Efficacy of commercial diets with varying levels of protein on growth performance, protein and lipid contents in carcass of Acehnese mahseer, *Tor tambra*

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Abstract

The Acehnese mahseer, locally known as keureling, *Tor tambra* is one of the highest economically valued freshwater fishes found in Aceh waters. The culture of *T. tambra* was initiated in Aceh Province, Indonesia more than five years ago. However, the growth rate in captivity has been low, probably due to feeding problems. Hence, the objective of the present study was to evaluate the optimum level of dietary protein in commercial feeds for growth improvement. Three commercial feeds with different levels of protein i.e. 20%, 25%, and 30%, were tested in this study. A total of nine semi-concrete ponds were used and each pond was stocked with 15 mahseer fingerlings. The experimental fish were fed at a ratio of 3% of body weight twice a day for 60 days. The ANOVA tests revealed that dietary protein levels significantly affected growth performance, protein efficiency ratio, protein retention efficiency and lipid content in carcass but did not significantly affect survival rate and protein content in the carcass (p>0.05). The results showed that the growth performance, survival rate, and protein content increased with increasing protein level in the diet and the best composition was 30% of protein.

Keywords: *Tor tambra*, Commercial diets, Acehnese mahseer, Carcass analysis, Feed conversion ratio

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Introduction

There are at least 114 species of freshwater fishes found in Aceh waters, Indonesia (Muchlisin and Siti-Azizah, 2009), 14 species of which have considerably higher economic values including the genus *Tor*, three species of which have been recorded in Aceh waters i.e. *T. soro*, *T. tambra* and *T. tambroides* (Muchlisin, 2013a). The genus *Tor* is a popular group and inhabits several rivers of Southeast Asia including Indonesia. In the past decade, wild populations decreased due to habitat degradation caused by agricultural development, overfishing (Ismail *et al*., 2011) and deforestation and in consequence the group is now endangered. The latter causes soil erosion on hilly rivers and increases in siltation (Hossain *et al*., 2002). Wild populations of Acehnese mahseer (*T. tambra*) have also decreased drastically because it has become a major target for fishing.

The basic information of the bio-ecology of Acehnese mahseer was reported by Muchlisin *et al*. (2015a) and the broodstock domestication technique was also documented by Muchlisin (2013b). In addition, studies on the parasites infestation on *T. tambra* were reported by Muchlisin *et al*. (2014) and Muchlisin *et al*. (2015b).

The mahseer has a great potential for the aquaculture industry (Misieng *et al*., 2011; Muchlisin, 2013a), especially in the wake of the recent push towards the culture of indigenous species (Singh *et al*., 2012). Therefore, mahseer fishing activity from the wild should be prohibited and fishermen should shift to alternative forms of income such as aquaculture so that the supply of capture fishes is sustainable. For this purpose, feeding technology needs to be studied comprehensively. Currently, fish farmers in Aceh Province catch wild mahseer fingerlings for growing out in flowing water ponds. However, the growth rate has been found to be low. Several studies have been conducted to overcome this obstacle, for example by addition of vitamins, enzymes and probiotics in diets (Muchlisin *et al*., 2016a; Muchlisin *et al*., 2016b; Muchlisin *et al*., 2017). However, these efforts have not been sufficiently effective in overcoming this problem. Probably this is because an optimum protein requirement for the *T. tambra* has not been evaluated.

Studies on protein requirements for several *Tor* species have been reported, for instance on *Labeobarbus douronensis*, the synonym for *T. douronensis* (Redjeki *et al*., 1999), *T. putitora* (Hossain *et al*., 2002; Islam and Tanaka, 2004) and *T. tambroides* (Misieng *et al*., 2011). However, the optimum protein requirement for Acehnese mahseer (*T. tambra*) has never been explored.

The optimum level of protein requirement is species dependent but most fish need protein in the range of 15-45% (Lee, 1981). Protein is the main and an expensive component in fish diets and contributes significantly to aquaculture operational costs reaching

to as much as 40-70% (Muchlisin, 2005). Protein is the main component of fish tissues (Watanabe, 1988). Its role is to develop and maintain tissue growth (Hepher, 1988). It is also essential in the formation of enzymes, hormones as well as vitamin metabolism (Furuichi, 1988; Brett and Groves, 2003). Energy from the diet is not only used for growth but also for basal metabolism and for daily activities and reproduction. Therefore, information on the minimum protein requirements for optimum growth of fish is crucial (Hossain *et al*., 2002) and it is a prerequisite to finding a cost-effective diet. This information is important for the culture of *T. tambra* fingerlings to support a systematic aquaculture program of this threatened species. Hence, the objective of the present study was to examine the optimum level of protein requirements of commercial diets for practical application.

Materials and methods

Diets and proximate analysis

Three commercial diets with different protein levels were examined in the study namely; (1) Turbo Feed T79 (20% dietary protein), (2) Hi Provit 784 (25% dietary protein), (3) Bintang 888s (30% dietary protein). The selected commercial diets were tested for their nutrient compositions (crude protein, crude lipid, and crude fiber); the analyses were carried out according to AOAC (2000). Dry matter content was calculated from weight loss after 72 h at 70^{o}C. Crude protein was measured using the Kjeldahl technique. A total of one gram of sample was weighed and placed into a Kjeldahl beaker, followed by 10 g catalyst and 25 mL sulfuric acid. The samples were heated to 250^{o}C for 20 minutes and carefully shaken, and the temperature was increased to 350 ^{o}C for 2 hours. Then, the samples were cooled for 10 minutes, and 300 mL of distilled water was added to the beaker. Diluted samples were distilled followed by titration using 0.1 N HCl. Crude lipid was measured after chloroform-methanol extraction. Samples were homogenized with a high-speed homogenizer for 5 min and lipid was determined gravimetrically after solvent separation and vacuum drying while ash was calculated from the weight loss after incineration of the sample for 24 h at 550^{o}C in a muffle furnace. The proximate composition of experimental diets is presented in Table 1.

Experimental fish and weaning process

A total of 135 *T. tambra* fingerlings with an average weight and length of 250 g and 19 cm, respectively were collected using casting nets from Nagan River, Indonesia. Fishes were acclimatized in a ground pond (5.0 x 5.0 x 1.2 m and water level 0.90 m) with water flowing continuously for one week. During acclimatization, the fish were kept unfed. After the acclimatization period, fish were weaned to the experimental diets (20% protein) over two weeks at a feeding

rate of 3% of total body weight twice a day (08.00 AM and 06.00 PM).

Stocking and feeding

After the weaning process, the fish were distributed randomly into nine outdoor semi- concrete ponds with water flowing continuously (5.0 m × 4.0 m × 1.2 m, and the water level of 0.90 m) and each tank was stocked with 15 fish. Fish were weighed and measured for initial weight and length prior to stocking. Feeding was carried out at a feeding rate of 5% of body weight per day for 60 days. A total of 10 fish were sampled randomly and their weights measured to adjust the feeding ration at two week intervals. After eight weeks of feeding, all the fish were measured and weighed for final weight and length, respectively.

Growth performance and carcass quality analysis

The survival rate was calculated based on Muchlisin *et al*. (2016a) as follows: SR (%)= [(No-Nt)/No] x 100, where SR= survival rate (%), Nt= total fish died during the experiment, No= total fish at the start of the experiment. The weight gain was examined using the formula: Total weight gain (g) = Final weight of fish (g) - Initial weight of fish (g); while, the daily growth rate was calculated as follows= Total weight gain (g)/ feeding duration (days); and the Specific growth rate (SGR) was calculated based on Biswas (1993) as follows: SGR= [(Ln Wt) – (Ln Wo)/ t] x 100, where SGR is specific growth rate (% day^{-1}), Wt is weigh of fish at the end of experiment (g), Wo is the initial weight of fish (g), and *t* is duration of experiment (days).

The protein retention efficiency and protein efficiency ratio were calculated based on Farhat and Khan (2011) as follows: Protein retention efficiency, PRE (%) = (protein gain/ protein fed) × 100 and Protein efficiency ratio (PER)= wet weight gain (g)/ protein fed (g). While, the proximate composition (protein and lipid) of the carcass were analyzed based on Association of Official Analytical Chemists (AOAC, 2000).

Statistical analyses

Percentage data were arcsine transformed prior to analysis. The one-way analysis of variance (ANOVA) followed by comparisons of means by Duncan's multiple range tests were used to analyze each variable. All statistical analyses were performed using the SPSS ver.17.

Results

The results indicated that the growth performance and survival rate of Acehnese mahseer, *T. tambra* fingerlings increased with increasing protein levels in the diet. The ANOVA tests showed that protein levels in the diet significantly affected weight gain, specific and daily growth rates, protein efficiency ratio, protein retention efficiency and lipid retention efficiency of mahseer fingerlings ($p<0.05$), but it did not have significant effects on the

survival rate (p>0.05). The Duncan multi-range test revealed that the highest weight gain, daily growth and specific growth rates, and protein efficiency ratio were found in fish fed 30% protein (diet C), and it was significantly different from that in fish fed 20% and 25% dietary protein (diet A and diet B). Highest survival rate was also observed in the group fed 30% protein (diet C), but this value was not different from that in other groups (Table 2).

In addition, the ANOVA test showed that the protein level in the diet did not significantly affect protein content of the carcass (p>0.05), but significantly affected lipid content of the carcass (p<0.05).

In general, the protein content of carcass increased with increasing protein levels but it was not significantly different among the diets.

The higher lipid content of carcass was found with the 25% protein diet, but it was not significantly different from the treatment fed 30% protein (Table 2). There were no significant differences in water quality parameters among the treatments, i.e. the water temperature ranged between 29.1 and 29.8^{o}C, pH ranged from 6.9 to 7.2, and dissolved oxygen ranged from 5.9 to 6.5 mg L^{-1}.

Table 1: Proximate composition of the commercial diets used in this study.

No.	Nutrient	Diet A (Turbo Feed T79)	Diet B (Hi Pro Vit 784)	Diet C (Bintang 888s)
1.	Crude protein (%)	20	25	30
2.	Crude lipid (%)	4	4	4
3.	Crude fiber (%)	8	6	6
4.	Ash (%)	12	12	13
5.	Moisture (%)	12	12	12

Table 2: Growth performance, survival rate, protein and lipid retentions of *T. tambra* carcass fed three levels of dietary protein for 60 days. Mean of values in the same row followed by a different superscript are significant different (p< 0.05).

No.	Parameters	Diet A (20% dietary protein)	Diet B (25% dietary protein)	Diet C (30% dietary protein)
1.	Weight gain (g)	14.50±3.93^{a}	15.14±7.93^{a}	39.78±20.69^{b}
2.	Length gain (cm)	5.14±0.42^{a}	4.92±0.89^{a}	7.90±2.94^{b}
3.	Daily growth (g day^{-1})	0.24±0.07^{a}	0.25±0.13^{a}	0.66±0.34^{b}
4.	Specific growth rate (% day^{-1})	0.13±0.04^{a}	0.18±0.08^{a}	0.44±0.25^{b}
5.	Survival rate (%)	82.22±7.20^{a}	85.00±10.00^{a}	90.00±4.71^{a}
6.	Protein content of carcass (%)	18.49 ± 2.57^{a}	20.63 ± 0.07^{a}	22.17±1.34^{a}
7.	Lipid content of carcass	1.35± 0.36^{a}	2.37 ± 0.35^{b}	1.44 ± 0.06^{ab}
8.	Protein efficiency ratio (PER)	0.73±0.08^{a}	0.61±0.11^{a}	1.33±0.17^{b}
9.	Protein retention efficiency (PRE%)	92.45 ± 2.57^{b}	82.52 ± 0.07^{ab}	73.9±1.34^{a}
10.	Lipid retention efficiency (LRE%)	33.75± 0.36^{a}	59.52 ± 0.35^{b}	36.00 ± 0.06^{ab}

Discussion

The study revealed that the growth and survival rates of Acehnese mahseer, *T. tambra* increased with increasing protein level in the diets from 20% to 30% protein, where the 30% protein was the best level for *T. tambra*. Hossain *et al*. (2002) and Misieng *et al*. (2011) reported that the optimum level of protein for *T. putitora* and *T. tambroides* was 40%, for both while Ng *et al*. (2008) reported that the *T. tambroides* fingerlings need about 45-50% dietary protein. In addition, Redjeki *et al*. (1999) reported that the growth performance of *Labeobarbus douronensis* (synonym name for *T. douronensis*) increased with increasing protein levels in the diet from 16% to 20%. Therefore, this study revealed that the crude protein requirement of *T. tambra* was lower compared to *T. putitora* and *T. tambroides*, but it was higher than that for *T. douronensis.*

In general, the optimum level of protein requirement is species and feeding habit dependent. For example, carnivorous fishes need higher protein content compared to herbivorous and omnivorous fishes. However, the protein requirement for broodstock is higher compared to younger fish in general as reported by Abidin *et al*. (2006). According to Muchlisin *et al*. (2015a), *T. tambra* is an omnivorous freshwater fish, with typical protein requirements in the range of 25-40% (Craig and Helfrich, 2002) as was also recorded in this study. Besides being influenced by feeding the growth performance of fish could also be affected by the environmental conditions such as water quality and photoperiod. The continuous flowing water system was used in this experiment which ensured good conditions for the fish to grow well (Marion, 1998; Mamedov *et al*., 2016).

This study revealed that the protein content in the carcass of *T. tambra* increased with increasing protein levels in the diet. Similar result were reported in *T. putitora* (Hossain *et al*., 2002; Islam and Tanaka, 2004), and bagrid catfish, *Mystus nemurus* (Abidin *et al*., 2006). The protein retention efficiency (PRE) of *T. tambra* decreased as protein in diets increased, shile the protein efficiency ratio (PER) was reduced when protein level was increased from 20% to 25%. However, it increased when protein level in the diet went up to 30%. A similar trend was recorded in juvenile Chinese sucker, *Myxocyprinus asiaticus* (Zhang *et al*., 2009). This phenomenon is probably due to the limiting efficiency of protein digestion and retention of the fish at a certain level. Thus, if the protein content in the diet exceeds or is lower than the digestion efficiency level, the protein cannot be digested and is stored optimally in the carcass and is released through the feces or urine.

Besides protein, lipid is also an important nutrient in fish diets as it is the key energy source and is composed of essential fatty acids. Lipids are needed for maintaining cell membranes, vitamin solvent and are a component

for hormone regulation and body buoyancy of fish (NRC, 1983). The results showed that the lipid content in the carcass increased from 1.35% to 2.37% with increasing protein levels in the diet from 20% to 25%, respectively, but then decreased to 1.44% when protein level increased to 30%. The present study indicates that lipid content of Acehnese mahseer carcass increased directly with protein dietary content up to a certain level but then decreased with increased uptake of protein. A similar result was reported in *T. putitora* where the lipid content in the carcass increased with increasing dietary protein from 25% to 40%, and then reduced slightly when dietary protein reached 45-50% (Islam and Tanaka, 2004). However, no significant differences of lipid content in the carcass of Malaysian mahseer, *T. tambroides* fed with dietary protein ranging between 30-50% were observed (Misieng *et al*., 2011).

Conclusions

The protein levels in the diet had significant effects on the growth performance, and lipid content in the carcass, protein efficiency ratio, protein retention efficiency and lipid retention efficiency of *T. tambra*, but it did not affect the survival rate and protein content in carcass significantly. The study revealed that commercial diets with 30% protein are optimum for Acehnese mahseer *T. tambra*.

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References

Abidin, M.Z., Hashim, R. and Chien, A.C.S., 2006. Influence of dietary protein levels on growth and egg quality in broodstock female bagrid catfish (*Mystus nemurus* Cuv. & Val.). *Aquaculture Research*, 37, 416-418.

Association of Official Analytical Chemists (AOAC), 2000. Official methods of analysis. Helrich, K. (ed) 15th eds. Association of Official Analytical Chemists, Arlington, Virginia.

Biswas, S.P., 1993. Manual of methods in fish biology. South Asian Publisher Pvt Ltd, New Delhi, Indian. 157P.

Brett, J.R. and Groves, T.D. 2003. Physiological energetics. *In*. Hoar, W.S., Randal, D.J., Brett, J.R. (Ed):

Fish Physiology. Academic Press, London.

Craig, S. and Helfrich, L.A., 2002. Understanding fish nutrition, feeds, and feeding. Virginia State University Publication, Virginia.

Farhat., Khan, M.A., 2011. Growth, feed conversion, and nutrient retention efficiency of African Catfish, *Clarias gariepinus*, (Burchell) fingerling fed diets with varying levels of protein. *Journal of Applied Aquaculture,* 23, 304–316.

Furuichi, M., 1988. Fish nutrition. *In*. Watanabe, T. (Ed). Fish nutrition and mariculture, JICA textbook, The General Aquaculture Course. Kanagawa International Fisheries Training Center, Tokyo.

Hepher, B., 1988. Nutrition of pond fishes. Cambridge University Press, New York.

Hossain, M.A., Hasan, N., Azadshah, A.K.M. and Hussain, M.G., 2002. Optimum dietary protein requirement of mahseer, *Tor putitora* (Hamilton) fingerlings. *Asian Fisheries Science*, 15(**3**), 203-214.

Islam, M.S. and Tanaka, N., 2004. Optimization of dietary protein requirement for pond-reared mahseer *Tor putitora* Hamilton (Cypriniformes: Cyprinidae). *Aquaculture Research*, 35, 1270-1276.

Ismail, M.F.S., Siraj, S.S., Daud, S.K. and Harmin, S.A., 2011. Association of annual hormonal profile of the gonad maturity of mahseer (*Tor tambroides*) in captivity. *General and Comparative Endocrinology*, 170, 125-130.

Lee, J.S., 1981. Commercial catfish farming. Chapman and Hall, New York.

Mamedov, C.A., Hajiyev, R.V. and Huseynli, S.V., 2016. Growing of sterlet *Acipenser ruthenus* in pool conditions at various modes of illumination. *Journal of Survey in Fisheries Sciences*, 2(**2**), 79-81.

Marion, J.E. 1998. Water quality for pond aquaculture. International Center for Aquaculture and Aquatic Environment, Alabama Agriculure Station, Auburn University, Alabama. 37P.

Misieng, J.D., Kamarudin, M.S. and Musa, M., 2011. Optimum dietary protein requirement of Malaysian mahseer (*Tor tambroides*) fingerling. *Pakistan Journal of Biological Sciences*, 14(**3**), 232-235.

Muchlisin, Z.A., 2005. Factors affect gonadal development and egg quality of female broodfish: a review. *Biology*, 4(**6**), 411-427.

Muchlisin, Z.A. and Siti-Azizah, M.N., 2009. Diversity and distribution of freshwater fishes in Aceh waters, northern Sumatera, Indonesia. *International Journal of Zoological Research*, 5(**2**), 62-79.

Muchlisin, Z.A., 2013a. Potency of freshwater fishes in Aceh waters as a basis for aquaculture development program. *Journal Iktiologi Indonesia*, 13(**1**), 91-96.

Muchlisin, Z.A., 2013b. A preliminary study on the domestication of wild

keureling broodfish (*Tor tambra*). Proceedings of The Third Annual International Conference Syiah Kuala University in Conjunction with The Second International Conference on Multidisciplinary Research, November 2-4, 2013, Banda Aceh, Indonesia.

Muchlisin, Z.A., Munazir, A.M., Fuady, Z., Winaruddin, W., Sugianto, S., Adlim, M., Fadli, N. and Hendri, A., 2014. Prevalence of ectoparasites on mahseer fish (*Tor tambra* Valenciennes, 1842) from aquaculture ponds and wild population of Nagan Raya District, Indonesia. *HVM Bioflux*, 6(**3**), 148-152.

Muchlisin, Z.A., Batubara, A.A., Siti-Azizah, M.N., Adlim, M., Hendri, A., Fadli, N., Muhammadar, A.A. and Sugianto, S., 2015a. Feeding habit and length weight relationship of keureling fish, *Tor tambra* Valenciennes, 1842 (Cyprinidae) from the western region of Aceh Province, Indonesia. *Biodiversitas*, 16(**1**), 89-94.

Muchlisin, Z.A., Fuadi, Z., Munazir, A.M., Fadli, N., Winaruddin, W., Defira, C.N. and Hendri, A., 2015b. First report on Asian fish tapeworm (*Bothriocephalus acheilognathi*) infection of indigenous mahseer (*Tor tambra*) from Nagan Raya District, Aceh Province, Indonesia. *Bulgarian Journal of Veterinary Medicine*, 18(**4**), 361-366.

Muchlisin, Z.A., Arisa, A.A., Muhammadar, A.A., Fadli, N., Arisa, I.I. and Siti-Azizah, M.N., 2016a. Growth performance and feed utilization of keureling (*Tor tambra*) fingerlings fed a formulated diet with different doses of vitamin E (alpha-tocopherol). *Archives of Polish Fisheries*, 23, 47-52.

Muchlisin, Z.A., Afrido, F., Murda, T., Fadli, N., Muhammadar, A.A., Jalil, Z. and Yulvizar, C., 2016b. The effectiveness of experimental diet with varying levels of papain on the growth performance, survival rate and feed utilization of keureling fish (*Tor tambra*). *Biosaintifika*, 8(**2**), 172-177.

Muchlisin, Z.A., Murda, T., Yulvizar, C., Dewiyanti, I., Fadli, N., Afrido, F., Siti-Azizah, M.N. and Muhammadar, A.A., 2017. Growth performance and feed utilization of keureling fish *Tor tambra* (Cyprinidae) fed formulated diet supplemented with enhanced probiotic. F1000Research, 6, 137.

National Research Council (NRC), 1983. Nutrient requirements of warm water fishes and shellfish. Nutritional Academy of Sciences, Washington DC.

Ng, W.K., Abdullah, N. and de Silva, S.S., 2008. The dietary protein requirement of the Malaysian mahseer, *Tor tambroides* (Bleeker) and the lack of protein-sparing action by dietary lipid. *Aquaculture*, 284, 201-206.

Redjeki, S., Diani, S. and Supriatna, A., 1999. Pengaruh pemberian pakan buatan dengan kadar protein berbeda pada pertumbuhan ikan kancra bodas (*Labeobarbus douronensis*). Limnotek, 6(**2**), 33-37.

Singh, S.K., Mishra, U., Roy, S.D., Chadha, N.K. and Venkateshwarlu, G., 2012. Effect of feeding enriched formulated diet and live feed on growth, survival and fatty acid profile of deccan mahseer, *Tor khudree* (Sykes) first feeding fry. *Aquaculture Research and Development*, 3(**4**), 1-6.

Watanabe, T., 1988. Fish nutrition and mariculture. Japan International Cooperation Agency, Tokyo.

Zhang, Z., Gong, S., Yuan, Y., Chu, Z. and Yuan, H., 2009. Dietary protein requirement for juvenile Chinese sucker (*Myxocyprinus asiaticus*). *Journal of Applied Ichthyology*, 25, 715–718.