

FATS AND PROTEINS RESEARCH FOUNDATION, INC.

FINAL REPORT

Final Report Submission Date: 12/October/2003

Submitted to: FATS AND PROTEINS RESEARCH FOUNDATION, INS.,

RP#2 BOX 298

BLOOMINGTON, IL 61704

Project Title: Potential Usefulness of Meat and Bone Meal and Poultry By-Products Meal in Diets for Gibel Carp (*Carassius auratus gibelio*)

Investigator: Shouqi XIE

Institute of Hydrobiology, the Chinese Academy of Sciences,

Wuhan, Hubei, 430072

P. R. China

Co-Investigators: Wu LEI, Xiaoming ZHU, Yunxia YANG, Wangbao GONG

Project Started: April 2002

Projected Completed: April 2003

Industry Summary

1. Introduction

Meat and bone meal (MBM) is an ingredient that has been shown to have a good nutritive value for livestock, poultry, and aquatic animals. Replacement of a large proportion of fish meal by MBM has been investigated by many authors (Tacon et al., 1983; Davies et al., 1989; Mansour, 1998; Bureau et al., 2000).

Poultry by-product meals (PBM) is known as an ingredients with very good nutritive value for fish while the effect on the growth and feed utilization by fish was variable (Higgs et al., 1979; Fowler 1982, 1991; Alexies et al., 1985; Steffen, 1987a,b; Davis and Arnold, 2000).

The reported work showed that MBM and PBM have the potential use in aquatic feeds. But most studies were conducted with salmonids and few were conducted with carp species (cyprinidae).

China is one of largest production of aquatic animals and the requirement of formulated feeds increases the request on dietary protein. Most cultured species in China are cyprinidae including gibel carp, common carp, grass carp etc.

Gibel carp is an important aquaculture species in China and in recent years, has almost been one of the main productions in aquaculture in China due to its higher growth rate. In Gibel carp, replacement of fishmeal by plant protein (soybean meal, rapeseed meal, peanut meal, potato protein concentrate, cotton seed meal) resulted in poor growth and feed utilization (Xie et al., 2001). A previous study from our laboratory (Cui et al., 2001) showed that 50% of the fish meal protein could be replaced by MBM or PBM protein without impact on the growth and feed efficiency of Gibel carp.

2. Objectives

The objective of this study was designed to investigate the effect of replacement of fish meal by MBM or PBM on the growth and feed utilization of gibel carp and to observe if the inclusion of MBM could improve the quality of soybean meal based diet.

3. Industry summary

The present study showed that decreased dietary fish meal protein resulted in

lower growth rate and it can help to decrease ammonia excretion. 6% replacement of fish meal protein by meat and bone meal (or poultry by product meal) protein decreased growth and feed utilization. High inclusion of meat and bone meal (10%) or poultry by-product meal (8%) could make the fish similar growth as soybean meal based diet. The use of MBM and PBM can help to decrease ammonia excretion of fish. Supplemental lysine and methionine did not improve the diet quality. In the diets containing less than 15% fish meal, inclusion of meat and bone meal can replace 10% fish meal without negative effect on growth and feed utilization.

Potential Usefulness of Meat and Bone Meal and Poultry By-Products Meal in Diets for Gibel Carp (*Carassius auratus gibelio*)

State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, the Chinese Academy of Sciences, Wuhan, Hubei, 430072, P. R. China

Abstract

Two growth trials were conducted in a semi-recirculation system to investigate the effect of inclusion of meat and bone meal (MBM) and poultry by-product meal (PBM) on the growth and feed utilization of gibel carp (*Carassius auratus gibelio*). All diets were formulated to be isonitrogenous and isoenergetic based on apparent digestibility. The results showed that decreased dietary fish meal protein resulted in lower growth rate and the use of MBM and PBM can help to decrease ammonia excretion. Supplemental lysine and methionine did not improve the diet quality. Inclusion of MBM can replace 10% fish meal without negative effect on growth and feed utilization. Inclusion of PBM can replace 10% fish meal without negative effect on growth and feed utilization.

Key words: Meat and bone meal, poultry by-product meal, gibel carp, replacement, fish meal

Introduction

The use of various economical protein sources in the diet of fish has been the topic of many studies. But most researches resulted in decreased growth and poor feed utilization (Dabrowski and Kozak, 1979; Tian et al., 1992; Xie et al., 2001). Viola et al. (1982) reported in common carp, when the diets were supplemented with lysine and/or methionine and oil, the fish meal could be completely replaced by soybean meal.

Meat and bone meal (MBM) is an ingredient that has been shown to have a good nutritive value for livestock, poultry, and aquatic animals. Replacement of a large proportion of fish meal by MBM has been investigated by many authors (Tacon et al., 1983; Davies et al., 1989; Mansour, 1998; Bureau et al., 2000). But Stone et al., (2000) replaced fishmeal by MBM at levels of 15%, 30% and found that the growth of Australian silver perch decreased when fishmeal content was less than 13% while feed conversion and protein retention efficiency was not affected.

Poultry by-product meals (PBM) is known as an ingredients with very good nutritive value for fish while the effect on the growth and feed utilization by fish was variable. Fowler (1982, 1991) replaced fish meal by PBM in the diets for Atlantic salmon and Chinook salmon and found there was no negative affect on growth and feed efficiency when the inclusion level was less than 20%. Higgs et al. (1979) reported that 28% fishmeal protein could be replaced by PBM in the diets for coho salmon while only 25% even with the addition of methionine in rainbow trout (Alexies et al., 1985). With the addition of lysine and methionine, 53% fishmeal could be replaced by PBM (Steffen, 1987a,b). Davis and Arnold (2000) reported in Pacific

white shrimp, that replacement of fish meal by PBM at the level of 80% did not affect the growth and feed utilisation while the protein conversion efficiency was higher than that of the control (all fish meal protein).

The reported work showed that MBM and PBM have the potential use in aquatic feeds. But most studies were conducted with salmonids and few were conducted with carp species (cyprinidae).

China is one of largest production of aquatic animals. Recent decades have seen a significant intensification of production and increase in the usage of complete (formulated) feeds which contain significant levels of fish meal and other high quality ingredients. But most cultured species in China are cyprinids including gibel carp, common carp, grass carp etc.

Gibel carp is an important aquaculture species in China and in recent years, has almost been one of the main productions in aquaculture in China due to its higher growth rate. In Gibel carp, replacement of fishmeal by plant protein (soybean meal, rapeseed meal, peanut meal, potato protein concentrate, cotton seed meal) resulted in poor growth and feed utilization (Xie et al., 2001). A previous study from our laboratory (Cui et al., 2001) showed that 50% of the fish meal protein could be replaced by MBM or PBM protein without impact on the growth and feed efficiency of Gibel carp.

The propose of the study is to investigate the optimal inclusion level of MBM and/or PBM and the use of additional amino acids in the diets to achieve highest benefit for Gibel carp culture.

The results from this study could also be useful for other cyprinids, such as common carp, crucian carp, which also represent very significant segments of the aquaculture production in China.

Materials and methods

Two growth trials were conducted with Gibel carp. In Experiment 1, nine diets formulated to be isonitrogenous and isoenergetic on apparent digestible basis. Different levels of MBM (or PBM) substituting soybean meal (Table 2). A high fish meal diet (Diet 1) and all fish meal protein diet (Diet 9) were formulated act as positive control. A commercial diet was also used for comparison.

In Experiment 2, MBM was selected to enhance the growth and feed utilisation of the diets containing low fish meal protein. Diet 1 contained high fish meal protein and Diet 9 contained all fish meal protein. Diet 2 to Diet 8 contained decreased levels of fish meal protein and increased levels of MBM. Supplemental lysine and methionine were provided to the levels of all fish meal protein diet.

Experiment 1

The experiment was conducted in fiberglass tanks in a recirculation system (Ø 70cm X 40cm, water volume: 100L). The gibel carp juveniles were collected from local fish farm and transferred into experimental tanks for acclimation one month before the experiment. At the beginning of the experiment, the fish were deprived of food for one day and batch-weighed. 30 fish were randomly allocated into each tank. Three samples were taken for chemical analysis of initial fish body composition.

During the experiment, the fish were fed to satiation twice a day (9:00 and 15:00) and uneaten feed were collected one hour after feeding. Feces were collected twice a day and dried at 60°C. About 10L/min new freshwater was introduced into the whole

system everyday. Aeration was provided intermittently for 1h every 2h. During the experiment, dissolved oxygen was more than 6mg/L. Water temperature was $29\pm 2^\circ\text{C}$ and pH was around 6.9 (range: 6.7-7.1). Ammonia-N was less than 0.1mg/L. Photoperiod was 12h L: 12D with the light period from 8:00-20:00.

The trial lasted for 8 weeks. At the end of the experiment, all fish were weighed after one-day starvation and 10 fish from each tank were sampled for chemical analysis.

Leaching rate of uneaten feed was measured by allocating three samples of feed into fish-free tanks and recollected one hour later, dried and reweighed.

Experimental 2

The experiment was conducted in fiberglass tanks in a semi-recirculation system (\varnothing 0.7 X 1.0m, water volume: 98L). The gibel carp juveniles were collected from local fish farm and transferred into experimental tanks for acclimation one month before the experiment. At the beginning of the experiment, the fish were deprived of food for one day and batch-weighed. 25 fish were randomly allocated into each tank. Three samples were taken for chemical analysis of initial fish body composition.

During the experiment, the fish were fed to satiation twice a day (9:00 and 15:00) and uneaten feed were collected one hour after feeding. Feces were collected twice a day and dried at 60°C . About 10L/min new freshwater was introduced into the whole system everyday. Aeration was provided intermittently for 1h every 2h. During the experiment, dissolved oxygen was more than 6mg/L. Water temperature was $27\pm 2^\circ\text{C}$ and pH was around 6.8 (range: 6.7-7.0). Ammonia-N was less than 0.1mg/L. Photoperiod was 12h L: 12D with the light period from 8:00-20:00.

The trial lasted for 8 weeks. At the end of the experiment, all fish were weighed after one-day starvation and 15 fish from each tank were sampled for chemical analysis.

Leaching rate of uneaten feed was measured by allocating three samples of feed into fish-free tanks and recollected one hour later, dried and reweighed.

Chemical analysis

Crude protein, lipid, ash and energy content were analyzed for the diet and fish body. Cr_2O_3 was analyzed for the diet and feces. Dry matter content was determined for fish and diet samples by drying to constant weight at 105°C . Nitrogen content was analyzed for fish and feeds by the Kjeldahl's method. Crude protein content was calculated from nitrogen content by multiplying by 6.25. Crude lipid was determined for fish and diets by ether extraction using a Soxtec System (Soxtec system HT6, Tecator, Hoganas, Sweden), ash for fish and diets by combustion at 550°C in muffle furnace, and energy by bomb calorimeter (Phillipson microbomb calorimeter, Gentry Instruments Inc., Aiken, U.S.A.). The content of Cr_2O_3 was determined as described by Bolin et al. (1952).

Statistica 5.0 for windows was used for data analysis. Differences of means between groups were analyzed by Duncan's multiple range tests after one-way ANOVA. Discrimination of values was identified at significance levels of $P < 0.05$.

Results

Experiment 1

Growth performance

Table 3 showed that the fish fed diets with high fish meal protein obtained higher final body weight and growth rate ($P < 0.05$) while low PBM and MBM inclusion diets (Diet 3 and Diet 4) and commercial diet showed lowest growth rate ($P < 0.05$). High fish meal diets (Diet 1 and Diet 9) showed significantly higher feed conversion efficiency ($P < 0.05$) while there was no significant difference between other groups ($P > 0.05$). Survival rate was significantly lower in fish fed Diet 7 (high MBM diet) ($P > 0.05$). Feeding rate was higher in commercial diet (Diet 10) and Diet 5 (10% MBM with supplemental lysine and methionine) ($P > 0.05$). Ammonia excretion was significantly higher in commercial diet and lower in Diet 3, Diet 5 and Diet 6 ($P > 0.05$). Phosphorus excretion per kg fish production was higher in Diet 7 and then Diet 3 and commercial diet while lower in Diet 9 and Diet 5. The feed cost per kg fish was lower in high fish meal diets (Diet 1 and Diet 9).

Table 5 showed that fish fed diets with high fish meal protein (Diet 1 and Diet 9) obtained higher final body weight and growth rate ($P < 0.05$). Final body weight and specific growth rate was lower in fish fed Diet 4 ($P < 0.05$) while there was no significant difference in other groups ($P > 0.05$). Feeding rate was significantly lower in Diet 7 ($P < 0.05$).

Discussion

The growth of gibel carp fed fish meal based diet in the present study showed similar growth rate to those reported by Qian et al (2001) and Xie et al. (2001a, b) and higher than that reported by Qian et al. (2000). There was no fish died during the experiment.

The present results showed that, for the diets with 5-15% fishmeal, the inclusion of MBM and PBM could not improve the growth performance of gibel carp and supplemental lysine and methionine was not effective. Different results have been achieved by different authors.

Fowler (1991) found that 10% (25% crude protein) replacement of fishmeal by PBM showed no negative affect on growth performance for Chinook salmon and 20% (50% crude protein) achieved similar growth while lower feed efficiency. Kureshy et al (2000) found that 16.6% crude protein (5.34% in dry matter) replacement of fish meal protein by MBM for red drum showed decreased growth and feed efficiency. 66.7% replacement of fish meal protein by PBM protein could obtain similar growth to fish meal.

Negas et al (1999) replaced 50% fishmeal protein by PBM without negative affect on growth and feed efficiency. Bureau et al (2000) replaced 7% and 14% fish meal by three kind of MBM at 12% and 24% and formulated diets based on apparent digestible level. Supplemental methionine could not improve growth performance. Robaina et al. (1997) reported no significant difference in the growth and feed utilization of gilthead seabream (*Sparus aurata*) fed diets with 20, 30 and 40% replacement of fish meal protein. Webster et al (2000) reported that MBM and PBM could replace 100% Menhaden fish meal and MBM showed better growth performance than PBM in sunshine bass *Morone chrysops* X *M. saxatilis*. Takagi et al (2000) reported that 70% fish meal protein replaced by PBM protein could obtain higher growth and feed efficiency for red sea bream.

Our previous studies in gibel carp showed that, when the diet contained more than 27.2% fish meal, the other protein could be provided by MBM or PBM without negative affect on growth and feed utilization (Yang et al., unpublished). Another kind of PBM could replace 100% fish meal and PBM based diets showed higher growth rate than the control fishmeal diet (Yang et al unpublished). It suggested that the quality of MBM or PBM did affect the dietary inclusion level. Improved MBM and PBM could even express better growth performance than fishmeal.

Conclusions

The present study showed that decreased dietary fish meal protein resulted in lower growth rate and it can help to decrease ammonia excretion. High inclusion of MBM (10%) or PBM (8%) could make the fish similar growth as soybean meal based diet. Supplemental lysine and methionine did not improve the diet quality. Inclusion of MBM can replace 10% fish meal without negative effect on growth and feed utilization.

Acknowledgements

The project was fund by FATS AND PROTEINS RESEARCH FOUNDATION, INC. and partly by the Asian Regional Office of the National Renderers Association (NRA), U.S.A.

References

- 1) Alexis, M.N., Papaparaskeva-Papoutsoglou, E. and Theochari, V., 1985. Formulation of practical diets for rainbow trout (*Salmon gairdner*) made by partial or complete substitution of fish meal by poultry by-products and certain plant products. *Aquaculture*, 50: 61-73.
- 2) Bolin, D. W., King, R.P. and Klosterman, E.W., 1952. A simplified method for the determination of chromic oxide (Cr₂O₃) when used as an index substance. *Science*, 116: 634.
- 3) Bureau, D.P., Harris, A.M., Bevan, D.J., Simmons, L.A., Azevedo, P.A. and Cho, C.Y., 2000. Feather meals and meat and bone meals from different origins as protein sources in rainbow trout, *Oncorhynchus mykiss* diets. *Aquaculture*, 181: 2000 281-291.
- 4) Cui Y, Xie, S., Zhu, X., Yang, Y. Y., Yang Y. X. and Yu Y., 2001. Animal protein meals successfully used in gibel carp diets. *Asian Aquaculture Magazine*, (Jan./Feb.) :27-28.
- 5) Dabrowski, K. and Kozak, B., 1979. The use of fish meal and soyabean meal as a protein source in the diet of grass carp. *Aquaculture* 18, 107-114.
- 6) Davies, S.J., Williamson, J., Robinson, M., Bateson, R.I., 1989. Practical inclusion levels of common animal by-products in complete diets for tilapia *Oreochromis mossambicus*, Peters . In: Proc. 3rd Intl. Symp. On Feeding and Nutr. Fish. Toba, Japan, pp. 325-332.
- 7) Davis, D. A. and Arnold, C.R., 2000. Replacement of fish meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture*, 185: 291-298.
- 8) Fowler, L.G., 1982. Tests with poultry by-product meal in fall Chinook salmon diets. Abernathy Salmon Cultural Development Center, Technical Transfer Series No. 82-1, 13pp.
- 9) Fowler, L. G., 1991. Poultry by -product meal as a dietary protein source in full Chinook salmon diets. *Aquaculture*, 99: 309-321.
- 10) Higgs, D. A., Markert, J. R., Macquarrie, D. W., McBride, J.R., Dosanjh, B.S., Nichols, C. and Hoskins, G., 1979. Development of practical diets for coho salmon, *Oncorhynchus kisutch*, using poultry by-product meal, feather meal, soybean meal and repaseed meal as major protein sources. In: J. E. Halver and K. Tiews (Editors), *Finfish Nutrition and Fish Feed Technology*, Vol., 2. Heenemann, Berlin, pp. 191-218.
- 11) Kureshy, N., Davis, D. A and Arnold, C. R., 2000. Partial replacement of fish meal with

- meat-and-bone meal, flash-dried poultry by-product meal, and enzyme-digested poultry by-product meal in practical diets for juvenile red drum. *North American Journal of Aquaculture*, 62: 266-272.
- 12) Mansour, C.R., 1998. Nutrient requirements of red tilapia fingerlings. MSc Thesis, Fac. Sci. Univ. Alex. Egypt, 121 pp.
 - 13) Nengas, I., Alexis, M. N., Davies, S.J., 1999. High inclusion levels of poultry meals and related byproducts in diets for gilthead seabream *Sparus aurata* L. *Aquaculture*, 179:13–23.
 - 14) Steffens, W., 1987a. Verwendung von Geflügelabfallmehl zum vollständigen Fischmehlersatz im Forellenbrut- und -aufzuchtfutter. *Archives of Animal Nutrition-Archiv Fur Tierernahrung*, 37: 98-103.
 - 15) Steffens, W., 1987b. Further results of complete replacement fo fish meal by means of poultry by-prodcuts meal in feed for trout fry and fingerling (*Salmo gairdner*). *Archives of Animal Nutrition-Archiv Fur Tierernahrung*, 37: 1135-1139.
 - 16) Qian, X., Cui, Y. Xiong, B. and Yang, Y. 2000. Compensatory growth, feed utilisation and activity in gibel carp, following feed deprivation. *Journal of Fish Biology* 56:228-232.
 - 17) Qian, X.; Cui, Y.; Xiong, B.; Xie, S.; Zhu, X.; Yang, Y. 2001. Spontaneous activity was unaffected by ration size in Nile tilapia and gibel carp. *Journal of Fish Biology*,58: 594-598.
 - 18) Robaina, L., Moyano, F.J., Izquierdo, M.S., Socorro, J., Vergara, J.M., Montero, D., 1997. Corn gluten and meat and bone meals as protein sources in diets for gillthead seabream *Sparus autata*: nutritional and histological implications. *Aquaculture*, 157: 347–359.
 - 19) Stone, D.A.J., Allan, G. L., Parkinson, S.,and Rowland, S. J., 2000. Replacement of fish meal in diets for Australian silver perch, *Bidyamus bidyamus* Ill. Digestibility and growth using meat meal products. *Aquaculture*, 186:311-326.
 - 20) Tacon, A.G.J. and Jackson,A., 1985. Utilization of conventional and unconventional protein sources in practical fish feeds. In: *Nutrition and Feeding in Fish*. Cowey,C.B., Mackie.A.M.M. and Bell,J.G. eds. Academic Press, London, pp.119-145.
 - 21) Tacon, A. G. J., Jauncey, K., Falaye, A., Pantah, M., MacGowen, I., Stafford, E., 1983. The use of meat and bone meal, hydrolyzed feather meal and soybean meal in practical fry and fingerling diets for *Oreochromis niloticus*. In: Fishelson, J., Yaron, Z. (Eds), Proc. 1st Intl. Symp. On Tilapia in Aquaculture. Tel Aviv Univ. Press, Israel, pp. 356-365.
 - 22) Takagi,S., Hosokawa, H., Shimeno, S. and Ukawa M., 2000. Utilization of poultry by-product meal in a diet for red sea bream (*Pagrus major*). *Nippon Suisan Gakkaishi*, 66:428-438.
 - 23) Tian, X., 1992. Toxic effect of dietary rapseed cake on common carp. *Guizhou Aquaculture* (2): 1-5.
 - 24) Viola, S., Mokady,S., Rappaport,V. and Ariell,Y., 1982. Partial and complete replacement of fish meal by soybean meal in feeds for intensive culture of carp. *Aquaculture*, 26, 223-236.
 - 25) Webster, C. D., Thompson, K. R., Morgan, A. M., Grisby, E. J. and Gannm, A. L., 2000. Use of hempseed meal, poultry by-product meal, and canola meal in practical diets without fish mela for sunshine bass (*Morone chrysops* X *M. saxatilis*). *Aquaculutre*, 188: 299-309.
 - 26) Xie, S., Zhu, X., Cui, Y. and Yang, Y., 2001a. Utilisation of several plant proteins by gibel carp (*Carassius auratus gibelio*). *Journal of Applied Ichthyology*, 17: 70-76.
 - 27) Xie, S., X. Zhu, Y. Cui, R. J. Wootton, W. Lei and Y. Yang, 2001b. Compensatory growth in the gibel carp following feed deprivation: temporal patterns in growth, nutrient deposition, feed intake and body composition. *Journal of Fish Biology*, 58(4): 999-1009.

Table 1 Chemical composition of ingredients (in dry weight)

Ingredient	Crude protein (%)	Digestible protein (%)	Gross Energy (kJ/g)	Digestible energy (kJ/g)	Lysine (%)	Digestible lysine (%)	Methionine (%)	Digestible methionine (%)	Ash (%)	Water (%)
White fishmeal (USA)	59.670	57.939	17.050	14.998	4.460	4.353	1.480	1.416	8.89	7.9100
Soybean meal (Hubei, China)	47.840	41.395	14.870	11.997	2.940	2.922	0.470	0.470	9.80	7.2500
Rapeseed meal(Hubei, China)	45.780	39.076	16.990	9.886	2.010	1.814	0.500	0.495	27.12	6.9600
MBM (NRA, USA)	67.480	42.607	15.680	9.510	3.520	2.880	0.960	0.875	17.50	4.5100
PBM (NRA, USA)	70.500	57.853	17.180	13.890	4.090	3.844	1.250	1.203	8.14	4.5300
Wheat bran (Hubei, China)	22.960	17.168	16.290	8.971	0.620	0.563	0.200	0.190		7.9100

Table 2 Formulation of different practical diets

Ingredient	1	2	3	4	5	6	7	8	9	10
Fishmeal	35	5	5	5	5	5	5	5	49	Commercial diet
Soybean meal	7.94	59.2	54.6	52.8	49.5	46	44	39	0	
Rapeseed meal	18.4	18.4	18.4	18.4	18.4	18.4	18.4	18.4	0	
Meat and bone meal	0	0	5	0	10	0	15	0	0	
Poultry byproduct Meal	0	0	0	4	0	8	0	12	0	
Wheat bran	27.8	5.36	4.47	7.66	4.31	10.46	4.31	13.43	41.84	
Plant oil	3	3.9	4.4	4	4.7	4	5.2	4	1.5	
Vitamin premix ¹	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	
Vitamin C	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	
Mineral premix ²	5	5	5	5	5	5	5	5	5	
Choline chloride	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	
Chromic oxide	1	1	1	1	1	1	1	1	1	
Lysine	0.1	0.07	0.08	0.1	0.07	0.13	0.09	0.18	0	
Methionine	0.1	0.41	0.39	0.38	0.36	0.35	0.34	0.33	0	
CMC	1	1	1	1	1	1	1	1	1	
Calculated composition										
DP (%)	35.53	35.51	35.59	35.57	35.58	35.55	35.43	35.48	35.57	
DE (kJ/g)	11.64	11.62	11.65	11.65	11.61	11.64	11.62	11.62	11.67	
MBM(PBM) replacement in protein	0	0	6	6	12	13	18	20	0	

¹ Vitamin premix contained the following vitamins per kg feed: vitamin A (as vitamin A acetate and vitamin A palmitate, 1:1), 5500 I.U.; vitamin D₃, 1000 I.U.; vitamin E (as dl- α -tocopheryl acetate), 50 I.U.; vitamin K₃ (as menadione sodium bisulfite), 10 I.U.; choline (as choline chloride), 550 mg; niacin, 100 mg; riboflavin, 20 mg; pyridoxine, 20mg; thiamin, 20mg; D-calcium pantothenate, 50 mg; biotin, 0.1 mg; folic acid, 5 mg; vitamin B₁₂, 20 mg; ascorbic acid, 100 mg; inositol, 100 mg.

² Mineral premix contained the following minerals as mg per kg feed: NaCl, 257; MgSO₄·7H₂O, 3855; Na₂H₂PO₄·2H₂O, 6425; KH₂PO₄, 8224; Ca(H₂PO₄)₂·H₂O, 5140; C₆H₁₀CaO₆·5H₂O, 899.5; FeC₆H₅O₇·5H₂O, 642.5; ZnSO₄·7H₂O, 90.7; MnSO₄·4H₂O, 41.6; CuSO₄·5H₂O, 7.97; CoCl₂·6H₂O, 0.26; KIO₃, 0.77.

Table 3 Effect of the diets with different formulations on growth and feed utilisation of gibel carp (Mean \pm S.E.)

Diet	IBW(g)		FBW(g)		SGR(%)		FCE(%)		Survival rate (%)		FR (%/g/d)		Ammonia(mg/g/d)		Phosphors excretion per kg fish production(g)		Feed cost per kg fish production (Chinese Yuan)
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
1	1.667	0.002	4.381a	0.014	1.790a	0.008	56.60a	1.82	91.11a	2.940	3.168a	0.048	0.114ab	0.006	94.1	6.40	
2	1.660	0.002	2.617bc	0.071	0.841bc	0.052	38.57b	0.93	85.56a	2.940	3.105a	0.071	0.169ab	0.054	93.9	7.21	
3	1.660	0.000	2.473c	0.179	0.728c	0.133	28.76b	2.75	70.00ab	5.092	2.953b	0.122	0.070b	0.021	110.5	9.70	
4	1.672	0.014	2.523bc	0.078	0.760c	0.041	34.14b	4.87	83.33a	5.092	3.077a	0.029	0.131ab	0.037	91.0	8.09	
5	1.663	0.002	2.699bc	0.026	0.896bc	0.017	33.69b	2.20	84.44a	6.759	3.384c	0.056	0.086b	0.021	68.2	8.25	
6	1.666	0.001	2.835bc	0.201	0.976bc	0.127	30.32b	9.68	83.33a	1.925	3.185a	0.153	0.075b	0.035	87.1	9.04	
7	1.659	0.003	2.688bc	0.246	0.879bc	0.164	18.90b	9.02	57.78b	6.759	3.070a	0.035	0.118ab	0.028	155.9	14.76	
8	1.661	0.001	2.977bc	0.090	1.079b	0.057	30.92b	5.46	68.89a	18.692	3.251a	0.046	0.105ab	0.023	81.0	8.77	
9	1.660	0.004	5.846d	0.154	2.330d	0.045	68.36a	9.99	88.89a	8.012	2.973b	0.103	0.123ab	0.012	31.9	6.25	
10	1.661	0.001	2.458c	0.128	0.720c	0.098	24.51b	1.76	82.22a	1.111	4.767d	0.208	0.248a	0.115	102.1	10.20	

Table 4 Formulation of different practical diets

Ingredient	1	2	3	4	5	6	7	8	9
Fishmeal	35	15	10	10	5	5	0	0	50
Soybean meal	15	48	55	55	48	48	42	42	0
Rapeseed meal	15	15	15	15	15	15	15	15	0
MBM	0	0	0	0	15	15	30	30	0
Wheat bran	25.54	14.34	12.34	11.92	8.34	7.94	1.84	1.96	40.04
Oil	1.6	0	0	0	1	1	3.5	3	2.3
Vitamin premix ¹	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
VC	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Mineral premix ²	5	5	5	5	5	5	5	5	5
Choline chloride	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Chromic oxide	1	1	1	1	1	1	1	1	1
Lys	0.1	0	0	0.05	0	0.05	0	0.05	0
Met	0.1	0	0	0.37	0	0.35	0	0.33	0
CMC	1	1	1	1	1	1	1	1	1
Calculated composition									
DP (%)	33.68	33.54	33.12	33.07	33.42	33.37	33.80	33.82	33.78
DE (kJ/g)	12.03	12.34	12.45	12.41	12.09	12.06	12.20	12.04	12.01
MBM replacement in protein (%)	0	0	0	0	19	19	38	38	0

¹ Vitamin premix contained the following vitamins per kg feed: vitamin A (as vitamin A acetate and vitamin A palmitate, 1:1), 5500 I.U.; vitamin D₃, 1000 I.U.; vitamin E (as dl- α -tocopheryl acetate), 50 I.U.; vitamin K₃ (as menadione sodium bisulfite), 10 I.U.; choline (as choline chloride), 550 mg; niacin, 100 mg; riboflavin, 20 mg; pyridoxine, 20mg; thiamin, 20mg; D-calcium pantothenate, 50 mg; biotin, 0.1 mg; foliacin, 5 mg; vitamin B₁₂, 20 mg; ascorbic acid, 100 mg; inositol, 100 mg.

² Mineral premix contained the following minerals as mg per kg feed: NaCl, 257; MgSO₄·7H₂O, 3855; Na₂H₂PO₄·2H₂O, 6425; KH₂PO₄, 8224; Ca(H₂PO₄)₂·H₂O, 5140; C₁₂H₁₀CaO₆·5H₂O, 899.5; FeC₆H₅O₇·5H₂O, 642.5; ZnSO₄·7H₂O, 90.7; MnSO₄·4H₂O, 41.6; CuSO₄·5H₂O, 7.97; CoCl₂·6H₂O, 0.26; KIO₃, 0.77.

Table 5 Growth performance of gibel carp fed different diets

Diet	IBW		FBW		FCE		SGR		FR	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	1.999	0.006	5.484a	0.183	47.24a	1.622	1.801b	0.063	3.390ab	0.054
2	2.015	0.011	4.090b	0.058	30.18b	1.217	1.264c	0.034	3.297ab	0.120
3	2.004	0.006	4.293b	0.201	33.05b	1.381	1.357c	0.078	3.454ab	0.086
4	2.001	0.010	3.420c	0.041	26.60b	1.435	0.957d	0.030	3.302ab	0.003
5	1.975	0.021	4.107b	0.164	32.81b	1.173	1.305c ✓	0.056	3.527a	0.057
6	2.007	0.004	3.762bc	0.100	28.25b	0.609	1.121cd ✓	0.050	3.467a	0.130
7	2.013	0.016	3.837bc	0.144	32.52b	3.179	1.149cd ✓	0.082	3.056b	0.043
8	2.011	0.004	4.327b	0.266	30.24b	4.261	1.362c ✓	0.116	3.491a	0.123
9	2.007	0.001	7.769d	0.144	62.26c	0.648	2.417a	0.034	3.345a	0.045

*FR(%/d): feeding rate = feed intake *100/((initial body weight + final body weight)/2 * days)

SGR (%/d): specific growth rate = (Ln(final body weight)-Ln(initial body weight))*100/days

FCE(%): feed conversion efficiency= weight gain *100/feed intake