and large yellow croaker of 47% CP (Duan et al., 2001), and giant croaker of 45% CP (Lee et al., 2001). Dietary protein requirement of cuneate drum appears to be similar to that of other sciaenids. Extending the comparison to other carnivorous fish species, protein requirement of cuneate drum is similar to that for small mouth bass (45% CP)(Anderson et al., 1981), European sea bass (44% to 45% CP) (Ballestrazzi et al., 1994; Pérez et al., 1997) and cobia (45% CP) (Chou et al., 2001).

In the present study, SGR and FBW of cuneate drum increased with the increase of dietary DE from 14 to 16 MJ kg^{-1}, and then appeared to decrease with the further increase of the dietary DE to 18 MJ kg^{-1}. The fish fed the feeds containing 16 MJ kg^{-1} DE showed relatively low FCR among the DE levels tested, suggesting dietary energy content of 16 MJ kg^{-1} DE was optimal for this fish.

Growth and metabolism of fish are sustained by the energy generated from the catabolism of either protein or non-protein (lipid and carbohydrate). Dietary protein requirements of fish are closely related to dietary energy levels, and by proper use of non-protein energy sources, such as lipid and carbohydrate, dietary protein in fish feed can be spared (Shiau and Lan, 1996). In the present study, protein and energy retention efficiencies increased with the increase of dietary DE from 14 to 16 MJ kg^{-1} at the same dietary DP level, this suggests dietary protein for cuneate drum can be spared by properly elevating of non-protein energy sources. Sparing dietary protein with non-protein energy sources has generally a beneficial effect on feed cost but also helps reduce nitrogen waste outputs. The ratio of protein to energy (P/E) in feeds is therefore an important consideration for the formulation of cost-effective and environment

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friendly fish feed. Dietary P/E varies among fish species, particularly between coldwater and warmwater fish. Coldwater fish, who can utilize high levels of dietary lipid for energy, require lower dietary P/E, e.g. 22 g MJ^{-1} for rainbow trout (Lee and Putnam, 1980) and 18 g MJ^{-1} for Atlantic salmon (Hillestad and Johnsen, 1994). In contrast, P/E for warmwater fish are relatively high, e.g. 31 g MJ^{-1} for grouper (Shiau and Lan, 1996), 28 g MJ^{-1} for Mediterranean yellow tail (Jover et al., 1999), and 28 g MJ^{-1} for red drum (McGoogan and Gatlin, 1999). In the present study, cuneate drum fed the feed containing 40% DP and 16 MJ g^{-1} DE (DP/DE=25 g MJ^{-1}) showed the highest SGR, FBW, NRE, ERE and better FCR, suggesting optimal P/E for the fish is similar or perhaps slightly lower than other warmwater carnivorous fish.

In the present study, cuneate drum fed the feeds containing dietary lipid of 15% to 17% showed higher SGR, FBW, NRE and ERE than those of fish fed the feeds containing dietary lipid of neither 9% to 10% or 22% to 24%. This suggests cuneate drum has a relatively good capacity to utilize dietary lipids as energy sources, and 15% to 17% dietary lipids appears optimal to the fish. This level is higher than values reported from other sciaenid species, such as Atlantic croaker (Davis and Arnold, 1997), red drum (McGoogan and Gatlin, 1999) and large yellow croaker (Duan et al., 2001). Fish fed feeds with high dietary energy exhibits high body lipid deposition (Millikin, 1983). In the present study, lipid content in carcass of fish increased with the increase in DP level, this is consistent with the results of previous studies on large yellow croaker (Duan et al., 2001), Atlantic croaker (Davis and Arnold, 1997) and red drum (Daniels and Robinson, 1986). Grouper fed formulated feeds exhibited higher weight gain and carcass lipid content than those fed raw fish (Milliamena, 2002). In the present study, cuneate drum fed the formulated feed containing 40% DP and 16 MJ kg^{-1} DE exhibited similar feed intake, SGR, FBW and FCR to those fed the raw fish, but showed higher NRE and ERE compared to those fed the raw fish, suggesting not only dietary protein and energy of the formulated feed are adequate to sustain rapid growth but also that nitrogen waste output from farming of this fish can be reduced by using formulated feeds.

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Replacement of fish meal by rendered animal protein ingredients in feeds for cuneate drum (*Nibea miichthioides*)

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Abstract

An 8-week feeding trial was carried out in floating net pens to examine the replacement of fish meal with three rendered animal protein ingredients, poultry by-product meal (PBM), meat and bone meal (MBM) and feather meal (FM) at various levels in practical feeds for cuneate drum. Triplicate groups of fish (initial body weight 27 g fish^{-1}) were fed nine isonitrogenous and isocaloric feeds formulated to contain 36% digestible protein and 15 MJ kg^{-1} digestible energy. The control feed contained 35% herring meal, whereas in the other eight feeds, PBM, MBM and FM, alone or in combination, directly replaced 10%, 30% or 50% of the fish meal. In addition, a raw fish feed was used as a comparison to assess growth performance of fish fed the formulated feeds. There were no significant differences in feed intake and feed conversion ratio (FCR) among fish fed the formulated feeds. Specific growth rate (SGR) and final body weight (FBW) of fish fed the feeds in which either PBM replaced 30% to 50 % of the fish meal or MBM replaced 30% of the fish meal were not significantly different from fish fed the control feed. Feather meal incorporation in the feeds resulted in lower SGR and FBW compared to those of fish fed the control feed. Replacing 50% of the fish meal by MBM significantly lowered SGR, FBW and nitrogen retention efficiency, whereas replacing 50% of the fish meal by a combination of PBM, MBM, FM, blood meal and soybean meal resulted in lower SGR and FBW. There were no significant differences in chemical composition of whole body among fish fed the formulated feeds. Results of the present study indicate that PBM can be used alone at 17% (to replace 50% of the fish meal), and MBM at 10% (to replace 30% of the fish meal) in feeds for cuneate drum.
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Keywords: Cuneate drum; Poultry by-product meal; Meat and bone meal; Feather meal; Growth; Nitrogen retention efficiency

1. Introduction

Cuneate drum is a carnivorous sciaenid native to near-shore waters of the China Sea (Chu and Wu, 1985), and has been widely cultured in net pens along the coast of the China Sea, from Zhanjiang to

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Lianyungang. Cuneate drum are generally fed raw fish on commercial operations and this results in high feed costs and cause serious environmental problems, due to the high amount of nitrogenous waste associated with feeding raw fish (Wang et al., in press). Feed formulae that have high nutritive value are cost-effective, and produce less waste outputs needed to improve economical and environmental sustainability of cuneate drum culture in China.

Fish meal is generally incorporated at levels between 30% and 60% in feeds for carnivorous marine fish. Fish meal is an expensive ingredient. Cost-effectiveness of the feed could be improved by replacing fish meal with more economical protein sources, such as rendered animal protein ingredients, e.g., poultry by-product meal (PBM), meat and bone meal (MBM) and feather meal (FM). These ingredients have been used successfully in feeds for various fish species, such as chinook salmon (Fowler, 1990, 1991), silver seabream (El-Sayed, 1994), rainbow trout (Steffens, 1994; Bureau et al., 2000), red drum (Moon and Gatlin, 1994; Kureshy et al., 2000), gilthead seabream (Robaina et al., 1997; Nengas et al., 1999), Indian major carp (Hasan et al., 1997), Australian snapper (Quartararo et al., 1998), Australian silver perch (Allan et al., 2000; Stone et al., 2000), Nile tilapia (El-Sayed, 1998), sunshine bass (Webster et al., 2000) and grouper (Milliamena, 2002). The suitability of these ingredients for cuneate drum has not been evaluated.

The present study was conducted to assess the effect of using rendered animal proteins, alone or in combination, as ingredients in practical feeds on growth, feed utilization, and body composition of cuneate drum.

2. Material and methods

2.1. Feed formulation and preparation

Poultry by product meal, MBM, FM and blood meal (BM) were obtained from various suppliers in the USA through the National Rendered Association. Other feed ingredients were obtained from a local feed company (Xinyang Feed, Shanghai, China). The proximate composition and gross energy content of the ingredients used in this study are presented in Table 1, and the amino acid profile in Table 2.

Nine dry feeds were formulated to contain 36% digestible protein (DP) and 15 MJ kg^{-1} digestible energy (DE), and a tenth feed consisted of raw fish (*Sardinella* spp.) and this feed served as a comparison to the formulated feeds. The control feed contained 350 g kg^{-1} herring meal. In the other eight feeds, the fish meal was directly replaced by PBM, MBM, FM alone, or with a combination (APM) of PBM, MBM, FM, BM, and soybean meal (SM). Dietary DP and DE of the feeds were calculated using the published apparent digestible coefficients (Bureau et al., 1999). The feeds were formulated isonitrogenous and isocaloric by adjusting proportion of BM, SM and wheat flour in formulation. The formulation and chemical composition of the test feeds are presented in Table 3, and amino acid profile in Table 4.

The dry ingredients were ground with a hammer grinder, passed through a 0.5 mm sieve, and mixed in

Table 1
Proximate composition (%) and gross energy content (MJ kg^{-1}) of the ingredients

Ingredients	Dry matter	Crude protein	Crude lipid	Ash	Gross energy
Meat and bone meal	94.6	60.2	11.0	23.7	19.4
Feather meal	92.0	83.0	11.7	2.9	22.8
Blood meal (spray-dried)	93.2	98.5	0.1	1.6	25.1
Poultry by product meal	95.3	67.4	15.9	12.4	23.2
Herring meal	89.9	72.5	8.6	16.0	18.4
Soybean meal (solvent-extracted)	87.6	50.1	0.9	6.2	18.5
Rapeseed meal	88.7	41.1	1.8	7.8	18.3
Wheat flour	85.9	13.1	1.0	0.6	17.7
APM	93.2	61.8	8.9	12.4	19.2

APM consist of 30% poultry by product meal, 29.5% meat and bone meal, 20% soybean meal, 10.5% blood meal and 10% feather meal.
Crude protein, crude lipid, ash and gross energy are expressed on a dry matter basis.

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t2.1 Table 2
t2.2 Essential amino acid (%) profile of the ingredients

t2.3 Ingredients	Thr	Val	Cys	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg
t2.4 Meat and bone meal	1.80	2.70	0.43	0.96	2.12	4.09	1.30	1.97	3.73	1.21	3.72
t2.5 Feather meal	2.80	5.80	3.10	0.62	3.67	6.32	1.78	3.22	1.97	0.80	4.80
t2.6 Blood meal (spray-dried)	3.18	7.73	0.82	0.99	1.51	12.31	2.24	5.48	8.54	5.80	3.80
t2.7 Poultry by product meal	1.92	2.95	0.55	1.13	2.45	4.52	1.57	2.24	3.84	1.35	4.78
t2.8 Herring meal	2.22	3.05	0.44	1.58	2.73	4.80	1.74	2.15	4.72	1.99	3.55
t2.9 Soybean meal (solvent-extracted)	1.40	2.14	0.38	0.38	2.05	3.45	1.05	1.92	2.83	1.04	2.94
t2.10 Rapeseed meal	1.50	2.14	0.56	0.44	1.68	2.99	0.89	1.39	2.16	0.95	
t2.11 APM	1.98	3.37	0.73	0.85	2.24	5.18	1.46	2.53	3.94	1.70	3.93

APM consist of 30% poultry by product meal, 29.5% meat and bone meal, 20% soybean meal, 10.5% blood meal and 10% feather meal. Threonine (Thr), Valine (Val), Cysteine (Cys), Methionine (Met), Isoleucine (Ile), Leucine (Leu), Tyrosine (Tyr), Phenylalanine (Phe), Lysine
t2.13 (Lys), Histidine (His) and Arginine (Arg) are expressed on a dry weight basis.

t3.1 Table 3
t3.2 Formulation (%), proximate composition (%) and energy content (MJ kg^{-1}) of the test feeds

t3.3 Ingredients	Feeds									
t3.4	Control	MBM1	MBM2	MBM3	FM1	FM2	PBM1	PBM2	MM	RF
t3.5 Herring meal	35.0	31.5	24.5	17.5	31.5	24.5	24.5	17.5	17.5	
t3.6 Poultry by product meal							10.5	17.5		
t3.7 Meat and bone meal		3.5	10.5	17.5						
t3.8 Feather meal					3.5	10.5				
t3.9 Blood meal	3.0	6.0	2.6	5.0	5.0	5.0	3.3	3.3	3.3	
t3.10 APM									0.17.5	
t3.11 Soybean meal	20.0	14.9	25.0	20.0	14.5	13.5	20.0	20.0	21.5	
t3.12 Rapeseed meal	8.0	8.0	8.2	9.0	9.0	9.0	9.0	9.0	9.0	
t3.13 Wheat flour	21.0	22.3	15.2	17.0	22.8	25.0	19.7	20.3	17.7	
t3.14 $CaHPO_4$	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	
t3.15 DL-Methionine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	
t3.16 Fish oil	9.0	9.8	10.0	10.0	9.7	8.5	9.0	8.4	9.5	
t3.17 Vitamin premix	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
t3.18 Mineral premix	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
t3.19 Nutrient and energy contents										
t3.20 Dry matter (%)	89.9	89.2	89.7	91.6	88.8	90.6	90.7	91.3	90.2	24.7
t3.21 Crude protein	42.6	41.3	40.9	41.8	41.3	42.6	42.3	42.5	42.7	72.5
t3.22 Crude lipid	12.3	12.0	13.9	14.2	13.7	12.1	13.2	13.1	13.2	8.5
t3.23 Ash	10.1	10.0	10.9	11.0	9.2	8.2	9.5	9.4	9.4	18.2
t3.24 Gross energy	17.8	18.2	18.3	18.5	18.2	18.2	18.3	18.4	18.4	17.9
t3.25 DDM (%)	68.4	68.9	68.2	68.0	68.3	67.3	67.8	67.4	67.5	
t3.26 DP (%)	35.8	35.9	35.8	35.5	35.6	35.4	35.8	35.5	35.4	
t3.27 DE	14.8	15.1	14.8	14.8	15.1	14.8	15.0	15.1	14.9	
t3.28 DP/DE (g MJ^{-1})	24.2	23.7	24.1	23.9	23.6	23.9	23.8	23.6	23.8	

APM consist of 30% poultry by product meal, 29.5% meat and bone meal, 20% soybean meal, 10.5% blood meal and 10% feather meal. Vitamin premix provides per kg of feed: retinyl acetate, 3000 IU; cholecalciferol, 2400 IU; all-rac-α-tocopheryl acetate, 60 IU; menadione sodium bisulfite, 1.2 mg; ascorbic acid monophosphate (49% ascorbic acid), 120 mg; cyanocobalamine, 0.024 mg; D-biotin, 0.168 mg; choline chloride, 1200 mg; folic acid, 1.2 mg; niacin, 12 mg; D-calcium pantothenate, 26 mg; pyridoxine.HCl, 6 mg; riboflavin, 7.2 mg; thiamin.HCl,
t3.30 1.2 mg.
Mineral premix provides per kg of feed: sodium chloride (39% Na, 61% Cl), 3077 mg; ferrous sulfate (20% Fe), 65 mg; manganese sulfate (36% Mn), 89 mg; zinc sulfate (40% Zn), 150 mg; copper sulfate (25% Cu), 28 mg; potassium iodide (24% K, 76% I), 11 mg; Celite AW521
t3.31 (acid-washed diatomaceous earth silica), 1000 mg.
t3.32 Crude protein, lipid, ash, gross energy, DP and DE are expressed on a dry matter basis and given as means (n=2).
t3.33 DDM=digestible dry matter; DP=digestible protein; DE=digestible energy; RF=raw fish.

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t4.1 Table 4
t4.2 Essential amino acid (%) profile of the test feeds

t4.3 Feeds	Thr	Val	Cys	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg
t4.4 Control	1.50	2.39	0.39	1.42	1.76	3.78	1.16	1.79	3.13	1.49	2.43
t4.5 MBM1	1.49	2.38	0.39	1.40	1.74	3.76	1.15	1.78	3.10	1.46	2.45
t4.6 MBM2	1.45	2.21	0.38	1.32	1.80	3.48	1.10	1.70	2.94	1.25	2.56
t4.7 MBM3	1.43	2.29	0.39	1.28	1.70	3.59	1.08	1.75	2.94	1.30	2.52
t4.8 FM1	1.49	2.36	0.47	1.38	1.78	3.70	1.14	1.77	2.94	1.39	2.44
t4.9 FM2	1.47	2.32	0.63	1.29	1.82	3.58	1.10	1.74	2.59	1.20	2.45
t4.10 PBM1	1.44	2.23	0.39	1.34	1.78	3.52	1.12	1.71	2.90	1.28	2.59
t4.11 PBM2	1.40	2.22	0.40	1.31	1.75	3.49	1.10	1.72	2.81	1.23	2.66
t4.12 MM	1.42	2.34	0.44	1.27	1.69	3.67	1.08	1.79	2.85	1.33	2.49

t4.13 Threonine (Thr), Valine (Val), Cysteine (Cys), Methionine (Met), Isoleucine (Ile), Leucine (Leu), Tyrosine (Tyr), Phenylalanine (Phe), Lysine (Lys), Histidine (His) and Arginine (Arg) are expressed on a dry weight basis.

a 30-l kitchen mixer. Slow sinking pellets were made using a laboratory-scale, single screw extruder (extruding temperature was controlled to be between 100 and 120 °C). The pellets (diameter 4 mm and length 8 mm) were cooled and dried at room temperature.

2.2. Fish, husbandry and feeding

An 8-week feeding trial was carried out in net pens in Shenao Bay, Shantou, China. Cuneate drum (*Nibea miichthioides*) fingerlings were obtained from a local marine fish hatchery (Qingao Bay Hatchery, Shantou, Guangdong, China). After transportation, the fish were reared in net pens (3 m × 3 m × 2 m), and gradually weaned from raw fish onto the control feed during an 8-week period. Two weeks prior to the trial, 1280 fish were selected and reared in 32 experimental pens (1 m × 1 m × 1.5 m) at 40 fish per pen, during the acclimation the fish were fed the control feed twice daily. At the start of the trial, the acclimated fish were deprived of feed for 24 h, pooled, and 30 groups each of 30 fish weighing 27.4 ± 0.2 g fish^{-1} (mean ± S.E., n = 30) were batch weighed, and randomly stocked into 30 experimental pens, with 3 replication of each treatment. Eight sub-samples of 3 fish each were removed from the remaining acclimated fish for the determination of initial body composition. The sampled fish were frozen at −20 °C until analysis.

During the trial, the fish were hand fed at 08:00 and 16:00 h daily except on days of strong waves or high temperatures. At each feeding, some pellets were dropped in each pen until no feeding activity of fish was observed. Dead fish were recorded and weighed for calculating feed conversion ratio (FCR). At the end of the trial, the fish were collected from each pen and batch weighed. Three fish were sampled from each pen for the determination of final body composition. The sampled fish were frozen at −20 °C until analysis.

Water temperature was measured daily and salinity weekly. Water temperature ranged from 25 to 32 °C, and salinity from 31‰ to 32‰ during the feeding trial.

2.3. Chemical analysis

The fish sampled at the start and end of the trial and raw fish sampled during the trial were autoclaved at 120 °C for 20 min, homogenized, and dried at 105 °C for 24 h prior to the chemical analysis. The samples of the ingredients, formulated feeds, raw fish and cuneate drum were ground into fine power with a laboratory grinder. Contents of moisture, crude protein, crude lipid, ash and gross energy, and amino acids in the ingredients, feeds and sampled fish were measured using the methods described in Wang et al. (in press).

2.4. Calculations and statistical analyses

Feed intake, specific growth rate (SGR), FCR and nitrogen retention efficiency (NRE) was calculated as below:

$$\text{Feed intake } (\%\text{day}^{-1}) = 100 \times I/[(W_0 + W_t)/2 \times t]$$

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$$\text{SGR } (\%\text{day}^{-1}) = [\text{Ln}(W_t/N_t) - \text{Ln}(W_0/N_0)]/t$$

$$\text{FCR } (\text{dry feed gain}^{-1}) = I/(W_t - W_0 + W_d)$$

$$\text{NRE } (\%) = 100 \times (W_t \times C_{Nt} - W_0 \times C_{N0} + W_d \times C_{N0}) / (I \times C_{Nf})$$

where I (g) is total amount of the feed consumed on a dry weight basis, W_0 (g) is total initial body weight and W_t (g) total final body weight, t (d) is duration of the feeding trial, N_t is number of fish at the end of the trial and N_0 at the start of the trial, W_d (g) is total body weight of the dead fish, C_{Nt} (%) is nitrogen content in whole fish body at the end of the trial and C_{N0} (%) at the start of the trial, C_{Nf} (%) is nitrogen content in the feeds.

One-way analysis of variance was performed to examine differences in survival, SGR, final body weight (FBW), feed intake, FCR, NRE and body components (contents of moisture, crude protein, crude lipid and ash) among fish fed the formulated feeds, and means between fish fed the control and other formulated feeds were examined using Tukey HSD test. Survival, SGR, NRE and body components were arcsine transformed. Differences in above variables between fish fed the raw fish and control feed were examined using Students t-test. Significance was accepted at $P<0.05$.

3. Results

Survival of fish in all the treatments was very high (greater than 94%) and there was no significant difference among fish fed the formulated feeds and between fish fed the raw fish and control feed.

Specific growth rate and FBW in fish fed the control feed was higher than fish fed the feeds in which the fish meal was replaced by 10% and 30% with FM, or by 10% and 50% with MBM, or by 50% with APM, but did not differ significantly from those fed the feeds in which the fish meal was replaced by 30% to 50% with PBM, or by 30% with MBM. There were no significant differences in feed intake and FCR among fish fed the formulated feeds. Replacing 50% of the fish meal by MBM resulted in lower NRE ($P<0.05$, Table 5).

There were no significant differences in moisture, crude protein, crude lipid and ash contents in whole body between fish fed the control and feeds in which the fish meal was replaced with rendered proteins at various levels. Fish fed the feed in which APM replaced 50% of the fish meal had higher crude protein content of whole body than that of fish fed the feed in which MBM replaced 50% of the fish meal ($P<0.05$, Table 6).

Fish fed the raw fish showed higher SGR ($P<0.05$), FBW, feed intake, whole body crude protein content, and lower NRE and whole body crude

Table 5
Final body weight (g fish^{-1}), specific growth rate (% day^{-1}), feed intake (% day^{-1}), feed conversion ratio (feed gain^{-1}) and nitrogen retention efficiency (%) of cuneate drum fed the test feeds (Mean ± S.E., $n=3$)

Feeds	Final body weight	Specific growth rate	Feed intake	Feed conversion ratio	Nitrogen retention efficiency
Control	93.8 ± 1.8^{aA}	2.21 ± 0.02^{aA}	2.03 ± 0.07^{A}	1.05 ± 0.03	35 ± 1^{aA}
MBM1	76.2 ± 3.8^{bc}	1.79 ± 0.09^{bc}	2.09 ± 0.09	1.35 ± 0.02	27 ± 2^{ab}
MBM2	83.7 ± 2.0^{ac}	2.00 ± 0.06^{ac}	1.94 ± 0.06	1.07 ± 0.06	35 ± 3^{a}
MBM3	66.8 ± 2.3^{b}	1.62 ± 0.11^{b}	2.03 ± 0.18	1.40 ± 0.06	19 ± 4^{b}
FM1	78.5 ± 5.5^{bc}	1.87 ± 0.09^{bc}	2.16 ± 0.19	1.34 ± 0.15	28 ± 3^{ab}
FM2	73.2 ± 4.4^{bc}	1.77 ± 0.11^{bc}	2.03 ± 0.10	1.27 ± 0.12	29 ± 3^{ab}
PBM1	82.3 ± 2.4^{abc}	1.97 ± 0.06^{abc}	1.88 ± 0.05	1.07 ± 0.04	35 ± 1^{a}
PBM2	83.5 ± 2.7^{ac}	1.98 ± 0.06^{abc}	1.95 ± 0.16	1.10 ± 0.06	33 ± 2^{ab}
MM	74.1 ± 1.8^{bc}	1.74 ± 0.04^{bc}	1.73 ± 0.09	1.13 ± 0.04	35 ± 4^{a}
RF	114.5 ± 1.7^{B}	2.54 ± 0.02^{B}	2.33 ± 0.04^{B}	1.10 ± 0.03	23 ± 1^{b}

RF = raw fish.
Feed intake and feed conversion ratio are expressed on a dry feed basis.
Values in the same column with different superscripts are statistically different at $P<0.05$.

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t6.1 Table 6
t6.2 Proximate composition (%) in whole body of cuneate drum fed the test feeds (Mean ± S.E., $n=3$)

t6.3 Feeds	Moisture	Crude protein	Crude lipid	Ash
t6.4 Initial	76.1 ± 0.1	16.2 ± 0.3	2.8 ± 0.1	4.5 ± 0.1
t6.5 Control	73.7 ± 0.3	15.7 ± 0.2^{abA}	6.4 ± 0.2^{A}	3.8 ± 0.1
t6.6 MBM1	74.2 ± 0.7	15.6 ± 0.4^{ab}	5.8 ± 0.1	4.0 ± 0.1
t6.7 MBM2	73.3 ± 0.6	15.6 ± 0.4^{ab}	6.8 ± 0.1	3.9 ± 0.1
t6.8 MBM3	77.6 ± 3.1	13.2 ± 1.8^{b}	5.4 ± 0.8	3.4 ± 0.4
t6.9 FM1	74.4 ± 0.4	15.4 ± 0.1^{ab}	6.1 ± 0.2	3.8 ± 0.1
t6.10 FM2	73.8 ± 0.2	15.8 ± 0.1^{ab}	6.3 ± 0.0	3.9 ± 0.0
t6.11 PBM1	73.1 ± 0.3	16.0 ± 0.1^{ab}	7.0 ± 0.1	3.9 ± 0.1
t6.12 PBM2	73.4 ± 0.2	15.7 ± 0.2^{ab}	6.9 ± 0.2	3.8 ± 0.0
t6.13 MM	73.2 ± 0.8	16.8 ± 0.8^{a}	6.7 ± 0.2	3.9 ± 0.1
t6.14 RF	74.9 ± 0.5	17.3 ± 0.5^{B}	3.5 ± 0.4^{B}	3.9 ± 0.0

t6.15 RF = raw fish.
t6.16 Crude protein, crude lipid and ash are expressed on a wet weight basis.
t6.17 Values in the same column with different superscripts are statistically different at $P<0.05$.

211 lipid content, than those of fish fed the control feed.
212 There were no significant differences in FCR and
213 moisture and ash contents in whole body between
214 fish fed the control and raw fish feed (Tables 5 and 6).

215 4. Discussion

216 In the present study, the test feeds were formulated
217 at DP and DE levels lower than the DP (40%) and DE
218 (16 MJ kg^{-1}) levels recently found optimal for cune-
219 ate drum (Wang et al., in press), based on a hypothesis
220 that suitability of the rendered proteins could be eval-
221 uated accurately when the fish fed at sub-optimal
222 dietary DP and DE levels. Cuneate drum fed the
223 control feed exhibited lower SGR and FBW than
224 fish fed the raw fish, but showed very similar growth
225 to fish fed the feed containing 36% DP and 16 MJ
226 kg^{-1} (Wang et al., in press), suggesting dietary DP of
227 36% and DE of 15 MJ kg^{-1} in the test feeds can
228 support adequate growth of cuneate drum. Fish fed the
229 control feed had higher NRE than fish fed the raw fish
230 in the present study, this further confirms the results of
231 a previous study (Wang et al., in press), and indicates
232 nitrogen waste outputs from cuneate drum farming
233 can be significantly reduced by using formulated
234 feed compared to raw fish.
235 The PBM used in the present study had high
236 protein and energy contents and balanced amino
237 acid. Incorporation of the PBM in feed formulation
238 at 10.5% to 17.5% (to replace 30% to 50% of the fish
239 meal) did not result in significantly negative effects on
240 SGR, FBW, FCR and NRE, suggesting the PBM is an
241 adequate protein ingredient for cuneate drum. In pre-
242 vious studies, PBM have been demonstrated success-
243 ful in use at 20% in feeds for chinook salmon (Fowler,
244 1991), 25% for silver seabream (El-Sayed, 1994),
245 21% for Australian snapper (Quartararo et al.,
246 1998), 71% for gilthead seabream (Nengas et al.,
247 1999), and 14% for red drum (Kureshy et al., 2000),
248 although declined growth performance was observed
249 in Australian silver perch (Allan et al., 2000) and
250 sunshine bass (Webster et al., 2000) fed feeds contain-
251 ing high PBM level. The results of the present study
252 confirm the conclusions of the previous studies on
253 chinook salmon (Fowler, 1991), silver seabream (El-
254 Sayed, 1994), Australian snapper (Quartararo et al.,
255 1998) and gilthead seabream (Nengas et al., 1999),
256 and indicate the PBM could be directly used at 17% in
257 feeds for cuneate drum.
258 In the present study, incorporating the MBM at
259 10.5% (to replace 30% of the fish meal) in feed
260 formulation for cuneate drum resulted in negligible
261 changes in SGR, FBW, FCR and NRE. This is in
262 agreement with the conclusion of a previous study
263 that indicated MBM could be used with success at
264 10% in feeds for red drum (Kureshy et al., 2000).
265 Incorporation of MBM at more than 24% in feeds for
266 gilthead seabream (Robaina et al., 1997), rainbow
267 trout (Bureau et al., 2000), sunshine bass (Webster
268 et al., 2000) and grouper (Milliamena, 2002) did not
269 result in negative effect on growth performance of
270 these fish. Cuneate drum fed the feed in which the
271 MBM was incorporated at 17.5% (to replace 50% of
272 the fish meal) in feed formulation, however, exhibited
273 significantly lower SGR, FBW and NRE than those
274 fed the control feed. As the test feeds used in the
275 present study were formulated isonitrogenous and
276 isocaloric and to have similar amino acid profile,
277 this implies the MBM was deficient at least in some
278 essential nutrients beside protein, energy and amino
279 acid. Results of the present study reveal the MBM
280 should not be used alone at an inclusion rate of more
281 than 10% in feeds for cuneate drum.
282 In the present study, cuneate drum fed the feeds in
283 which the FM was incorporated at 3.5% to 10.5% (to
284 replace 10% to 30% of the fish meal) in feed formula-

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tion exhibited lower SGR and FBW than those of fish fed the control feed, indicating the FM is not a good substitute for fish meal in feeds for cuneate drum. This disagrees with the results of the previous studies that indicated that chinook salmon (Fowler, 1990) and rainbow trout (Bureau et al., 2000) grew well when fed feeds containing 5% to 15% FM (replacing up to 30% of the fish meal). The FM used in the present study had high contents of protein and energy, low ash content, but was probably deficient in methionine and lysine. The amino acid of the tested feeds used in the present study was well balanced by adding crystal amino acid. The low nutritional value of the FM may be attributed to low availability of protein in the ingredient (Bureau et al., 1999).

Nutritional benefits of using combinations of various animal or plant ingredients, such as PBM, FM and BM (Fowler, 1991), PBM and FM (Steffens, 1994), PBM and SM (Quartararo et al., 1998), MBM and SM (Webster et al., 2000), MBM and BM (Milliamena, 2002) have been demonstrated for many fish species. In the present study, a combination of PBM, MBM, SM, BM and FM, of which protein content and amino acid profiled were formulated similar to that of the fish meal, were incorporated at 17.5% (to replace 50% of the fish meal) in feed formulation. Fish fed the feed had lower SGR and FBW than fish fed the control feed, but did not show any difference in SGR and FBW compared with fish fed the feeds in which either PBM replaced 30% to 50% of the fish meal or MBM replaced 30% of the fish meal.

Theoretically, the replacement level of fish meal by substitute proteins in fish feeds are partially dependent on the amount of the fish meal used in the basal feed. Abnormally high replacement level of fish meal may be achieved when the fish meal are used in excess in the basal feed. Fish meal introduced in basal feeds was more than 50% in the studies on chinook salmon (Fowler, 1990), rainbow trout (Steffens, 1994; Bureau et al., 2000), silver seabream (El-Sayed, 1994), gilthead seabream (Robaina et al., 1997; Nengas et al., 1999) and Australian snapper (Quartararo et al., 1998), and 30% to 40% in the studies on chinook salmon (Fowler, 1991), red drum (Moon and Gatlin, 1994; Kureshy et al., 2000) and grouper (Milliamena, 2002). The lower fish meal replacement level, by MBM and FM, determined in the present study, compared to those determined in the studies on chinook salmon (Fowler, 1990), gilthed seabream (Robaina et al., 1997) and rainbow trout (Bureau et al., 2000), may be due to lower amount of fish meal (35% herring meal) used in the basal feed in the present study, rather than lower capacity for cuneate drum to utilize MBM and FM. By formulating the test feeds at sub-optimal dietary protein and energy levels, and by formulating the basal feed to contain fish meal at a relatively low level, therefore, the fish meal replacement levels determined in the present study reliably reflect the potential use of PBM, MBM and FM in feeds for cuneate drum.

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