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# The potential of land animal protein ingredients to replace fish meal in diets for cuneate drum, *Nibea miichthioides*, is affected by dietary protein level

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## Abstract

A net pen experiment was carried out to examine the effect of dietary protein level on the potential of land animal protein ingredients as fish meal substitutes in practical diets for cuneate drum *Nibea miichthioides*. Two isocaloric basal (control) diets were formulated to contain 400 g $kg^{-1}$ herring meal but two different digestible protein (DP) levels (400 versus 350 g $kg^{-1}$). At each DP level, dietary fish meal level was reduced from 400 to 280, 200, 80 and 0 g $kg^{-1}$ by incorporating a blend that comprised of 600 g $kg^{-1}$ poultry by-products meal (PBM), 200 g $kg^{-1}$ meat and bone meal (MBM), 100 g $kg^{-1}$ feather meal (FEM) and 100 g $kg^{-1}$ blood meal (BLM). Cuneate drum fingerling (initial weight 42 g $fish^{-1}$) were fed the test diets for 8 weeks. Fish fed the test diets exhibited similar feed intake. Final body weight, feed conversion ratio and nitrogen retention efficiency was not significantly different between fish fed the basal diets containing 350 and 400 g $kg^{-1}$ DP. Weight gain decreased linearly with the reduction of dietary fish meal level at the 350 g $kg^{-1}$ DP level, but did not decrease with the reduction of dietary fish meal level at the 400 g $kg^{-1}$ DP level. Results of the present study suggest that fish meal in cuneate drum diets can be completely replaced with the blend of PBM, MBM, FEM and BLM at the 400 g $kg^{-1}$ DP level, based on a mechanism that excessive dietary protein compensate lower contents of bio-available essential amino acid in the land animal protein ingredients relative to fish meal.

**KEY WORDS**: amino acid, cuneate drum, dietary protein level, fish meal replacement, growth, nitrogen retention efficiency

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## Introduction

Limited supply, increasing demand and high price of fish meal are challenges to the sustainable development of fish culture industry, notably marine carnivorous fish who are frequently fed feeds with high fish meal levels. Wider use of more economical plant or animal protein ingredients is needed for the formulation cost-effectiveness of marine fish diets. Numerous studies focused on assessing the potential to reduce fish meal level in fish diets over the past four decades (Cho *et al*. 1974; Wilson & Poe 1985; Fowler 1991; El-Sayed 1994; Steffens 1994; Kaushik *et al*. 1995; Adelizi *et al*. 1998; Bureau *et al*. 2000; Kureshy *et al*. 2000; Webster *et al*. 2000; Milliamena 2002; Gaylord & Rawles 2005; Wang *et al*. 2006b; Guo *et al*. 2007). The magnitude of fish meal replacement by economical protein sources varies greatly among literature because of great variability of fish meal and protein levels used in the basal (control) diets, as well as great variability of chemical composition and nutritive value of the alternate protein ingredients (Cho *et al*. 1974; Steffens 1994; Kaushik *et al*. 1995; Adelizi *et al*. 1998; Bureau *et al*. 2000). Contents of the essential amino acids (EAA), especially lysine and sulphur amino acids, are generally lower in economical protein sources from plant or terrestrial animal origins than fish meal. The formulation of basal diets with high fish meal and/or protein level may have greater 'safety margins' in terms of EAA greatly in excess of requirements, and result in more 'optimistic' evaluation of the potential to replace fish meal with economical protein sources relative to using basal diets formulated at lower fish meal and/or protein level (Wang *et al*. 2006b). Cuneate drum, a carnivorous sciaenid native to the China Sea and with commercial importance to near shore marine culture in China, can perform well when fed the diets formulated to contain significant levels of land animal protein ingredients (Wang *et al*. 2006a; Guo *et al*.

2007). Complete replacement of fish meal in cuneate drum diets has not been achieved.

Traditionally, EAA requirements of fish and other animals have been expressed as % of diet (NRC, 1993). A common view amongst fish nutritionists is that EAA requirements should be expressed as a proportion of dietary protein (Cowey & Cho 1993). Meeting EAA requirements of fish in the diets formulated with the economical but nutritionally imperfect protein sources could be achieved by (1) supplementing the diets with synthetic amino acids, (2) using the protein ingredients with complementary amino acid profiles or (3) formulating the diets at protein levels higher than the dietary protein requirement so that absolute dietary EAA contents are above the requirements. Urdaneta-Rincon *et al*. (2005) mentioned that, in broiler chicken, lysine requirement (% of diet) increased with the increase of dietary protein level. If EAA requirements of fish are a function of the dietary protein level, elevating dietary protein level may have limited value to enhance the potential of fish meal replacement by nutritionally imperfect protein sources. Few studies have examined this hypothesis in fish. Ballestrazzi *et al*. (1994) reported that the effectiveness of corn gluten meal to replace fish meal in diets for sea bass was not different at two different dietary protein levels. In their experiment, however, fish meal level of the basal diet was high and magnitude of fish meal replacement was limited. There is a need to examine the effect of dietary protein level over a wide range of fish meal replacement levels. The objective of the present study was, therefore, to evaluate the potential of a blend of poultry by-products meal (PBM), meat and bone meal (MBM), feather meal (FEM) and blood meal (BLM) as fish meal substitute in practical diets for cuneate drum reared in net pens, at two dietary protein levels.

## Materials and methods

### *Ingredients, diet formulation and diet preparation*

Poultry by-products meal, MBM, BLM and FEM were supplied by National Renderers Association (Hong Kong Office). Other ingredients were purchased from Xinnong Feed Company (Shanghai, China). The diet treatments were designed following a factorial layout, including 10 isocaloric [15 MJ $kg^{-1}$ digestible energy (DE)] diets formulated at two dietary protein levels [either 400 or 350 g $kg^{-1}$ digestible protein (DP)] and five fish meal levels (400, 280, 200, 80 and 0 g $kg^{-1}$). At each of the DP levels, a basal (control) diet contained 400 g $kg^{-1}$ herring meal, and a blend of 600 g $kg^{-1}$ PBM, 200 g $kg^{-1}$ MBM, 100 g $kg^{-1}$ FEM and 100 g $kg^{-1}$ BLM (Guo *et al*. 2007) was used to replace 30%, 50%, 80% and 100% of the fish meal in the basal diet. Formulation, proximate composition and energy content of the test diets are shown in Table 1, and amino acid profile in Table 2. Protein and energy levels of the test diets cover the adequate dietary protein (400 g $kg^{-1}$ DP) and energy (16 MJ $kg^{-1}$ DE) levels for growth of cuneate drum reared in net pens (Wang *et al*. 2006a).

The dry ingredients were ground with a hammer grinder, and mixed with a 30-L Hobart kitchen mixer. The test diets were made into slow sinking pellets using a laboratory scale, single screw extruder (extruding temperature 100–120 °C), and dried under room temperature.

### *Feeding, sampling and chemical analysis*

The experiment was carried out in Shenao Bay, Shantou, China. Cuneate drum (*Nibea miichthioides*) fingerlings were collected from a local marine fish hatchery and reared in net pens (3 m × 3 m × 3 m) for 12 weeks, during which the fish were gradually weaned from chopped raw fish onto the basal diet containing 400 g $kg^{-1}$ DP. Prior to the start of the experiment, 750 fish were sorted into 30 experimental pens (1 m × 1 m × 1.5 m) at 25 fish per pen, and fed the basal diet containing 400 g $kg^{-1}$ DP twice daily for 2 weeks. At the start of the experiment, the acclimatized fish were deprived of diet for 24 h and then pooled. Thirty groups each of 20 fish were bulk weighed, and randomly distributed into the experimental pens, with three replicates for each diet treatment. Initial body weight of the fish was 42 ± 0.3 g (mean ± SE, $n$ = 30). Three groups each of six fishes were killed for the determination of initial carcass composition. The sampled fish were stored at −20 °C until analysed.

During the experiment, the fish were hand fed at 08:00 and 16:00 hours daily as described in Wang *et al*. (2006a). Water temperature was monitored daily (fluctuated within 25–32 °C), and salinity weekly (fluctuated within 26–33 ppt). At the end of the experiment, fish in each pen were bulk weighed, and then six fish were killed for the determination of carcass composition.

The sampled fish were autoclaved at 120 °C for 20 min prior to the chemical analysis. Contents of moisture (dried at 105 °C for 24 h), crude protein (Kjeldahl method), crude lipid (ether extract), ash (combusted at 550 °C for 6 h) and gross energy (Parr 1281 bomb calorimeter, Moline, Illinois, USA) of the ingredients, diets and fish were analysed as described in Wang *et al*. (2006a).

**Table 1** Formulation (g $kg^{-1}$), chemical composition (g $kg^{-1}$) and energy content (MJ $kg^{-1}$) of the test diets

| | Diets | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Ingredient | HC | HR1 | HR2 | HR3 | HR4 | LC | LR1 | LR2 | LR3 | LR4 |
| Fish meal | 400 | 280 | 200 | 80 | | 400 | 280 | 200 | 80 | |
| Protein blend[1] | | 105 | 177 | 282 | 352 | | 105 | 177 | 282 | 352 |
| Blood meal | 74 | 61 | 76 | 72 | 65 | 10 | 8 | 6 | 11 | 15 |
| Soybean meal | 200 | 230 | 200 | 216 | 243 | 180 | 200 | 200 | 200 | 200 |
| Rapeseed meal | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| Wheat flour | 186 | 180 | 212 | 210 | 200 | 240 | 248 | 252 | 257 | 263 |
| $CaHPO_4$ | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 |
| DL-Met | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Fish oil | 50 | 50 | 45 | 50 | 50 | 80 | 69 | 75 | 80 | 80 |
| Vitamin premix | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Mineral premix | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Dry matter | 911 | 903 | 905 | 903 | 903 | 909 | 905 | 902 | 906 | 902 |
| Crude protein | 472 | 476 | 490 | 469 | 474 | 416 | 418 | 391 | 412 | 360 |
| Crude lipid | 86 | 82 | 91 | 97 | 95 | 140 | 130 | 136 | 136 | 145 |
| Ash | 113 | 106 | 100 | 93 | 88 | 113 | 107 | 102 | 92 | 86 |
| Gross energy | 17.2 | 17.5 | 17.7 | 18.2 | 18.4 | 17.5 | 17.6 | 18.0 | 18.5 | 18.7 |
| DP[2] | 396 | 395 | 395 | 393 | 393 | 347 | 349 | 347 | 347 | 347 |
| DE[2] | 14.4 | 14.4 | 14.5 | 14.7 | 14.7 | 14.7 | 14.5 | 14.8 | 15.1 | 15.2 |
| DP/DE (g $MJ^{-1}$) | 27.6 | 27.4 | 27.3 | 26.8 | 26.7 | 23.6 | 24.0 | 23.5 | 23.0 | 22.9 |

Diet HC, HR1, HR2, HR3 and HR4 contained 400 g $kg^{-1}$ digestible protein, and diet LC, LR1, LR2, LR3 and LR4 contained 350 g $kg^{-1}$ digestible protein. At each of the protein levels, a basal diet (HC or LC) contained 400 g $kg^{-1}$ herring meal, and 30% (HR1 or LR1), 50% (HR2 or LR2), 80% (HR3 or LR3) and 100% (HR4 or LR4) of the fish meal in the basal diet was replaced with a protein blend.

Vitamin premix and mineral premix are described as Wang *et al.* (2006a). Crude protein, crude lipid, ash and gross energy are expressed on a dry matter basis and given as means ($n$ = 2).

[1] Protein blend comprises of 600 g $kg^{-1}$ poultry by product meal, 200 g $kg^{-1}$ meat and bone meal, 100 g $kg^{-1}$ feather meal and 100 g $kg^{-1}$ blood meal.

[2] DP, digestible protein; DE, digestible energy. DP and DE are calculated using the method described as Wang *et al.* (2006a), and expressed on a dry matter basis.

**Table 2** Essential amino acid profile of the test diets

| Feeds | Thr | Val | Cys | Met | Ile | Leu | Tyr | Phe | Lys | His | Arg |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HC | 17.1 (36.2) | 19.6 (41.5) | 2.0 (4.2) | 11.1 (23.5) | 14.6 (30.9) | 34.9 (73.9) | 11.6 (24.6) | 19.8 (41.9) | 28.7 (60.8) | 16.3 (34.5) | 26.2 (55.5) |
| HR1 | 17.7 (37.2) | 19.9 (41.8) | 2.0 (4.2) | 11.5 (24.2) | 15.5 (32.6) | 36.3 (76.3) | 12.1 (25.4) | 21.0 (44.1) | 30.8 (64.7) | 17.2 (36.1) | 26.7 (56.1) |
| HR2 | 17.1 (34.9) | 20.1 (41.0) | 3.4 (6.9) | 9.5 (19.4) | 14.5 (29.6) | 36.0 (73.5) | 11.3 (23.1) | 20.5 (41.8) | 28.4 (58.0) | 17.7 (36.1) | 26.8 (54.7) |
| HR3 | 16.5 (35.2) | 20.0 (42.6) | 4.2 (9.0) | 9.0 (19.2) | 14.1 (30.1) | 35.5 (75.7) | 10.3 (22.0) | 19.9 (42.4) | 26.5 (56.5) | 17.1 (36.5) | 27.5 (58.6) |
| HR4 | 16.5 (34.8) | 19.7 (41.6) | 3.3 (7.0) | 9.1 (19.2) | 13.7 (28.9) | 35.1 (74.1) | 10.1 (21.3) | 19.9 (42.0) | 25.7 (54.2) | 17.5 (36.9) | 27.6 (58.2) |
| LC | 15.0 (36.1) | 15.5 (37.3) | 2.7 (6.5) | 12.2 (29.3) | 14.0 (33.7) | 27.9 (67.1) | 10.6 (25.5) | 16.9 (40.6) | 24.7 (59.4) | 12.8 (30.8) | 22.9 (55.0) |
| LR1 | 14.8 (35.4) | 15.5 (37.1) | 2.3 (5.5) | 11.6 (27.8) | 14.0 (33.5) | 28.0 (67.0) | 10.0 (23.9) | 16.0 (38.3) | 23.5 (56.2) | 13.5 (32.3) | 22.6 (54.1) |
| LR2 | 14.5 (37.1) | 15.5 (39.6) | 2.5 (6.4) | 6.6 (16.9) | 13.1 (33.5) | 27.5 (70.3) | 9.5 (24.3) | 16.5 (42.2) | 22.4 (57.3) | 13.3 (34.0) | 22.9 (58.6) |
| LR3 | 14.2 (34.5) | 15.9 (38.6) | 3.7 (9.0) | 13.4 (32.5) | 12.6 (30.6) | 27.6 (67.0) | 9.3 (22.6) | 15.9 (38.6) | 20.9 (50.7) | 13.6 (33.0) | 23.3 (56.6) |
| LR4 | 13.4 (37.2) | 13.7 (38.1) | 1.7 (4.7) | 9.6 (26.7) | 12.2 (33.9) | 24.3 (67.5) | 9.0 (25.0) | 14.5 (40.3) | 16.1 (44.7) | 11.5 (31.9) | 22.1 (61.4) |

Diet HC, HR1, HR2, HR3 and HR4 contained 400 g $kg^{-1}$ digestible protein, and diet LC, LR1, LR2, LR3 and LR4 contained 350 g $kg^{-1}$ digestible protein. At each of the protein levels, a basal diet (HC or LC) contained 400 g $kg^{-1}$ herring meal, and 30% (HR1 or LR1), 50% (HR2 or LR2), 80% (HR3 or LR3) and 100% (HR4 or LR4) of the fish meal in the basal diet was replaced with a protein blend.
Data are expressed on a dry weight basis (g $kg^{-1}$) as % of diet or (% of protein).

### *Data calculation and statistics*

Feed intake (g $fish^{-1}$), weight gain (WG, g $fish^{-1}$), feed conversion ratio (FCR, dry $gain^{-1}$) and nitrogen retention efficiency (NRE, %) were calculated as described in Wang *et al.* (2006a). Ratio of fish meal consumption to fish production (RCP) was calculated as: $RCP\ (g\ g^{-1}) = WG \times FCR \times FL/(FBW \times DMF_t - IBW \times DMF_0)$, where FL is dietary fish

meal level (g kg$^{-1}$), FBW is final body weight (g) and IBW initial body weight (g), $DMF_t$ (g kg$^{-1}$) is dry matter content in carcass of fish at the end of the experiment and $DMF_0$ (g kg$^{-1}$) at the start.

Differences in survival, feed intake, WG, FBW, FCR, NRE, carcass components (moisture, crude protein, crude lipid and ash) and RCP among the diet treatments were analysed with ANOVA for factorial layout, and mean values of these variables were examined with the Turkey honestly significant differenced (HSD) test. Survival, NRE and carcass components were arcsine transformed. Relationships between WG and dietary fish meal level at the same DP level was examined using multiple linear regression. Significance was considered at $P < 0.05$.

## Results

All the diet treatments had excellent survival (>99%) in the present experiment. All fish accepted the test diets well and feed intake was not significantly different among the diet treatments ($P > 0.05$, Table 3). WG, FBW and FCR were dependent on dietary protein and fish meal levels ($P < 0.05$). WG linearly decreased with the reduction of dietary fish meal level at the 350 g kg$^{-1}$ DP level ($n = 15$, $r^2 = 0.367$, $P < 0.05$, Fig. 1), while no significant correlation was found between WG and dietary fish meal level at the 400 g kg$^{-1}$ DP level ($n = 15$, $r^2 = 0.019$, $P > 0.05$). There were no significant differences in WG, FBW and NRE among fish fed the diets containing various levels of fish meal at the same DP level (either 400 or 350 g kg$^{-1}$, $P > 0.05$, Table 3). Fish fed the basal diet had lower FCR than that of fish fed the diet containing 0 g kg$^{-1}$ fish meal at the 350 g kg$^{-1}$ DP level ($P < 0.05$), but no significant difference was found in FCR among fish fed the diets containing various levels of fish meal at the 400 g kg$^{-1}$ DP level ($P > 0.05$). FCR of fish fed the diet containing 400 g kg$^{-1}$ DP and 0 g kg$^{-1}$ fish meal was lower than that of fish fed the diet containing 350 g kg$^{-1}$ DP

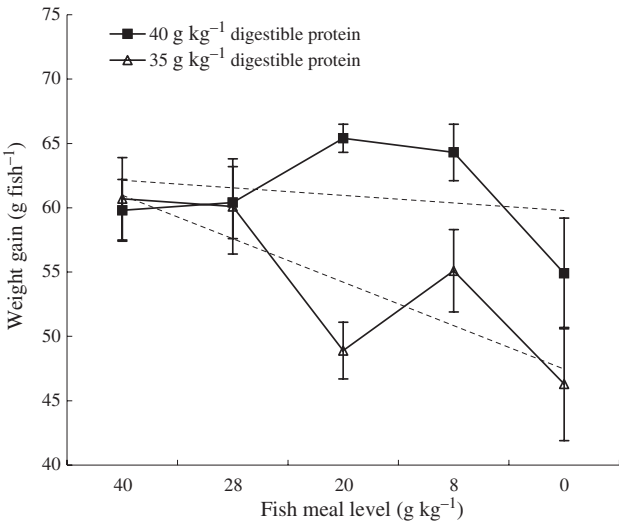


**Figure 1** Weight gain of cuneate drum fed the test diets as a function of fish meal level at two dietary protein levels.

**Table 3** Body weight (g fish$^{-1}$), feed intake (g fish$^{-1}$), feed conversion ratio, nitrogen retention efficiency (%) and ratio of fish meal consumption to fish production of cuneate drum fed the test diets

| Feeds | Initial body weight | Final body weight | Feed intake | Feed conversion ratio | Nitrogen retention efficiency | Ratio of fish meal consumption to fish production |
|---|---|---|---|---|---|---|
| HC | 41.6 ± 0.5 | 101.4 ± 2.5$^{ab}$ | 66.1 ± 3.6 | 1.12 ± 0.03$^{a}$ | 34.8 ± 0.8$^{ab}$ | 1.62 ± 0.03$^{a}$ |
| HR1 | 43.5 ± 0.8 | 103.9 ± 3.3$^{ab}$ | 66.2 ± 3.0 | [1]1.10 ± 0.00$^{a}$ | 35.0 ± 0.3$^{ab}$ | 1.16 ± 0.02$^{b}$ |
| HR2 | 42.2 ± 1.1 | 107.6 ± 2.1$^{ab}$ | 68.8 ± 2.3 | 1.06 ± 0.03$^{a}$ | 35.4 ± 1.3$^{ab}$ | 0.78 ± 0.04$^{c}$ |
| HR3 | 42.1 ± 0.8 | 106.3 ± 2.8$^{a}$ | 69.2 ± 1.0 | 1.12 ± 0.02$^{a}$ | 36.3 ± 0.2$^{ab}$ | 0.32 ± 0.00$^{d,1}$ |
| HR4 | 40.8 ± 0.2 | 95.8 ± 4.1$^{ab}$ | 65.2 ± 2.3 | 1.21 ± 0.06$^{ab}$ | 31.4 ± 1.5$^{b}$ | 0$^{e}$ |
| LC | 41.9 ± 0.9 | 102.6 ± 2.9$^{ab}$ | 71.5 ± 4.2 | 1.21 ± 0.03$^{ab}$ | 35.5 ± 0.9$^{ab}$ | 1.70 ± 0.07$^{a}$ |
| LR1 | 41.8 ± 1.1 | 101.9 ± 3.9$^{ab}$ | 66.5 ± 3.4 | 1.14 ± 0.02$^{ab}$ | 37.1 ± 0.6$^{a}$ | 1.17 ± 0.03$^{b}$ |
| LR2 | 42.7 ± 1.3 | 91.6 ± 2.3$^{b}$ | 65.6 ± 1.2 | 1.35 ± 0.04$^{bc}$ | 33.8 ± 1.0$^{ab}$ | 0.92 ± 0.01$^{c}$ |
| LR3 | 42.9 ± 1.0 | 98.1 ± 2.5$^{ab}$ | 67.1 ± 1.3 | 1.22 ± 0.05$^{ab}$ | 34.5 ± 0.9$^{ab}$ | 0.36 ± 0.00$^{d,1}$ |
| LR4 | 42.2 ± 1.2 | 88.5 ± 3.9$^{b}$ | 66.0 ± 2.7 | 1.46 ± 0.08$^{c}$ | 33.1 ± 1.7$^{ab}$ | 0$^{e}$ |

Diet HC, HR1, HR2, HR3 and HR4 contained 400 g kg$^{-1}$ digestible protein, and diet LC, LR1, LR2, LR3 and LR4 contained 350 g kg$^{-1}$ digestible protein. At each of the protein levels, a basal diet (HC or LC) contained 400 g kg$^{-1}$ herring meal, and 30% (HR1 or LR1), 50% (HR2 or LR2), 80% (HR3 or LR3) and 100% (HR4 or LR4) of the fish meal in the basal diet was replaced with a protein blend.

Letters indicate results of Turkey HSD test. The values in same column with different superscripts are significantly different at 0.05 level.

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

[1] SEM < 0.005.

The values are represented as mean ± SEM, $n = 3$.

**Table 4** Proximate composition (g $kg^{-1}$) in carcass of cuneate drum fed the test diets

| Feeds | Moisture | Crude protein | Crude lipid | Ash |
|---|---|---|---|---|
| Initial | 751 ± 2 | 180 ± 2 | 22 ± 2 | 49 ± 1 |
| HC | 735 ± 3 | 182 ± $1^{ab}$ | 44 ± $1^{ab}$ | 42 ± $0^{ab,1}$ |
| HR1 | 742 ± 2 | 182 ± $1^{ab}$ | 38 ± $3^{a}$ | 42 ± $1^{ab}$ |
| HR2 | 738 ± 4 | 183 ± $2^{ab}$ | 41 ± $5^{ab}$ | 41 ± $1^{a}$ |
| HR3 | 732 ± 2 | 186 ± $2^{b}$ | 44 ± $0^{ab,1}$ | 42 ± $1^{ab}$ |
| HR4 | 739 ± 2 | 180 ± $1^{ab}$ | 40 ± $2^{ab}$ | 44 ± $1^{b}$ |
| LC | 730 ± 5 | 179 ± $2^{ab}$ | 49 ± $4^{ab}$ | 43 ± $0^{ab,1}$ |
| LR1 | 737 ± 3 | 178 ± $1^{a}$ | 44 ± $2^{ab}$ | 43 ± $1^{ab}$ |
| LR2 | 728 ± 3 | 179 ± $0^{ab,1}$ | 54 ± $5^{b}$ | 44 ± $1^{b}$ |
| LR3 | 737 ± 4 | 176 ± $3^{a}$ | 47 ± $2^{ab}$ | 43 ± $1^{ab}$ |
| LR4 | 733 ± 3 | 176 ± $1^{a}$ | 47 ± $3^{ab}$ | 44 ± $0^{b,1}$ |

Diet HC, HR1, HR2, HR3 and HR4 contained 400 g $kg^{-1}$ digestible protein, and diet LC, LR1, LR2, LR3 and LR4 contained 350 g $kg^{-1}$ digestible protein. At each of the protein levels, a basal diet (HC or LC) contained 400 g $kg^{-1}$ herring meal, and 30% (HR1 or LR1), 50% (HR2 or LR2), 80% (HR3 or LR3) and 100% (HR4 or LR4) of the fish meal in the basal diet was replaced with a protein blend.

Letters indicate results of Turkey HSD test. The values in same column with different superscripts are significantly different at 0.05 level.

Crude protein, crude lipid and ash are expressed on a wet weight basis.

[1] SEM < 0.5.

The values are represented as mean ± SEM, $n = 3$.

and 0 g $kg^{-1}$ fish meal ($P < 0.05$). Ratio of fish meal consumption to fish production were dependent on dietary protein and fish meal levels ($P < 0.05$), and decreased with the reduction of dietary fish meal level at the same DP level ($P < 0.05$). Dietary fish meal consumption was lower than fish production (RCP < 1) for fish fed the diets containing 200, 80 or 0 g $kg^{-1}$ fish meal. At the end of the experiment, there were no significant differences in carcass composition (moisture, crude protein, crude lipid and ash) among fish fed the diets containing various fish meal levels at the same DP level ($P > 0.05$, Table 4).

## Discussion

Feeding 19 g cuneate drum the diets containing 16 MJ $kg^{-1}$ DE but different contents of DP (360, 380 and 400 g $kg^{-1}$ DP) did not result in statistical differences in FBW, FCR and NRE, although FBW and NRE of the fish increased with the increase of dietary DP level (Wang *et al.* 2006a). In the present study, there were no significant differences in WG, FBW, FCR and NRE between fish fed the basal diets containing 400 and 350 g $kg^{-1}$ DP. Feed intake, WG, FBW, NRE and carcass composition of fish fed the basal diet were not significantly different from that of fish fed the diet containing 0 g $kg^{-1}$ fish meal at the 400 g $kg^{-1}$ DP level, while FBW was higher in fish fed the basal diet than fish fed the diet containing 0 g $kg^{-1}$ fish meal at the 350 g $kg^{-1}$ DP level. This suggests elevating dietary protein level could enhance potential of the blend of PBM, MBM, FEM and BLM as fish meal substitutes in diets for cuneate drum, and fish meal level could be reduced from 80 to 0 g $kg^{-1}$ with increasing dietary protein level from 350 to 400 g $kg^{-1}$ DP. Results of the present study is consistent with earlier conclusion (Guo *et al.* 2007) that indicated dietary fish meal level for 28 g cuneate drum could be reduced to 80 g $kg^{-1}$ by using blended PBM, MBM, FEM and BLM as fish meal substitutes at dietary protein level of 360 g $kg^{-1}$ DP. Dietary fish meal consumption was lower than fish production (RCP < 1) for fish fed the diets containing 0, 80 and 200 g $kg^{-1}$ fish meal, indicating net fish production could be achieved in cuneate drum farming by using low fish meal diets (dietary fish meal level <200 g $kg^{-1}$). Therefore, using economic protein sources as fish meal substitutes in fish diets can overcome the excessive fish meal consumption in fish culture.

Deficiency in EAA has been considered one of the factors limiting the use of economic protein ingredients in fish diets (Glencross *et al.* 2007). There are significant differences in opinion as to the most appropriate mode of expressing EAA (Wilson 1985; Cowey 1994; Rodehutscord *et al.* 1997). EAA requirements of fish and other animals have been traditionally expressed as % of diet (NRC, 1993), but were sometimes expressed as g $MJ^{-1}$ DE (Pfeffer *et al.* 1992; Rodehutscord *et al.* 1995, 1997) or % of dietary protein (Cowey & Cho 1993). Expressing EAA requirements as % of diet presents the least dietary EAA contents, but fails to reflect the proportion of dietary EAA to protein. In opposite, EAA requirements expressed as % of protein reflects the least proportion of dietary EAA to protein, but the least dietary EAA contents changes at different dietary protein levels. EAA requirements determined by dose response studies have been frequently expressed as % of protein. In such studies, to ensure that the tested EAA is the first limiting EAA at all the tested levels, the remaining EAA and unessential amino acids are supplied in excess of their requirements. This relative oversupply of dietary amino acids should result in an underestimation of EAA requirements when expressed as % of protein. Moreover, the use of diets with excessive protein for a particular life-stage of a fish species is likely to have underestimated EAA requirements expressed as % of protein (Hauler & Carter 2001). Expressing EAA requirements as % of protein, would nevertheless, make sense in the context of a low protein diet formulated based on the ideal protein concept where each EAA is equally limiting to protein accretion. It needs to

establish the requirements for both balanced protein and amino acid composition of ideal protein. Encarnação *et al.* (2006) reported that increasing dietary DP level with different amino acids had no effect on protein accretion or efficiency of lysine utilization at marginal levels of lysine intake. This indicates that the catabolism of excess amino acid in the diet does not affect the inevitable catabolism of the first limiting EAA. Expression of EAA requirements in relation to protein may result in a need for amino acid supplementation in diets formulated to high DP (and DE) levels with imbalanced economical protein sources. The need to balance the EAA content of the diet, at high protein levels, is still very much a matter of debate. In the present study, the gap in EAA contents between the diets containing 400 and 350 g $kg^{-1}$ DP was higher when expressed as % of the diet than that expressed as % of protein. Thus expressing EAA contents as % of diet, rather than % of protein, is rational to explain the difference in growth performance between fish fed the diets containing 400 or 350 g $kg^{-1}$ DP.

Fish meal are generally considered the most important protein ingredients for formulating carnivorous fish diet because of their excellent palatability, digestibility and bioavailable EAA. In the present study, a linear correlation between WG and dietary fish meal level occurred at the 350 g $kg^{-1}$ DP level, but not at the 400 g $kg^{-1}$ DP level. WG and FBW of fish fed the diets containing 200–0 g $kg^{-1}$ fish meal were lower at the 350 g $kg^{-1}$ DP level than that at the 400 g $kg^{-1}$ DP level, suggesting elevation of dietary protein level improved growth of cuneate drum fed the low fish meal diets. Ballestrazzi *et al.* (1994) indicated replacing fish meal with corn gluten meal did not negatively affect growth of sea bass at three dietary protein levels, however, high fish meal level (280 g $kg^{-1}$) in their test diets might provide enough EAA to meet all requirements. Urdaneta-Rincon *et al.* (2005) observed that lysine requirement (g $kg^{-1}$ of diet) for broiler chicken increased with the increase of dietary protein level. If this is also true in fish, formulating diets to higher protein levels may have limited value in enhancing potential of fish meal replacement by economic protein ingredients. EAA requirements for cuneate drum have not been reported, and EAA contents (expressed as g $kg^{-1}$ of dry weight) in whole body of cuneate drum were Thr 22.1, Val 27.9, Cyst 2.1, Met 17.7, Ile 24.6, Leu 42.8, Tyr 15.4, Phe 22.5, Lys 45.6, His 12.3 and Arg 39.5 (Wang *et al.* 2006a). The proportion of the dietary Lys content in the present study to the Lys content in whole body of cuneate drum was 35–68%, and the proportion for the other EAA are Thr 61–80%, Val 49–72%, Cyst + Met 57–86%, Ile 50–63%, Leu 57–82%, Tyr 58–78%, Phe 64–93%, Lys, His 93–132% and Arg 56–70%. Higher dietary Lys content (% of diet) at the 400 g $kg^{-1}$ DP level relative to that at the 350 g $kg^{-1}$ DP level indicated Lys deficiency might be an reason responsible to the lower WG and higher FCR of fish fed the diets containing 0–200 g $kg^{-1}$ fish meal at the 350 g $kg^{-1}$ DP level, relative to fish fed the diets containing same levels of fish meal at the 400 g $kg^{-1}$ DP level. Results of the present study reveal that EAA requirements of cuneate drum should not be a function of dietary protein level, and formulating the diet to a higher DP level could, by consequently increasing digestible EAA level, reduce fish meal requirement.

In the published studies, potential of fish meal replacement was usually termed as the highest percentage of the fish meal of the basal diet replaced by economic protein ingredients (Fowler 1991; El-Sayed 1994; Steffens 1994; Kureshy *et al.* 2000; Wang *et al.* 2006b). This 'fish meal replacement potential' is dependent on many factors, such as dietary DP level, EAA level and fish meal level of the basal diet, etc. Excessive fish meal or protein level of the basal diet, as well as the improper indicator (using the highest percentage of the fish meal replaced from the basal diet to quantify fish meal replacement level), may result in significant overestimation of ability of economic protein ingredients as fish meal substitutes.

In conclusion, results of the present study indicate (1) high dietary protein level can enhance the potential of land animal protein ingredients as fish meal substitutes in diets for cuneate drum, and fish meal can be completely replaced by blended PBM, MBM, FEM and BLM at the 400 g $kg^{-1}$ DP level, (2) fish production will be over fish meal consumption in cuneate drum farming when dietary fish meal level was reduced to 200 g $kg^{-1}$ or less, (3) the least dietary fish meal level and ratio of fish meal consumption to fish production are useful indicators for evaluating the potential of fish meal replacement by economic protein sources and (4) realistically assessing nutritive value of economic protein ingredients should be based on more rational approaches, notably of the assessment of availability of EAA of the ingredients and the EAA requirements of the fish studied.

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