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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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1
2 Effects of dietary protein and energy levels on growth, feed
3 utilization and body composition of cuneate drum
4 (*Nibea miichthioides*)

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10 Abstract

11 An 8-week experiment was conducted in net pens to assess the effects of dietary protein and energy levels on growth, feed
12 utilization and body composition of cuneate drum. Cuneate drum (initial body weight 19 g fish⁻¹) were fed 9 feeds formulated
13 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).
14 Groups of fish were fed raw fish (*Sardinella* spp.) to serve as a commercial control. Specific growth rate (SGR), final body
15 weight (FBW), feed intake, feed conversion ratio (FCR), energy retention efficiency (ERE), and moisture and protein contents
16 in carcass of the fish were significantly affected by DP and DE levels. Nitrogen retention efficiency (NRE) was dependent on
17 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
18 feeds with the same DE level increased when DP increased from 36% to 40%, whereas SGR and FBW of fish fed the feeds
19 formulated at the same DP level increased when dietary DE increased from 14 to 16 MJ kg⁻¹. No improvement, or even a slight
20 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
21 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
22 increased with increases in DE level. There were no significant differences in SGR, FBW, feed intake, FCR, and protein and
23 lipid contents in carcass of fish fed the raw fish and feed containing 40% DP and 16 MJ kg⁻¹ DE. Nitrogen retention efficiency
24 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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26 **Keywords:** Cuneate drum; Protein; Energy; Growth; Body composition

27
28 1. Introduction

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

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32 able characteristics, such as good fillet quality, fast
33 growth, and high resistance against diseases. Wild
34 cuneate drum are predators of small finfish and shell-
35 fish (Chu and Wu, 1985), and fish reared in commercial
36 pens are generally fed raw fish. Feeding raw fish results
37 in high feed costs for commercial operations, as well as
38 high waste outputs, an increasingly important concern
39 in some regions due to the high farm densities encoun-
40 tered. Wider use of high quality formulated feed could
41 help improve the economical and environmental sus-
42 tainability of the Chinese cuneate drum industry, as it
43 has been the case for other species in various countries.

44 Growth, feed utilization and body composition of
45 fish closely depend upon contents of protein and
46 energy in feed. Although dietary protein and energy
47 requirements have been studied in many fish species,
48 information of nutritional requirements of sciaenid fish
49 species is still limited, with most studies having been
50 limited to red drum (Daniels and Robinson, 1986;
51 Williams and Robinson, 1988; Ellis and Reigh, 1991;
52 Serrano et al., 1992; Moon and Gatlin, 1994; McGoo-
53 gan and Gatlin, 1998, 1999; Thoman et al., 1999),
54 Atlantic croaker (Davis and Arnold, 1997), giant croa-
55 ker (Lee et al., 2001), and large yellow croaker (Duan et
56 al., 2001). Dietary protein and energy requirements for
57 cuneate drum have not been determined. In the present
58 study, we examined the effects of protein and energy
59 levels in practical feeds on growth, feed utilization and
60 body composition of cuneate drum.

61 2. Material and methods

62 2.1. Test feeds

63 A 3 × 3 factorial layout including 3 levels of diges-
64 tible dietary protein (DP 36%, 38% and 40%) and 3
65 levels of digestible dietary energy (DE 14, 16 and 18
66 MJ kg⁻¹) was established. In addition, frozen *Sardi-*
67 *nella* spp., a raw fish feed widely used in commercial
68 cuneate drum farming, served as a comparison to the
69 formulated feeds. A total of 10 feed treatments (9
70 formulated feeds and 1 raw fish diet) were, therefore,
71 examined in this experiment. Contents of DP and DE
72 in the formulated feeds were estimated using pub-
73 lished digestible coefficients (Bureau et al., 1999).
74 Amino acids in whole body of cuneate drum were
75 analyzed, served as a reference to establish adequate

76 dietary amino acid levels. Amino acids (expressed on
77 a dry weight basis) in whole body of cuneate drum
78 included: threonine 2.21%, valine 2.79%, cysteine
79 0.21%, methionine 1.77%, isoleucine 2.46%, leucine
80 4.28%, tyrosine 1.54%, phenylalanine 2.25%, lysine
81 4.56%, histidine 1.23%, arginine 3.95%. Synthetic
82 methionine (DL-methionine) was added to the formu-
83 lated feeds as it was predicted to be the first limiting
84 amino acid. Formulation, chemical composition and
85 energy content of the feeds are shown in Table 1, and
86 amino acid profile in Table 2.

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

95 2.2. Feeding and sampling

96 An 8-week experiment was carried out in net pens
97 in Shenao Bay, Nanao, China. Cuneate drum (*Nibea*
98 *mitchthioides*) fingerlings were collected from Raop-
99 ing marine fish hatchery, and transported by boat to
100 the experimental site. The fish were reared in net pens
101 (3 m × 3 m × 2 m) for 4 weeks, during which the fish
102 were gradually weaned from raw fish onto the for-
103 mulated feed containing 38% DP and 16 MJ kg⁻¹
104 DE. After the pre-acclimation period, 1200 fish, with
105 similar body size, were moved into 30 experimental
106 pens (1 m × 1 m × 1.5 m) at 40 fish per pen, and
107 acclimated to the formulated feed (38% DP and 16 MJ
108 kg⁻¹ DE) for 2 weeks.

109 At the start of the experiment, fish were deprived
110 of feed 24 h and pooled. Thirty groups of 30 fish each,
111 with initial body weight of 19.1 ± 0.2 g fish⁻¹ (mean
112 ± S.E., n=30), were batch weighed and randomly
113 distributed into 30 experimental pens. Each feed treat-
114 ment had 3 replications. Eight sub-samples of 3 fish
115 each were randomly collected from the remaining
116 acclimated fish, and sacrificed for calculating condi-
117 tion factor (CF) and hepatosomatic index (HSI), and
118 for analysis of proximate composition in whole body
119 and carcass. Total length and body weight of the fish
120 sampled were measured, and then liver of 4 sub-
121 samples were dissected and weighed. Whole body

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	<i>Feed formulations</i>										
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	<i>Feed nutrient and energy contents^{c,d}</i>										
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 D₃, 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; folic acid, 1; vitamin B₁₂, 0.02; ascorbic acid, 50.

t1.29 ^b Mineral mixture provided (mg per kg of feed): NaCl, 1200; FeSO₄, 13; ZnSO₄, 60; MnSO₄, 32; CuSO₄, 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

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

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

2.3. Chemical analysis 140

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

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

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

155 2.4. Calculation and statistical analysis

156 Feed intake, specific growth rate (SGR), FCR, nitro-
 157 gen retention efficiency (NRE), energy retention effi-
 158 ciency (ERE), CF and HSI were calculated as below:

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

$$159 \text{ SGR (\%day}^{-1}\text{)} = [\text{Ln}(W_t/N_t) - \text{Ln}(W_0/N_0)] / t$$

$$160 \text{ FCR (dry feed gain}^{-1}\text{)} = I / (W_t - W_0 + W_d)$$

$$161 \text{ NRE (\%)} = 100 \times (W_t \times C_{Nt} - W_0 \times C_{N0} + W_d \times C_{N0}) / (I \times C_{Nf})$$

$$162 \text{ ERE (\%)} = 100 \times (W_t \times C_{Et} - W_0 \times C_{E0} + W_d \times C_{E0}) / (I \times C_{Ef})$$

$$163 \text{ CF (g cm}^{-3}\text{)} = 100 \times W_s / L_s^3$$

$$164 \text{ HSI (\%)} = 100 \times W_l / W_s$$

166 where I (g) is total amount of the consumed feed on a
 167 dry weight basis, W_t (g) is total final body weight and

W_0 (g) total initial body weight, t (day) is duration of 168
 the experiment, N_t is number of fish at the end of the 169
 experiment and N_0 at the start of the experiment, W_d (g) 170
 is total body weight of the dead fish, C_{Nt} (%) is nitrogen 171
 content in whole fish body at the end of the experiment 172
 and C_{N0} (%) at the start of the experiment, C_{Et} (kJ g⁻¹) 173
 is energy content in whole fish body at the end of the 174
 experiment and C_{E0} (kJ g⁻¹) at the start of the experi- 175
 ment, C_{Nf} (%) is nitrogen content in the feeds and C_{Ef} 176
 (kJ g⁻¹) energy content, W_s (g fish⁻¹) is body weight 177
 of the fish dissected at the end of the experiment and L_s 178
 (cm) total length, W_l (g) is liver weight of the fish 179
 dissected at the end of the experiment. Survival, 180
 SGR, final body weight (FBW), feed intake, FCR, 181
 NRE, ERE, CF, HSI, and contents of components 182
 (moisture, crude protein, crude lipid and ash) in car- 183
 cass, among fish fed the formulated feeds, were exam- 184
 ined using the variance of analysis for factorial layout, 185
 and mean comparison between the treatments were 186
 performed using Tukey HSD test. Survival, SGR, 187
 feed intake, NRE, ERE, CF, HSI, and components in 188
 carcass were arcsine transformed prior to the variance 189
 of analysis. Differences in above variables between fish 190
 fed the raw fish and formulated feed containing 40% 191
 DP and 16 MJ kg⁻¹ DE were examined using Student's 192
 t -test. Correlation between HSI and carcass lipid con- 193
 tent was examined. $P < 0.05$ was regarded significantly 194
 different. 195

3. Results 196

Survival was above 98% for all treatments. There 197
 was no significant difference in survival among fish 198
 fed the formulated feeds, or between fish fed the raw 199
 fish and formulated feed containing 40% DP and 16 200
 MJ kg⁻¹ DE. 201

t3.1 Table 3

t3.2 Summary of analysis of variance in variables among fish fed the formulated feeds

t3.3 Sources of variance	df	Feed conversion ratio, energy retention efficiency	Specific growth rate, final body weight, feed intake, moisture and crude protein contents in carcass	Significances			
t3.4				Protein retention efficiency	Lipid and ash contents in carcass	Condition factor	Hepatosomatic index
t3.5 DP	2	$P < 0.01$	$P < 0.01$	$P < 0.05$	NS	NS	NS
t3.6 DE	2	$P < 0.01$	$P < 0.01$	NS	$P < 0.01$	$P = 0.046$	NS
t3.7 DP*DE	4	$P < 0.05$	NS	NS	NS	NS	NS

t3.8 DP=dietary digestible protein; DE=dietary digestible energy.

t4.1 Table 4

t4.2 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
t4.4 L1	92.4 ± 3.3 ^{bc}	2.86 ± 0.06 ^{bcd}	2.9 ± 0.1 ^a	1.24 ± 0.05 ^{bc}
t4.5 L2	99.2 ± 1.5 ^{ac}	2.99 ± 0.05 ^{ad}	2.7 ± 0.1 ^{ab}	1.11 ± 0.03 ^{bc}
t4.6 L3	81.1 ± 3.9 ^b	2.60 ± 0.07 ^b	2.8 ± 0.1 ^{ab}	1.38 ± 0.08 ^a
t4.7 M1	99.7 ± 2.5 ^{ac}	2.88 ± 0.05 ^{cd}	2.9 ± 0.1 ^a	1.25 ± 0.03 ^{bc}
t4.8 M2	102.0 ± 4.0 ^{ac}	2.98 ± 0.01 ^{ad}	2.8 ± 0.1 ^{ab}	1.16 ± 0.05 ^{abc}
t4.9 M3	89.6 ± 6.4 ^{bc}	2.69 ± 0.08 ^{bc}	2.6 ± 0.2 ^{ab}	1.14 ± 0.09 ^{abc}
t4.10 H1	103.7 ± 1.2 ^{ac}	3.02 ± 0.05 ^{ad}	2.7 ± 0.0 ^{ab}	1.13 ± 0.06 ^{bc}
t4.11 H2	115.8 ± 0.6 ^a	3.24 ± 0.02 ^a	2.5 ± 0.0 ^b	0.95 ± 0.02 ^b
t4.12 H3	104.9 ± 4.9 ^{ac}	3.06 ± 0.07 ^{ad}	2.3 ± 0.0 ^b	0.92 ± 0.01 ^b
t4.13 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

t4.14 *P* < 0.05.

t4.15 Feed intake and feed conversion ratio are expressed on a dry feed basis.

202 Dietary DP and DE levels both significantly
203 affected SGR and FBW of fish fed the formulated
204 feeds (Table 3). Specific growth rate and FBW of fish
205 fed the feeds formulated to contain the same DE
206 content increased with the increase of DP from 36%
207 to 40%. For fish fed the feeds formulated at the same
208 DP level, SGR and FBW increased with the increase
209 of DE from 14 to 16 MJ kg⁻¹. Further increase of
210 dietary DE to 18 MJ kg⁻¹ resulted in no improvement
211 or even a slight decline in SGR and FBW. Fish fed the
212 feed containing 40% DP and 16 MJ kg⁻¹ DE exhib-
213 ited the highest SGR and FBW among fish fed the
214 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

t5.1 Table 5

t5.2 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
t5.4 L1	32.11 ± 1.50 ^{ab}	29.56 ± 1.15 ^{bc}
t5.5 L2	35.03 ± 1.43 ^{ab}	32.79 ± 1.54 ^{ac}
t5.6 L3	27.84 ± 1.26 ^b	26.03 ± 1.05 ^b
t5.7 M1	30.62 ± 0.81 ^{ab}	29.58 ± 0.50 ^{bc}
t5.8 M2	31.84 ± 1.75 ^{ab}	30.00 ± 1.40 ^{bcd}
t5.9 M3	31.20 ± 2.63 ^{ab}	29.66 ± 1.45 ^{bc}
t5.10 H1	32.07 ± 1.90 ^{ab}	31.78 ± 0.97 ^{acd}
t5.11 H2	36.32 ± 0.33 ^{aA}	37.16 ± 0.41 ^{aA}
t5.12 H3	36.00 ± 0.74 ^a	35.46 ± 0.66 ^{ad}
t5.13 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.

t5.14

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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t6.1
t6.2
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246 kg⁻¹ at the same DP level. Fish fed the feeds contain-
247 ing DP of 40% and DE of 16 and 18 MJ kg⁻¹
248 exhibited higher NRE ($P < 0.05$) and ERE ($P < 0.05$)
249 relative to fish fed the feeds containing 36% DP and
250 18 MJ kg⁻¹ DE (Table 5). Levels of DP and DE in the
251 formulated feeds did not significantly affect CF and
252 HSI ($P < 0.05$, Tables 3 and 6).

253 At the end of the experiment, moisture and pro-
254 tein contents in carcass of fish were affected by DP
255 and DE levels, while crude lipid and ash contents in
256 carcass were affected by DE level (Table 3). Lipid
257 content in carcass of fish fed the feeds with the
258 same DP level increased with increase in DE. Fish
259 fed the feed containing 36% DP and 18 MJ kg⁻¹
260 DE exhibited the highest lipid content ($P < 0.05$,
261 Table 7). There were no correlation between HSI
262 and lipid content in carcass for fish fed the formu-
263 lated feeds.

264 There were no significant differences in SGR,
265 FBW, feed intake, FCR (Table 4), CF (Table 6), and
266 protein and lipid contents (Table 7) between fish fed
267 the raw fish and feed containing 40% DP and 16 MJ
268 kg⁻¹ DE. Fish fed the feed containing 40% DP and 16
269 MJ kg⁻¹ DE showed higher NRE ($P < 0.05$), ERE
270 ($P < 0.05$, Table 5), HSI ($P < 0.05$, Table 6) and car-
271 cass ash content ($P < 0.05$, Table 7) than those of fish
272 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 ^{bc}	4.4 ± 0.07 ^{ab}
t7.10	M3	72.2 ± 0.30 ^{bc}	16.1 ± 0.15 ^{bc}	5.9 ± 0.31 ^{bcd}	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 Contents of crude protein, crude lipid and ash are expressed on a
t7.16 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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320 friendly fish feed. Dietary P/E varies among fish
321 species, particularly between coldwater and warm-
322 water fish. Coldwater fish, who can utilize high levels
323 of dietary lipid for energy, require lower dietary P/E,
324 e.g. 22 g MJ⁻¹ for rainbow trout (Lee and Putnam,
325 1980) and 18 g MJ⁻¹ for Atlantic salmon (Hillestad
326 and Johnsen, 1994). In contrast, P/E for warmwater
327 fish are relatively high, e.g. 31 g MJ⁻¹ for grouper
328 (Shiau and Lan, 1996), 28 g MJ⁻¹ for Mediterranean
329 yellow tail (Jover et al., 1999), and 28 g MJ⁻¹ for red
330 drum (McGoogan and Gatlin, 1999). In the present
331 study, cuneate drum fed the feed containing 40% DP
332 and 16 MJ g⁻¹ DE (DP/DE=25 g MJ⁻¹) showed the
333 highest SGR, FBW, NRE, ERE and better FCR,
334 suggesting optimal P/E for the fish is similar or
335 perhaps slightly lower than other warmwater carni-
336 vorous fish.

337 In the present study, cuneate drum fed the feeds
338 containing dietary lipid of 15% to 17% showed
339 higher SGR, FBW, NRE and ERE than those of
340 fish fed the feeds containing dietary lipid of neither
341 9% to 10% or 22% to 24%. This suggests cuneate
342 drum has a relatively good capacity to utilize dietary
343 lipids as energy sources, and 15% to 17% dietary
344 lipids appears optimal to the fish. This level is higher
345 than values reported from other sciaenid species,
346 such as Atlantic croaker (Davis and Arnold, 1997),
347 red drum (McGoogan and Gatlin, 1999) and large
348 yellow croaker (Duan et al., 2001). Fish fed feeds
349 with high dietary energy exhibits high body lipid
350 deposition (Millikin, 1983). In the present study,
351 lipid content in carcass of fish increased with the
352 increase in DP level, this is consistent with the
353 results of previous studies on large yellow croaker
354 (Duan et al., 2001), Atlantic croaker (Davis and
355 Arnold, 1997) and red drum (Daniels and Robinson,
356 1986). Grouper fed formulated feeds exhibited higher
357 weight gain and carcass lipid content than those fed
358 raw fish (Milliamena, 2002). In the present study,
359 cuneate drum fed the formulated feed containing
360 40% DP and 16 MJ kg⁻¹ DE exhibited similar
361 feed intake, SGR, FBW and FCR to those fed the
362 raw fish, but showed higher NRE and ERE com-
363 pared to those fed the raw fish, suggesting not only
364 dietary protein and energy of the formulated feed are
365 adequate to sustain rapid growth but also that nitro-
366 gen waste output from farming of this fish can be
367 reduced by using formulated feeds.

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2 Replacement of fish meal by rendered animal protein ingredients in 3 feeds for cuneate drum (*Nibea miichthioides*)

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8

9 Abstract

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

27

1. Introduction

28

Cuneate drum is a carnivorous sciaenid native to
29 near-shore waters of the China Sea (Chu and Wu,
30 1985), and has been widely cultured in net pens
31 along the coast of the China Sea, from Zhanjiang to
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33 Lianyungang. Cuneate drum are generally fed raw
34 fish on commercial operations and this results in
35 high feed costs and cause serious environmental
36 problems, due to the high amount of nitrogenous
37 waste associated with feeding raw fish (Wang et al.,
38 in press). Feed formulae that have high nutritive
39 value are cost-effective, and produce less waste out-
40 puts needed to improve economical and environ-
41 mental sustainability of cuneate drum culture in
42 China.

43 Fish meal is generally incorporated at levels
44 between 30% and 60% in feeds for carnivorous mar-
45 ine fish. Fish meal is an expensive ingredient. Cost-
46 effectiveness of the feed could be improved by repla-
47 cing fish meal with more economical protein sources,
48 such as rendered animal protein ingredients, e.g.,
49 poultry by-product meal (PBM), meat and bone
50 meal (MBM) and feather meal (FM). These ingredi-
51 ents have been used successfully in feeds for various
52 fish species, such as chinook salmon (Fowler, 1990,
53 1991), silver seabream (El-Sayed, 1994), rainbow
54 trout (Steffens, 1994; Bureau et al., 2000), red drum
55 (Moon and Gatlin, 1994; Kureshy et al., 2000), gilt-
56 head seabream (Robaina et al., 1997; Nengas et al.,
57 1999), Indian major carp (Hasan et al., 1997), Aus-
58 tralian snapper (Quartararo et al., 1998), Australian
59 silver perch (Allan et al., 2000; Stone et al., 2000),
60 Nile tilapia (El-Sayed, 1998), sunshine bass (Webster
61 et al., 2000) and grouper (Milliamena, 2002). The
62 suitability of these ingredients for cuneate drum has
63 not been evaluated.

64 The present study was conducted to assess the
65 effect of using rendered animal proteins, alone or in
66 combination, as ingredients in practical feeds on

67 growth, feed utilization, and body composition of
68 cuneate drum.

2. Material and methods 69

2.1. Feed formulation and preparation 70

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

79 Nine dry feeds were formulated to contain 36%
80 digestible protein (DP) and 15 MJ kg⁻¹ digestible
81 energy (DE), and a tenth feed consisted of raw fish
82 (*Sardinella* spp.) and this feed served as a comparison
83 to the formulated feeds. The control feed contained
84 350 g kg⁻¹ herring meal. In the other eight feeds, the
85 fish meal was directly replaced by PBM, MBM, FM
86 alone, or with a combination (APM) of PBM, MBM,
87 FM, BM, and soybean meal (SM). Dietary DP and DE
88 of the feeds were calculated using the published
89 apparent digestible coefficients (Bureau et al., 1999).
90 The feeds were formulated isonitrogenous and isocalo-
91 ric by adjusting proportion of BM, SM and wheat
92 flour in formulation. The formulation and chemical
93 composition of the test feeds are presented in Table 3,
94 and amino acid profile in Table 4.

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

t1.1 Table 1
t1.2 Proximate composition (%) and gross energy content (MJ kg⁻¹) of the ingredients

Ingredients	Dry matter	Crude protein	Crude lipid	Ash	Gross energy
t1.4 Meat and bone meal	94.6	60.2	11.0	23.7	19.4
t1.5 Feather meal	92.0	83.0	11.7	2.9	22.8
t1.6 Blood meal (spray-dried)	93.2	98.5	0.1	1.6	25.1
t1.7 Poultry by product meal	95.3	67.4	15.9	12.4	23.2
t1.8 Herring meal	89.9	72.5	8.6	16.0	18.4
t1.9 Soybean meal (solvent-extracted)	87.6	50.1	0.9	6.2	18.5
t1.10 Rapeseed meal	88.7	41.1	1.8	7.8	18.3
t1.11 Wheat flour	85.9	13.1	1.0	0.6	17.7
t1.12 APM	93.2	61.8	8.9	12.4	19.2

t1.14 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.

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 (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; menadiolone 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, 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 (acid-washed diatomaceous earth silica), 1000 mg.

t3.31 Crude protein, lipid, ash, gross energy, DP and DE are expressed on a dry matter basis and given as means ($n=2$).

t3.32 DDM=digestible dry matter; DP=digestible protein; DE=digestible energy; RF=raw fish.

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.

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

103 2.2. Fish, husbandry and feeding

104 An 8-week feeding trial was carried out in net
105 pens in Shenao Bay, Shantou, China. Cuneate drum
106 (*Nibea miichthioides*) fingerlings were obtained
107 from a local marine fish hatchery (Qingao Bay
108 Hatchery, Shantou, Guangdong, China). After trans-
109 portation, the fish were reared in net pens (3 m × 3
110 m × 2 m), and gradually weaned from raw fish onto
111 the control feed during an 8-week period. Two
112 weeks prior to the trial, 1280 fish were selected
113 and reared in 32 experimental pens (1 m × 1
114 m × 1.5 m) at 40 fish per pen, during the acclima-
115 tion the fish were fed the control feed twice daily.
116 At the start of the trial, the acclimated fish were
117 deprived of feed for 24 h, pooled, and 30 groups
118 each of 30 fish weighing 27.4 ± 0.2 g fish⁻¹ (mean-
119 \pm S.E., $n=30$) were batch weighed, and randomly
120 stocked into 30 experimental pens, with 3 replica-
121 tion of each treatment. Eight sub-samples of 3 fish
122 each were removed from the remaining acclimated
123 fish for the determination of initial body composi-
124 tion. The sampled fish were frozen at -20 °C until
125 analysis.

126 During the trial, the fish were hand fed at 08:00
127 and 16:00 h daily except on days of strong waves or
128 high temperatures. At each feeding, some pellets were

dropped in each pen until no feeding activity of fish 129
was observed. Dead fish were recorded and weighed 130
for calculating feed conversion ratio (FCR). At the 131
end of the trial, the fish were collected from each pen 132
and batch weighed. Three fish were sampled from 133
each pen for the determination of final body composi- 134
tion. The sampled fish were frozen at -20 °C until 135
analysis. 136

Water temperature was measured daily and salinity 137
weekly. Water temperature ranged from 25 to 32 °C, 138
and salinity from 31‰ to 32‰ during the feeding 139
trial. 140

2.3. Chemical analysis 141

The fish sampled at the start and end of the trial 142
and raw fish sampled during the trial were autoclaved 143
at 120 °C for 20 min, homogenized, and dried at 105 144
°C for 24 h prior to the chemical analysis. The sam- 145
ples of the ingredients, formulated feeds, raw fish and 146
cuneate drum were ground into fine power with a 147
laboratory grinder. Contents of moisture, crude pro- 148
tein, crude lipid, ash and gross energy, and amino 149
acids in the ingredients, feeds and sampled fish were 150
measured using the methods described in Wang et al. 151
(in press). 152

2.4. Calculations and statistical analyses 153

Feed intake, specific growth rate (SGR), FCR and 154
nitrogen retention efficiency (NRE) was calculated as 155
below: 156

$$\text{Feed intake (\%day}^{-1}\text{)} = 100 \times I / [(W_0 + W_1) / 2 \times t]$$

$$157 \text{ SGR } (\% \text{ day}^{-1}) = [\text{Ln}(W_1/N_1) - \text{Ln}(W_0/N_0)]/t$$

$$158 \text{ FCR } (\text{dry feed gain}^{-1}) = I/(W_1 - W_0 + W_d)$$

$$159 \text{ NRE } (\%) = 100 \times (W_1 \times C_{N1} - W_0 \times C_{N0} + W_d \times C_{N0}) / (I \times C_{Nf})$$

160 where I (g) is total amount of the feed consumed on a
162 dry weight basis, W_0 (g) is total initial body weight
163 and W_1 (g) total final body weight, t (d) is duration of
164 the feeding trial, N_1 is number of fish at the end of the
165 trial and N_0 at the start of the trial, W_d (g) is total body
166 weight of the dead fish, C_{N1} (%) is nitrogen content in
167 whole fish body at the end of the trial and C_{N0} (%) at
168 the start of the trial, C_{Nf} (%) is nitrogen content in the
169 feeds.

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

3. Results

182 Survival of fish in all the treatments was very
183 high (greater than 94%) and there was no signifi-
184 cant difference among fish fed the formulated
185 feeds and between fish fed the raw fish and con-
186 trol feed.
187

188 Specific growth rate and FBW in fish fed the
189 control feed was higher than fish fed the feeds in
190 which the fish meal was replaced by 10% and 30%
191 with FM, or by 10% and 50% with MBM, or by 50%
192 with APM, but did not differ significantly from those
193 fed the feeds in which the fish meal was replaced by
194 30% to 50% with PBM, or by 30% with MBM. There
195 were no significant differences in feed intake and FCR
196 among fish fed the formulated feeds. Replacing 50%
197 of the fish meal by MBM resulted in lower NRE
198 ($P < 0.05$, Table 5).

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

208 Fish fed the raw fish showed higher SGR
209 ($P < 0.05$), FBW, feed intake, whole body crude pro-
210 tein content, and lower NRE and whole body crude

t5.1 Table 5

t5.2 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 \pm S.E., $n=3$)

t5.3	Feeds	Final body weight	Specific growth rate	Feed intake	Feed conversion ratio	Nitrogen retention efficiency
t5.4	Control	93.8 \pm 1.8 ^{na}	2.21 \pm 0.02 ^{na}	2.03 \pm 0.07 ^A	1.05 \pm 0.03	35 \pm 1 ^{aA}
t5.5	MBM1	76.2 \pm 3.8 ^{bc}	1.79 \pm 0.09 ^{bc}	2.09 \pm 0.09	1.35 \pm 0.02	27 \pm 2 ^{bh}
t5.6	MBM2	83.7 \pm 2.0 ^{bc}	2.00 \pm 0.06 ^{ac}	1.94 \pm 0.06	1.07 \pm 0.06	35 \pm 3 ^a
t5.7	MBM3	66.8 \pm 2.3 ^b	1.62 \pm 0.11 ^b	2.03 \pm 0.18	1.40 \pm 0.06	19 \pm 4 ^b
t5.8	FM1	78.5 \pm 5.5 ^{bc}	1.87 \pm 0.09 ^{bc}	2.16 \pm 0.19	1.34 \pm 0.15	28 \pm 3 ^{ab}
t5.9	FM2	73.2 \pm 4.4 ^{bc}	1.77 \pm 0.11 ^{bc}	2.03 \pm 0.10	1.27 \pm 0.12	29 \pm 3 ^{ab}
t5.10	PBM1	82.3 \pm 2.4 ^{abc}	1.97 \pm 0.06 ^{abc}	1.88 \pm 0.05	1.07 \pm 0.04	35 \pm 1 ^a
t5.11	PBM2	83.5 \pm 2.7 ^{ac}	1.98 \pm 0.06 ^{abc}	1.95 \pm 0.16	1.10 \pm 0.06	33 \pm 2 ^{ab}
t5.12	MM	74.1 \pm 1.8 ^{bc}	1.74 \pm 0.04 ^{bc}	1.73 \pm 0.09	1.13 \pm 0.04	35 \pm 4 ^a
t5.13	RF	114.5 \pm 1.7 ^h	2.54 \pm 0.02 ^d	2.33 \pm 0.04 ^B	1.10 \pm 0.03	23 \pm 1 ^b

t5.14 RF=raw fish.

t5.15 Feed intake and feed conversion ratio are expressed on a dry feed basis.

t5.16 Values in the same column with different superscripts are statistically different at $P < 0.05$.

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

t6.3	Feeds	Moisture	Crude protein	Crude lipid	Ash
t6.4	Initial	76.1 \pm 0.1	16.2 \pm 0.3	2.8 \pm 0.1	4.5 \pm 0.1
t6.5	Control	73.7 \pm 0.3	15.7 \pm 0.2 ^{abA}	6.4 \pm 0.2 ^A	3.8 \pm 0.1
t6.6	MBM1	74.2 \pm 0.7	15.6 \pm 0.4 ^{ab}	5.8 \pm 0.1	4.0 \pm 0.1
t6.7	MBM2	73.3 \pm 0.6	15.6 \pm 0.4 ^{ab}	6.8 \pm 0.1	3.9 \pm 0.1
t6.8	MBM3	77.6 \pm 3.1	13.2 \pm 1.8 ^b	5.4 \pm 0.8	3.4 \pm 0.4
t6.9	FM1	74.4 \pm 0.4	15.4 \pm 0.1 ^{ab}	6.1 \pm 0.2	3.8 \pm 0.1
t6.10	FM2	73.8 \pm 0.2	15.8 \pm 0.1 ^{ab}	6.3 \pm 0.0	3.9 \pm 0.0
t6.11	PBM1	73.1 \pm 0.3	16.0 \pm 0.1 ^{ab}	7.0 \pm 0.1	3.9 \pm 0.1
t6.12	PBM2	73.4 \pm 0.2	15.7 \pm 0.2 ^{ab}	6.9 \pm 0.2	3.8 \pm 0.0
t6.13	MM	73.2 \pm 0.8	16.8 \pm 0.8 ^a	6.7 \pm 0.2	3.9 \pm 0.1
t6.14	RF	74.9 \pm 0.5	17.3 \pm 0.5 ¹¹	3.5 \pm 0.4 ¹¹	3.9 \pm 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 cuneate
219 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

acid. Incorporation of the PBM in feed formulation 237
at 10.5% to 17.5% (to replace 30% to 50% of the fish 238
meal) did not result in significantly negative effects on 239
SGR, FBW, FCR and NRE, suggesting the PBM is an 240
adequate protein ingredient for cuneate drum. In pre- 241
vious studies, PBM have been demonstrated success- 242
ful in use at 20% in feeds for chinook salmon (Fowler, 243
1991), 25% for silver seabream (El-Sayed, 1994), 244
21% for Australian snapper (Quartararo et al., 245
1998), 71% for gilthead seabream (Nengas et al., 246
1999), and 14% for red drum (Kureshy et al., 2000), 247
although declined growth performance was observed 248
in Australian silver perch (Allan et al., 2000) and 249
sunshine bass (Webster et al., 2000) fed feeds contain- 250
ing high PBM level. The results of the present study 251
confirm the conclusions of the previous studies on 252
chinook salmon (Fowler, 1991), silver seabream (El- 253
Sayed, 1994), Australian snapper (Quartararo et al., 254
1998) and gilthead seabream (Nengas et al., 1999), 255
and indicate the PBM could be directly used at 17% in 256
feeds for cuneate drum. 257

In the present study, incorporating the MBM at 258
10.5% (to replace 30% of the fish meal) in feed 259
formulation for cuneate drum resulted in negligible 260
changes in SGR, FBW, FCR and NRE. This is in 261
agreement with the conclusion of a previous study 262
that indicated MBM could be used with success at 263
10% in feeds for red drum (Kureshy et al., 2000). 264
Incorporation of MBM at more than 24% in feeds for 265
gilthead seabream (Robaina et al., 1997), rainbow 266
trout (Bureau et al., 2000), sunshine bass (Webster 267
et al., 2000) and grouper (Milliamena, 2002) did not 268
result in negative effect on growth performance of 269
these fish. Cuneate drum fed the feed in which the 270
MBM was incorporated at 17.5% (to replace 50% of 271
the fish meal) in feed formulation, however, exhibited 272
significantly lower SGR, FBW and NRE than those 273
fed the control feed. As the test feeds used in the 274
present study were formulated isonitrogenous and 275
isocaloric and to have similar amino acid profile, 276
this implies the MBM was deficient at least in some 277
essential nutrients beside protein, energy and amino 278
acid. Results of the present study reveal the MBM 279
should not be used alone at an inclusion rate of more 280
than 10% in feeds for cuneate drum. 281

In the present study, cuneate drum fed the feeds in 282
which the FM was incorporated at 3.5% to 10.5% (to 283
replace 10% to 30% of the fish meal) in feed formula- 284

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 profile 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), gilt-head 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, com-

pared to those determined in the studies on chinook salmon (Fowler, 1990), gilt-head 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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