# DIGESTIBILITY OF SELECTED FEED INGREDIENTS FOR TIGER GROUPER, *Epinephelus fuscoguttatus*

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**ABSTRACT**

The apparent digestibility (AD) of eight feed ingredients are widely available in Indonesia was determined. In each of two 5x5 latin-square experimental, tiger grouper *Epinephelus fuscoguttatus* juveniles (100—150 g) were fed a reference diet and four test diets in accordance with the latin-square design. Test feed ingredients were substituted at rates of 40% for animal meals or 30% for plant meals. Chromic oxide was used as the digestibility marker. In determining the ingredient AD, the substitution ratio was calculated as the proportion of the nutrient (or energy) contributed by the test ingredient on an 'as-is' basis. Digestibility tanks were steeply slope 200 L cylindro-conical tanks with a bottom outlet to facilitate faecal collection, which was carried out at 3-hourly intervals throughout the day. Each collection period took 5—7 days with a similar acclimatization time between diets. A combined ANOVA of the data for both experimental showed no difference (P>0.05) in the AD's for each reference diets. Thus for comparative purpose, the derived AD's of the test ingredients were analysed as a single ANOVA. The digestibility of animal meals was generally high (>59% for dry matter, >83% for protein, >65% for lipid, and >70 for gross energy) while that of plant meals was slow (<53% for dry matter, <53% for protein, <66% for lipid, and <46% for gross energy). This information will enable grow-out feeds for tiger grouper to be formulated on a least-cost digestible nutrient basis.

**KEYWORDS: nutrition, feed ingredient**

## INTRODUCTION

Marine feed ingredients and specifically fish meal, are in high demand as protein sources for fish feeds. Fish meal is regarded as the best protein source for fish feeds, being high in protein, has a close to ideal profile of essential amino acids and few if any anti-nutritional factors (Allan *et al.*, 2000). The world demand for fish meal keeps increasing. However, world production of fish meal has not increased beyond a production of about 6.5 million tonnes per year since the early 1990's (Shepherd *et al.*, 2005).

Tiger grouper require high dietary protein of 47%—50% (Laining *et al.*, 2003a; Giri *et al.*, 2004), and typically manufactured diets contain from 40 to 62% of expensive fish meal. Replacement of fish meal with a cheaper, high-quality source of protein might reduce the production costs of culturing high tropic level species such as tiger grouper, *Epinephelus fuscoguttatus*. The assessment of different sources of protein for fish feed is an international and increasing research priority (Manzi, 1989; Hardly & Kissil 1997 *in* Allan *et al.*, 2000).

Apart from considerations of palatability and anti-nutritional factors, the apparent digestibility of an ingredient determines how much of the nutrients in the ingredient can be utilized by the animal. The higher the digestibility of the ingredient, more of its nutrient content will be available to support growth and other metabolic functions of the animal. Consequently, the digestibility of an ingredient is an important measure of an ingredient's nutrient quality.

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The potential sources of protein available in large quantities in Indonesia are poultry offal, golden snail, green mussel and mysid meal. Yellow and white corn meal, rice bran, and sorghum meal are also carbohydrate sources that are in plentiful supply in Indonesia. This paper reports a digestibility study to determine the apparent digestibility of these feed ingredients because of their potential to be used in the manufacture of cost-effective feeds for tiger grouper.

## MATERIALS AND METHODS

### *Experiment Overview*

The study comprised two independent 5 x 5 latin square experiment with five diets, a reference and four test feed ingredient diets, being examined in each experiment. The reference diet was the same for each experiment with four test feed ingredients of animal origin - poultry offal meal, mysid meal, golden snail meal and green mussel meal - being examined in Experiment 1and four of plant origin - fine rice bran, corn meal (yellow and white) and sorghum meal - being examined in Experiment 2. Test feed ingredients were substituted into the reference diet at the rate of either 40% or 30% for ingredients of animal or plant origin, respectively (Table 1). Chromic oxide was added at an inclusion rate of 1% of the diet as the digestibility marker (Takeuchi, 1988; De Silva & Anderson, 1995).

All test feed ingredients were obtained locally. Each set involved five faecal collection periods with all fish, randomly in turn, being fed each of the five diets in accordance with the latin-square design of the experiment. The proximate nutrient and gross energy composition of the test ingredients are shown in Table 2.

Table 1. Formulation of diets used in determining the apparent digestibility of feed ingredients in Experiment 1 and 2

| Ingredient | Diet type | | |
|---|---|---|---|
| | Refference | 40% substituted[1] | 30% substituted[2] |
| | As used product (g) | | |
| Trash fish (fresh)[3] | 90 | 54 | 63 |
| Fish meal | 30.5 | 18.3 | 21.4 |
| Shrimp head meal | 8 | 4.8 | 5.6 |
| Squid liver meal | 9 | 5.4 | 6.3 |
| Soybean meal | 8 | 4.8 | 5.6 |
| Wheat flour | 12 | 7.2 | 8.4 |
| Animal test ingredient | 0 | 40 | 0 |
| Plant test ingredient | 0 | 0 | 30 |
| Fish oil | 3 | 1.8 | 2.1 |
| Soybean oil | 1.5 | 0.9 | 1.1 |
| Vitamin premix[4] | 3 | 1.8 | 2.1 |
| Mineral premix[5] | 1.5 | 0.9 | 1.1 |
| Chromic oxide | 1 | 1 | 1 |
| Total (as used)[6] | 167.5 | 141 | 147.7 |

[1] For poultry offal meal, mysid meal, golden snail meal and green mussel meal
[2] For fine rice bran, yellow and corn meal and sorghum meal
[3] Dry matter content of 25%
[4] Vitamin mix provided (mg/kg diet): Thiamin-HCl, 50; riboflavin, 50; Ca-panthothenate, 100; niacin, 20; pyridoxine-HCl, 40; biotin, 6; folic acid, 15; inositol, 2000; para-aminobenzoic acid, 50; astaxanthin, 150; menadione, 40; calciferol, 19; µ–tocopherol, 200; ascorbic acid, 1500; cyanocobalamin, 1; and choline, 1000
[5] Trace mineral provided (mg/kg diet): $FeCl_3.4H_2O$, 1660; $ZnSO_4$, 100; $MnSO_4$, 67.5; $CuSO_4$, 20; KI, 1.5; and $CoSO_4.7H_2O$, 1.0
[6] Equates to approximately 100 g on a dry basis

Table 2. The proximate nutrient and gross energy composition of air-dry test feed ingredients

| Test feed ingredient | Dry matter | Crude protein | Total lipid | Crude fibre | Ash | Gross energy (kJ/g) |
|---|---|---|---|---|---|---|
| | (%) | | | | | |
| Poultry offal meal | 94.5 | 59.2 | 16.2 | 1.8 | 5 | 22.5 |
| Mysid meal | 93.9 | 57.6 | 9.1 | 3 | 15 | 18.8 |
| Golden snail meal | 94.3 | 53.7 | 4.9 | 2.6 | 11 | 18.5 |
| Green mussel meal | 92.8 | 52.9 | 12.4 | 1.9 | 9 | 20.2 |
| Rice bran | 93.8 | 13.7 | 14.9 | 5.8 | 8.8 | 18.0 |
| Corn meal (yellow) | 92.4 | 10.2 | 3.8 | 2.2 | 1.7 | 16.8 |
| Corn meal (white) | 91.3 | 10.2 | 4.6 | 2.1 | 1.6 | 16.8 |
| Sorghum | 91.7 | 9.4 | 1.4 | 1.1 | 1.9 | 16.4 |

One cohort of juvenile tiger grouper, *Epinephelus fuscoguttatus*, bred at the Gondol RIM, Bali hatchery was air-transported to South Sulawesi and acclimated to the floating net cage facility at Awerange Bay, Barru Regency, South Sulawesi. Prior to starting on the experiment, fish were held for several months in 2x2x2 m floating net cages and acclimatized to being fed a moist reference diet. Afterwards, the fish were selected on the basis of freedom from defects, initial weight of 100—150 g and then distributed into 5 floating net cages of 1x1x2 m at a density of 25 fish/cage. For each collection period, the fish were acclimated to their test diet for 5 d and fed twice daily, morning and late afternoon. On the 6$^{th}$ day, 20 fish from each cage were carefully transferred into 200 L conical, 35% slope, bottom tank for faecal collection. In each period, faeces were collected over 5—7 days, which enabled a sufficient quantity of faeces to be collected for chemical analysis.

### *Chemical Analyses and Calculations*

A representative sample of feed or dried faeces was homogenized using a mortar and pestle and analyzed essentially by AOAC International (1999) procedures: dry matter (DM) by oven drying at 105ºC for 16 h; ash by ignition in a muffle furnace at 550ºC for 24 h and crude protein by micro-Kjeldahl analysis with distillation into 4% boric acid and titration with sulphuric acid using methyl red indicator for end point determination. Total lipid was determined gravimetrically following chloroform: methanol extraction of the sample (Bligh & Dyer, 1959); energy by bomb calorimetry and chromium after acid digestion using a Shimadzu UV-VIS 2401 PC spectrophotometer.

The apparent digestibility coefficient (ADC) of dry matter (DM), crude protein (CP), total lipid (TL) and gross energy (GE) for the reference and test diets were calculated using the equation:

$$ADC\ (\%) = 100 * \left[1 - \left(\frac{M_D * A_F}{M_F * A_D}\right)\right]$$

Where $M_D$ and $M_F$ are the concentrations (on a DM basis) of the marker in the ingested diet and faeces, respectively and $A_D$ and $A_F$ are the concentrations (on a DM basis) of the nutrient (or analyte) in the ingested diet and faeces, respectively. The AD of the test feed ingredient was calculated using Pfeffer *et al*. (1995) equation:

$$ADC_{NI} = \frac{1}{\alpha}\ [AD_{NT} - (1 - \alpha)AD_{NR}]$$

Where $AD_{NI}$, $AD_{NT}$ and $AD_{NR}$ are apparent digestibility of the nutrient in the ingredient, the test diet and the reference diet, respectively. The substitution rate (*a*) was calculated as the proportion of the nutrient (or energy) contributed by the test ingredient in the reference diet on an 'as-fed wet' basis (Bureau *et al.,* 1999).

The ADC data for diets and those derived for the test feed ingredient in each experiment were subjected to a least squares analysis of variance for a latin square design, isolating effect due to column (fish) and row (collection period). A combined analysis of the ADC's for

diets for the two experiments found no significant difference between the reference diets in each experiment (P>0.05). Thus, the derived ADC for the test feed ingredients were analysed as a single factor ANOVA with differences between treatments (ingredients) tested for significance using Fisher's protected *t*-test (Snedecor & Cochran, 1989) wherein differences between means were examined only when the *F*-test of the ANOVA was significant (P<0.05).

## RESULTS AND DISCUSSION

Digestibility coefficients for the diets fed in experimental 1 and 2 are detailed in Tables 3 and 4, respectively. A statistical analysis of the data for both experiments, isolating the effects of experiment, diet and associated interactions, showed that the observed ADCs for each of the reference diets were not significantly different (P>0.05) from one another. Thus, the ADCs derived for each of the test feed ingredients in each experiment were analysed as a combined ANOVA and the results presented in Table 5.

The apparent digestibility of the protein of animal meals was high (77% to 89%) and not significantly different from each other but significantly higher than for the plant meals (41% to 52%), which were statistically not different from each other. Total lipid digestibility tended to follow a similar pattern as for protein digestibility but differed in that golden snail meal was lower than for the other

Table 3. The dry matter (DM), crude protein (CP), total lipid (TL) and gross energy (GE) apparent digestibility coefficients of the diets examined in Experiment 1

| Diets label | DM | CP | TL | GE |
|---|---|---|---|---|
| | Apparent digestibility coefficient (%) | | | |
| Reference | 66.1$^{a}$ | 80.4$^{cd}$ | 88.4$^{b}$ | 79.7$^{ab}$ |
| Poultry offal | 65.8$^{b}$ | 82.4$^{b}$ | 86.1$^{c}$ | 80.1$^{ab}$ |
| Mysid | 68.3$^{a}$ | 85.0$^{a}$ | 90.0$^{a}$ | 81.0$^{a}$ |
| Golden snail | 63.4$^{c}$ | 78.6$^{d}$ | 84.2$^{d}$ | 76.2$^{c}$ |
| Green mussel | 60.1$^{b}$ | 82.0$^{bc}$ | 88.6$^{b}$ | 79.1$^{b}$ |
| ± sem | 0.63 | 0.66 | 0.42 | 0.57 |

$^{A,B,C,D}$ Within columns, means without a common letter differ (P<0.05)

Table 4. The dry matter (DM), crude protein (CP), total lipid (L) and gross energy (GE) apparent digestibility coefficients of diets examined in Experiment 2

| Diet label | DM | CP | TL | GE |
|---|---|---|---|---|
| | Apparent digestibility coefficient (%) | | | |
| Reference | 65.0$^{a}$ | 79.4$^{a}$ | 86.7$^{a}$ | 78.9$^{a}$ |
| Rice bran | 56.5$^{c}$ | 75.9$^{d}$ | 79.2$^{c}$ | 67.4$^{c}$ |
| Maize – yellow | 59.9$^{b}$ | 76.7$^{c}$ | 81.6$^{b}$ | 68.8$^{bc}$ |
| Maize – white | 60.3$^{b}$ | 76.7$^{c}$ | 80.6$^{bc}$ | 68.9$^{bc}$ |
| Sorghum | 61.4$^{b}$ | 77.6$^{b}$ | 84.6$^{a}$ | 70.0$^{b}$ |
| ± sem | 0.57 | 0.22 | 0.53 | 0.61 |

$^{A,B,C,D}$ Within columns, means without a common letter differ (P<0.05)

Table 5. The dry matter (DM), crude protein (CP), total lipid (TL) and gross energy (GE) apparent digestibility coefficients of the test feed ingredients derived from diets examined in Experiment 1 and 2

| Test feed ingredient | DM | CP | TL | GE |
|---|---|---|---|---|
| | Apparent digestibility coefficient (%) | | | |
| Poultry offal meal | 65.4$^{ab}$ | 84.0$^{a}$ | 84.1$^{ab}$ | 80.5$^{a}$ |
| Mysid meal | 69.9$^{a}$ | 88.9$^{a}$ | 93.9$^{a}$ | 82.8$^{a}$ |
| Golden snail meal | 59.4$^{b}$ | 76.9$^{a}$ | 62.0$^{c}$ | 70.8$^{b}$ |
| Green mussel meal | 66.0$^{a}$ | 83.4$^{a}$ | 88.9$^{a}$ | 78.3$^{a}$ |
| Rice bran | 36.3$^{d}$ | 43.7$^{b}$ | 65.8$^{bc}$ | 39.1$^{d}$ |
| Maize – yellow | 47.8$^{c}$ | 41.9$^{b}$ | 30.4$^{d}$ | 41.3$^{cd}$ |
| Maize – white | 48.8$^{c}$ | 41.3$^{b}$ | 30.9$^{d}$ | 41.7$^{cd}$ |
| Sorghum | 52.5$^{c}$ | 52.1$^{b}$ | 23.7$^{d}$ | 45.1$^{c}$ |
| ± sem | 1.64 | 5.23 | 6.9 | 1.72 |

$^{A,B,C,D}$ Within columns, means without a common letter differ (P<0.05)

animal meals (62 cf 84%-94%, respectively) while the rice bran was higher than for the other plant meals (66 cf 24%-31%, respectively). Dry matter and energy digestibilities of poultry offal meal, mysid meal and green mussel meal were not significantly different from each other and significantly higher in the case of the mysid and mussel meals than the golden snail meal, which was significantly higher than the plant meals. For the plant meals, rice bran had the lowest, and sorghum the highest, DM and energy apparent digestibilities.

Tiger grouper digested the animal feed ingredients more efficiently than the plant feed ingredients with DM, CP, total lipid and energy ADC's generally being significantly higher. Of the animal meals, golden snail meal was the least digestible while the fibre-rich rice bran had the lowest DM, CP and energy digestibilities of the plant meals. However, the lipid digestibility of the rice bran was comparatively high, much higher than all other plant meals and equivalent to that of golden snail meal. Similar results wherein animal meals have high digestibility and plant meals much lower digestibilities have been reported for other carnivorous fish such as rainbow trout *Oncorhynchus mykiss* (Austreng *et al*., 1977), red drum *Sciaenops ocellatus* (Gaylord & Gatlin, 1996; McGoogan & Reigh, 1996), yellow tail *Seriola quinqueradiata* (Masumoto *et al*., 1996) humpback *Cromileptes altivelis* and gold-spot *Epinephelus coioides* groupers (Laining *et al*., 2003b; Eusebio *et al*., 2004), catfish *Clarias spp* (Usman *et at*., 2003) and cobia *Rachycentron canadum* (Zhou *et al*., 2004). The low ADC's of these plant ingredients were probably due to their high content of nitrogen free extract (NFE), particularly the complex carbohydrate components of seeds, which are poorly digested by piscivorous fish (Lupatsch *et al*., 1997; Cowey & Walton, 1989).

The low protein ADC of the plant meals was not unexpected as studies with other grouper species have shown similar low digestibilities. For instance, tiger grouper digested only about 47% of the protein of rice bran in this study, which was in the range of values of 43% for gold-spot grouper (Eusebio *et al*., 2004) and 59% for humpback grouper (Laining *et al*. 2003b). However, higher protein digestibilities for rice bran were reported by Sullivan & Reigh (1995) for hybrid striped bass *Morone saxatilis x M. chrysops* (71%) and by McGoogan & Reigh (1996) for red drum (77%). Such effects may indicate differences between species in their capacity to digest the fibre matrix of rice bran although differences in how the rice bran was handle and processed may equally have played a role. In our study, the rice bran originated from a local rice mill and was used without any further refining. Importantly, all diets were fed as moist pellets and manufactured using a cold extrusion process. Cooking of the meal, either

that which results from steam conditioning of the mix prior to pelleting or that occurring during hot extrusion processing can increase starch gelatinization and improve digestibility (De Silva & Anderson, 1995; Davis & Arnold, 1995). The rice bran examined in the above cited hybrid striped bass and red drum studies was finely ground prior to mixing and dried after pelleting. This processing may have improved the digestibility of the rice bran.

The digestibility of unsaturated lipids, including those from plants, is usually high for carnivorous fish with estimates typically between 90 and 100% (Caballero *et al*., 2002; Carter *et al*., 2003; Usmani *et al*., 2003; Zhou *et al*., 2004). In the present study, lipid digestibility was high (>84%) for each of the animal meals other than for golden snail meal, which at 62% was slightly lower than rice bran (66%). The comparatively low lipid digestibility of the snail meal was surprising since about two thirds of its lipid comprises unsaturated fatty acids and of these one third are fatty acids with three or more unsaturated bonds (Bombeo-Tuburant *et al*., 1995). It is well estabilished that the digestibility of lipid in fish, and animals generally, is influenced by chain length and saturation of the constituent fatty acids, decreasing with increasing length and increasing with increasing degree of unsaturation (Olsen *et al*., 1998; Johnsen *et al*., 2000; Menoyo *et al*., 2003; Ng *et al*., 2004). Perhaps the quantitatively low content of lipid in the snail meal (4.9%) might explain the apparent anomalous result although the comparatively low DM and protein digestibility values for snail meals suggests that other factors may also have had a role in reducing overall digestibility. The low lipid digestibility of the cereal grains (24% to 31%) is also an anomalous result but probably due to their low lipid contents (1% to 5%) and the associated mathematical errors in deriving the estimate rather than the lipid in these meals being poorly digestible.

There was a clear distinction between the animal and plant meals in the amount of digestible energy they provided: 71% to 83% for the animal meals and 39% to 45% for the plant meals. This was to be expected given the low protein, DM and lipid digestibility of the plant meals and the comparatively high digestibility of the animal meals. A similar result has been reported for other carnivorous fish (Sullivan & Reigh, 1995; Burel *et al*., 2000; Booth *et al*., 2005).

In conclusion, the study has shown that tiger grouper efficiently digest animal feed ingredients, indicating that the ingredients have potential to be used as dietary replacements for fish meal. Some caution is advised for golden snail meal since its overall digestibility was inexplicably poor, perhaps indicating that unknown factors may be affecting its nutritional value. While the plant meals were not easily digested, nevertheless they are an integral part of compounded diets and data on their digestibility are important when formulating diets to satisfy the animal's requirements for digestible nutrients. However, steam conditioning of these meals prior to pelleting or using hot extrusion manufacturing procedures may increase their digestibility for tiger grouper. The high digestibility of green mussel suggests its polyculture with fish may provide an additional local source of protein for the fish while helping to alleviate nutrient output from farms. Until more eco-friendly pelleted feeds become commercially available, such integrated systems may be an effective way of reducing the environmental impacts of cage aquaculture.

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## REFERENCES

Allan, G.L., S. Parkinson, M.A. Booth, D.A.J. Stone, S.J. Rowland, J. Frances, and R. Warner-Smith. 2000. Replacement of fishmeal in diets for Australian silver perch, *Bidyanus bidyanus*: I. Digestibility of alternative ingredients. Aquaculture. 186: 293—310.

AOAC International. 1999. Official Methods of Analysis, 16th edn. Association of Official Analytical Chemists International, Gaithersberg, Maryland, USA. 1,141 pp.

Austreng, E., S. Risa, D.J. Edwards, and H. Hvidsten. 1977. Carbohydrate in rainbow trout diets: II. Influence of carbohydrate levels on composition and feed utilization of fish from different families. Aquaculture. 11: 39—50.

Bligh, E.G. and W.J. Dyer. 1959. A rapid method of total lipid extraction and purification.

Canadian Journal of Biochemistry and Physiology. 37: 911—917.

Bombeo-Tuburan, I., S. Fukumoto, and E.M. Rodriguez.. 1995. Use of the golden apple snail, cassava, and maize as feeds for the tiger shrimp, *Penaeus monodon*, in ponds. Aquaculture. 131: 91—100.

Booth, M.A., G.L. Allan, and A.J. Anderson. 2005. Investigation of the nutritional requirements of Australian snapper *Pagrus auratus* (Bloch & Schneider, 1801): apparent digestibility of protein and energy sources. Aquacult. Res. 36: 378—390.

Bureau, D.P., A.M. Harris, and C.Y. Cho. 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 180: 345—358.

Burel, C., T. Boujard, F. Tulli, and S.J. Kaushik. 2000. Digestibility of extruded peas, extruded lupin, and rapeseed meal in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). Aquaculture. 188: 285—298.

Caballero, M.J., A. Obach, G. Rosenlund, D. Montero, M. Gisvold, and M.S. Izquierdo. 2002. Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. Aquaculture. 214: 253—271.

Carter, C.G., T.E. Lewis, and P.D. Nichols. 2003. Comparison of cholestane and yttrium oxide as digestibility markers for lipid components in Atlantic salmon (*Salmo salar* L.) diets. Aquaculture. 225: 341—351.

Cowey, C.B. and M.J. Walton. 1989. Intermediary metabolism. In: Halver, J.E. (Ed.) Fish Nutrition, 2nd edn. Academic Press, New York. NY. p. 259—329.

Davis, D.A. and C.R. Arnold. 1995. Effects of two extrusion processing conditions on the digestibility of four cereal grains for *Penaeus vannamei*. Aquaculture. 133: 287—294.

De Silva, S.S. and T.A. Anderson. 1995. Fish Nutrition in Aquaculture. Chapman & Hall, London, UK. 319 pp.

Eusebio, P.S., R.M. Coloso, and R.E.P. Mamauag. 2004. Apparent digestibility of selected ingredients in diets for juvenile grouper, *Epinephelus coioides* (Hamilton). Aquacult. Res. 35: 1,261—1,269.

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

Giri, N.A., K. Suwirya, and M. Marzuqi. 2004. Optimum level of dietary protein and lipid for rearing juvenile tiger grouper (*Epinephelus fuscoguttatus*). In: Rimmer, M.A., McBride, S. & Williams, K.C. (Eds.) Advances in Grouper Aquaculture. Australian Centre for International Agricultural Research, Canberra. p. 92—94.

Johnsen, R.I., O. Grahl-Nielsen, and A. Roem. 2000. Relative absorption of fatty acids by Atlantic salmon *Salmo salar* from different diets, as evaluated by multivariate statistics. Aquacult. Nutr. 6: 255—261.

Laining, A., N. Kabangnga, and Usman. (2003a) Pengaruh protein pakan yang berbeda terhadap koefisien kecernaan nutrien serta performansi biologis kerapu macan, *Epinephelus fuscoguttatus* dalam keramba jaring apung. J. Pen. Perik. Indonesia. 9(2): 29—34.

Laining, A., Rachmansyah, T. Ahmad, and K. Williams. 2003b. Apparent digestibility of selected feed ingredient for humpback grouper, *Cromileptes altivelis*. Aquaculture. 218: 529—538.

Lupatsch, I., G.W. Kissil, D. Sklan, and E. Pfeffer. 1997. Apparent digestibility coefficients of feed ingredients and their predictability in compound diets for gilthead seabream, *Sparus aurata* L. Aquacult. Nutr. 3: 81—89.

Manzi, J.J. 1989. Aquaculture research priorities for the 1990s. World Aquacult. 20: 29—32.

Masumoto, T., T. Ruchimat, Y. Ito, H. Hosokawa, and Shimeno, S. 1996. Amino acid availability values for several protein sources for yellowtail (*Seriola quinqueradiata*). Aquculture. 146: 109—119.

McGoogan, B.B. and R.C. Reigh. 1996. Apparent digestibility of selected ingredient in red drum (*Sciaenops ocellatus*) diets. Aquaculture. 141: 233—244.

Menoyo, D., C.J. Lopez-Bote, Bautista, J.M. and A. Obach. 2003. Growth, digestibility and fatty acid utilization in large Atlantic salmon (*Salmo salar*) fed varying levels of n-3 and saturated fatty acids. Aquaculture. 225: 295—307.

Ng. W-K., T. Sigholt, and J.G. Bell. 2004. The influence of environmental temperature on the apparent nutrient and fatty acid digestibility in Atlantic salmon (*Salmo salar* L.) fed finishing diets containing different

blends of fish oil, rapeseed oil and palm oil. Aquacult. Res. 35: 1,228—1,237.

Olsen, R.E., R.J. Henderson, and E. Ringo. 1998. The digestion and selective absorption of dietary fatty acids in Arctic charr, *Salvelinus alpinus*. Aquacult. Nutr. 4: 13—21.

Pfeffer, E., S. Kinzinger, and M. Rodehutscord. 1995. Influence of the proportion of poultry slaughter by-products and of untreated or hydrothermically treated legume seeds in diets for rainbow trout, *Oncorhynchus mykiss* (Walbaum), on apparent digestibilities of their energy and organic compounds. Aquacult. Nutr. 1: 111—117.

Shepherd, C.J., I.H. Pike, and S.M. Barlow. 2005. Sustainable feed resources of marine origin. In: B. Howell & R. Floss (eds.) EAS Special Publication No. 35. pp. 59-66. European Aquaculture Society, The Hague, The Netherlands.

Snedecor, G.W. and W.G. Cochran. 1989. Statistical Methods, 8th edn. Iowa State University Press, Ames, Iowa, USA. 503 pp.

Sullivan, J.A. and R.C. Reigh. 1995. Apparent digestibility of selected feedstuff in diets for hybrid striped bass (*Moreno saxatilis* ♀ *x Moreno chrysops* ♂). Aquaculture. 138: 313—322.

Takeuchi, T. 1988. Laboratory work-chemical evaluation of dietary nutrients. In: Watanabe, T (ed.) Fish Nutrition and Mariculture. JICA Kanagawa International Fisheries Training Centre, Tokyo. p. 179—233.

Usmani, N., A.K. Jafri, and M.A. Khan. 2003. Nutrient digestibility studies in *Heteropneustes fossilis* (Bloch), *Clarias batrachus* (Linnaeus) and *C. gariepinus* (Burchell). Aquacult. Res. 34: 1,247—1,253.

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