Effect of faecal collection interval and dietary meat and bone meal levels on digestibility of nutrients in gibel carp (Carassius auratus gibelio)

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Abstract: The present study was undertaken to evaluate the effects of faecal collection interval (using a settling column) and the levels of dietary meat and bone meals (MBM) on the apparent digestibility coefficient (ADC) for dry matter, protein, energy and phosphorus in gibel carp. Six iso-nitrogenous (crude protein: 410 g/kg) and iso-energetic (gross energy: 18 kJ/g) diets were used in which fish meal (FM) protein was gradually replaced by MBM at 0 %, 20 %, 40 %, 60 %, 80 % and 100 %. These diet combinations were fed to juvenile gibel carp for 11 weeks. The faecal samples were collected two weeks after the beginning of the experiment using a settlement column starting 1 min after excretion started, or 4h and 16h after feeding. The results showed that the ADCs of dry matter, protein, energy and phosphorus increased significantly as the time from excretion to faecal collections increased while not being significant for the high MBM inclusion diets. ADCs of dry matter, crude protein, energy and phosphorus decreased linearly or almost linearly with the increase in dietary MBM levels. These findings suggest that faeces should be collected soon after they had settled under the conditions of this study for digestibility determination while rapid leaching is apparently responsible for this false reading of digestibility with increasing sampling interval. It is also apparent that the digestibility is one of the problems affecting the use of MBM in juvenile gibel carp.

Key words: Fecal collection interval; Meat and bone meal; Digestibility; *Carassius auratus gibelio*
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Determination of the apparent digestibility of feedstuffs is required to formulate economically optimized feeds to meet also the nutritional demands of the animal and to minimize environmental impact derived from excretion[1,2]. The measurement of digestibility for aquatic species is technically more difficult than that for terrestrial animals because nutrients from the faeces can quickly leach into the water, depending on the contact time in the water[3,4]. A number of techniques have been used to overcome this problem, including stripping of the contents of the hindgut[5—10], removal of faeces after intestinal dissection[6,7,9,11—13], and anal suction of faeces using a specific device[7,14,15]. However, some partially digested food, urine, sperm, eggs or intestinal epithelial cells can contaminate the samples and result in an underestimation of the apparent digestibility coefficient (ADC)[16—19]. Furthermore, such methods require frequent handling of the fish which is stressful and is not suitable for obtaining faeces from small fish[20]. Also, these methods can not be used for continuous assessment of digestibility in combination with growth trials[18].

Other researchers collected faeces in the water by pipetting immediately after release[21], netting[7], decantation column[22,23], continuous effluent filtra-

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tion[24,25] and the use of metabolic chambers[26]. These methods can avoid the problems caused by collection of un-defaecated intestinal contents. However, methods that rely on the removal of feces from the water tend to overestimate the ADC as a result of nutrients leaching from the feces[7,27—30] Thus, in order to minimize the error of this source, the faecal particles should be collected quickly after being voided from the fish. Accurate data were obtained in the study of Spyridakis, *et al*.[20] when faeces were separated from water in about 30s by using immediate pipetting and continuous filtration. However, immediate pipetting requests the investigator to keep close watch over the tanks, which is labour intensive[20]. And as for the method of continuous filtration, the devices are expensive to install and which will restrict the magnitude of the experiment[31].

Settlement column (Guelph system) collection, a convenient and economical method, seems to be more suitable in practice than other methods. Satoh *et al*.[32] proved that the Guelph system is a suitable apparatus for collecting faeces from salmonid fish to test digestibility. The results of Allan *et al*.[4] demonstrated that collection of faeces by settlement is a suitable method for determining digestibility in juvenile silver perch, although faeces were collected several hours after the final feeding. Since Possompes[33] indicated that the kinetics of nitrogen leaching from fish faeces was found to be rapid during the first 5 minutes of immersion and tended to stabilize after 1h, faeces should be removed from the water within seconds when the Guelph System is employed.

With the levelling of fish meal (FM) production and the world-wide increase of aquaculture yield in the, the search for alternatives of FM has become an international research priority[34,35]. Using alternative protein sources as the partial or total replacement of FM, the quality of the new protein sources should be evaluated in terms of chemical composition as well as their bioavailability based on their digestibility and biological values[36]. Meat and bone meal (MBM), an animal protein source, had successfully been used as alternatives of fish meal by some researchers[36—39]. In their studies, digestibility of nutrients differed under different replacement levels. And so it is suggested that a correlation may exist between nutrient digestibility and replacement levels. However, no such confirmation has been published previously. It is well known that phosphorus in effluent water is a key nutrient in causing excessive algal blooms and can contribute to the eutrophication of natural waters[40]. Generally, MBM contains a high level of phosphorus and the indigestible dietary phosphorus will be excreted by the fish. So it is necessary to investigate the digestibility of phosphorus in order to alleviate environmental deterioration.

The gibel carp, *Carassius auratus gibelio*, is a subspecies of goldfish or Crucian carp and has almost replaced Crucian carp in aquaculture in China because of its excellent taste and high growth rate[41]. So far, little is known, however, about the digestibility of nutrients in diets where fish meal has been replaced by other protein sources when using gibel carp. Also, the settlement methods have not been examined when using these new feeds.

The aims of present experiment, therefore, were (1) to determine the effect of fecal collection interval with the method of settlement on the digestibility of nutrients in the diets of gibel carp; (2) to examine the effect of gradual replacement of fish meal with MBM on nutrient digestibility, and to find the correlations between replacement levels and apparent digestibility coefficients (ADCs).

1. Materials and methods

1.1 Experimental diets White fish meal protein was replaced at 0 %, 20 %, 40 %, 60 %, 80 % and 100 % by MBM (provided by the National Renderers Association (NRA), USA) in six iso-nitrogenous and iso-energetic practical diets (Tab. 1). The chemical composition of ingredients is shown in Tab. 2. Phosphorus concentration in the experimental diets increased with the increase of dietary MBM and ranged from 1.85 % to 2.83 %. 1 % chromium oxide (Cr_2O_3) was used as inert dietary marker at identical concentrations in all diet formulations employed in these digestibility measurements. Diets were made into sinking pellets (2 mm in diameter) by a laboratory pelleter, oven-dried at 60 ℃ and stored at - 20 ℃ until use.

Tab. 1　Composition of the experimental diets with gradual replacement of fishmeal by meat and bone meal (MBM) (% in dry matter). **Indices indicate the protein level in replacements**

Ingredients	Control	MBM_{20}	MBM_{40}	MBM_{60}	MBM_{80}	MBM_{100}
White fishmeal[1]	43.22	34.58	25.93	17.29	8.64	0.00
Defatted soybean meal[2]	15.00	15.00	15.00	15.00	15.00	15.00
Meat and bone meal[3]	0.00	11.01	22.02	33.03	44.05	55.06
Corn starch	18.98	18.89	18.81	18.72	18.64	18.53
Fish oil	4.77	4.29	3.81	3.34	2.86	2.38
Vitamin premix[4]	0.40	0.40	0.40	0.40	0.40	0.40
Mineral premix[5]	4.00	4.00	4.00	4.00	4.00	4.00
$NaH_2PO_4 \cdot 2H_2O$	2.52	2.52	2.52	2.52	2.52	2.52
Carboxymethyl cellulose	1.00	1.00	1.00	1.00	1.00	1.00
Choline chloride	0.11	0.11	0.11	0.11	0.11	0.11
Cellulose	9.00	7.20	5.39	3.59	1.78	0.00
Chromic oxide	1.00	1.00	1.00	1.00	1.00	1.00
Chemical composition (% dry matter)						
Moisture	7.66	6.85	7.86	7.56	6.89	5.81
Crude protein	41.47	41.47	41.27	41.42	41.45	41.43
Crude lipid	8.04	7.81	8.08	8.38	7.92	8.17
Ash	11.11	12.60	14.03	15.17	17.07	18.54
Nitrogen-free extract	27.16	27.10	27.23	27.28	27.02	27.30
Phosphorus	1.60	1.85	2.06	2.33	2.56	2.83
Digestible energy (kJ/g)	18.17	18.42	18.38	18.37	18.21	17.84

[1]Source: Golden Alaska, USA

[2]Source: Coland Feed Company, Wuhan, China

[3]Source: the National Renderers Association (NRA), USA

[4]Vitamin premix (mg/1000 g 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

[5]Mineral premix (mg/kg 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

Tab. 2　Proximate composition of the proteins used in the experimental diets in which fishmeal is gradually replaced by meat and bone meal (g 100/g dry matter)

Ingredient	White fish meal (Golden Alaska, USA)	Meat and bone meal (NRA, USA)	Defatted soybean meal (Coland Feed Company, Wuhan, China)
Crude protein	72.00	56.52	49.22
Crude lipid	9.37	11.69	1.22
Ash	12.69	23.81	6.78
Moisture	4.26	4.78	8.10
Phosphorus	2.18	3.77	0.79
Gross energy (kJ/g)	19.06	17.94	18.44

Tab. 3 Effect of fecal collection interval on the apparent digestibility coefficient of nutrients in gibel carp diets with different levels of MBM (means ±SE) * **Authors, we need the n = number of determinations behind the statistics, to be identified in the caption**

Diet	Feces collection period	Apparent digestibility coefficient (%)			
		Dry matter[1]	Crude protein[2]	Gross energy[3]	Phosphorus[4]
Control	1min	68.99 ±0.76^{a}	92.46 ±0.72^{a}	79.51 ±0.26^{a}	38.80 ±0.68^{a}
	4h	71.08 ±0.40^{b}	94.04 ±0.21^{b}	83.62 ±0.64^{b}	43.73 ±1.17^{b}
	16h	71.49 ±0.54^{b}	95.82 ±0.25^{c}	84.42 ±0.65^{b}	41.03 ±1.57^{ab}
MBM_{20}	1min	69.68 ±0.69^{a}	91.51 ±0.66^{a}	80.14 ±1.37	35.78 ±0.91^{a}
	4h	71.32 ±0.64^{ab}	93.10 ±0.29^{b}	83.29 ±1.12	38.02 ±0.90^{a}
	16h	72.92 ±0.05^{b}	94.75 ±0.11^{c}	83.53 ±0.42	43.93 ±1.02^{b}
MBM_{40}	1min	67.90 ±0.41^{a}	89.25 ±0.27^{a}	80.14 ±0.83^{a}	27.20 ±1.21^{a}
	4h	67.62 ±0.14^{a}	90.48 ±0.13^{b}	82.25 ±0.51^{ab}	35.43 ±1.37^{b}
	16h	70.64 ±0.28^{b}	93.05 ±0.18^{c}	84.06 ±0.61^{b}	38.72 ±1.07^{b}
MBM_{60}	1min	66.62 ±0.51	88.02 ±0.29^{a}	79.99 ±0.33^{a}	23.14 ±1.38^{a}
	4h	66.19 ±0.76	87.42 ±0.49^{a}	83.00 ±0.29^{b}	27.08 ±0.61^{b}
	16h	68.98 ±1.16	91.02 ±0.47^{b}	83.09 ±0.54^{b}	30.89 ±0.99^{c}
MBM_{80}	1min	62.03 ±0.24	83.89 ±0.70^{a}	77.82 ±0.85	20.32 ±1.56^{a}
	4h	63.66 ±0.18	85.85 ±0.12^{b}	80.89 ±1.1	23.27 ±1.21^{ab}
	16h	64.64 ±1.34	87.98 ±0.63^{c}	80.93 ±1.20	26.13 ±0.65^{b}
MBM_{100}	1min	63.11 ±1.03^{ab}	83.26 ±0.73^{a}	78.37 ±0.12^{a}	17.57 ±1.32
	4h	61.64 ±0.86^{a}	82.38 ±0.56^{a}	80.49 ±0.37^{b}	20.73 ±0.98
	16h	65.48 ±0.56^{b}	87.20 ±0.56^{b}	81.17 ±0.21^{b}	21.24 ±0.87

*Means in the same column with different superscripts are significantly different ($p < 0.05$)

[1]ADCd: 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]ADCpro: 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] ADCe: 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]ADCp: ADC of P (%) = 100[1 - (Cr_2O_3 in the diet/ Cr_2O_3in the feces) ×(P in feces/ P in the diet)]

1.2 Experimental facilities The digestibility system used in the present study was modified from the collection apparatus described by Allan *et al*.[4] (Fig. 1). 18 fibreglass digestibility tanks (diameter: 80 cm, water volume: 300L) with cylindro-conical bottoms (conical base sloped at an angle of 40°) were used in this experiment. There was a 60-mm diameter faecal settling column which tapered into a 20-mm diameter, 80mm length of removable translucent collection container at the bottom. The effluent pipe connected to the faecal settling column was acclivitous but not horizontal, which was different from that of Allan, *et al*.[4]. The system was equipped with a filter tank filled with zeolite and active carbon for dechlorination of the tap water. Water flow-through rate in the tanks was set at 2.5L/min with the theoretical water renewal rate of once every two hours. During the experiment, continuous aeration was supplied to each tank, water temperature varied between 21.8 ℃ and 28.0 ℃ (mean 24.8 ℃, n = 77). The photoperiod was 12L∶12D with the light period from 08:00 to 20:00. (by a 9 Watt lamp 60cm above the water surface). Dissolved oxygen was above 6mg/L and toal ammonia-N (NH_4^+ —N plus NH_3 —

N) was maintained below 0.1mg/L, while pH was around 6.8. The phosphorus concentration in the rearing water was less than 0.05mg/L during the entire experimental period.

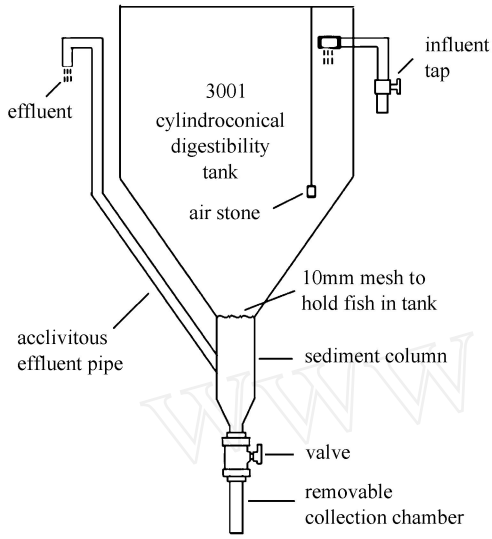

Fig. 1　Principle layout of the digestibility tank system used in this study. Dimensions are not to scale

1.3　Fish and feeding　Young-of-the-year juvenile gibel carp were obtained from the hatchery farm of the Institute of Hydrobiology, the Chinese Academy of Sciences, Wuhan. Prior to the experiments, fish were acclimated to laboratory conditions for 6 weeks. In the first 4 weeks fish were fed a practical diet and then an equal mixture of experimental diets was given for 2 weeks to satiation twice a day at 09:00 and 15:00 h. At the beginning of the experiment, the fish (average body weight 3.6g; n = 210) were pooled and batch weighed after deprivation of feed for 1 day and randomly distributed into 18 tanks (30 fish per tank). Triplicate tanks were randomly assigned to each of the experimental diets. During the experiment, fish were hand-fed to apparent satiation twice daily (09:00 and 15:00 h), seven days a week for 11 weeks.

1.4　Faecal collection procedures　The faecal samples were collected starting at two weeks after the beginning of the experiment. Feeding ceased each afternoon, and at that time the walls of the tanks, the settling columns and the translucent collection containers were thoroughly cleaned to remove uneaten feed and faecal residues from the systems. Thereafter, samples were withdrawn from the collection container during three periods: (1) **1-min samples.** Within 1h after the system was cleaned, the investigators monitored the system carefully and the faeces in the translucent collection containers were removed immediately after they were settled at the terminal end of the container. This system allowed a separation of feces from water within 1min after they were voided by the fish, so the samples were called 1min sample. (2) **4h samples.** The mixture of faeces and water in the translucent collection containers was collected 4h post feeding, viz. at 8:00 pm. (3) **16h samples.** The mixture of faeces and water in the translucent collection container was removed 16h after feeding, viz. at 8:00 am the next day, encompassing a collection period from 4 to 16hs after feeding. Faecal samples were frozen immediately after collection and then freeze dried to constant weight. Samples of different collection times were carried out in the same manner for each tank over time.

1.5　Analytical methods and calculations　Protein content of the feeds and faecal 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 ℃, 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[42]. P concentrations in the diets and feces were analysed 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 ℃ to constant weight[43]. At least duplicate measurements were made for each sample.

The apparent digestibility coefficient (ADC) of dry matter was calculated as described by Cho & Kaushik (1990)[44]:

$$ADC(\%) = 100 - 100 \times \frac{\%Cr_2O_3 \text{ in diet}}{\%Cr_2O_3 \text{ in feces}}$$

The apparent digestibility coefficients (ADCs) for protein, energy and phosphorus were calculated according to the formula:

$$ADC(\%) = 100 - 100 \times \frac{\%Cr_2O_3 \text{ in diet} \times \%\text{nutrient in feces}}{\%Cr_2O_3 \text{ in feces} \times \%\text{nutrient in diet}}$$

1.6 Statistical analysis Data are presented as means of duplicate analyses from three replicate tanks. Duncan's multiple range test was used to compare the difference between means after one-way analysis of variance. Statistica 6.0 was used to perform statistical calculations. Differences were considered significant at $p < 0.05$.

2 Results

The effect of faecal collection interval on ADCs of nutrients was presented in Tab. 3. The ADC of dry matter in each diet increased significantly with the increase of time that faeces were exposed in water ($p < 0.05$) while there were no statistically significant differences between each faecal collection time in MBM_{60} and MBM_{80} group ($p > 0.05$). Increasing the faecal collection interval resulted in a significant ($p < 0.05$) increase in ADC of protein, but no significant differences ($p > 0.05$) were observed between the ADC of protein of the 4h samples and 1min samples in MBM_{60} and MBM_{100} group ($p > 0.05$). ADCs of energy and phosphorus exhibited the same trend as dry matter and crude protein. The ADC obtained from the 1 min samples were much lower than that determined with feces collected at 4h or more.

ADCs of dry matter, crude protein, energy and phosphorus decreased linearly or near linearly as dietary MBM levels increased (Fig. 2—Fig. 5). The relationships between ADC and replacement level of FM protein by MBM protein (MBMP) can be described as follows:

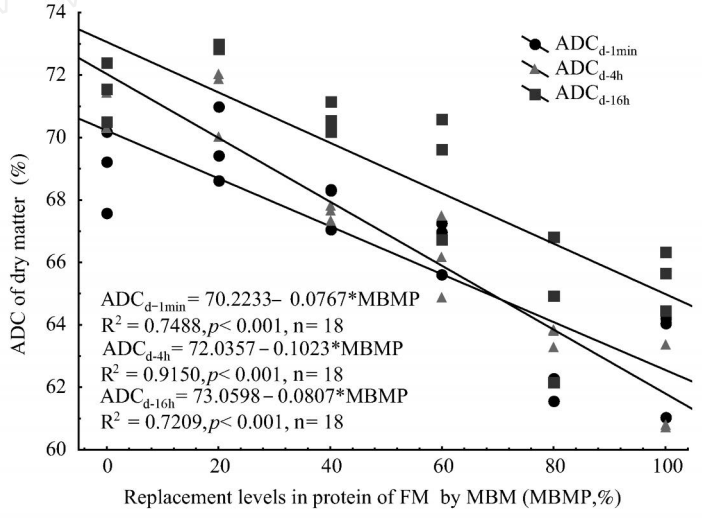

Fig. 2 Relationship between ADC of dry matter and dietary MBM protein levels as affected by different time intervals elapsed from the start of faeces collection until after 16 hours

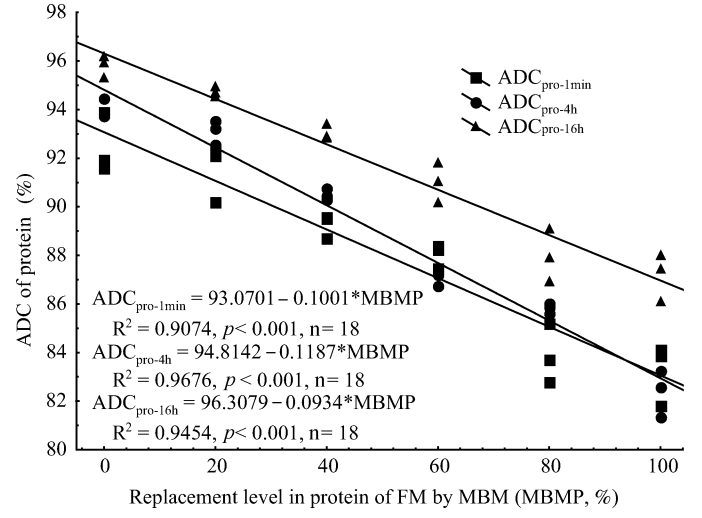

Fig. 3 Relationship between ADC of protein and dietary MBM protein levels and the effects of delayed faeces sampling of faeces (1min, 4 and 16 hours after start of faeces collection)

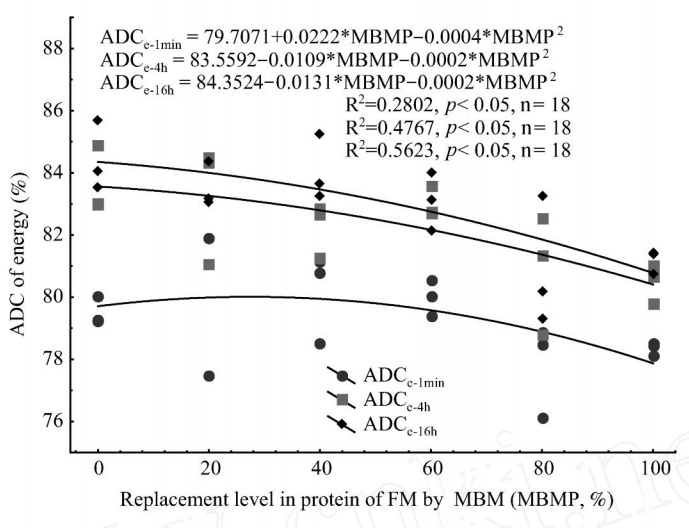

Fig. 4　Relationship between ADC of energy and dietary MBM protein levels

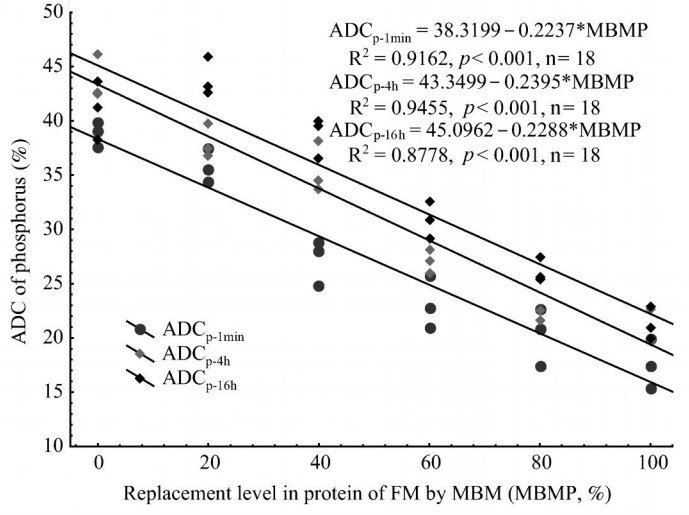

Fig. 5　Relationship between ADC of phosphorus and dietary MBM protein levels

The relationships between ADC of dry matter and replacement levels in protein of FM by MBM were:

$ADC_{d\text{-}1min} = 70.2233 - 0.0767^{*}MBMP$

$R^2 = 0.7488$, $p < 0.001$, $n = 18$

$ADC_{d\text{-}4h} = 72.0357 - 0.1023^{*}MBMP$

$R^2 = 0.9150$, $p < 0.001$, $n = 18$

$ADC_{d\text{-}16h} = 73.0598 - 0.0807^{*}MBMP$

$R^2 = 0.7209$, $p < 0.001$, $n = 18$

where $ADC_{d\text{-}1min}$, $ADC_{d\text{-}4h}$ and $ADC_{d\text{-}16h}$ (%) are the apparent digestibility coefficient of dry matter determined with 1min samples, 4h samples and 16h samples, respectively. MBMP (%) is the replacement level of FM protein by MBM protein.

The relationships between ADC of protein and replacement levels in protein of FM by MBM were:

$ADC_{pro\text{-}1min} = 93.0701 - 0.1001^{*}MBMP$

$R^2 = 0.9074$, $p < 0.001$, $n = 18$

$ADC_{pro\text{-}4h} = 94.8142 - 0.1187^{*}MBMP$

$R^2 = 0.9676$, $p < 0.001$, $n = 18$

$ADC_{pro\text{-}16h} = 96.3079 - 0.0934^{*}MBMP$

$R^2 = 0.9454$, $p < 0.001$, $n = 18$

where $ADC_{pro\text{-}1min}$, $ADC_{pro\text{-}4h}$ and $ADC_{pro\text{-}16h}$ (%) are the apparent digestibility coefficient of protein determined with 1min samples, 4h samples and 16h samples, respectively.

The relationships between ADC of energy and replacement levels in protein of FM by MBM were:

$ADC_{e\text{-}1min} = 79.7071 + 0.0222^{*}MBMP - 0.0004^{*}$

$MBMP^2$;

$R^2 = 0.2802$, $p < 0.05$, n = 18

$ADC_{e\text{-}4h} = 83.5592 - 0.0109 * MBMP - 0.0002 * MBMP^2$;

$R^2 = 0.4767$, $p < 0.05$, n = 18

$ADC_{e\text{-}16h} = 84.3524 - 0.0131 * MBMP - 0.0002 * MBMP^2$;

$R^2 = 0.5623$, $p < 0.05$, n = 18

where $ADC_{e\text{-}1min}$, $ADC_{e\text{-}4h}$ and $ADC_{e\text{-}16h}$ (%) is the apparent digestibility coefficient of energy determined with 1min samples, 4h samples and 16h samples, respectively.

The relationships between ADC of phosphorus and replacement levels in protein of FM by MBM were:

$ADC_{p\text{-}1min} = 38.3199 - 0.2237 * MBMP$

$R^2 = 0.9162$, $p < 0.001$, n = 18

$ADC_{p\text{-}4h} = 43.3499 - 0.2395 * MBMP$

$R^2 = 0.9455$, $p < 0.001$, n = 18

$ADC_{p\text{-}16h} = 45.0962 - 0.2288 * MBMP$

$R^2 = 0.8778$, $p < 0.001$, n = 18

where $ADC_{p\text{-}1min}$, $ADC_{p\text{-}4h}$ and $ADC_{p\text{-}16h}$ (%) are the apparent digestibility coefficient of phosphorus determined with 1min samples, 4h samples and 16h samples, respectively.

3 Discussion

It is generally accepted that collecting faeces from the water tend to overestimate the ADCs as a result of nutrient leaching from the faeces. The leached material should be added to the non-metabolized portion. However, since the amount is unknown it is often simply forgotten and therefore contributes to the error of the presumed converted material Windell *et al*.[7] demonstrated that most of the nutrient leaching from faeces occurred during the first hour after defaecation. Similar results were observed by Possompes[40]. In the study of Watanabe *et al*.[30], leaching was considered to explain the increase in ADC of digestible energy for rainbow trout using faeces collected at increasing time intervals after feeding. Conversely, Cho *et al*.[45] claimed that nutrient leaching is minimal when the faeces remain undisturbed and unbroken until faecal pellets are collected. There was no significant difference in the ADC of dry matter, protein, lipid and energy for rainbow trout faeces determined with the Guelph System in a serious of extended collection intervals (3, 6, 9, 12 or 15h after the final feeding)[32]. The data of Allan *et al*.[4] indicated that leaching did not affect the digestibility coefficients after faeces had settled and concluded that collection of faeces by settlement over 18h is a suitable method for determining overall digestibility in juvenile silver perch. However, both Satoh *et al*.[32] and Allan *et al*.[4] collected faeces at least 2h after feeding. It was the very time that most leaching of nutrients from feces had occurred[7,33]. In the present study, 1min samples showed the lowest ADC values while 16h samples obtained the highest, which were agreeable to the previous reports. Certainly, leaching from faeces is also dependent on the consistency and compactness of the faecal material. Numerous fish species produce faeces with a relatively strong peritrophic membrane which keeps the faecal material togethere while other species produce faeces that extremely quickly desintigrate and release a lot of soluble organics and nutrients immediately into the water. Therefore, differences in ADC values should be expected even when using the same sampling technique.

Besides the species-specific differences in faecal stability, diet formulation was also reported to affect the faecal stability and resistance to leaching. Satoh *et al*.[32] used two different diets with settling columns for digestibility studies. Herring meal, white FM, soybean meal and corn gluten meal were the protein sources of the diet 1 while the diet 2 contained only white FM as the protein source. The results showed that digestibility of crude protein in the diet 1 determined in faeces collected at 3h was significantly lower than that collected after 9h while no significant difference due to sampling time was found in the diet 2. Similarly, ADCs of nutrients in the control diet determined in the 4h samples were significantly higher than that determined with the 1min samples in the present study, while it was not the case when MBM was added to the five other diets. Brinker *et al*.[46] found that the addition of binders would enhance faecal stability and improve shear resistance.

In order to reduce the leaching of nutrients, any disturbance to the faeces after they have been released by the fish should be avoided as far as possible[32]. In the present study, the mixture of faeces and surrounding water

from the bottom of the column was collected to avoid the handling loss. However, the ADC values of nutrients indicated that the loss of nutrients from the faeces was still significant. Because the faecal settling column was connected with the effluent pipe, the leached nutrients of the faeces in the collection container may partially be removed by the water outflow. Thus, faeces should be taken quickly after they were excreted when using the present faecal collection system for gibel carp. Furthermore, the effluent pipe should be set at upper position of the faecal settling column and/or the faecal settling column should be prolonged in the future work to reduce the disturbance of water outlet to the settled faeces. Another option to be considered further would be a collecting double-conveyor belt by which the faeces are preserved transported in timed intervals of 5—10 minutes to a preservation chamber while being packaged between the two layers of the belts and cooled to avoid metabolic activity[8].

In the study of Spyridakis *et al*.[20] some faecal material often stood momentarily in the connection pipe between the tank bottom and the decantation column. ADC was overestimated after faeces were exposed to a constant flow of water, which was also observed by Hajen *et al*.[9] when a substantial amount of nutrients and organic materials were washed out. The foregoing problem was solved in later experiments[47] by reducing the cross-section of the connecting pipe which could increase water velocity and thus carry the faeces to the settling column more quickly. Allan *et al*.[4] modified the faecal collection system by taking out the connection pipe between the tank bottom and the settling column so that the faeces only sink to the settlement chamber. Using this system, the faecal pellets could settle to the terminal end of the collecting chambers within 20—30s while being voided by fish. In the present study, the traveling time of faeces from the fish after release to the bottom was also within 30s. Furthermore, the effluent pipe connected to the faecal settling column was acclivitous but not horizontal, which would reduce the leaching of faeces by the water outflow.

ADC showed dial variations and differences at different periods of the experiment[8,10,48,49]. To reduce the variability, digestibility studies should be based on pooled faecal material collected over a number of days, if not weeks, and include the majority of faeces produced from each meal[4,49]. In the present study, faecal samples were obtained because the faeces were collected in combination with growth trials for about 9 weeks (except the first two weeks of the experiment), thereby reflecting the overall apparent digestibility of whole experimental period. Certainly, this assumes that during this period metabolic efficiency has not changed with growth, an assumption that may have to be specifically studied when investigating very young fish.

Decreased apparent digestibility has been reported in gibel carp and turtle with the increase in dietary inclusion of MBM[39,50]. Zhou *et al*.[51] reported lower digestibility of dry matter, protein, lipid, phosphorus and energy of MBM than that of FM. Lower apparent protein digestibility of MBM compared to that of FM had also been reported in other fishes or shrimps[36,39,50—57]. MBM contains a high level of ash content and it was proved to correlate negatively with protein digestibility[39,52,58,59]. In the present study, apparent digestibility of dry matter, protein and phosphorus decreased linearly with the increase in dietary MBM levels. Low digestibility of MBM could be one of the problems affecting the use in aquafeed. New processing technology should be developed to improve the digestibility so as to improve the quality of MBM.

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References:

[1] Vandenberg G W & De la Noüe J. Apparent digestibility comparison in rainbow trout (*Oncorhynchus mykiss*) assessed using three methods of faeces collection and three digestibility markers [J]. *Aquaculture Nutrition*, 2001, **7**: 237—245

[2] Irvin S J & Tabrett S J. A novel method of collecting fecal samples from spiny lobsters [J]. *Aquaculture*, 2005, **243**: 269—272

[3] De la Noüe J & Choubert G. Digestibility in rainbow trout: comparison of the direct and indirect methods of measurement [J]. *The Progressive Fish-Culturist*, 1986, **48**: 190—195

[4] Allan G L, Rowland S J, Parkinson S, Stone D A J & Jantrarotai W. Nutrient digestibility for juvenile silver perch *Bidyanus bidyanus*: development of methods [J]. *Aquaculture*, 1999, **170**: 131—145

[5] Nose T. On the digestion of food proteins by gold-fish (*Carassius auratus* L.) and rainbow trout (*Salmo irideus* G.). *Bulletin of Freshwater Fisheries Research Laboratory*, 1960, **10**: 11—22

[6] Austreng E. Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract [J]. *Aquaculture*, 1978, **13**: 265—272

[7] Windell J T, Foltz J W & Sarokon J A. Methods of fecal collection

and nutrient leaching in digestibility studies [J]. *The Progressive Fish-Culturist*, 1978, **40**: 51—55

[8] Vens-Cappell B. Methodical studies on digestion in trout: I. Reliability of digestion coefficients in relation to methods for digesta collection [J]. *Aquacultural Engineering*, 1985, **4**: 33—49

[9] Hajen W E, Beames R M, Higgs D A & Dosanjh B S. Digestibility of various feedstuffs by post-juvenile chinook salmon (*Oncorhynchus tshawytscha*) in sea water: 1. Validation of technique [J]. *Aquaculture*, 1993a, **112**: 321—332

[10] Percival S & Lee P. Development of more cost-effective feeds for the Tasmanian Atlantic salmon industry. Final Report to FRDC, Project No. 93/126. Salmon Enterprises Tasmania (SALTAS). Hobart, Tasmania, 1996, 55

[11] Smith B W & Lovell R T. Digestibility of nutrients in semipurified rations by channel catfish in stainless steel troughs [J]. *Proceedings of the Annual Conference Southeast Association of Game and Fish Commissioners*, 1971, **25**: 425—459

[12] Smith B W & Lovell R T. Determination of apparent protein digestibility in feeds for channel catfish [J]. *Transactions of the American Fisheries Society*, 1973, **102**: 831—835

[13] Henken A M, Kleingeld D W & Tijssen P A T. The effect of feeding level on apparent digestibility of dietary dry matter, crude protein and gross energy in the African catfish *Clarias gariepinus* (Burchell, 1822) [J]. *Aquaculture*, 1985, **51**: 1—11

[14] Lovell R T. Digestibility of nutrients in feedstuffs for catfish. In: R R Stickney and R T Lowell (Eds.), *Nutrition and Feeding of Channel Catfish*. *Southern Cooperative Series Bulletin*. 1977, **218**: 33—37

[15] Brown B P, Strange R J & Robbins K R. Protein digestibility coefficients for yearling channel catfish fed high protein feedstuffs [J]. *The Progressive Fish-Culturist*, 1985, **47**: 94—97

[16] Smith R R. Methods for determination of digestibility and metabolisable energy of feedstuffs for finfish. In: *Proceedings of the World Symposium on Finfish Nutrition and Fishfeed Technology*. Vol. II, Hamburg 20—23 June, 1978. Heenemann Verlagsgessellschaft, Berlin, 1979, 453—459

[17] National Research Council (NRC), *Nutrient Requirements of Fish*. National Academy Press, Washington D C. 1993, 114

[18] Storebakken T, Kvien I S, Shearer K D, Grisdale-Helland B, Helland S J, Berge G M. The apparent digestibility of diets containing fish meal, soybean meal or bacterial meal fed to Atlantic salmon (*Salmo salar*): evaluation of different faecal collection methods [J]. *Aquaculture*, 1998, **169**: 195—210

[19] Ramsay J M, Castell J D, Anderson D M & Hebb C D. Effects of fecal collection methods on estimation of digestibility of protein feedstuffs by winter flounder [J]. *North American Journal of Aquaculture*, 2000, **62**: 168—173

[20] Spyridakis P, Metailler R, Gabaudan J & Riaza A. Studies on nutrient digestibility in European sea bass (*Dicentrarchus labrax*): 1. Methodological aspects concerning faeces collection [J]. *Aquaculture*, 1989, **77**: 61—70

[21] Alliot E, Pastoureaud A, Pelaez-Hudlet J & Métailler R. Utilisation des farines végétales et des levures cultivées sur alcanes pour l'alimentation du bar (*Dicentrarchus labrax*). In: Proceedings of the World Symposium on Finfish Nutrition and Fishfeed Technology. Hamburg. Heenemann, Berlin, 1978, Vol. **2**: 229—238

[22] Cho C Y, Slinger S J. Apparent digestibility measurement in feedstuffs for rainbow trout. In: Proceedings of the World Symposium on Finfish Nutrition and Fishfeed Technology. Hamburg, Germany. Vol. II, Hamburg, 20—23 June, 1978. Heenemann Verlagsgesellschaft, Berlin. 1979, 239—247

[23] Cho C Y, Slinger S J & Bayley H S. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity [J]. *Comparative Biochemistry and Physiology*, 1982, **73B**: 25—41

[24] Choubert G Jr, de la Noüe J & Luquet P. Continuous quantitative automatic collector for fish feces [J]. *The Progressive Fish-Culturist*, 1979, **41**: 64—67

[25] Choubert G Jr, de la Noüe J & Luquet P. Digestibility in fish: Improved device for the automatic collection of feces [J]. *Aquaculture*, 1982, **29**: 185—189

[26] Smith B R. A method for measuring digestibility and metabolisable energy of fish feeds [J]. *The Progressive Fish-Culturist*, 1971, **33**: 132—134

[27] Smith R R, Peterson M C & Allred C. Effect of leaching on apparent digestibility coefficients of feedstuffs for salmonids [J]. *The Progressive Fish-Culturist*, 1980, **42**: 195—199

[28] Lied E, Julshamn K & Braekkan O R. Determination of protein digestibility in Atlantic cod (*Gadus morhua*) with internal and external indicators [J]. *Canadian Journal of Fisheries and Aquatic Sciences*, 1982, **39**: 854—861

[29] Brown P B & Robinson E H. Nutrient concentrations of catfish feces and practical diets after immersion in water [J]. *Journal of the World Aquaculture Society*, 1989, **20**: 245—249

[30] Watanabe T, Takeuchi T, Satoh S & Kiron V. Digestible energy: methodological influences and a mode of calculation [J]. *Fisheries Science*, 1996, **62**: 288—292

[31] Sales J & Britz P J. Evaluation of different markers to determine apparent nutrient digestibility coefficients of feed ingredients for South African abalone (*Haliotis midae* L.) [J]. *Aquaculture*, 2001, **202**: 113—129

[32] Satoh S, Cho C Y & Watanabe T. Effect of fecal retrieval timing on digestibility of nutrients in rainbow trout diet with the Guelph and TUF feces collection systems [J]. *Nippon Suisan Gakkaishi*, 1992, **58**: 1123—1127

[33] Possompes B P. Influence de la température sur les besoins en protéines, le transit alimentaire et la digestibilité chez la truite arc-en-ciel (*Salmo gairdneri* R.). Thèse de 3e Cycle, Université de Paris VI, 1973, 70

[34] Hardy R W, Kissil G W M. Trends in aquaculture feeding [J]. *Feed Mix*, 1997, **5**: 31—34

[35] Lee S M. Apparent digestibility coefficients of various feed ingredients for juvenile and grower rockfish (*Sebastes schlegeli*) [J]. *Aquaculture*, 2002, **207**: 79—95

[36] Watanabe T, Pongmaneerat J. Quality evaluation of some animal protein sources for rainbow trout *Oncorhynchus mykiss* [J]. *Nippon*

Suisan Gakkaishi, 1991, **57**: 495—501

[37] Davies S J, Williamson J, Robinson M, Bateson R I. Practical inclusion levels of common animal by-products in complete diets for tilapia *Oreochromis mossambicus*, Peters, 1989. In: The Current Status of Fish Nutrition in Aquaculture, Proceedings of the Third International Symposium on Feeding and Nutrition in Fish, Toba, Japan, 28 August-1 September, Japan, Takeda M & Watanabe T (Eds.), 1989, 325—332

[38] Millamena O M. Replacement of fish meal by animal by-product meals in a practical diet for grow-out culture of grouper *Epinephelus coioides* [J]. *Aquaculture*, 2002, **204**: 75—84

[39] Yang Y, Xie S Q, Cui Y, Lei W, Zhu X, Yang Y & Yu Y. Effect of replacement of dietary fish meal by meat and bone meal and poultry by-product meal on growth and feed utilization of gibel carp, *Carassius auratus gibelio* [J]. *Aquaculture Nutrition*, 2004, **10**: 289—294

[40] Environmental Protection Agency. Pollution as a result of fish culture activities. USAEP, EPA-R3-73-009, Washington, D C, U S A. 1973

[41] Xue M & Cui Y B. Effect of several feeding stimulants on diet preference by juvenile gibel carp *Carassius auratus gibelio*, fed diets with or without partial replacement of fish meal by meat and bone meal [J]. *Aquaculture*, 2001, **198**: 281—292

[42] Bolin D W, King R P & Klosterman E W. A simplified method for the determination of chromic oxide (Cr_2O_3) when used as an index substance [J]. *Science*, 1952, **116**: 634—635

[43] Association of Official Analytical Chemists (AOAC) In: Official Methods of Analysis, 14thed (Williams S ed). AOAC, Washington. 1984

[44] Cho C Y & Kaushik S J. Nutritional energetics in fish: energy and protein utilisation in rainbow trout (*Salmo gairdneri*) [J]. In: Aspects of Food Production, Consumption and Energy Values. Bourne G H (Eds.), World Review of Nutrition and Dietetics, 1990, **61**: 132—172

[45] Cho C Y, Cowey C B & Watanabe T. Methodological approaches to research and development. In: *Finfish Nutrition in Asia*, Cho C Y, Cowey C B & Watanabe T (Eds.), 1985, 10—80. IDRC, Ottawa, Canada

[46] Brinker A, Koppe W & Rösch R. Optimised effluent treatment by stabilised trout faeces [J]. *Aquaculture*, 2005, **249**: 125—144

[47] Hajen W E, Higgs D A, Beaines R M & Dosanjh B S. Digestibility of various feedstuffs by post-juvenile Chinook salmon (*Oncorhynchus tshawytscha*) in sea water. 2. Measurement of digestibility [J]. *Aquaculture*, 1993b, **112**: 333—348

[48] De Silva S S & Perera M K. Digestibility of aquatic macrophyte by the cichlid *Etroplus suratensis* (Bloch) with observations on the relative merits of three indigenous components as markers and daily changes in protein digestibility [J]. *Journal of Fish Biology*, 1983, **23**: 675—684

[49] De Silva S S & Perera M K. Digestibility in *Sarotherodon niloticus* fry: effect of dietary protein level and salinity with further observations on variability in daily digestibility [J]. *Aquaculture*, 1984, **38**: 293—306

[50] Yang Y. A comparative study on the utilization of meat and bone meal (MBM) and poultry by-product meal (PBM) in oriental river prawn, gibel carp and soft-shell turtle. Doctor's thesis, Institute of Hydrobiology, Chinese Academy of Sciences, China, 2004, 46—59 (in Chinese with English abstract)

[51] Zhou Q C, Tan B P, Mai K S, Liu Y J. Apparent digestibility of selected feed ingredients for juvenile cobia *Rachycentron canadum* [J]. *Aquaculture*, 2004, **241**: 441—451

[52] Pongmaneerat J, Watanabe T. Nutritive value of protein of feed ingredients for carp *Cyprinus carpio* [J]. *Nippon Suisan Gakkaishi*, 1991, **57**: 503—510

[53] Bruce B M & Reigh R C. Apparent digestibility of selected ingredients in red drum (*Sciaenops ocellatus*) diets [J]. *Aquaculture*, 1996, **141**: 233—244

[54] Gaylord T G, Gatlin D M. Determination of digestibility coefficients of various feedstuffs for red drum (*Sciaenops ocellatus*) [J]. *Aquaculture*, 1996, **139**: 303—314

[55] McGoogan B B & Reigh R C. Apparent digestibility of selected ingredients in red drum (*Sciaenops ocellatus*) diets [J]. *Aquaculture*, 1996, **141**: 233—244

[56] Brunson J F, Romaire R P & Reigh R C. Apparent digestibility of selected ingredients in diets for white shrimp *Penaeus setiferus* L. [J]. *Aquaculture Nutrition*, 1997, **3**: 9—16

[57] Forster I P, Dominy W, Obaldo L & Tacon A G J. Rendered meat and bone meals as ingredients of diets for shrimp *Litopenaeus vannamei* (Boone, 1931) [J]. *Aquaculture*, 2003, **219**: 655—670

[58] National Research Council (NRC) *Nutrient Requirements of Coldwater Fishes and Shellfishes*. National Academy Press, Washington D C. 1983, 63

[59] Kureshy N, Davis D A & Arnold C R. Partial replacement of fish meal with meat and bone meal, ash-dried poultry by-product meal, and enzyme-digested poultry by-product meal in practical diets for juvenile red drum [J]. *North American Journal of Aquaculture*, 2000, **62**: 266—272

粪便收集时间和饲料中肉骨粉含量对异育银鲫(Carassius auratus gibelio) 消化率的影响

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摘要:本研究探讨了通过收集器不同粪便收集时间和饲料中肉骨粉(MBM)含量对异育银鲫干物质、蛋白、能量、磷的表观消化率 (ADC) 影响。不同梯度(0, 20, 40, 60, 80 ,100 %)的肉骨粉替代鱼粉(FM) 蛋白配制成六种等氮(粗蛋白: 410 g/ kg) 等能 (总能: 18 kJ/ g) 的饲料,通过 11 周的饲养实验,实验开始后 2 周开始收集粪便,收集时间分别是:排粪后 1 min, 投喂后 4h 和 16h。结果表明,干物质、蛋白、能量、磷的消化率明显随粪便收集时间增加而升高($p < 0.05$),但在高肉骨粉替代饲料中差异不显著($p > 0.05$)。干物质、蛋白、能量、磷的消化率随饲料中肉骨粉含量的增加呈线性或近线性下降。因此,在消化率测定中应该尽快收集排出的粪便以保证消化率的真实性。消化率是影响肉骨粉利用一个因素。

关键词:粪便收集时间;肉骨粉;消化率;异育银鲫