

Application of fish bone meal from byproducts of fish processing industry in diets of juvenile striped catfish, *Pangasianodon hypophthalmus*

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Abstract. Fish bone meal from fish processing industry has been suggested to be a better candidate for fish meal replacement compared to alternative plant proteins in aquafeed. Nutritional composition analysis of bones from fish processing industry in Terengganu, Malaysia, showed that the fish bone meal contains high level of protein and lipid. The fish bone meal was then used in fish meal replacement study for Asian catfish, *Pangasianodon hypophthalmus*. Four diets were formulated to be iso-nitrogenous (35% crude protein) with 0%, 25%, 50% and 75% of the protein comes from laboratory prepared fish bone meal. The experimental diets were fed to juvenile *P. hypophthalmus* for 60 days. Detrimental effects were not apparent in survival of juvenile *P. hypophthalmus*. Weight gain and specific growth rate of the fish were not significantly ($P>0.05$) affected by the dietary treatments, however, increases in weight gain was demonstrated when dietary fish bone meal were up to 50% inclusion level. Whole body and muscle protein were significantly ($P<0.05$) higher in 25% and 50% fish bone meal based diets compared to the 75% fish bone meal based diet. Somatic parameters and blood parameters of the fish remain parallel over all dietary treatments. The results indicated that juvenile *P. hypophthalmus* could well utilize fish bone meal as protein source in their diets, subsequently reducing cost while optimizing their culture productivity. Based on the present experimental condition, it is concluded that at least half of the dietary fish meal could be replaced with fish bone meal in *P. hypophthalmus*.

Key Words: alternative protein, fish meal, iso-nitrogenous, specific growth rate, fish body composition.

Introduction. In aquaculture industry, fish meal are dedicatedly sought after for their high and well balanced essential amino acids and fatty acids, low carbohydrates, high digestibility and low levels of anti-nutritional factors (Tacon & Metian 2008; Lee et al 2010). However, with fish meal prices increase concomitantly in the past decades, and the questionable continuance of the source, the usage of fish meal as a sole protein source in aquafeeds will no longer be sustainable in long run (Tacon & Metian 2008). Protein derived from plants has been suggested to be more sustainable protein source due to their high availability and generally less expensive (Francis et al 2001).

However, other than lack of certain essential amino acids and fatty acids (Carter & Hauler 2000), the use of plant derived materials as alternative protein is limited by the presence of variety anti-nutritional substances (Lee et al 2010). Anti-nutritional compounds such as protease enzyme inhibitors, phytic acid and saponins were found in most commercially used plant protein such as soy bean meal, rapeseed meal and cotton seed meal (Francis et al 2001). In addition, almost 70% important minerals such as phosphorus in plant sources are bound as phytate (a salt form from phytic acid) that could not be digested or absorbed by fish (Lim & Lee 2008). Phytic acid also can chelate other divalent and trivalent cations beside phosphorus, such as calcium, magnesium, zinc, copper and iron, resulting in significantly decreased bioavailability of these minerals (Wise 1983). These resulted in more complicated research trying to balance the amino

acids profiles by providing several protein ingredients in the diets (Tacon & Metian 2008; Kader & Koshio 2012).

In recent years, fishery products marketed are in the supply of frozen fish fillets, shrimps and prawns in the form of ready to cook or ready to eat meals to cater consumer demands. The fish flesh was collected, leaving the head, bone and viscera to waste. In estimation, fish processing waste plus by catch is approximately 90% of the annual harvest of fish and could be used for meal production (New 1996). Bones constitute a significant part of the waste; with approximately 10-15% of total fish biomass is bones from the head and vertebrae (Toppe et al 2007). As it represent a significant part of the cut offs from the filleting industries, a better utilization of this raw material for various applications is a matter of great scientific interest.

Fish bone meal from fish processing industry has been suggested to have high nutritional value such as high level of protein, lipids and minerals (Malde et al 2010; Lee et al 2010; Toppe et al 2007). Protein and lipid content of fish bone meal tested in 13 different marine fish varied from 29% to 42% and from 2% to 50%, respectively (Toppe et al 2007). The amino acids profiles also shows that fish bone meal contain all 10 essential amino acids such as arginine, methionine and leucine while the fatty acids profiles show a high level of unsaturated fatty acids, mainly DHA (docosahexaenoic acid) and EPA (ecosapentaenoic acid). As the same in fish meal, fish bone meal also contains high macro-minerals such as calcium, phosphorus, magnesium and zinc (Toppe et al 2007). Although, feeding trials using fish bone meal as partial or fully replacement of fish meal or plant protein has shown positive outcomes in growth and survival of rainbow trout (*Oncorhynchus mykiss*) (Lee et al 2010) and juvenile coho salmon (*Oncorhynchus kisutch*) (Rathbone et al 2001), there is lack of scientific documentation concerning content and function of fish bone meal in fish culture.

Hence, the objective of this study is to investigate the suitability and the effects of using fish bone meal from by-products of fisheries industry in aquafeeds. *Pangasianodon hypophthalmus* was chosen as the aquaculture species candidate to be supplemented with dietary fish bone meal as it is one of the important species cultured in Asian region including Vietnam, Malaysia, Indonesia, Thailand and Bangladesh (Griffiths et al 2010).

Material and Method

Diet preparation. Four diets were formulated to contain increasing level of fish bone meal at the expense of fish meal between 0 and 75% (Table 1) and the diets were also made isolipidic and isonitrogenous by balancing level of fish meal, fish bone meal and rice bran.

Table 1
Proximate composition of key ingredients used in experimental diets (dry matter basis, %)

Ingredients	Parameters			
	Dry matter	Crude protein	Crude lipid	Ash
Fishmeal	86.95	81.32	13.19	11.75
Fish bone meal	91.61	57.06	18.64	21.46
Soybean meal	85.73	44.7	0.78	5.88

All of the ingredients used were supplied by Sri Purta Sdn Bhd with the exception of fish bone collected from local fisherman. The fish bone were sun dried for one hour to remove excess liquid and dried in oven at 60°C for further 48 h. The fish bone then were grinded and sieved with 100 µm to make the meal. All dry and wet ingredients of the diets were mixed well separately before the two were combined and thoroughly blended in an electric mixer. Water was included at approximately 30% of the ingredients until it becomes homogenous dough and the dough was made into pellet in a laboratory pellet mill through a 2 mm sieve. The pellets were then oven dried for overnight at 60°C and

stored in refrigerator until used. Prior to formulation of experimental diets, all protein based ingredients were analyzed for proximate composition.

Feeding trial. The feeding trial was conducted at freshwater hatchery of School of Fisheries and Aquaculture Sciences, Universiti Malaysia Terengganu (UMT). Twelve 50 L tanks were prepared to contain dechlorinated fresh water and an individual unit of filtration system each. 500 juvenile *P. hypophthalmus* were purchased from supplier and reared in similar tanks and condition for acclimatization. Three replicates were set up for each dietary treatment with 12 juvenile *P. hypophthalmus* per replicates. The tanks were also provided with aeration throughout the experimental period and the water quality was maintained by changing 50% of the water in the tank every other day before each feeding time to ensure good water quality. Any mortality was recorded daily during feeding time and all fish were fed with the experimental diets by hand, to apparent visual satiation, twice daily for 60 days at 08:00 and 18:00.

Composition analysis of ingredients and experimental diets. The nutritional composition of the ingredients and the experimental diets were determined for crude protein by Kjeldahl method through the process of digestion, distillation and titration. The percent of nitrogen in the samples was then multiply with protein factor of 6.25 for animal based samples and 5.38 for plant materials. Ash of the samples were determined by dry ashing method, where the samples were burned in muffle furnace at 600°C for 6 hours while moisture content was calculated by the loss of water content upon drying of samples at 105°C for 4 h. Total lipid was calculated by using petroleum ether in Soxhlet Extraction Machine (AOAC 2001).

Data collection and statistical analysis. To assess growth over time, specific growth rate (SGR) based on weight and standard length of juvenile *P. hypophthalmus* were calculated using following equation:

$$\text{SGR} = (\ln F - \ln I) / T \times 100$$

Where *F* is the final value of weight or standard length, *I* is the initial value of weight or standard length and *T* is total culture period (days).

Survival, final weight, standard length and SGR data were analyzed using one-way ANOVA after confirmation of normality and homogeneity of variances. Survival data were arcsine transformation before analysis. Significant differences between treatments were determined using Tukey's test and the level of significance difference was set at $p < 0.05$. All statistics were performed using the SPSS statistic software version 16.

Results. The analyzed protein contents of the experimental diets used in present study are shown in Table 1 and Table 2. All diets contained similar level of protein with minimal deviation.

Survival and growth parameter. Survival of juvenile *P. hypophthalmus* was relatively high for all diet treatments and ranged from 88-100% (Table 3). The highest survival was recorded for fish fed the diet containing 0% FBM while the lowest survival was recorded for the diet with 50% FBM. However, no significant differences were detected between these dietary treatments ($p > 0.05$). Following the same pattern as survival, no significant differences were found for final weight (g), specific growth rates (SGR) (% day⁻¹) and weight gain (%) among all treatments. However, the mean cumulative growth for juvenile *P. hypophthalmus* was consistently higher when the fish were fed with diets supplemented with 50% FBM. Approximately 20% less weight gain was obtained for fish fed diets that contained 25% FBM or lower (Table 3).

Table 2

Composition of the experimental diets (% dry matter basis) with different levels of fish bone meal (FBM) and fish meal fed to juvenile *Pangasianodon hypophthalmus*

Ingredients	0% FBM	25% FBM	50% FBM	75% FBM
	Diet 1	Diet 2	Diet 3	Diet 4
Fish meal	28	21	14	7
Fishbone meal	0	9.1	18.2	27.3
Soybean meal	18	18	18	18
Fish oil	6	5.5	5.0	4.5
Wheat flour	25	25	25	25
Vit-min premix	3	3	3	3
Mollases	2	2	2	2
CMC	1	1	1	1
Rice bran	17	15.4	13.8	12.2
Analyzed nutritional composition (%)				
Crude protein	35.91	35.18	35.78	35.17
Crude lipid	8.90	9.68	10.49	10.33
Ash	8.77	8.62	11.16	12.39

With the exception of self-prepared fish bone meal, all ingredients were supplied by Sri Purta Sdn Bhd, Kedah, Malaysia.

Table 3

The mean (\pm SE) of survival, initial weight (In wt), final weight (Fn wt), specific growth rates (SGR) and weight gain (Wg) of juvenile *Pangasianodon hypophthalmus* fed with different levels of fish meal and fish bone meal (FBM)

Parameters	0% FBM	25% FBM	50% FBM	75% FBM
	Diet 1	Diet 2	Diet 3	Diet 4
Survival (%)	100 \pm 0.00	97.33 \pm 4.62	88.67 \pm 9.82	97.33 \pm 4.62
In wt (g)	7.57 \pm 0.15	8.12 \pm 0.82	7.55 \pm 0.30	7.12 \pm 0.16
Fn wt (g)	11.73 \pm 0.16	12.47 \pm 0.35	13.33 \pm 1.20	11.91 \pm 1.34
SGR (% day ⁻¹)	0.89 \pm 0.07	0.88 \pm 0.26	1.31 \pm 0.29	1.04 \pm 0.26
Wg (%)	55.05 \pm 5.20	54.85 \pm 19.26	76.41 \pm 8.83	67.39 \pm 20.51

Values are means of triplicate groups \pm S.E. Within a row, means with the same letters are not significantly different ($P>0.05$).

Whole body composition, somatic parameters, haematological parameters and bactericidal activity. Mean crude protein and crude lipid of whole body composition was significantly higher for fish fed with 25% FBM, however for protein concentrated in fish muscle, the highest significant value was obtained when the fish were fed with 50% FBM ($p<0.05$). Whole body moisture was also significantly increased with the increasing level of fish meal replacement. No significant differences were found for somatic parameters, haematological parameters (Table 4) and bactericidal activity (Figure 1) of juvenile *P. hypophthalmus* blood between the treatments.

Table 4

The whole body composition, muscle content and somatic parameters of juvenile *Pangasianodon hypophthalmus* fed with different level of fish meal and fish bone meal (FBM)

Parameters	0% FBM	25% FBM	50% FBM	75% FBM
	Diet 1	Diet 2	Diet 3	Diet 4
<i>Whole body composition (% wet basis)</i>				
Moisture	73.56±0.35 ^a	72.78±1.08 ^a	76.06±4.16 ^{ab}	78.84±1.42 ^b
Crude protein	15.70±0.21 ^{ab}	17.46±0.70 ^b	13.56±1.41 ^a	13.49±1.35 ^a
Crude lipid	6.52±0.12 ^a	6.68±0.30 ^b	6.35±0.74 ^{ab}	5.60±0.37 ^a
Ash	4.39±0.15	4.56±0.22	3.55±0.43	3.71±0.61
<i>Muscle content (%)</i>				
Crude protein	16.07±0.48 ^a	16.75± 1.14 ^a	18.57± 0.34 ^b	15.72± 0.98 ^a
Ash	9.40± 0.02	8.74± 0.46	8.95± 0.46	8.96± 0.66
<i>Somatic parameters (%)</i>				
CF	0.75±0.50	0.75±0.12	0.77±0.01	0.71±0.73
HSI	1.25±0.61	0.83±0.06	0.93±0.1	1.00±0.12
VSI	2.34±1.13	1.59±0.09	2.01±0.32	2.17±0.12
<i>Haematological parameters</i>				
Ht (%)	39.00±2.65	37.33±3.51	40.00±2.83	33.7±9.50
RBC count (mm ⁻³)	4.69±7.68	5.27±2.64	3.77±1.09	3.33±1.23
EL (µm)	9.77±0.88	9.44±0.43	8.47±0.16	9.16±0.35
EW (µm)	7.89±0.77	7.22±0.38	7.29±1.09	6.61±0.31
ES (µm ²)	60.81±10.48	53.58±4.97	48.44±6.33	47.58±4.02

Values are means of triplicate groups ±S.E.

Within a row, means with the same letters are not significantly different (P>0.05).

CF - Condition factor, HIS - Hepatosomatic index, VSI - Viscerasomatic index, Ht - Hematocrit, RBC - red blood cell, EL - erythrocyte length, EW - erythrocyte width, ES - erythrocyte size.

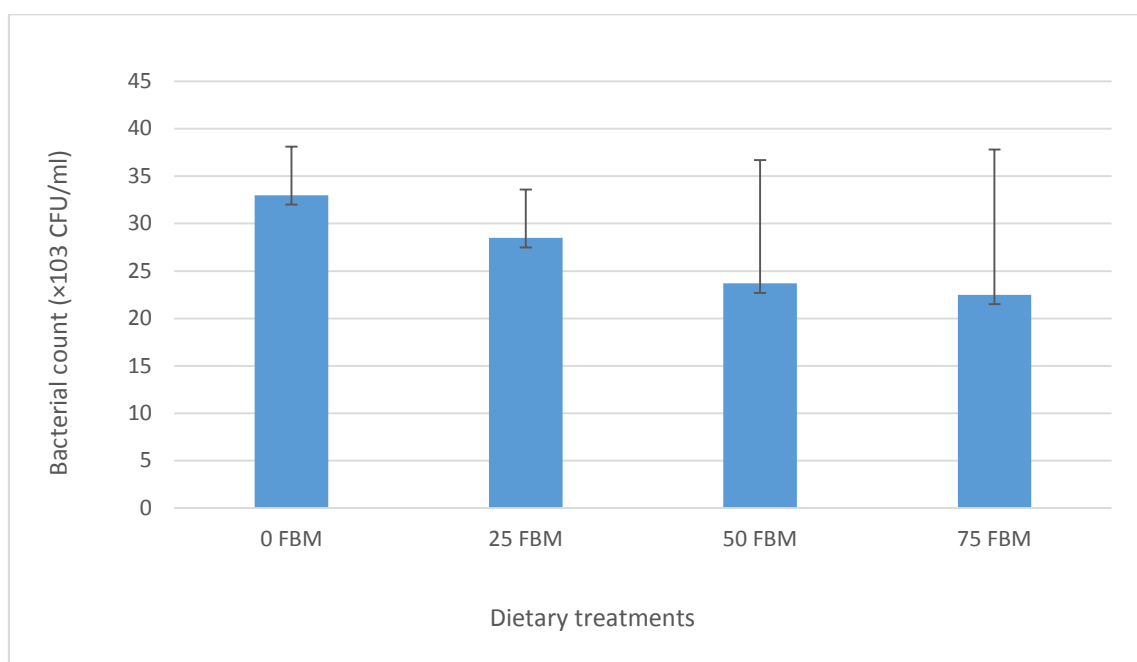


Figure 1. The bactericidal activity of juvenile *Pangasianodon hypophthalmus* juvenile fed with different level of fish meal and fish bone meal (FBM).

Discussion. In concomitant with dramatic aquaculture expansion, it is necessary to come up with alternative protein sources or a mix of various plant proteins (Hardy & Tacon 2002; Gatlin et al 2007). The optimal protein sources for aquaculture industry

should satisfy three main criteria which are cost effective, high nutrient contents and sustainable. Many studies has focused on using plant protein as an alternative to fish meal (Collins et al 2013) however alternative plant proteins are often correlated with cons factors of low protein content, imbalance essential and non-essential amino acid and the presence of anti-nutritional factors (Carter & Hauler 2000).

Fish bone is largely composed of calcium phosphate and it is made from seafood containing high level of phosphorus and calcium at 6.5-9.7% and 13-18%, respectively (Lee et al 2010). Fish bone meal used as a dietary ingredient in a study on Atlantic cod, *Gadus morhua* was produced by separation of the bone fraction from a commercial fish meal (Toppe et al 2007). The study reported that almost 45% of dietary fish meal protein can be replaced by the fish bone meal containing 56% crude protein. The use of fish bone meal as a replacement to the fish meal can be promising alternative source of protein in aquafeed, especially when *P. hypophthalmus* has shown significant increase in growth performances when they were fed with alternative proteins of meat and bone meal (Kader et al 2011), soybean meal (Phumee et al 2010) and rice bran (Da et al 2011). In present study, replacement of fish meal with fish bone meal up to 50% in diet of juvenile *P. hypophthalmus* yield the highest growth and the growth was only comprised when the fish contain 25% fish bone meal or lower.

Chemical analysis (crude protein, crude lipid, moisture and crude ash) on whole body composition of *P. hypophthalmus* at the end of feeding trial is used to determine the influence of feed on fish composition. Body composition of fish can be affected by endogenous factors (size, sexes and stage of life cycle) and exogenous factors (diet composition, feeding frequency and temperature) (Soltan et al 2008). As other endogenous factors were consistently maintained, it is expected that the composition of the feed in present study that contained various level of fish meal and fish bone meal will influence the body composition of the fish. Replacement of fish meal with 25% fish bone meal significantly improved crude protein and crude lipid content of whole body composition of juvenile *P. hypophthalmus* while replacement of fish meal with 50% fish bone meal significantly improved the crude protein content of fish muscle. In contrast, highest accumulation of muscle protein was observed in juvenile *P. hypophthalmus* fed diets with fish meal replaced by 0% and 15% soybean meal only while higher replacement of fish meal with soybean meal slightly declined the protein in muscle (Phumee et al 2010). The differences between these two studies on the same species suggested that fish bone meal when used to replace fish meal have higher nutrient retention efficiencies when compared to plant protein, soybean meal. Lowest level of crude lipid in whole body composition was obtained when the juvenile *P. hypophthalmus* fed diet that contained the highest replacement of fish meal; 75% fish bone meal. Since all of the experimental diets were formulated to contain same level of lipid, the decrease lipid deposition in the body composition suggested lower utilization of lipid with increasing fish bone meal. Condition factor (CF), hepasomatic index (HSI) and viscerasomatic index (VSI) usually are used to reflect degree of nourishment and commonly associated with sexual maturity (Williams 2000). No significant differences between the CF, HSI and VSI in present study could well be explained that increasing level of fish bone meal in the diets does not have any detrimental effects on the somatic parameters.

Blood parameters had been use widely as indicators of the physiological health and nutritional status of fish, and major changes that could during these conditions includes fluctuation of red blood cells (RBC), white blood cells (WBC), hormones, hematocrit, hemoglobin concentration, leukocytes counts and other basic components (Jahanbakshi et al 2013). No obvious pattern on the blood parameters of juvenile *P. hypophthalmus* with exception of erythrocytes size that appears to be decreasing as level of dietary fish bone meal increased. Similarly, when *Escherichia coli* was used to trigger the bactericidal activity of serum collected from juvenile *P. hypophthalmus* used in present study, the bactericidal activity appears to be decreasing as dietary fish bone meal increased. As erythrocytes play major roles in transporting oxygen (Morera & Mackenzie 2011) and bactericidal activities are considered one of the important defense mechanism during microbial infections (Hollebecq et al 1995), further research are needed to clarify

whether inclusion of higher level fish bone meal in diets of *P. hypophthalmus* could negatively impact their culture performances in long run.

Conclusions. The search for fish meal replacement is inevitable as the pressure on this mostly sought ingredient is no longer reliable. Fish processing industry produces huge amount of by products that have great potential to be used as fish meal substitutes in aquafeed. The present study has demonstrated that fish bone meal have a huge potential as alternative protein and could replace at least half of the dietary fish meal from the diets of fish like *P. hypophthalmus*. The research should be expanded to other commercial aquaculture fish to aid in sustaining world of aquaculture industry.

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