# Meat and bone meal replacement in diets for juvenile gibel carp (*Carassius auratus gibelio*): effects on growth performance, phosphorus and nitrogen loading

S. ZHANG[1,3], S. XIE[1,2], X. ZHU[1], W. LEI[1], Y. YANG[1] & M. ZHAO[1,3]

[1] *State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, The Chinese Academy of Sciences, Wuhan, Hubei, P.R. China;* [2] *Aquaculture Division, E-Institute of Shanghai Universities, Shanghai, P.R. China;* [3] *Graduate School of the Chinese Academy of Sciences, Beijing, P.R. China*

## Abstract

A 11-week growth trial was conducted in a flow-through system with juvenile gibel carp *Carassius auratus gibelio* to evaluate the effects of gradual replacement of fish meal (FM) by meat and bone meal (MBM) on growth performance, phosphorus (P) and nitrogen (N) loading. Six isonitrogenous (crude protein: 410 g $kg^{-1}$) and isoenergetic (gross energy: 18 kJ $g^{-1}$) diets were formulated. FM was used as the control protein. In the other five diets, 20, 40, 60, 80 and 100% FM protein was substituted with $MBM_{20}$, $MBM_{40}$, $MBM_{60}$, $MBM_{80}$, $MBM_{100}$, respectively. Total P content in the diets ranged from 16.0 to 28.3 g $kg^{-1}$ and the available P was 5.0–6.6 g $kg^{-1}$. The results showed that the best growth was achieved with fish fed on the control diet and $MBM_{20}$. Final body weight, weight gain, feed efficiency, protein retention efficiency and energy retention efficiency decreased with increased dietary MBM. No significant differences were found in the feeding rate and hepatosomatic index between the groups. Apparent digestibility coefficient (ADC) of dry matter, protein and P decreased with increase in dietary MBM, while there were no significant differences in the ADC of energy. P and N retention decreased linearly while P and N loading increased linearly with the increased dietary MBM levels. No significant differences were observed in the activity of alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase, as well as pyruvate kinase in liver or in serum. Total superoxide dismutase activity in $MBM_{20}$ was significantly higher than that of $MBM_{100}$.

KEY WORDS: *Carassius auratus gibelio*, growth performance, loading, meat and bone meal, nitrogen, phosphorus, retention

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*Correspondence: S. Xie, State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, Hubei, 430072, P.R. China. Email: sqxie@ihb.ac.cn*

## Introduction

Fish meal (FM) is the most common protein source in aquafeeds (Xue & Cui 2001). But with increasing human population and fisheries pressure, the global production of FM has been declining (Starkey 1994). There is also a competitive demand for marine protein sources by human and other livestock (Millamena 2002). Thus, the search for alternatives to FM in aquafeeds is an international research priority (Hardy & Kissil 1997; Lee 2002). Meat and bone meal (MBM), which has higher protein content and better essential amino acid profiles than plant protein, could be used as an economical alternative protein source of FM (Tan *et al.* 2005). Davies *et al.* (1989); Watanabe & Pongmaneerat (1991) and Millamena (2002) had successfully replaced partially the FM protein with MBM combined with other protein sources in diets for Mozambique tilapia *Oreochromis mossambicus*, grouper *Epinephelus coioide* and rainbow trout *Oncorhynchus mykiss*, respectively. Recently, Yang *et al.* (2004) reported that the replacement level of FM by MBM in diets for gibel carp at 500 g $kg^{-1}$ protein resulted in similar growth and feed utilization to the FM diet. However, only two replacement levels were designed in the experiment (Yang *et al.* 2004). The effects on gibel carp, when higher levels of FM are substituted by MBM is still unknown.

Environmental pollution associated with aquaculture effluent is a critical issue for the sustainability and expansion of aquaculture (Sugiura *et al.* 2000). Intensive production results in the release of organic wastes and soluble inorganic nutrients, such as phosphorus (P) and nitrogen (N), which can enrich and generate eutrophication in natural aquatic eco-

systems (GESAMP 1996). P waste produced by aquaculture can be reduced in a number of ways, one of which is the use of FM substitutes that contains less and more available P (Cho & Bureau 2001). However, the P concentration of MBM is about 3.5% to 5.5% (Lall 1991), which is even higher than that of FM. Therefore, it is necessary to investigate the P utilization when using MBM as an alternative protein of FM. So far, only little is known about it in practice. Most of the concerned studies mainly focused on growth performance, not the P loading or retention. Apparent digestibility coefficients (ADC) of P have been observed in different feedstuffs including MBM and FM (Ye & He 1991; Sugiura *et al*. 2000; Zhou *et al*. 2004). Similarly, a few researches had been conducted on N utilization in diets with the replacement of FM by MBM and different results had been obtained. Bureau *et al*. (2000) fed either 12% or 24% of the diet as MBM and reported no significant difference in the retention of N in rainbow trout fed on any of the three sources of MBM compared with fish fed on a control diet. The values of N-$NH_4^+$ excretion obtained from diets containing 20% or 30% MBM was similar to that from the control diet and the statistical difference could only be found when comparing values of excretion from the highest substitution level (40%) (Robaina *et al*. 1997). Most of the former evaluations incorporated MBM at relatively low levels in the diets of only few fish species and therefore, further studies should be carried out to solve the problems. P or N retention (or loading) differed under different replacement levels in some studies (Robaina *et al*. 1997; Jahan *et al*. 2000), and hence it is suggested that a correlation may exist between nutrient retention (or loading) and replacement levels. However, no such confirmation has been published previously.

Gibel carp, *Carassius auratus gibelio*, is an improved strain of crucian carp, which is becoming increasingly popular as an aquaculture species in China (Yang *et al*. 2004). The objectives of the present study are to evaluate the effects of gradual replacement of FM with MBM on growth performance, feed utilization, P and N loading of gibel carp.

## Materials and methods

### *Experimental diets*

The MBM was provided by the National Renderers Association (NRA), USA. It was composed of 80% beef, 10% pork and 10% poultry. The chemical composition and amino acid profiles of its ingredients are shown in Table 1. Six isonitrogenous (crude protein: 410 g $kg^{-1}$) and isoenergetic (gross energy: 18 kJ $g^{-1}$) practical diets were formulated. White FM was used as the control protein. In the other five diets, 20, 40, 60, 80 and 100% FM protein was substituted with MBM. Monosodium phosphate ($NaH_2PO_4$) was supplemented to meet the P requirement for gibel carp (6.9 g $kg^{-1}$ total P, or 6.0 g $kg^{-1}$ available P; Ye et al., unpublished data). P concentration in the control diet is 16.0 g $kg^{-1}$ total P, or 6.2 g $kg^{-1}$ available P. As MBM contains a higher concentration of P than FM, Diet 2–Diet 6 included increasing levels of P from 18.5 to 28.3 g $kg^{-1}$.

**Table 1** Proximate composition and amino acid profiles of ingredients used in the experimental diets (g $kg^{-1}$dry matter)

| Ingredients | White fish meal (Golden Alaska, USA) | Meat and bone meal (NRA, USA) | Defatted soybean meal (Coland Feed Company, Wuhan, China) |
|---|---|---|---|
| Crude protein | 720.0 | 565.2 | 492.2 |
| Crude lipid | 93.7 | 116.9 | 12.2 |
| Ash | 126.9 | 238.1 | 67.8 |
| Moisture | 42.6 | 47.8 | 81.0 |
| Calcium | 34.7 | 80.8 | 8.7 |
| Phosphorus | 21.8 | 37.7 | 7.9 |
| Gross energy (kJ $g^{-1}$) | 19.1 | 17.9 | 18.4 |
| *Amino acid (g $kg^{-1}$)* | | | |
| Aspargine | 62.9 | 41.2 | 48.2 |
| Threonine | 28.9 | 18.6 | 16.6 |
| Serine | 43.6 | 28.0 | 31.4 |
| Glutamine | 104.2 | 76.2 | 88.9 |
| Proline | 28.7 | 39.0 | 23.6 |
| Glycine | 44.2 | 63.6 | 20.5 |
| Alanine | 43.8 | 40.6 | 20.2 |
| Cystine | 5.4 | 3.5 | 4.6 |
| Valine | 35.9 | 25.1 | 20.2 |
| Methionine | 22.9 | 11.4 | 5.6 |
| Isoleucine | 29.8 | 18.6 | 19.2 |
| Leucine | 58.8 | 39.8 | 37.4 |
| Tyrosine | 28.8 | 16.8 | 18.6 |
| Phenylalanine | 29.5 | 22.0 | 23.6 |
| Lysine | 60.3 | 35.6 | 29.7 |
| Histidine | 15.4 | 12.2 | 12.4 |
| Arginine | 47.0 | 38.0 | 33.9 |

Chromium oxide ($Cr_2O_3$) (1%) was used as inert marker for digestibility measurement. All diets were turned into sinking pellets (2 mm in diameter) by a laboratory pelleter, oven-dried at 60 °C and stored at −20 °C. Diet formulation and chemical composition are shown in Table 2.

### *Experimental conditions, fish and feeding*

The growth trial was carried out in 18 fiberglass tanks with cylindro-conical bottoms (diameter: 80 cm, water volume: 300 L). The experimental system was equipped with a big concrete filter tank filled with zeolite and active carbon for

**Table 2** Formulation and chemical composition of the experimental diets (g $kg^{-1}$in dry matter)

| Ingredients | Control | $MBM_{20}$ | $MBM_{40}$ | $MBM_{60}$ | $MBM_{80}$ | $MBM_{100}$ |
|---|---|---|---|---|---|---|
| White fishmeal | 432.2 | 345.8 | 259.3 | 172.9 | 86.4 | 0.0 |
| Soybean meal | 150.0 | 150.0 | 150.0 | 150.0 | 150.0 | 150.0 |
| Meat and bone meal | 0.0 | 110.1 | 220.2 | 330.3 | 440.5 | 550.6 |
| Corn starch | 189.8 | 188.9 | 188.1 | 187.2 | 186.4 | 185.3 |
| Fish oil[1] | 47.7 | 42.9 | 38.1 | 33.4 | 28.6 | 23.8 |
| Mineral premix[3] | 40.0 | 40.0 | 40.0 | 40.0 | 40.0 | 40.0 |
| NaH2PO4.2H2O | 25.2 | 25.2 | 25.2 | 25.2 | 25.2 | 25.2 |
| Vitamin premix[2] | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| Carboxymethyl cellulose | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| Choline chloride | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 | 1.1 |
| Cellulose | 90.0 | 72.0 | 53.9 | 35.9 | 17.8 | 0.0 |
| Chromic oxide | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| *Chemical composition (g $kg^{-1}$)* | | | | | | |
| Moisture | 76.6 | 68.5 | 78.6 | 75.6 | 68.9 | 58.1 |
| Crude protein | 414.7 | 414.7 | 412.7 | 414.2 | 414.5 | 414.3 |
| Crude lipid | 80.4 | 78.1 | 80.8 | 83.8 | 79.2 | 81.7 |
| Ash | 111.1 | 126.0 | 140.3 | 151.7 | 170.7 | 185.4 |
| Nitrogen-free extract | 271.6 | 271.0 | 272.3 | 272.8 | 270.2 | 273.0 |
| Calcium | 18.4 | 23.9 | 29.7 | 35.9 | 41.9 | 48.1 |
| Total phosphorus | 16.0 | 18.5 | 20.6 | 23.3 | 25.6 | 28.3 |
| Available phosphorus | 6.2 | 6.6 | 5.6 | 5.4 | 5.2 | 5.0 |
| Digestible energy(KJ g-1) | 18.2 | 18.4 | 18.4 | 18.4 | 18.2 | 17.8 |

MBM, meat and bone meal.

[1] Coland Feed Company, Wuhan, China.

[2] Vitamin premix (mg $kg^{-1}$ diets): vitamin A, 1.83; vitamin D, 0.5; vitamin E, 10; vitamin K, 10; niacin, 100; riboflavin, 20; pyridoxine, 20; thiamin, 20; D-calcium pantothenate, 50; biotin, 1.0; folic acid, 5; vitamin $B_{12}$, 2; ascorbic acid, 400; inositol, 100.

[3] Mineral premix (mg $kg^{-1}$ diets): NaCl, 400; $MgSO_4 \cdot H_2O$, 3365.6; $Na_2SO_4$, 9120; KCl, 7000; $CaSO_4$, 5460; $FeSO_4 \cdot H_2O$, 1118.4; $(CH_2CHCOO)_2Ca \cdot 5H_2O$, 1400; $ZnSO_4 \cdot H_2O$,88; $MnSO_4 \cdot H_2O$, 48.8; $CuSO_4 \cdot 5H_2O$, 12.4; $CoSO_4 \cdot H_2O$, 40; KI, 1.2; cellulose, 209.6.

dechlorination of the water. Filtered municipal water was supplied to each tank at a rate of 2.5 L $min^{-1}$ in an indoor flow-through system with the water renewed once every 2 h. Continuous aeration was supplied via airstones in each tank. During the trial, water temperature was recorded daily and ranged from 21.8 to 28 °C (mean 24.8 °C). The photoperiod was 12 L : 12 D with the light period from 08:00 to 20:00 h and the light intensity between 120 and 160 lx on the water surface. Dissolved oxygen was above 6 mg $L^{-1}$ and ammonia-*N* ($NH_4^+$–N + $NH_3$–N) was less than 0.1 mg $L^{-1}$, pH was around 6.8. The P concentration in the rearing water was less than 0.05 mg $L^{-1}$ during the experimental period.

Juvenile gibel carp was obtained from the hatchery farm of the Institute of Hydrobiology, the Chinese Academy of Sciences. Prior to the growth trial, fish were held in the experimental system at 100 fish per tank for acclimatization. During this period, fish were fed with an FM-based diet for 4 weeks and then an equal mixture of experimental diets for 2 weeks to satiation twice a day (09:00 and 15:00 h). At the beginning of the experiment, the fish (average body weight 3.6 g) were deprived of food for 1 day and pooled. Then they were randomly distributed into 18 tanks (30 fish each tank). Triplicate tanks were randomly assigned to each of the experimental diets and the fishes were hand-fed to apparent satiation two times per day (09:00 and 15:00 h), 7 days a week for 11 weeks. At each feeding, an excess quantity of the diet was provided and uneaten diet was collected 1 h after feeding, dried to constant weight at 70 °C and reweighed. The leaching rate of the uneaten diet was estimated by placing weighed feed in tanks without fish for 1 h and then collecting, drying and weighing. Leaching rate was used to calibrate the amount of feed intake. The fecal collection system used in this study was modified from the settlement method described by Allan *et al.* (1999). The system collected feces from the removable collection chamber connected to the conical bottom as soon as the feces were discharged by the fish. Feces were collected from the second week of the trial and throughout the experiment period. To minimize nutrient leaching in the feces, the mixture of feces and the water in the column were dried at 70 °C and stored for chemical analysis.

### *Sample collection*

At the beginning of the experiment, 30 fish were randomly selected from the stock. Of the 30 fish, 15 were used to collect vertebrae for bone ash and Ca, P concentration analysis. The

remaining 15 fish were used for the analysis of body composition.

At the end of the experiment, the fish were batch-weighted each tank after 24 h deprivation of feed. Ten fish per tank were quickly anesthetized with MS-222, weighed, and blood sample was taken from the caudal vein using a syringe. The blood was then transferred into a 1.5-mL microfuge tube, centrifuged at 3000 g for 10 min at 25 °C and serum was removed and stored at −20 °C until analysis. Another ten fish from each tank were killed with sharp blow to the head. Of the ten fish, five were individually weighed and the liver was then removed. After weighing, the liver was stored immediately in liquid nitrogen for enzyme determination. Fish carcasses were cooked in a microwave oven for 2 min, and the surrounding tissues were removed from the vertebrae. Then the vertebraes were rinsed with deionized water, dried and ground for ash and mineral analyses. The remaining five fish were taken for final fish body composition analysis.

### *Analytical methods and calculations*

Samples of liver were homogenized in 0.9% physiological saline, and then the homogenates were centrifuged to remove debris and the resultant supernatants were used for enzyme assays. Alkaline phosphatase (AKP) activity was determined spectrophotometrically using an p-nitrophenyl-phosphate substrate assay (Tietz 1986). Liver superoxide dismutase (SOD) activity was determined using the method of McCord & Fridovich (1969). One unit of SOD activity is described as the amount of sample required to cause 50% inhibition of the rate of reduction of cytochrome c by $O_2^-$. The activity of aspartate aminotransferase (GOT) and alanine aminotransferase (GPT) were measured according to Hørder & Rej (1981). Pyruvate kinase (PK) was assayed according to the procedure used by Thibeault *et al.* (1997).

The ash content of vertebrae was measured using the method described in Roy & Lall (2003). P and Ca concentrations in the diets, bones, feces and whole fish body were analyzed by inductively coupled plasma emission spectrophotometry (IRIS advantage, TJA solutions, USA) at HuBei Agricultural Academy of Sciences of China. Dry matter was determined by drying at 105 °C to constant weight (AOAC 1984). The protein content of the feeds and fecal samples were measured using 2300 Kjeltec Analyzer Unit (FOSS TECATOR, made in Sweden). Lipid was determined by petroleum ether extraction using a Soxtec system (Soxtec System HT6; Tecator, Hoganas, Sweden), ash by combustion at 550 °C, gross energy by bomb calorimetry (Phillipson Microbomb Calorimeter; Gentry Instruments Inc., Aiken, USA), and $Cr_2O_3$ content by concentrated nitric and perchloric acid digestion (Bolin *et al.* 1952). At least duplicate measurements were made for each sample. The ADC were calculated according to the formula from Maynard & Loosli (1969) and NRC (1993). Apparent retention and loading of P and N were calculated as given by Green *et al.* (2002).

### *Statistical analysis*

All data were subjected to one-way analysis of variance (ANOVA) followed by the Duncan's multiple range test. Statistica 6.0 was used to perform statistical calculations. Differences were considered significant at $P < 0.05$. The tables present the mean values of three replicate tanks.

## Results

### *Growth and feed utilization*

Table 3 shows that final body weight (FBW), weight gain (WG), feed efficiency (FE), protein retention efficiency (PRE) and energy retention efficiency (ERE) decreased with increased dietary replacement of FM by MBM ($P < 0.05$), while there was no significant difference between the control and $MBM_{20}$ group ($P > 0.05$). No significant difference was found in the feeding rate (FR) and hepatosomatic index (HSI) ($P > 0.05$).

### *Apparent digestibility coefficient*

Table 4 shows that the ADC of dry matter ($ADC_d$), protein ($ADC_p$) and P ($ADC_{ph}$) decreased with the increase in dietary MBM levels ($P < 0.05$), while there was no significant difference in the ADC of gross energy ($ADC_e$) ($P > 0.05$).

### *Body composition*

The proximate composition of the whole body and bone (vertebra) for the initial and the final fish is presented in Table 5. Dry matter, crude protein, ash, P and Ca contents in the final fish body were not significantly affected by the inclusion of MBM ($P > 0.05$). The final body crude lipid and gross energy was higher at $MBM_{100}$ group ($P < 0.05$).

### *Phosphorus and nitrogen loading*

Figure 1 shows that both P and N retention rate decreased linearly with the increase in dietary MBM levels ($P < 0.05$). The relationship between P retention ($P_R$, %), N retention

**Table 3** Growth and feed utilization of gibel carp fed on diets with different levels of meat and bone meal (MBM) (means ± SE)*

| | Control | $MBM_{20}$ | $MBM_{40}$ | $MBM_{60}$ | $MBM_{80}$ | $MBM_{100}$ |
|---|---|---|---|---|---|---|
| IBW[1] | 3.56 ± 0.00 | 3.56 ± 0.00 | 3.57 ± 0.00 | 3.58 ± 0.00 | 3.56 ± 0.00 | 3.58 ± 0.01 |
| FBW[2] | 9.62 ± 0.17$^{a}$ | 9.07 ± 0.35$^{a}$ | 8.16 ± 0.50$^{b}$ | 8.21 ± 0.12$^{b}$ | 7.29 ± 0.07$^{c}$ | 7.23 ± 0.12$^{c}$ |
| FR[3] | 1.95 ± 0.02 | 1.91 ± 0.02 | 1.91 ± 0.09 | 2.04 ± 0.04 | 1.92 ± 0.04 | 2.04 ± 0.03 |
| WG[4] | 170.15 ± 4.97$^{a}$ | 155.16 ± 9.98$^{a}$ | 128.60 ± 14.07$^{b}$ | 129.36 ± 3.16$^{b}$ | 104.80 ± 1.92$^{c}$ | 102.18 ± 3.22$^{c}$ |
| FE[5] | 61.33 ± 1.13$^{a}$ | 59.86 ± 2.13$^{a}$ | 52.97 ± 1.18$^{b}$ | 50.29 ± 0.64$^{b}$ | 46.51 ± 0.62$^{c}$ | 43.10 ± 0.44$^{c}$ |
| HSI[6] | 11.30 ± 0.12 | 11.72 ± 0.30 | 12.04 ± 0.15 | 10.92 ± 0.18 | 11.00 ± 0.29 | 11.06 ± 0.65 |
| PRE[7] | 35.13 ± 0.65$^{a}$ | 35.22 ± 0.16$^{a}$ | 34.09 ± 0.92$^{ab}$ | 32.03 ± 1.10$^{b}$ | 32.96 ± 0.11$^{b}$ | 29.75 ± 0.22$^{c}$ |
| ERE[8] | 24.35 ± 0.57$^{a}$ | 23.8 ± 1.54$^{ab}$ | 21.77 ± 0.46$^{bc}$ | 21.10 ± 0.61$^{c}$ | 19.42 ± 0.35$^{c}$ | 19.53 ± 0.29$^{c}$ |

*Figures in the same column with different superscripts are significantly different ($P < 0.05$).
[1] IBW, initial body weight (g).
[2] FBW, final body weight (g).
[3] FR, feeding rate (% BW $day^{-1}$) = 100 × dry feed intake/(days ×[FBW + IBW]/2).
[4] WG, weight gain (%) = [(final body weight − initial body weight)/initial body weight] × 100.
[5] FE, feed efficiency (%) = 100 × (wet weight gain/dry feed intake).
[6] HSI, hepatosomatic index (%) = 100 × (liver weight/body weight).
[7] PRE, protein retention efficiency (%) = 100 × (protein retained in fish body/protein intake).
[8] ERE, energy retention efficiency (%) = 100 × (energy retained in fish body/energy intake).

**Table 4** Apparent digestibility (% ADC) of experimental diets in gibel carp (means ± SE)*

| Diet | $ADC_d$[1] | $ADC_p$[2] | $ADC_e$[3] | $ADC_{ph}$[4] |
|---|---|---|---|---|
| Control | 68.99 ± 0.76$^{a}$ | 92.46 ± 0.72$^{a}$ | 79.51 ± 0.26 | 38.80 ± 0.68$^{a}$ |
| $MBM_{20}$ | 69.68 ± 0.69$^{a}$ | 91.51 ± 0.66$^{a}$ | 80.14 ± 1.37 | 35.78 ± 0.91$^{a}$ |
| $MBM_{40}$ | 67.90 ± 0.41$^{ab}$ | 89.25 ± 0.27$^{b}$ | 80.14 ± 0.83 | 27.20 ± 1.21$^{b}$ |
| $MBM_{60}$ | 66.62 ± 0.51$^{b}$ | 88.02 ± 0.29$^{b}$ | 79.99 ± 0.33 | 23.14 ± 1.38$^{c}$ |
| $MBM_{80}$ | 62.03 ± 0.24$^{c}$ | 83.89 ± 0.70$^{c}$ | 77.82 ± 0.85 | 20.32 ± 1.56$^{cd}$ |
| $MBM_{100}$ | 63.11 ± 1.03$^{c}$ | 83.26 ± 0.73$^{c}$ | 78.37 ± 0.12 | 17.57 ± 1.32$^{d}$ |

MBM, meat and bone meal.
*Figures in the same column with different superscripts are significantly different ($P < 0.05$).
[1] $ADC_d$, ADC of dry matter (%) = 100(1 − [$Cr_2O_3$ in the diet/$Cr_2O_3$ in the feces] × [dry matter in feces/dry matter in the diet]).
[2] $ADC_p$, ADC of protein (%) = 100(1 − [$Cr_2O_3$ in the diet/$Cr_2O_3$ in the feces] × [crude protein in feces/crude protein in the diet]).
[3] $ADC_e$, ADC of energy (%) = 100(1 − [$Cr_2O_3$ in the diet/$Cr_2O_3$ in the feces] × (energy in feces/energy in the diet]).
[4] $ADC_{ph}$, ADC of phosphorus (%) = 100(1 − [$Cr_2O_3$ in the diet/$Cr_2O_3$ in the faeces) × (P in feces/P in the diet]).

**Table 5** Body and bone (vertebra) composition of gibel carp fed on diets with different levels of meat and bone meal (MBM) (in wet weight) (means ± SE)*

| Diet | Initial | Control | $MBM_{20}$ | $MBM_{40}$ | $MBM_{60}$ | $MBM_{80}$ | $MBM_{100}$ |
|---|---|---|---|---|---|---|---|
| Body | | | | | | | |
| Dry matter (%) | 25.86 ± 0.35$^{a}$ | 28.57 ± 0.14$^{b}$ | 28.99 ± 0.22$^{b}$ | 29.06 ± 0.26$^{b}$ | 28.92 ± 0.23$^{b}$ | 28.79 ± 0.22$^{b}$ | 29.08 ± 0.33$^{b}$ |
| Crude protein (%) | 14.87 ± 0.11$^{a}$ | 14.45 ± 0.14$^{ab}$ | 14.36 ± 0.13$^{ab}$ | 14.45 ± 0.15$^{ab}$ | 14.49 ± 0.16$^{ab}$ | 14.58 ± 0.23$^{ab}$ | 14.12 ± 0.18$^{b}$ |
| Crude lipid (%) | 5.83 ± 0.13$^{a}$ | 7.90 ± 0.20$^{b}$ | 8.21 ± 0.47$^{b}$ | 8.08 ± 0.37$^{b}$ | 7.89 ± 0.24$^{b}$ | 7.91 ± 0.13$^{b}$ | 9.32 ± 0.17$^{c}$ |
| Ash (%) | 2.78 ± 0.09$^{a}$ | 2.32 ± 0.10$^{b}$ | 2.35 ± 0.03$^{b}$ | 2.39 ± 0.08$^{b}$ | 2.28 ± 0.03$^{b}$ | 2.42 ± 0.02$^{b}$ | 2.33 ± 0.00$^{b}$ |
| Gross energy (kJ $g^{-1}$) | 5.78 ± 0.03$^{a}$ | 6.54 ± 0.04$^{b}$ | 6.60 ± 0.15$^{bc}$ | 6.68 ± 0.07$^{bc}$ | 6.77 ± 0.09$^{bc}$ | 6.62 ± 0.12$^{bc}$ | 6.86 ± 0.03$^{c}$ |
| Phosphorus (%) | 0.64 ± 0.02 | 0.63 ± 0.03 | 0.65 ± 0.01 | 0.65 ± 0.01 | 0.63 ± 0.01 | 0.65 ± 0.00 | 0.63 ± 0.00 |
| Calcium (%) | 0.96 ± 0.04$^{a}$ | 0.62 ± 0.03$^{b}$ | 0.62 ± 0.02$^{b}$ | 0.63 ± 0.01$^{b}$ | 0.60 ± 0.01$^{b}$ | 0.63 ± 0.01$^{b}$ | 0.60 ± 0.00$^{b}$ |
| Bone | | | | | | | |
| Ash (%) | 54.60 ± 0.95$^{a}$ | 51.06 ± 0.36$^{b}$ | 49.63 ± 0.84$^{b}$ | 50.09 ± 1.23$^{b}$ | 48.18 ± 0.97$^{b}$ | 49.11 ± 1.74$^{b}$ | 48.73 ± 1.19$^{b}$ |
| Phosphorus (%) | 11.83 ± 0.63$^{a}$ | 10.36 ± 0.23$^{b}$ | 10.76 ± 0.11$^{b}$ | 10.46 ± 0.08$^{b}$ | 10.02 ± 0.38$^{b}$ | 10.30 ± 0.13$^{b}$ | 9.86 ± 0.37$^{b}$ |
| Calcium (%) | 24.97 ± 0.66$^{a}$ | 21.74 ± 0.95$^{b}$ | 21.94 ± 0.20$^{b}$ | 20.91 ± 0.08$^{b}$ | 20.23 ± 0.55$^{b}$ | 21.19 ± 0.16$^{b}$ | 20.02 ± 0.77$^{b}$ |

*Figures in the same column with different superscripts are significantly different ($P < 0.05$).

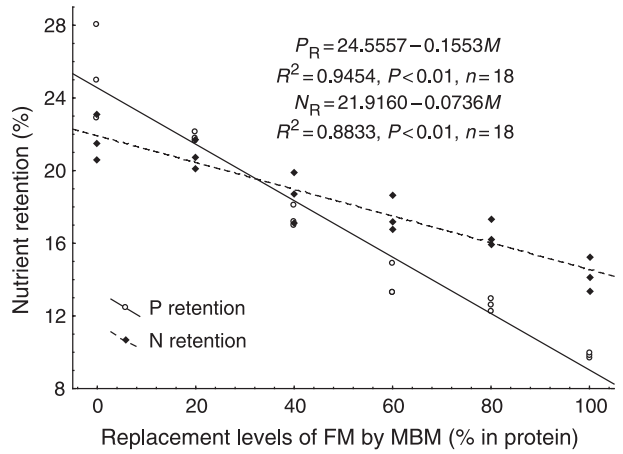


**Figure 1** Relationship between phosphorus (P) and nitrogen (N) retention rate and dietary meat and bone meal (MBM) protein levels in gibel carp fed on test diets for 11 weeks.

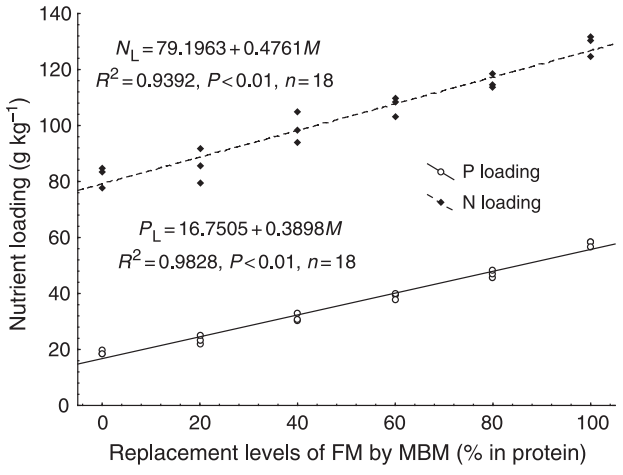


**Figure 2** Relationship between phosphorus (P) and nitrogen (N) loading and dietary meat and bone meal (MBM) protein levels in gibel carp fed on test diets for 11 weeks.

($N_R$, %) and dietary MBM levels ($M$, % replacement levels) could be described as:

$$P_R = 24.5557 - 0.1553 \times M \quad R^2 = 0.9454, P < 0.01, n = 18$$

$$N_R = 21.9160 - 0.0736 \times M \quad R^2 = 0.8833, P < 0.01, n = 18$$

Figure 2 shows the P and N loading, which were excreted as the waste increased linearly with the increase in dietary MBM levels ($P < 0.05$). The relationship between P loading ($P_L$, %), N loading ($N_L$,%) and dietary MBM levels ($M$, % replacement levels) could be described as:

$$N_L = 79.1963 + 0.4761 \times M \quad R^2 = 0.9392, P < 0.01, n = 18$$

$$P_L = 16.7505 + 0.3898 \times M \quad R^2 = 0.9828, P < 0.01, n = 18$$

### *Enzyme activities*

Table 6 shows that the replacement of FM by MBM in the diets had no significant effect on the activity of AKP, GOT, GPT or PK ($P > 0.05$). The total SOD activity in $MBM_{20}$ was significantly higher than that of $MBM_{100}$ ($P < 0.05$).

## Discussion

MBM has been used as an alternative protein of FM or other protein sources in diets for rainbow trout *O. mykiss* (Watanabe & Pongmaneerat 1991; Bureau *et al.* 2000), Mozambique tilapia *O. mossambicus* (Davies *et al.* 1989), Nile tilapia *Oreochromis niloticus* (L.) (El-Sayed 1998; Wu *et al.* 1999), gibel carp *C. auratus gibelio* (Xue & Cui 2001; Yang *et al.* 2004), common carp *Cyprinus carpio* L. (Pongmaneerat & Watanabe 1991), Japanese flounder *Paralichthys olivaceus* (Kikuchi *et al.* 1997), yellowtail *Seriola quinqueradiata* (Shimeno *et al.* 1993), hybrid striped bass *Morone saxatilis* × *Morone chrysops* (Bharadwaj *et al.* 2002), gilthead

**Table 6** Enzyme activities in liver and serum of gibel carp fed on diets with different levels of meat and bone meal (MBM) (means ± SE)*

| | In plasma | In liver | | | | |
|---|---|---|---|---|---|---|
| Diet | AKP (U L$^{-1}$) | AKP (U g$^{-1}$ protein) | SOD (U mg$^{-1}$ protein) | GOT (U g$^{-1}$ protein) | GPT (U g$^{-1}$ protein) | PK (U g$^{-1}$ protein) |
| Control | 15.83 ± 1.97 | 112.85 ± 10.32 | 283.87 ± 14.43$^{ab}$ | 313.87 ± 42.69 | 267.57 ± 28.59 | 295.03 ± 40.39 |
| $MBM_{20}$ | 12.21 ± 2.07 | 111.45 ± 8.67 | 360.26 ± 39.63$^{a}$ | 256.74 ± 9.11 | 152.41 ± 16.02 | 329.76 ± 41.29 |
| $MBM_{40}$ | 11.30 ± 3.17 | 94.33 ± 4.69 | 313.51 ± 18.36$^{ab}$ | 383.27 ± 61.46 | 220.94 ± 25.06 | 315.84 ± 51.02 |
| $MBM_{60}$ | 10.85 ± 1.57 | 106.71 ± 16.05 | 293.12 ± 22.53$^{ab}$ | 311.37 ± 14.26 | 256.62 ± 108.23 | 374.47 ± 21.49 |
| $MBM_{80}$ | 12.66 ± 0.90 | 105.91 ± 14.73 | 322.70 ± 26.76$^{ab}$ | 336.35 ± 68.49 | 199.35 ± 44.92 | 361.32 ± 59.12 |
| $MBM_{100}$ | 11.76 ± 0.90 | 105.74 ± 14.09 | 244.88 ± 34.86$^{b}$ | 370.26 ± 14.42 | 208.46 ± 30.37 | 383.78 ± 74.78 |

*Figures in the same column with different superscripts are significantly different ($P < 0.05$).
AKP, alkaline phosphatase; SOD, superoxide dismutase; GOT, aspartate aminotransferase; GPT, alanine aminotransferase; PK, pyruvate kinase.

seabream *Sparus aurata* (Robaina *et al.* 1997), red drum *Sciaenops ocellatus* (Kureshy *et al.* 2000) and shrimp *Litopenaeus vannamei* (Forster *et al.* 2003) and different results have been obtained. In the research of Kureshy *et al.* (2000), all four MBM diets produced significantly lower weight gain compared with fish fed on FM-based diet. Several species have exhibited a low tolerance to the inclusion of MBM (192–250 g $kg^{-1}$ FM protein) (Shimeno *et al.* 1993; Kikuchi *et al.* 1997; Forster *et al.* 2003). Similarly, gibel carp in the present study can tolerate up to 20% MBM protein. However, Watanabe & Pongmaneerat (1991) successfully incorporated MBM at a relatively high level (78% in diet) in the diet of rainbow trout without any negative effect on growth performance. The same level was used in the diet of common carp and a positive result was obtained (Pongmaneerat & Watanabe 1991). Yang *et al.* (2004) reported that growth performance of gibel carp fed on diets with 50% MBM protein was similar to that fed on the control diet, while 15% MBM protein diets showed better growth than the control. The different results might be due to the fact that there was still 27.2% FM in the diet containing 50% MBM, and the dietary protein was a little higher than the control (Yang *et al.* 2004). The diets containing MBM or high MBM/bone meal ratios (3 : 1 and 2 : 3) were found to be superior to FM, even at a 100% substitution level for Mozambique tilapia (Davies *et al.* 1989). However, although some researches had shown that a great part of FM could be substituted by MBM, high dietary MBM level is still not recommended due to relatively poor amino acid profiles (Watanabe & Pongmaneerat 1991) and palatability (Xue & Cui 2001).

Previous studies indicated that there is variability in the ability of MBM to replace FM in aquafeeds. The possible reasons were as follows: (1) The composition of MBM obtained from different commercial producers, produced by different process techniques, differed in proximate composition, amino acid, particle size distribution, energy content and elemental analysis (Forster *et al.* 2003). The apparent digestibility, especially ADC of lysine in MBM, was lower than the other ingredients tested for juvenile cobia (Zhou *et al.* 2004). This could be due to heat damage to lysine during the rendering process (Opsvedt *et al.* 1984) or possibly reduced digestibility of protein in bone fragments. (2) Difference in palatability of MBM resulted in different feed intake. The positive effect obtained could largely be due to good palatability of MBM, which showed increased feed intake (Mohson & Lovell 1990; Watanabe *et al.* 1993; Robaina *et al.* 1997; Wu *et al.* 1999; Yang *et al.* 2004). However, the palatability of MBM in the present work was not as good as those used by Robaina *et al.* (1997) and Yang *et al.* (2004). (3) Research has shown that nutrient utilization can be improved by combining different animal and plant protein sources (Bureau *et al.* 2000; Webster *et al.* 2000; Coyle *et al.* 2004; Xue *et al.* 2004). Generally, the inclusion of MBM at levels above 5% of the diet is not recommended (Kureshy *et al.* 2000). Davies *et al.* (1989); Watanabe & Pongmaneerat (1991) and Millamena (2002) successfully replaced 750, 800 and 900 g $kg^{-1}$ FM protein with MBM combined with other protein sources in diets for Mozambique tilapia, grouper *E. coioide* and rainbow trout, respectively. (4) The fish species also showed different abilities of utilizing MBM. Different protein digestibility values were noted for rainbow trout (75%) (Watanabe & Pongmaneerat 1991) and carp (54%) (Pongmaneerat & Watanabe 1991) with the same MBM. This may be due to higher protease activity in the digestive tract of rainbow trout (Phillips 1969).

In the present study, $ADC_p$ decreased with increase in dietary MBM protein levels (Fig. 3). It has been reported that the $ADC_p$ of FM is generally higher than that of MBM (Kureshy *et al.* 2000), which seemed to be related to the high ash content of MBM (Robaina *et al.* 1997). A negative relationship was found between $ADC_p$ and dietary ash content in the present study (Fig. 4), which was in agreement with the results of previous studies (NRC 1983; Pongmaneerat & Watanabe 1991; Kureshy *et al.* 2000; Yang *et al.* 2004).

It was determined in rainbow trout that $ADC_{ph}$ was 26.9% in MBM and 36.0% in whitefish meal (deboned) (Sugiura *et al.* 2000). However, similar $ADC_{ph}$ of FM (18%) and

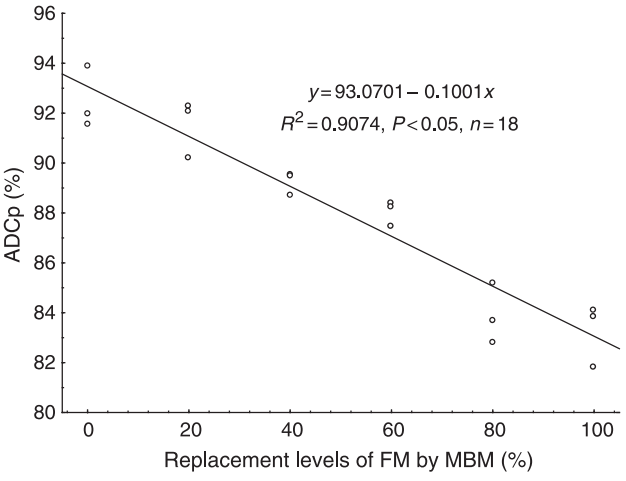


**Figure 3** Relationship between protein digestibility and dietary meat and bone meal (MBM) levels.

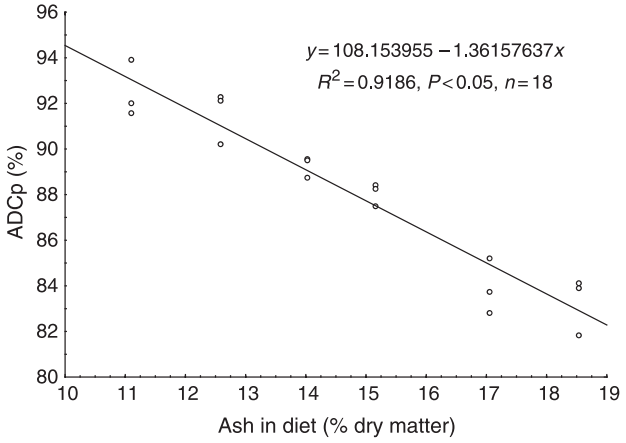


**Figure 4** Relationship between protein digestibility and dietary ash content.

MBM (16%) were observed by Ye & He (1991). The different data obtained in previous researches might be attributed to the differences in fish size and/or MBM quality (Sugiura *et al.* 2000). Furthermore, ADC of bone P appears to be much lower in stomachless fish, such as gibel carp, than that for monogastric fish (Cho & Bureau 2001). In the present study, $ADC_{ph}$ in the control diet is 38.80%, which is more than twice the $MBM_{100}$ group (17.6%). But the available P content in the control diet is similar to the $MBM_{100}$ group due to the fact that the total P level in the control diet (1.60%) was much lower than that of the $MBM_{100}$ group (2.83%).

It was reported that reducing the fraction of high ash (high P) ingredients or using low ash ones was an essential approach to develop low-pollution feeds (Riche & Brown 1999; Sugiura *et al.* 2000; Jahan *et al.* 2003). MBM, however, has a relatively higher ash content (P content) and lower digestibility than FM and it could be the reason why the P loading increased with increase in dietary MBM levels in the present study.

Some reports showed that protein quality and dietary available P content directly affected the N utilization efficiency and the N loading (Watanabe *et al.* 1987; Jahan *et al.* 2000, 2003). Accordingly, the N loading was proportional to the dietary FM levels due to the good quality and better amino acid balance of FM compared with MBM (Dong *et al.* 1993; Wang *et al.* 1997). However, in the present study, the slightly higher available P content in $MBM_{20}$ group compared with the control may compensate the poor protein quality of MBM and, thus result in a similar N loading and growth.

The AKP activity of the plasma/serum or body tissues was proved to be a useful diagnostic tool in determining P status of some species (Skonberg *et al.* 1997; Tan *et al.* 2001). Nevertheless, the relationship between dietary P levels and AKP have not been defined accurately. Sakamoto & Yone (1979) found that the AKP activity in the serum increased as the availability of P decreased. However, fish fed on the lowest P diet was also reported a low AKP activity in the plasma (Skonberg *et al.* 1997). The results of Tan *et al.* (2001) found that for abalone, the AKP activity of the whole soft body was sensitive to dietary P levels, while plasma AKP values could only be used to diagnose extreme P-deficiency states based on the results of Skonberg *et al.* (1997). In the present study, no significant differences were observed for the activity of AKP in serum and liver between different diets, since the available P content in different diets was similar (from 0.50% to 0.66%).

Onishi *et al.* (1981) had examined the change in hepatopancreatic enzyme activities in carp with dietary P levels and found that the activity of PK decreased remarkably with the decrease of dietary P levels. In the present study, the available P levels in different diets varied slightly and it might result in no difference in the PK activity.

In the present study, SOD activity in $MBM_{20}$ group was significantly higher than that of $MBM_{100}$ group. Increased SOD has been considered a health index in carp (Cai *et al.* 2001). In the present study, fish fed on $MBM_{20}$ showed higher SOD activity and higher growth performance compared with other groups with MBM.

## Conclusion

The present study indicated that 20% of FM protein could be substituted by MBM without negative effects on growth performance and N loading, while the loading of P increased significantly. Enzyme activity examination of liver and serum showed that metabolic responses of gibel carp were not influenced by the inclusion of MBM. In the eye of environmental protection, the inclusion of MBM in diet is not recommended. An extended study should focus on the increase in the utilization of dietary P in MBM.

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