Available online at www.sciencedirect.com

ScienceDirect

Agriculture and Agricultural Science Procedia 9 (2016) 403 – 410

Agriculture and Agricultural Science Procedia

International Conference on Food, Agriculture and Natural Resources, IC-FANRes 2015

Effects of Feeding Diets Containing *Azolla Pinnata* and Probiotic on the Growth and Nutritional Content of Patin Fish (*Pangasius djambal*)

Ika Oktavianawati*, Dodik Andinata, Alviona Noer Isnaeni, Meirinda Hermiastuti, Novita Rahmawati, Wuriyanti Handayani, I Nyoman Adi Winata

Dept. Chemistry, Fac. Math and Sciences, University of Jember, Jl. Kalimantan III/25 Jember, 68121, East Java, Indonesia

Abstract

The main aim of this research is to explore the effect of supplementary feeding, *Azolla piñata* and probiotics, on the quality and profile of fatty acids in patin fish oil extracts, protein and amino acids content. In this research, patin fish were divided into three different feeding treatments: pellets only (P1) as a control; pellets and *A. piñata* (P2); pellets and probiotics (P3). These fishes were characterized for their growth profile, fish oil quality, protein and amino acids content. P3 shows the best growth profile indicated with the heaviest bodyweight compare to other fish samples. Quality of fish lipids was determined by an analysis of acid value, saponification number, peroxide value, iodine number, along with an analysis of the composition of fatty acids via gas chromatography. The results show that the highest oil yield from patin fish meal extracted using dry rendering method was obtained from P3. P3 also shows the lowest saponification number and peroxide value. Oleic acid and palmitic acid are the major constituent of unsaturated fatty acid and saturated one, respectively, in all these fish samples. The highest crude protein content which was determined using Kjeldahl method also presented in P3. The amount and the dominant amino acids content in the protein of P1, P2, and P3, in the order, are 13 amino acids: tryptophan, 14 amino acids: asparagine, and 13 amino acids: glutamic acids.

Peer-review under responsibility of the organizing committee of IC-FANRes 2015

Keywords: patin; fish oil; crude protein; amino acids

* Corresponding author. Tel.: +62-331-334293; fax: +62-331-330225.
E-mail address: ika.fmipa@unej.ac.id

Peer-review under responsibility of the organizing committee of IC-FANRes 2015
doi:10.1016/j.aaspro.2016.02.156

1. Introduction

Patin fish is originally from Indonesia (Ariyanto & Utami, 2006) and is one of the most popular freshwater fish to consume in the world (Thuy et al., 2002). This fish has high freshness level, lack of fish scent (Domiszewski et al., 2011), low lipid content (Orban et al., 2008) and low cholesterol level (Rahardja et al., 2011). In detail, this catfish contains protein, fat, carbohydrate, ash and water up to 68.8%, 5.8%, 1.5%, 5.0% and 75.7%, respectively (Panagan et al., 2011; Rahardja et al., 2011; Suryaningrum et al., 2010).

Patin fish (*Pangasius djambal*) is a kind of an aquaculture fish grown in pond, which is also commonly called as fish farming. Feeding is an important factor on fish farming since the nutrient content and feed composition will influence the performance of patin fish. The common main feed for patin fish are pellet, crustaceae, insect and molusc (Susanto & Amri, 2002; Rahardja et al., 2011), while the feed additive are rotifers, probiotic, small fish and water plants. Probiotic is food supplements containing microorganisms to help fermentation in digestion tract so nutrition would be easier to be absorbed (Andriyanto et al., 2010; Yeo and Kim, 1997; Mahdavi et al., 2005). *Azolla pinnata* is a kind of water plant containing protein up to 30% (Manin, 1997) and rich on essential amino acids such as threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, histidine, arginine, tryptophan (Cohen et al., 2002; Basak et al., 2002).

Normally cold water fishes from northern hemisphere are rich on omega-3 (ω3) of polyunsaturated fatty acid (PUFA) in their fish oils, such as docosahexaenoic acid (DHA) and eicosapentanoic acid (EPA). Increasing of temperature and decreasing of water salinity in fish environment result in higher amount of ω6 PUFA such as arachidonic acid. Tropical freshwater fish *Monopterus albus* has been reported to contain arachidonic acid and DHA (Razak et al., 2011). As essential fatty acids, ω3 and ω6 PUFA have attracted a great interest nowadays for their utilization as medicine and nutraceutical components.

According to those statements, this research explores the influence of feeding diets containing *A. pinnata* and probiotic on the growth and nutritional content of patin fish. In this study, protein was isolated and then was hydrolyzed to obtain the profile of amino acids analyzed by Liquid Chromatography Mass Spectrometry (LCMS). Patin fish oil was extracted using dry rendering method (Oktavianawati et al., 2014) which applies heating on material under low temperature (70℃). The profile of fatty acids from this fish oil was analyzed using Gas Chromatography Mass Spectrometry (GCMS).

2. Materials and Methods

2.1. Materials

The fish samples were patin jambal (*Pangasius djambal*) wich were cultured for seven months. The sample for analysis was fillet of body parts of those fishes. The pellet used in this research has a trade name of Hi-Pro-Vite 781-3 which contained of 31-33% of protein, 4% of fat, 5% of fiber, 13% of ash, and 12% of wazter. The chemicals used in this research were obtained from Sigma and Merck.

2.2. Fish Culture and the Treatment of Feeding

Three pools, each sized 3×5×1 m^3, with a stocking density of 500 fish/15 m^3 were treated by different feeding: pellets (300 g/meal); pellets coated with probiotic; pellets plus supplement of *A. pinnata* (3:1). Feeding was given for twice a day. The sampling of catfish was taken as much as 5% of the fish population (Singh, 2006) at the seventh month of fish culture.

2.3. Isolation and Quantification of Protein Content

Isolation of protein from the patin fish was done based on the method prepared by Moayedi et al. (2010). A 50 g sample of fish fillet was blended in ice until be a homogeneous mixture. 2N NaOH was added to the fish slurry in order to get pH 10.5 and then was kept at 4℃ for 30 minutes. The mixture was centrifuged at 12,000 rpm and 4℃

for 20 minutes to obtain three layers mixture. The middle layer was separated carefully by pipetting and then was adjusted to pH 5.2 using 2 N HCl.

Determination of crude protein content was performed using Kjeldahl method adopted from AOAC (1995), while determination of soluble protein was carried out using the Bradford method (Bradford, 1976).

2.4. Hydrolysis of Protein and Determination of Amino Acids Content

Hydrolysis of proteins was prepared based on the method stated by Sudarmadji (2007). A certain amount of protein was put in a test tube with screw cap. Then 6N HCl was added to the sample followed by purging of nitrogen gas for 1 minute. After sealing the tube, the mixture was heated in an oven at 110°C for 24 hours. Then the mixture was filtered and washed with 0.01N HCl. The mixture was dried by evaporating the solvent and then was added with 0.01N NaOH and kept open at room temperature for 4 hours. Finally 0.02N HCl was added to produce a hydrolysate of protein. The amino acids content in protein hydrolysate was analyzed using LCMS.

2.5. Dry Rendering Method

250 g of sample was placed on a shelf of pan and roasted in vacuum oven at 70°C for 3 hours. The oil would drop on the pan and be mentioned as first extract. After three hours on heating, the hot fish meat was then pressed to obtain the remaining oil (second extract). First and second extracts were mixed and purified using separating funnel in the presence of 2.5% sodium chloride. After heating at 50°C, the oil extract was then centrifuged at 7000 rpm for 20 minutes. The supernatant was specified as fish oil.

2.6. Characteristic of Fish Oil

The oil yield was calculated as dry fish oil after deducting from its water content. Chemical properties of the oil including acid number, saponification value, peroxide value and iodine number of fish oil extracts were determined using standard method (AOAC, 1995).

2.7. Analysis of Fatty Acids in Fish Oil Extract

Analysis of fatty acids was carried out using GCMS-QP2010S from Shimadzu with Agilent J&W DB-1 column (height x ID = 30 m x 0.25 mm), helium as the gas carrier, oven column temperature at 80.0°C, injection temperature at 310.00°C, flow control mode 16.5 kPa, total flow of 40.0 mL/min, and column rate 0.50 mL/min. Oven temperature of program rate, temperature, hold time are (-, 80.0°C, 5.00 min) and (10.0, 305.0°C, 32.50 min). MS conditions were ion source temperature of 250.00°C and interface temperature of 305.00°C.

3. Results and Discussion

In this research, we specify the fish fed with pellet only, is P1, while fish fed with pellet and supplementary feeding *A. pinnata* and probiotic as P2 and P3.

3.1. The Influence of Feeding on the Fish Performance

All kind of fish samples were given pellet only until starting the age of two month, P2 and P3 were given an additive feed *A. pinnata* and probiotic. Figure 1 shows that P3 produces the heaviest weight of patin fish compare to the others. Probiotic helps body to ferment and convert the complex compounds into small compounds. As a consequence, it makes those nutrition are easily to be absorbed hence spur the fish growth.

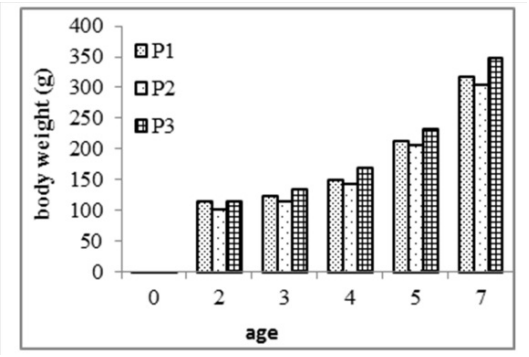

Figure 1. The performance (body weight) of patin fish during seven month of culture

A. pinnata contains high crude fiber contents (Christiyanto & Subrata, 2005) which are hard to absorb since it requires certain enzymes and microorganisms to digest. This feed additive leads to apparent satiety on P2, hence produce a patin fish with the lowest body weight compare to the other two, P1 and P3.

3.2. The Influence of Feeding on the Protein Content

Isolation of protein was carried out based on Moayedi method (2010). This method has been previously optimized for its extraction pH, that is at pH 10.5 (Oktavianawati et al., 2014).

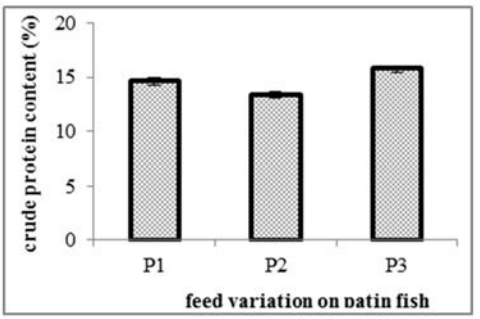

Figure 2. The crude protein content in patin fish

Figure 2 illustrates that P3 contains a higher content of crude protein than P1 and P2. This may due to the fact that probiotic decrease the pH of digestive tract helping the breakdown of food nutrition into simple molecules which is easily to absorb in the fish intestine (Arief et al., 2008).

3.3. The Profile of Amino Acids in Patin Fish

The protein isolate was hydrolyzed to obtain information of amino acids content in patin fish. Hydrolysis was conducted by adding 6M HCL for 6 hours at 110°C into tube containing protein isolate in inert condition (purge with N2). The formation of free amino acids was measured using Bradford method and the result was illustrated in Figure 3.

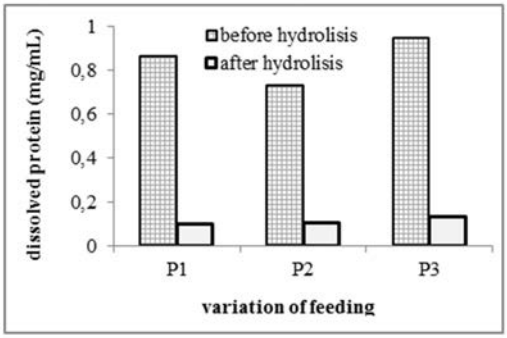

Figure 3. The level of water soluble protein before and after protein hydrolysis

Furthermore, those free amino acids from hydrolysis of protein isolates were analyzed using LCMS. Table 1 shows the comparison of amino acids content among the patin fishes. Normally, P1 contains amino acids in a higher amount compare to P2 and P3. It is interesting to note that aspartic acid is present only in P1, while asparagine and glutamic acid does not present in P2 and P3. However, tryptophan is only obtained from P2. Totally, P1 contains 13 amino acids, while P2 and P3 contain 14 and 13 amino acids, respectively. However, serine, glysine, sisteine and alanine are amino acids that do not present in patin fishes even in control fish, P1.

Table 1. Types and the amount of amino acids in patin fish.

No.	Amino Acid	Relative %		
		P1	P2	P3
1	Methionine	48,95	25,72	25,32
2	Triptophane	72,68	27,31	-
3	Histidine	38,83	32,42	28,75
4	Threonine	33,61	36,34	30,05
5	Isoleucine/Leucine	55,68	23,25	21,07
6	Phenylalanine	35,20	25,24	39,56
7	Lysine	47,65	29,40	22,95
8	Arginine	41,09	28,31	30,60
9	Valine	63,72	22,30	13,98
10	Tyrosine	42,29	42,50	15,21
11	Serine	-	-	-
12	Glysine	-	-	-
13	Cysteine	-	-	-
14	Alanine	-	-	-
15	Aspartic acid	100,00	-	-
16	Asparagine	-	46,50	53,50
17	Glutamic acid	-	40,92	59,07
18	Glutamine	45,86	33,36	20,77
19	Proline	44,66	13,99	41,34

3.4. The Profile of Protein in Patin Fish

The profile of protein in patin fish is determined by running SDS-PAGE of the protein extracts (Figure 4). The estimation of molecular weight for each of protein is done by comparing mobility distance between the protein sample and protein marker in the SDS PAGE chromatogram. Relative molecular weight is determined by plotting *Rf* (retention factor) versus log of molecular weight in the graph to obtain an equation of log molecular weight.

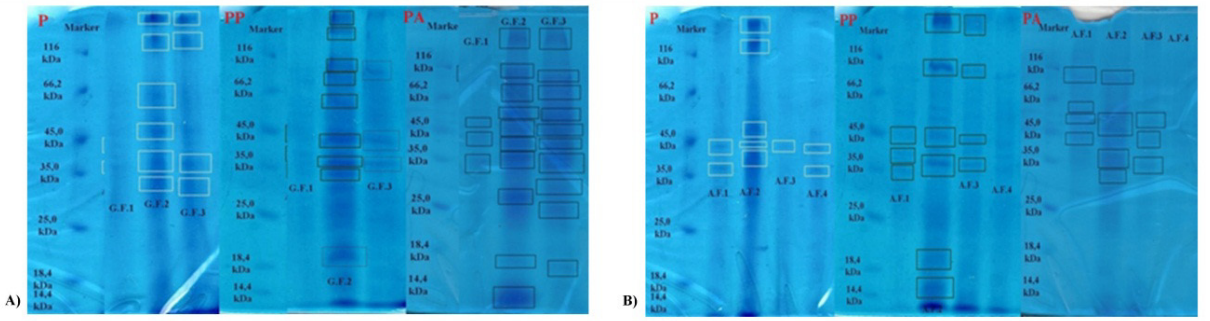

Figure 4. Electrophoregram of protein isolate of patin fish: (A) water soluble protein; (B) salt soluble protein. P: P1; PA: P2; PP: P3.

The figure illustrates that commonly in all patin fish: P1, P2 and P3, there are eight protein bands with molecular weight in a range of 39.29 to 124.38 kDa; 12 protein bands in a range of 15.03 to 133.05; and 10 protein bands in a range of 32.10 to 79.42.

3.5. The Influence of Feeding on Fish Oil Characteristic

As previously described (Oktavianawati et al., 2014), fish oil extraction method from these patin fish was best obtained using dry rendering extraction method. As a consequence of the low heat treatment, dry rendering method results in better quality of fish oil compare to wet rendering method. However, the profile of their fatty acids content was not significantly different. Further analysis was conducted based on dry rendering extraction-based fish oil product (Table 2).

Table 2. Characteristic of fish oil

Characteristics	**Patin Fish**		
	P1	P2	P3
Yield (%)	1.47 ± 0.41	2.33 ± 0.07	2.81 ± 0.38
Acid number (mg KOH/g)	2.10 ± 0.07	1.92 ± 0.06	2.18 ± 0.01
Saponification value (mg KOH/g)	117.49 ± 0.75	145.06 ± 1.45	92.15 ± 0.46
Peroxide value (mek/kg)	3.19 ± 0.13	3.56 ± 0.13	2.87 ± 0.12
Iodine number	106.91 ± 1.05	101.70 ± 0.53	102.96 ± 0.83

Basically, technological process of fish oil extractions may influence the characteristic of fish oil itself. All fats containing some free fatty acid, but these are removed by refining process. The amount of free fatty acid in oil indicates the degree of spoilage has occurred. The lower acid number would be the better quality of fish oil. The table shows that fish oil from P2 has the lowest acid number, meaning that P2 contains the lowest free fatty acid, compare to other fish oils. Saponification value is a measure of the average molecular weight (or chain length) of all the fatty acids present in fish oil. Fish oil from P3 may contain longer chain of fatty acids compare to other fish oils because only a fewer number of carboxylic functional groups per unit mass which can be saponified by base.

Peroxide value determines the rancidity of fish oil which can be caused by oxidation and hydrolysis. P3 produces the lowest peroxide value indicating the good stability of fish oil from autoxidation.

Among these chemical properties lists, iodine number is the most representative characteristic of fish oil since it show the nature or real characteristic of fish oil. Degree of unsaturation in fish oil demonstrated as iodine number was determined using iodometry titration. P1 as a control shows the best quality of fish oil in terms of iodine number characteristic.

Table 3. The profile of fatty acids from fish oil of patin fish

Carbon number	Fatty Acids	P1	P2	P3
Saturated Fatty Acids				
C_{12}	Lauric Acid	-	0.15	-
C_{14}	Myristic Acid	4.69	5.19	5.25
C_{16}	Palmitic Acid	30.65	30.25	27.88
C_{17}	Margaric Acid	0.33	0.36	-
C_{18}	Stearic Acid	8.45	7.87	6.34
C_{20}	Arachidat Acid	-	0.26	-
Total of Saturated Fatty Acids		44.12	44.08	39.47
Unsaturated Fatty Acids				
$C_{16:1}\Delta^{9}$	Palmitoleic Acid	2.69	2.66	2.79
$C_{18}\Delta^{9}\omega9$	Oleic Acid	32.34	32.49	31.90
$C_{20:1}\Delta^{11}\omega9$	Gondoic Acid	1.00	1.44	0.70
$C_{22:1}\Delta^{13}\omega9$	Erucic Acid	-	0.38	-
$C_{18:3}\Delta^{6,9,12}\omega6$	γ-Linolenic Acid	-	0.28	-
$C_{18:2}\Delta^{9,12}\omega6$	Linoleic Acid	11.90	12.6	10.6
$C_{20:2}\Delta^{11,14}\omega6$	Eicosadienoic Acid	-	0.35	-
$C_{20:4}\Delta^{5,8,11,14}\omega6$	Arachidonic Acid	0.86	-	-
$C_{18:3}\Delta^{9,12,15}\omega3$	γ-Linoleic Acid	0.07	0.65	-
$C_{20:5}\Delta^{5,8,11,14,17}\omega3$	Eicosapentanoic Acid	1.52	1.9	1.33
Total of Unsaturated Fatty Acids		50.38	52.75	47.32

The profile of fatty acids, as shown in table 3, was determined using GCMS. The fish oil was saponified to isolate free fatty acids and then those fatty acid compositions can be determined. In a glance, fatty acid content among three fish oils from P1, P2 and P3 are similar. The most dominant saturated fatty acid in all fish oils is palmitic acid, while the major unsaturated fatty acids is ω9 PUFA, oleic acid. In detail, fish oil from P1 contains EPA and arachidonic acid, while P2 contains EPA and linoleic acid in the highest percentage compare to P1 and P3. Therefore, it can be stated that patin fish P2 is a potential source of PUFA ω3, ω6 and ω9.

4. Conclusion

Patin fish fed with supplementary feeding of probiotic shows the best growth performance and the highest content of crude protein compare to control and patin fish supplemented with *A. pinnata*. However, patin fish supplemented with *A. pinnata* shows a better profile of fatty acids compare to control and fish fed with pellet plus probiotic.

Acknowledgements

We would like to thank to team of Fendra N. from Program Mahasiswa Wirausaha UNEJ 2012 for providing the patin fish as the sample for this research.

References

Andriyanto, S., Listyanto, N., Rahmawati, R., 2010. Pengaruh pemberian probiotik dengan dosis yang berbeda terhadap sintasan dan pertumbuhan benih patin jambal (Pangasius djambal). Prosiding Forum Inovasi Teknologi Akuakultur, 117-122.

AOAC, 1995. Official Methods of Analysis of the Association of Official Analytical Chemist. AOAC Int. Washington.

Arief, M., Mufidah, Kusriningrum, 2008. Pengaruh Penambahan Probiotik pada Pakan Buatan terhadap Pertumbuhan dan Rasio Konversi Pakan. Jurnal Berkala Ilmiah Perikanan 3(2), 53-58.

Ariyanto, D., and Utami, R., 2006. Evaluasi Laju Pertumbuhan, Keragaman Genetik dan Estimasi Heterosis pada Persilangan antar Spesies Ikan Patin (Pangasius sp.). Jurnal Perikanan VIII (1), 81-86.

Basak, B., Pramanik, M.A.H., Rahman, M.S., Tarafdar, S.U., Roy, B.C., 2002. Azolla (*Azolla pinnata*) as a feed ingredient in broiler ration. International Journal of Poultry Science 1(1), 29-34.

Bradford, 1976. A Rappid and Sensitive Methode for Quantitation of Protein Utilization. The Principle of Protein-Dye Binding. Analytical Biochemistry 72, 248-254.

Cohen, Meziane1, Tsuchiya, and Yamasaki, 2002. Feeding deterrence of Azolla in relation to deoxyanthocyanin and fatty acid composition. Aquatic Botany 74, 181-187.

Domiszewski, Z., Bienkiewicz, G., and Plust, D., 2011, Effects of Different Heat Treatments on Lipid Quality of Striped Catfish (Pangasius hypophthalmus). Acta Scientiarum Polonorum, Technol. Aliment 10(3), 359-373.

Mahdavi, A.H., Rahmani, H.R., and Fourreza, J., 2005. Effect of probiotic supplements on egg quality and laying hen's performance. International Journal of Poultry Science 4(7), 488-492.

Manin, 1997. Penggunaan Tepung Eceng Gondok (*Eichornia crassipes Mart*) dan Azolla (*Azolla pinnata Brown*) dalam Ransum Ternak Itik Periode Pertumbuhan. Jurnal Peternakan Lingkungan 3(2), 13-20.

Moayedi, Omana, Chan, Xu, Betti, 2010. Alkali-aided protein extraction of chicken dark meat: composition and stability to lipid oxidation of the recovered proteins. Poultry Science Association Inc. 89, 766-775.

Oktavianawati, I., Andinata, D., Isnaeni, A., Winata, I.N.A., 2014. Characteristic of fish oil from patin fish (Pangasius djambal) extracted by dry and wet rendering methods. Proceeding of International Seminar on Science and Technology 2014, 103-106.

Oktavianawati, I., Hermiastuti, M., Rahmawati, N., Handayani, W., Winata, I.N.A., 2014. The influence of supplementary feeding (probiotic and Azolla pinnata) on protein and amino acids content in patin fish. Proceeding of Indonesian Protein Society.

Orban, E., Nevigato, T., Lena, G.D., Masci, M., Casini, I., Gambelli, L., and Caproni, R., 2008. New Trend in the Seafood Market. Sutchi Catfish (Pangasius hypopthalmus) Filet from Vietnam: Nutritional Quality and Safety Aspect. Food Chemistry 110(2), 383-389.

Panagan, A.T., Yohandini, H., Gultom, J.A., 2011. Analisis Kualitatif dan Kuantitatif Asam Lemak Tak Jenuh Omega-3 dari Minyak Ikan Patin (Pangasius pangasius) dengan Metode Kromatografi Gas. Jurnal Penelitian Sains 14(4), 38-42.

Rahardja, B.S., Sari, D., Alamsjah, M.A., 2011. Pengaruh Penggunaan Tepung daging Bekicot (Achatina fulica) pada Pakan Buatan terhadap Pertumbuhan, Rasio, Konversi Pakan dan Tingkat Kelulushidupan Benih Ikan Patin (Pangasius pangasius). Jurnal Ilmiah Perikanan dan Kelautan 3(1), 117-122.

Razak, Z.K.A., Basri, M., Dzulkefly, K., Razak, C.N.A., and Salleh, A.B., 2001. Extraction and Characterization of Fish Oil from Monopterus Albus. Malaysian Journal of Analytical Sciences 7(1), 217-220.

Singh, Y. K. 2006. Fundamental of Research Methodology and Statistics. New Delhi: New Age International (P) Limited, Publisher.

Sudarmaji. 2007. Analisa untuk bahan Pangan dan pertanian. Yogyakarta : Liberti.

Suryaningrum, T.D., Muljanah, I., and Tahapari, E., 2010. Profil Sensori dan Nilai Gizi beberapa Jenis Ikan Patin dan Hibrid Nasutus. Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan 5(2), 153-164.

Susanto, H., and Amri, 2002. Budidaya Ikan Patin. Jakarta: Penebar Swadaya.

Thuy, N.T., Loc, N.T., Linberg, J.E., Ogle, B., 2002. Survey of the production, processing and nutritive value of catfish by-product meals in the Mekong Delta of Vietnam. Published in *Louisiana Agriculture*.

Yeo, J., and Kim, K.I., 1997. Effect of feeding diets containing an antibiotic, a probiotic, or Yucca extract on growth and intestinal urease activity in broiler chicks. Poultry Science 76, 381-385.