Composition and amino acid profile of fish meal processed using probiotics and prebiotic sources

E Hendalia, F Manin, Adriani, Mairizal and A R Admiral

Faculty of Animal Husbandry Universitas Jambi

Email: ellahendalia@gmail.com

Abstract. The study evaluated the composition and amino acid profile of trash fish meal (TFM) processed using Probio_FM probiotics (containing Lactobacillus sp.) combined with coconut meal (CM) or palm kernel meal (PKM) as prebiotics sources. The study was arranged in 2x3 factorial design with four replications. The first factor was the prebiotic sources (CM and PKM) and the second factor was the Probio_FM levels (0, 1 and 2%). The TFM was processed by mixing trash fish with CM or PKM and Probio_FM, then it was milled and dried. The TFM processed using CM or PKM combined with 1 - 2% Probio_FM contained 43.77 - 45.81% crude protein. Most essential amino acids, except methionine, were inferior compared to the NRC 1994 table. Arginine, valine and tryptophan was the first, second and third limiting amino acid in TFM, respectively. The chemical score of TFM ranged from 20 to 28%. Combination of PKM with 2% Probio_FM resulted the highest crude protein (45.81%), methionine (2.15%) and chemical score (28%). It was concluded that the TFM processed using PKM combined with 2% Probio_FM had a potential to be used as a source of protein and methionine as well as a source of probiotics.

1. Introduction

In developing countries, including Indonesia, fish meal is generally produced from trash fish or side catches from fishermen that are less suitable for human consumption. Fish meal is conventionally processed by steaming or boiling the raw fish, followed by pressing and drying [1]. Steaming, boiling and pressing may cause protein denaturation and nutritional loss, such as amino acids, fatty acids, vitamins and minerals. Besides requiring a lot of water and energy, this method also produces waste and bad odor that pollutes the environment. An alternative method to overcome this problem is by processing raw fish using Probio_FM probiotics (containing lactic acid bacteria) combined with prebiotic sources such as coconut meal (CM) or palm kernel meal (PKM) to produce trash fish meal (TFM) containing probiotics, that is potentially used as a source of protein and probiotics [2]. The probiotics exert antimicrobial effect against pathogenic bacteria via production of metabolites, such as short chain fatty acids (SCFA) and bacteriocins [3].

The principle of the application of probiotics and prebiotics in processing fish meal was the utilization of the LAB to inhibit the growth of decomposing bacteria because the LAB has an ability to produce lactic acid and bacteriocins [4]. The LAB can also utilize ammonia derived from protein decomposition and use fat as its energy source, thus it can reduce bad odor and keep the fish meal from being rancid easily [2]. In order to maintain the viability of the LAB, addition of suitable prebiotic sources is needed. Both CM and PKM contain mannan-oligosaccharides (MOS) which can be used by the LAB as prebiotics [5,6].
Previous study showed that TFM processed using Probio_FM combined with CM or PKM, contained Lactobacillus sp. at $10^{10}$ cfu/g was free from Salmonella, had a distinctive aroma of FM and did not indicate rancidity after four months storage [2]. The aim of the present work was to evaluate the composition and amino acids profile of the TFM containing Lactobacillus sp. to be used as a source of protein and probiotics.

2. Material and methods

2.1. Material

Materials used in this study were obtained from Jambi Province, consisting of fresh trash fish, Probio_FM, CM and PKM. The trash fish were obtained from fishermen in Tanjung Jabung Timur Regency. The CM was obtained from coconut processing factories in Tanjung Jabung Barat Regency; while the PKM were from palm oil processing plants in Sarolangun Regency. The probiotics used were Probio_FM produced by Faculty of Animal Husbandry Universitas Jambi, containing $10^{10} - 10^{11}$ cfu/ml of Lactobacillus sp. [7].

2.2. Methods

2.2.1. General. The TFM processing was carried out according to Hendalia et al. [8]. The treatment was designed to 2×3 factorials with four replications. The first factor was two types of prebiotic sources (CM and PKM) and the second factor was three levels of Probio_FM probiotics (0, 1 and 2%).

Trash fish were separated from poisonous fish, then rinsed and drained. The trash fish was milled, then CM or PKM (10% of the fish weight) and Probio_FM (0%, 1% and 2%) was added. The mixture was homogeneously blended and placed into a tray to be dried. The dried TFM were analyzed to determine crude protein (CP) content according to AOAC methods and amino acid profile using an elution gradient in HPLC system. The quality of TFM protein was evaluated by comparing the content of essential amino acids (EAA) in the TFM with the EAA listed in NRC 1994 table [9]. Biological value of the TFM was predicted by calculating chemical score [10], by dividing the content of EAA in the TFM with the EAA content in fish meal according to NRC [9] as protein reference.

2.2.2. Statistic. The data were statistically analyzed by analysis of variance (ANOVA) with Duncan’s post hoc at 5% probability using SPSS version 24.0 by IBM. Amino acid composition and chemical scores were analyzed descriptively.

3. Results and discussion

3.1. Crude protein content

Crude protein (CP) content of the TFM processed using CM or PKM and Probio_FM probiotic is presented in Table 1. The combination of Probio_FM and CM decreased crude protein content of the TFM (P<0.05), but combination of Probio_FM and PKM increased crude protein content (P<0.05). This contradiction was related to the content and availability of MOS in CM and PKM, where the MOS in PKM more widely available [5–6–11]. The increasing of Probio_FM level did not cause a breakdown of fish protein, because the LAB would use ammonia from decaying process of the fish as a source of nutrients [12]. The CM-PRO 0% had the highest CP content (47.57 ± 0.86%), however, Salmonella was detected from previous study [2]. Therefore, processing TFM without Probio_FM was not recommended. Based on the results, the TFM with the highest protein and free of Salmonella were found in PKM-PRO 2% (45.81±0.41%) and CM-PRO 2% (45.38±2.09%).

Overall, CP content in TFM is lower than CP content of fish meal produced by commercial industries. According to the NRC [9], protein content of fish meal ranged from 61.30% to 72.0 %. Protein contents of fishmeal varied from 50.51% to 61.26% [1], and 53.90 to 61.79% [13], while the
protein content of trash fish meal treated with cooking varied from 51.47% (steamed); 55.93% (pressure cooker) to 58.02% (boiled) [14].

**Table 1.** Crude protein content of the trash fish meal (% DM)

| Prebiotic Sources | Probio_FM Levels | Average |
|-------------------|------------------|---------|
|                   | 0%               |         |
|                   | 1%               |         |
|                   | 2%               |         |
| Coconut meal (CM) | 47.57 ± 0.86ab   | 47.63 ± 2.09 | 45.96 ± 1.41 |
| Palm kernel meal (PKM) | 42.40 ± 1.34ab | 43.77 ± 0.41 | 43.99 ± 1.72 |
| Average           | 44.98 ± 3.66ab   | 44.35 ± 0.82 | 45.60 ± 0.30 |

* Different letters in the same column or row indicate significant differences (P<0.05)
*1 Found *Salmonella* (10^7 cfu/g)

3.2. Composition and profile of essential amino acids

Protein quality of fish meal is largely determined by a completeness and balance of its EAA, such as methionine (Met), arginine (Arg), threonine (Thr), tryptophan (Trp), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), valine (Val), and phenylalanine (Phe). Composition and profile of the EAA in the TFM compared to the EAA in fish meal those listed in NRC Table (FM-NRC) [9] can be seen in Table 2.

**Table 2.** Composition and chemical scores of the EAA in TFM compared to the EAA in NRC

| TFM          | (a) Composition of EAA in TFM (% DM) | (b) Chemical Scores of EAA in TFM Compared to the EAA Listed in NRC |
|--------------|-------------------------------------|------------------------------------------------------------------|
|               | Met       | Arg       | Thr       | Trp       | His       | Ile       | Lys       | Val       | Phe       | Met       | Arg       | Thr       | Trp       | His       | Ile       | Lys       | Val       | Phe       |
| CM-PRO 1%    | 1.63      | 0.81      | 1.41      | 0.15      | 0.96      | 1.52      | 3.70      | 2.91      | 1.02      | 1.31      | 0.92      | 0.20      | 0.53      | 0.29      | 0.62      | 0.82      | 0.82      | 0.59      | 0.34      | 0.55      |
| CM-PRO 2%    | 1.69      | 0.85      | 1.43      | 0.14      | 0.95      | 1.56      | 3.75      | 3.03      | 0.91      | 1.33      | 0.96      | 0.21      | 0.53      | 0.37      | 0.64      | 0.83      | 0.83      | 0.59      | 0.25      | 0.54      |
| PKM-PRO 1%   | 1.73      | 0.87      | 1.42      | 0.20      | 0.99      | 1.51      | 3.76      | 2.90      | 0.74      | 1.31      | 0.98      | 0.22      | 0.53      | 0.37      | 0.64      | 0.83      | 0.83      | 0.59      | 0.25      | 0.54      |
| PKM-PRO 2%   | 2.15      | 1.12      | 1.74      | 0.21      | 1.21      | 1.67      | 4.30      | 3.28      | 0.93      | 1.66      | 1.22      | 0.28      | 0.65      | 0.40      | 0.79      | 0.95      | 0.95      | 0.67      | 0.31      | 0.69      |

Data in Table 2 shows that the TFM had a complete composition of EAA, but most of the EAA, except methionine, were inferior compared to those listed in NRC [9]. Methionine content of PKM-PRO 2% showed the highest value, even it was higher than its content in FM-NRC [9]. Overall, methionine content in the TFM ranged from 1.63 to 2.15%. It was closed to the methionine content in the FM-NRC (1.63% to 2.16%). A high content of methionine in PKM-PRO 2% is in line with a high content of CP in those treatment. Previous study found that methionine content of FM varied greatly according to the CP content, ranged from 0.55 to 1.39% in FM containing 53 to 61.79% of CP [13], and 1.72 to 2.70% in FM containing 58 to 61.79% CP [15]. The high methionine content of the TFM in this study plays a very significant role in feed formulation, because most of animal feed, especially corn and soybeans are deficient in methionine [9].

The content of arginine, valine and tryptophan in the TFM were lower than in the FM-NRC, while histidine, threonine, isoleucine, leucine and lysine content were closer to the FM-NRC. A low composition of the EAA in the TFM is related to the low of its CP content (43.77 to 45.81%). Based on its chemical scores (ratio between the EAA content of the TFM and EAA in FM-NRC as protein...
of the amino acids, except methionine, as protein and methionine content was higher than methionine content in processed using probiotics produce. Processing trash fish using coconut meal or palm kernel meal contained 43.77 to 45.81% crude protein and had a complete essential amino acid content. However, most of the amino acids, except methionine, were inferior compared with those listed in the NRC. Arginine was the main limiting amino acid, with chemical scores of 20 - 28%, while valine and tryptophan were the second and/or the third limiting amino acid. The fish meal processed using 10% palm kernel meal and 2% Probio_FM had the highest crude protein and methionine content and showed the highest chemical score. Methionine content of the fish meal was higher than methionine content in the NRC, so that the fish meal had a potential to be used as a source of protein and methionine as well as a source of probiotics.

4. Conclusion
Processing trash fish using coconut meal or palm kernel meal combined with 1 to 2% Probio_FM probiotics produced fish meal containing 43.77 to 45.81% crude protein and had a complete essential amino acid content. However, most of the amino acids, except methionine, were inferior compared with those listed in the NRC. Arginine was the main limiting amino acid, with chemical scores of 20 - 28%, while valine and tryptophan were the second and/or the third limiting amino acid. The fish meal processed using 10% palm kernel meal and 2% Probio_FM had the highest crude protein and methionine content and showed the highest chemical score. Methionine content of the fish meal was higher than methionine content in the NRC, so that the fish meal had a potential to be used as a source of protein and methionine as well as a source of probiotics.

Acknowledgment
The author would like to thank to the Director of Research and Community Services, Directorate General of Strengthening Research and Development, Ministry of Research, Technology and Higher Education who funded this research and the Head of the Institute for Research and Community Services, Universitas Jambi which facilitated the implementation of this research.

References
[1] Rahim A, Abbas G, Naeem M, Ferrando S, Gallus L, Khan N, Hafeez-Ur-rehman M, Ghaffar A and Mateen A 2017 Pak. J. Zool. 49 337–44
[2] Hendalia E, Manin F and Adriani 2018 Peningkatan Kualitas Tepung Ikan Rucah Berprobiotik Sebagai Pakan Sumber Protein (Jambi)
[3] Park Y H, Hamidon F, Rajangan C, Soh K P, Gan C Y, Lim T S, Abdullah W N W and Liaong M T 2016 Korean J. Food Sci. Anim. Resour. 36 567–76
[4] Lopez J 2000 Asian-Australian J. Anim. Sci. 55 1238–46
[5] Ariff A, Mohd Nor N ‘Ain N, Marikkar M N, Mustafa S, Abdul Manap M Y, Amid M, Lamasudin D U and Abbasiliasi S 2016 J. Food Sci. Technol. 54 164–73
[6] Sundu B, Kumar A and Dingle J 2006 Worlds. Poulit. Sci. J. 62 316–25
[7] Manin F, Hendalia E, Asra R and Helda 2014 Pengembangan industri produk probiotik Probio_FM berbasis kemirian. (Jambi)
[8] Hendalia E, Manin F and Insulisistiyowati A 2016 Ekspose Hasil Penelitian dan Seminar Hasil Pengabdian (Jambi: Universitas Jambi) pp 276–89
[9] National Research Council 1994 Nutrient Requirement of Poultry (Washington DC: National Academy Press.)
[10] Block R J and Mitchell H H 1946 Nutr. Abstr. Rev. 16 249–78
[11] Manin F, Hendalia E, Yatno and Rahayu P . 2014 J. Ilmu Ternak 1 7–11
[12] Hendalia E, Manin F, Sapidianto A and Pratama P 2017 Prosiding Seminar Nasional Industri Petteranak 1 pp 248–51
[13] Prado P J de S, Marcelino J, Cavalheiro O, Andrade J, Cavalheiro B and Vanessa F 2016 Gaia Sci. 10 347–60
[14] Assadad L, Hakim A R, Widianto T N . 2015 Prosiding Seminar Tahunan XII Hasil Penelitian Perikanan dan Kelautan pp 53–62
[15] Montoya-Martinez C, Nolasco-Soria H, Carillo-Farnes O, Civera-Cerecedo R, Alvarez-Gonzales C 2016 Lat. Am. J. Aquat. Res 44 835–44