ジャイアントグーラミィ,Osphronemus gouramy用飼料における脱脂大豆油粕による魚粉代替

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Defatted Soybean Meal as an Alternative Source to Substitute Fish Meal in the Feed of Giant Gouramy, *Osphronemus gouramy* Lac.

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Abstract: This experiment was conducted to examine the utilization of defatted soybean meal (SBM) as a fish meal (FM) substitute in the diets for giant gouramy, *Osphronemus gouramy* Lac. The levels of substitution of FM by SBM were 0, 25, 50, 75 and 100% of dietary protein, respectively. The diets were isoenergetic, isonitrogenous and isolipid. Daily growth rate, feed efficiency, protein and lipid retention, apparent digestibility of protein, lipid and carbohydrate, ammonia excretions and respiratory quotient were used as parameters to evaluate the differences among treatments.

A substitution level of 50% resulted in the highest daily growth rate, feed efficiency, and protein and lipid retention ($p < 0.05$). This treatment also resulted in the lowest nitrogen excretion. Digestibility of protein, lipid and carbohydrate decreased with an increase in SBM, due to the increase in fiber content and insoluble carbohydrate. The respiratory quotient of the experimental fish ranged from 0.85 to 0.99, indicated that both protein and carbohydrate were catabolyzed as the main sources of energy.

Based on the evaluation of these parameters, it is concluded that SBM can substitute 50% of the protein for FM in the diets of giant gouramy without impairment of performance.

Key words: Defatted soybean meal; Alternative protein source; Giant gouramy, *Osphronemus gouramy*.

Fish meal (FM) has been used as the main source of protein in various fish diets because of its high digestibility of protein and energy, high palatability, richness in minerals and in available lysine and methionine, which are deficient in plant feedstuffs[1]. Watanabe *et al.*[2] reported that FM is the widely sought after fish meal ingredient because it is a rich source of essential amino acids, essential fatty acids, energy and minerals. Moreover it is very palatable and highly digestible to most freshwater and marine fishes. However as the shortage of quality fish meal due to the decline in the landing of meal grade fish, it is important to look for alternative protein sources to substitute FM[3-5]. Among the substitution candidates of plant sources, the less expensive and more abundant, soybean meal and its products are of vital interest. One of them, defatted soybean meal (SBM) is a by-product of the soybean oil industry with a high protein content and low price compared with FM, and it is high in availability[4].

It is well known that soybean protein has one of the best amino acid profiles of all protein-rich

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plant foodstuffs for meeting the essential amino acid requirements of fish. On the other hand, soybean meal has also some nutritional limitations when being considered as a replacement in fish feed due to the low digestibility of nitrogen and energy, deficiency in minerals, indigestible components such as oligosaccharides and some anti-nutritional factors, and its feeding results in poor growth when being considered as a partial or total substitute for fish meal[2].

Previous studies demonstrated that soybean protein can replace part of the FM in fish feed for yellowtail, *Seriola quinqueradiata*[1,6,7], carp, *Cyprinus carpio*[8], and cod, *Gradus macrocephalus*[9]. Contrary to these, Halver[10] showed that soybean protein is not deficient in any essential amino acid on channel catfish. However no experiments have been done for giant gouramy, so this experiment was conducted to examine the utilization of SBM as a FM substitute and to determine the optimum SBM level in the diets of giant gouramy, *Osphronemus gouramy* Lac.

Materials and Methods

Diet Composition

The composition and proximate analysis of the experimental diets are shown in Table 1. Five experimental diets formulated to be isonitrogenous, isolipidic and isoenergetic were used in this experiment. Diets were in paste form with a 32% protein content and calorie per protein ratio of 33.44 Kj DE/g protein[11]. All of the protein content in diet A came from FM (CP 71.08%, CL 8.61%, Subur Poultry Shop). In diets B, C and D, SBM was used to replace 25%, 50% and 75% of the protein from FM, respectively. Diet E contained SBM (CP 49.82%, CL 3.08%, Subur Poultry Shop) as the sole protein source.

Fish Rearing

Giant gouramy weighing 16 to 21 g were used in the experiment. Each fish was placed in one of 15 recirculation fiberglass tanks radius 13 cm and height 38 cm (18 *l* in volume). The period of feeding was 50 days and fish were fed three times daily (7:00, 13:00 and 17:00), each time to apparent satiation level (0.84-2.14% body weight). The individual body weight of each group was determined every 10 days. During rearing periods the flow of circulation was 7.5 *l*/h and every 10 days all the water in each tank was changed and the filter was washed. The water temperature in each tank was 29-30.5°C.

Table 1. The composition of the experimental diets (%)

Ingredient	Substitution of FM*1 by SBM*1 in dietary protein				
	A (0%)	B (25%)	C (50%)	D (75%)	E (100%)
Fish meal	45.22	33.91	22.61	11.3	0.00
Defatted soybean meal	0.00	16.13	32.26	48.38	64.51
Dextrin	40.21	34.61	28.97	23.35	17.14
CLO+CO*2	1.11	1.58	2.06	2.54	3.01
Vitamin mixture	2.50	2.50	2.50	2.50	2.50
Mineral mixture	5.87	5.87	5.87	5.87	5.87
CMC	2.83	2.83	2.83	2.83	2.83
Cellulose	1.66	1.97	2.30	2.63	2.94
Proximate composition (% dry weight)					
Crude protein	33.07	33.18	31.85	32.26	32.72
Crude lipid	6.32	6.50	6.34	6.45	6.65
Crude ash	10.14	9.87	9.48	8.70	8.30
Fiber	1.96	5.68	7.22	9.74	10.58
NFE*3	48.51	44.68	45.11	42.75	41.75
Total energy (Mj/kg)	12.05	11.74	11.52	11.37	11.40

*1 FM, fish meal; SBM, defatted soybean meal.
*2 CLO+CO; pollack liver oil : corn oil (1 : 1.6).
*3 Nitrogen free extract.

Ten fiberglass tanks (radius 13 cm and height 38 cm) were used to collect the feces in order to determine nutrient digestibility. The diet formulation was the same as in Table 1, except 0.6% CMC was replaced with 0.6% Cr_2O_3. One fish were placed into each fiberglass tank and all the treatments were duplicated.

Twelve aquariums (50 × 40 × 35 cm) were used to determined oxygen consumption, CO_2 production and ammonia excretion. Two in twelve aquriums were used as controls without fish. Three fish were placed in each of the rest ten aquarium. Fish were fed to satiation with the test diets before the water samples were taken. The water samples were taken five times every one hour for measurement of oxygen consumption, CO_2 production and ammonia excretions.

Chemical Analysis

The analytical methods for proximate composition and chromic oxide; the calculation formula for apparent digestibility; protein and lipid retention (PR and RL) and protein efficiency ratio (PER) were all described previously[12]; Ammonia and carbon dioxide were measured by the indophenol and phenolphthalein methods respectively. Dissolved oxygen was measured by oxygen meter, a YSI Model 57 (YSI Industries, Yellow Springs, OH). Amino acid compositions of the whole body, FM and SBM were analyzed by the HPLC method (Dept. of Biochemistry, Bogor Agricultural Univ.).

Evaluating Parameters

The daily growth rate (DGR), feed efficiency (FE), PR and LR, apparent digestibility of protein, lipid and carbohydrate, ammonia excretion and respiratory quotient (RQ) were used as the parameters to evaluate differences among dietary treatments.

Statistical Analysis

This research used a completely randomized design with five treatments and three replications. Tukey test was performed to compare the mean value of body weight and DGR and feed performance of the SBM substitution in dietary protein used[13].

Results

The apparent digestibility of the nutrients in test diets by giant gouramy is shown in Table 2. The increasing protein contribution from SBM in the diets decreased the digestibility of nutrients

Table 2. Apparent nutrient digestibility and fiber content of the dietary treatments

	Apparent digestibility (%)			Fiber content (%)
	Protein	Lipid	Carbohydrate	
Diet A	92.12	97.45	96.17	4.96
Diet B	87.92	96.96	94.76	5.85
Diet C	87.53	95.80	91.87	7.72
Diet D	86.71	95.80	86.81	9.74
Diet E	80.71	94.10	84.35	10.58

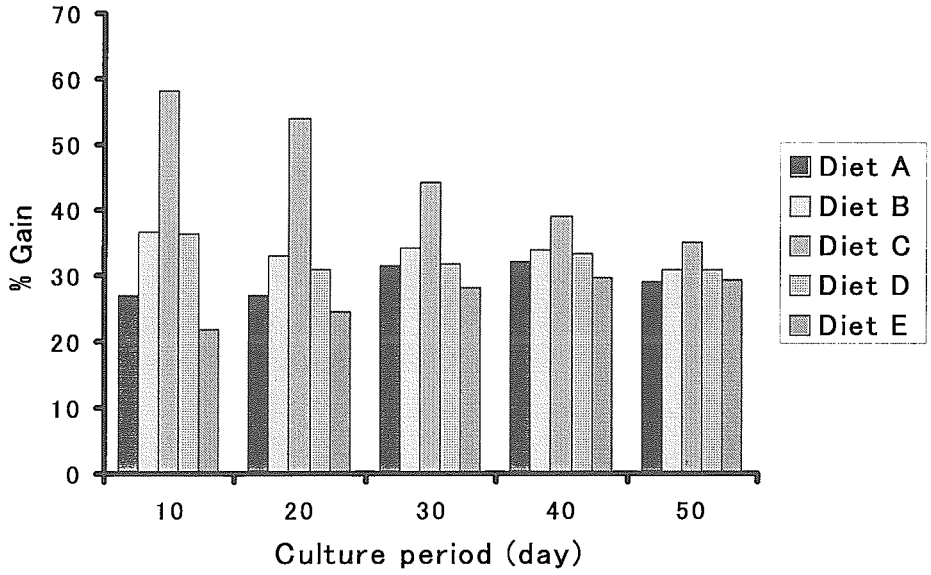

Fig. 1. Weight gain (%) of giant gouramy for 50-days culture.

Table 3. Result of a 50-day feeding experiment of giant gouramy

	Average body weight (g)*1		% Gain	Daily feed intake*2 (%)	Daily growth*2 rate (%)	Feed efficiency*2 (%)
	Initial	Final				
Diet A	20.3±2.03	74.5±15.01	267.5	1.7±0.55^{ab}	2.6±0.45^{b}	37.9±5.68^{a}
Diet B	18.3±0.64	78.9±12.20	332.1	1.7±0.31^{ab}	2.9±0.32^{ab}	42.4±6.80^{a}
Diet C	16.1±0.80	106.0± 5.52	560.0	2.1±0.20^{b}	3.9±0.03^{ab}	64.2±3.39^{b}
Diet D	17.2±1.58	70.7±19.67	311.7	1.5±0.57^{ab}	2.8±0.49^{a}	41.0±8.49^{a}
Diet E	15.6±0.75	52.0± 6.65	237.0	0.8±0.16^{a}	2.5±0.37^{a}	36.7±4.84^{a}

*1 Means±SD (n=3).

*2 Value with different subscript in the same column are significantly different ($p<0.05$).

due to the increase of the fiber content and soluble carbohydrate from SBM in the diets. Generally, the digestibility of vegetable feedstuffs was lower than that of the animal ones, especially to carnivorous fish[14,15].

Fish fed the test diets except for diet E produced good growth and high feed performance, as show in Fig. 1 and Table 3.

Fish fed diet A (FM) showed the same DGR as fish fed diet D (contained 75% SBM dietary protein) and diet E (contained 100% SBM dietary protein); but it was lower than that of fish fed diet B (contained 25% SBM dietary protein) and diet C (contained 50% SBM dietary protein) ($p<0.05$). Furthermore diet C showed the highest FE value compared to the other diets. It is suggested that giant gouramy can use SBM as well as FM as dietary protein sources.

Increasing SBM content more than 75% reduced the palatability and acceptability ($p<0.05$). However, good palatability and acceptability showed till 50% substitution of SBM in dietary protein (Table 3).

The average values of PR, LR, PER and ammonia excreted from fish fed the test diets are shown in Table 4. The PR tendency was the same as that of DGR and FE, in which diet A produced the same PR as diets B, D and E, however the value was lower than that of diet C. ($p<0.05$). The LR tendency was slightly different from PR; diets A, B and D showed a higher LR value than diet E; but their LR value were lower than diet C ($p<0.05$).

The fish fed diet C (contained 50% SBM dietary protein) produced the lowest ammonia value compared to the fish fed the other diets. In this experiment, ammonia value ranged from 0.0134 to 0.0292 mg/g body weight/hour.

Fig. 2 shows the comparison of the essential amino acids in the test diet and the whole body of giant gouramy. Shigueno[16] suggested that the whole body essential amino acid profile (by comparing the essential amino acids in the test diet and the whole body of fish) can be used to determine the essential amino acid requirement. And the deviation of the essential amino acid profile of the test diet can be used as an indicator of protein quality. As shown in Fig 2, arginine was found as the most limiting amino acid in the diet, indicated by the lowest availability among essential amino acids in the test diets.

RQ value was 0.71 (diet E), 0.88 (diet D), 0.85 (diet C), 0.88 (diet B) and 0.99 (diet A), respectively.

Table 4. Protein and lipid retention, protein efficiency ratio (PER) and ammonia production

	Retention (%)*		PER	Ammonia production (mg/kg/h)
	Protein	Lipid		
Diet A	36.0±5.19^{a}	85.9±12.30^{ab}	2.18	0.0185
Diet B	43.6±6.68^{a}	106.5±16.00^{b}	2.53	0.0292
Diet C	66.2±3.41^{b}	157.7±8.00^{c}	3.61	0.0134
Diet D	37.5±7.66^{a}	74.3±16.50^{ab}	2.31	0.0164
Diet E	33.5±4.35^{a}	65.7±9.40^{a}	2.09	0.0151

* Values with the different subscript in the same column are significantly different ($p<0.05$).

Discussion

Good palatability shows until 50% substitution of SBM in dietary protein. The increase of SBM content beyond 75% reduced the palatability and acceptability. It has been demonstrated that high replacement of FM by SBM reduced palatability and acceptability in general[17]. Palatability closely correlated with attractability depends on the free amino acids such as betain, glutamic acid, alanine

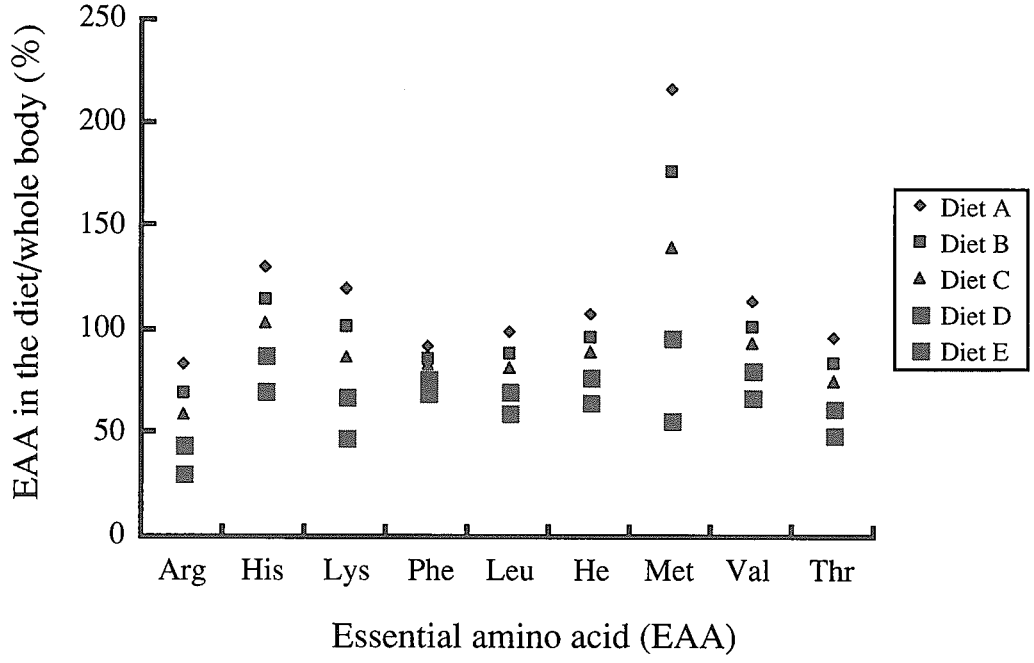

Fig. 2. The comparison (%) of essential amino acids in the test diet and the whole body of giant gouramy.

and glycine, which affects searching response[18]. The increase of SBM in dietary protein decrease the contents of alanine, glycine and glutamic acid as well; glutamic acid, glycine and alanine in the diet dropped to 26.8%, 74.4% and 34.9% from the whole body. Chhorn and David[19] also showed that poor palatability and water stability were observed in fish fed a soybean meal diet. It was suggested that SBM has a low free amino acid content besides sulfur containing amino acids and anti-nutritionals factor (heat stable group) such as saphonin and lysinolamine[20]. In this case, like *Penneaus duorarum*[19] giant gouramy showed good tolerance to accept SBM as a single source of to replace FM compared to other fish such as yellowtail[6,7], rainbow trout[2,21] and cod[9].

The increased protein contribution from SBM in the diet impacts on the decrease in digestibility of nutrients due to the increase of fiber content in the diets besides the unique oligosaccharide one[20]. Generally, the digestibility of vegetable sources is lower compared to animal sources, especially in carnivorous fish[14]. The digestibility of nutrients from SBM was much better compared to the other vegetable sources such as coconut and peanut meal[22].

The PR range of this experiment was between 33.52 and 66.20%. In the contrary, previous research[9,17] showed that the replacement of FM by SBM reduced protein retention.

The protein retained in the body in fact depended on the level of proteins in the diet; total energy in the test diet correlated to non-protein energy such as carbohydrates and lipids; and protein quality correlated to the essential amino acids. In this experiment, all diets were formulated to be isonitrogenous, isolipid and isoenergetic (12.05 Mj/Kg). It can be predicted that the difference in PR and PER is due to protein quality, especially the essential amino acid profile. Fig. 2 show that the essential amino acid profile in diet C (contained 50% SBM dietary protein) is near that in the whole body, compared to the other diets, and diet C showed a high PR and PER value. And it is also demonstrated that a combination of protein sources could fulfill the requirement of essential amino acid[2,10,23]. The low PER and PR in diets A, B, D, and E are caused by the high deviation in essential amino acids in the diet compared to the whole body.

Fig. 2 also show that arginine is the most limiting amino acid in the diet. The increasing arginine deficiency could be handled to certain level. It was suggested that young giant gouramy could diminish the arginine deficiency by using endogenous body protein. Meske and

Pfeffer[24] demonstrated that carp can utilize endogenous body protein up to 25.71%. Another suggestion indicated that young giant gouramy like *elasmobranchi* and *dipnio* can cover the arginine deficiency from the ornithine cycle[25]. Some warmwater species excrete a part of nitrogenous waste in urea form that is linked to the ornithine cycle, such as *Carasius auratus*, (71 mg urea/kg body weight/day) and *Leptocorcus armatus* (14 mg urea/kg body weight/day)[26].

Ammonia production can be used as an indicator of protein quality. Ammonia produced by fish is caused by an imbalance of energy, low content in non-protein energy sources (lipid and carbohydrate)[27,28]. The imbalance of amino acid content in the diet also reduced the protein synthesis per unit of protein digested[1]. The ammonia value observed in this research ranged from 0.0134 to 0.0292, and the low ammonia excretions yielding by fish fed diet C (0.0134) resulted in the highest protein retention.

Most metabolized energy used by fish correlated with feeding, came from protein, lipid and carbohydrate. Huisman[29] shows that RQ value was limited to 1 and 0.7; one indicated protein and carbohydrate metabolism and 0.7 lipid metabolism. From the RQ value, it is suggested that most metabolized energy used by giant gouramy came from protein followed by carbohydrate.

These results indicated that giant gouramy can use SBM as a source of protein up to 50%. It is concluded that SBM could substituted FM in the diet of giant gouramy. Furthermore a research can be conducted to adding arginine as the most limiting essential amino acid and free amino acid to increase the SBM contribution in dietary protein; protein utilization and palatability.

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ジャイアントグーラミィ，*Osphronemus gouramy* 用飼料における脱脂大豆油粕による魚粉代替

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グーラミィ用飼料における脱脂大豆油粕 (SBM) による魚粉の代替率を求めることを目的に，5種類の SBM の添加率が異なる飼料を用いて，16～20 g の魚を50日間飼育し，成長，飼料効率，タンパク効率，各栄養素の消化率，タンパクおよび脂質の魚体蓄積率さらにアンモニア排泄量などを求めた。その結果，SBM で飼料中のタンパク質のうちの50%ほどを代替した区が最も優れた飼育成績と低い窒素排泄量を示した。一方，50%以上代替した区では，摂餌率の低下やタンパク質と炭水化物の消化率の低下が顕著に見られた。今回の結果から，グーラミィ用飼料中のタンパク源のうち50%は SBM で代替できることがわかった。