# Effect of earthworm (Lumbricus rubellus) in feed formulation to improve fatty acids profile in eel (Anguilla bicolor) meat

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# Effect of earthworm (*Lumbricus rubellus*) in feed formulation to improve fatty acids profile in eel (*Anguilla bicolor*) meat

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**Abstract.** Eel requires unsaturated fatty acids of linolenic acid for growth. Which can be supplied from earthworms. In this study, addition of earthworm in formulation feed aimsed to improve the fatty acid profile eel meat. This research used experimental method and randomized complete design method with five treatments. Each treatment was repeated four times. The use of earthworms in feeding treatment formulation was done for 21 days with different level i.e: 0 % (P0), 25 % (P1), 50 % (P2), 75 % (P3) and 100 % (P4). The result showed that the addition of eartworm significantly influenced the omega 3 contents (EPA & DHA) of eel meat.

## 1. Introduction

Eel (*Anguilla bicolor*) is a freshwater fish that has a large potential to be developed. According to Hameed *et al.* [1], eel contains 48.430 % saturated fatty acids, 50.639% unsaturated fatty acids. In addition, there is a 0.461 % EPA, 1.294 % DHA, 9.134 % linoleic acid and 0.472 % arachidonic acid. Eelcontains 1.337 mg/100 g DHA and 742mg/100g EPA. Consumers' demand for eels has increased due to its high nutrient content. Demand for eels in international markets has reached 300,000 tons/year. The market demand for eels is increasing because people consider the meat is savory and rich beneficial for the body [2]. Eels areknown as a fishery commodity that is rich in protein, fat, minerals, and vitamins compared to other fish species [3].

Polyunsaturated fatty acids functions as a nutrient in the body, such as EPA and DHA that give benefits to human health. EPA and DHA contained in fatty fish and they cannot be synthesized in the human body [4]. The ratio between omega-3 and omega-6 fatty acidsis a good indicator for comparing the relative nutritional value of different species of fish. The ratio of omega-3 and omega-6 fatty acids helps to prevent coronary heart disease by reducing the levels of plasma lipids and risk of cancers[5]. Omega-3 and omega-6 fatty acids are polyunsaturated are important components of cell membranes and are precursors to many other substances in the body such as those involved with regulating blood

pressure and inflammatory responses, thus theymust be obtained through food [6]. Therefore, this research was aimed to know effect of addition of earthworms (*Lumbricus rubellus*) in feed formula on fatty acid profile of eels.

## 2. Methodology

### *2.1 Preparation of earthworm (Lumbricus rubellus)*

Earthworms were obtained from Malang, East Java. They were mixed with feed formulation (commercial fishmeal) in doses of 0, 25, 50, 75, and 100 % and shaped like pasta. The feed then went through proximate analysis as shown in table 1.

**Table 1.** Nutritional feed ingredients.

| Feed Ingredients | Dry Ingredients | Crude Protein (%) | Crude Fat (%) | Crude Fiber (%) | Ash (%) | NFE (%) |
|---|---|---|---|---|---|---|
| Formulation Feed | 80.029 | 42.067 | 13.011 | 6.806 | 14.228 | 3.916 |
| Earthworms | 22.896 | 13.634 | 5.749 | 0.548 | 1.589 | 1.376 |

### *2.2 Experimental design*

Eels used were from fingerling stage weighing 20-25 g with the total of 100 g and they were supplied from Malang, East Java. Eelswere selected and then acclimatized for 30 min. Furthermore,eels wasadapted for a week. For 21 days, the eels were given feed containing 0 %, 25 %, 50 %, 75 %, and 100 % earthworm.

### *2.3 Fish composition*

The levels of fat, protein, fiber, and energy ingredients without nitrogen (BETN) in eel meat were analyzed based on AOAL methods [7].

### *2.4 Fatty acid derivatisation*

Eel meat was cut, chopped, weighed for 1 g, and put into test tubes. Sodium chloride (0.5 g) and 4 mL hexane were added and the mixture was vortexed for 2 min until it was clear. Clear hexane layer was taken and transferred into the next derivatisation tube and drained with stream of nitrogen. NaOH 2 % (2 mL) was added to methanol then close to temperate at 90 °C for 5 min. The result was left to cool before added with 2 mL methanol in BF3 further heated again for 30 min. Samples were then extracted with 3 mL of hexane to final stage. Extract was analyzed by GC-MS[8].

### *2.5 Gas chromatography (GC)*

Sampleswere analyzed using gas chromatograph Shimadzu GC-2014 with helium as a carrier gas and SGE forte BPX 70 column (film thickness of 30 m x 0.25 mmID x 0.25µm) (SGE Europe Ltd. Milton Keynes.UK) as the analytical column. The peaks were identified using standardmix of 38 external FAME (FAME Mix C4-C24. Supelco; Sigma – Aldrich). Initial column temperature was set at 50°C for 1 min. Temperature was raised at 2 °C/min until it reached 188 °Cand maintained for 10 min. next, the temperature was increased further to 240 °C and maintained for 4 minbefore it was returned to the initial temperature [9].

### *2.6 Statistical analysis*

The data were expressed as mean ± standard deviation. The data were analyzed using SPSS version 16.0 (SPSS Inc., Chicago, IL). Differences between means were analyzed by Analysis of Variance followed by Duncan's multiple comparison test. Significantdifferent was set at $p < 0.05$.

## 3. Results

### *3.1 Proximate analysis of eel meat*

ANOVA showed that there were no significant effect of different feed formulations ($p>0.05$) (Figure 1) on nutritional content of eel meat. The use of earthworms led to increasing levels of fat on eel meat with 1.612 % in P0, 1.242 % in P1, 1.256 % in P2, 1.690 % in P3, and 1.505 % in P4.Protein levels in eel meat were 17.073 % in P0, 16.957 % in P1, 16.599 % in P2, 17.413 % in P3, and17.846 % in P4. Meanwhile, ash content ranged from 0.083 % to 1.148 % and the energy content was within the range of 0.95 to 1.014 %.

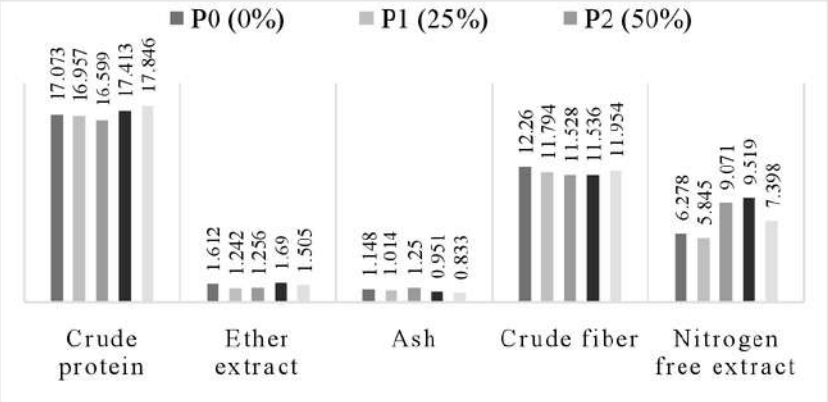


**Figure 1.** Proximatecomposition of eel meat as affected by different feed formula added with earthwormson the feed formulation against fish eel meat content.

### *3.2 Fatty acid profile*

There were no significant effect given by the different feed formula on saturated fatty acids, unsaturatedfatty acids, and omega 6 fatty acids of eel meat. Meanwhile, the treatments gave significant differences in EPA and DHA content of eel meat, where P4 had significantly higher EPA and DHA contents than P0 (table 1).

**Table 2.** Fatty acid profile of eel meat.

| FAMEs | Treatment (%) | | | | |
|---|---|---|---|---|---|
| | P0 | P1 | P2 | P3 | P4 |
| C12:0 Lauric acid | 0.347 | 1.003 | 0.624 | 0.397 | 0.830 |
| C14:0 Myristic acid | 5.291 | 5.417 | 5.507 | 4.942 | 5.465 |
| C13:0 Pentadecanoic acid | 0.665 | 0.696 | 0.629 | 0.701 | 0.739 |
| C16:0 Palmitic acid | 25.347 | 25.057 | 25.410 | 24.529 | 25.164 |
| C18:0 Stearic acid | 5.317 | 5.471 | 5.010 | 5.834 | 5.552 |
| ∑Saturated Fatty Acid (SFA) | 36.967 | 37.644 | 37.180 | 36.403 | 37.750 |
| C16:1Palmitoleic acid | 6.219 | 6.217 | 6.445 | 5.699 | 6.070 |
| C18:1 Oleic acid (ώ9) | 32.478 | 31.880 | 32.177 | 32.193 | 31.859 |
| C18:1 Elaidic acid | 3.894 | 4.179 | 3.978 | 3.898 | 4.099 |
| C20:1 cis 11 Eicosenoic acid | 2.268 | 2.648 | 2.521 | 3.403 | 2.638 |

| | | | | | |
|---|---|---|---|---|---|
| C22:1 Erucic acid | 0.293 | 0.256 | 0.372 | 0.458 | 0.273 |
| ∑Mono Unsaturated Fatty Acid (MUFA) | 45.152 | 45.180 | 45.493 | 45.651 | 44.939 |
| 8:2 Linoleic acid (LA) (ώ6) | 8.139 | 8.946 | 8.593 | 9.1635 | 8.661 |
| C20:4 Arachidonic acid (ώ6) | 1.432 | 1.481 | 1.314 | 1.488 | 1.392 |
| C20:5 Eicosapentaenoic acid (EPA) (ώ3) | $1.243^{a}$ | $1.014^{c}$ | $1.194^{ab}$ | $1.101^{abc}$ | $1.073^{bc}$ |
| C20: 3 Cis 8. 11. 14 Eicosatrienoic acid (hGLA) (ώ6) | 0.712 | 0.771 | 0.673 | 0.792 | 0.749 |
| C18:3 α Linolenic (ώ3) | 0.600 | 0.541 | 0.577 | 0.600 | 0.543 |
| C22:6 Docosahexaenoic (DHA) (ώ3) | $4.599^{a}$ | $3.257^{b}$ | $4.045^{ab}$ | $3.655^{ab}$ | $3.790^{ab}$ |
| ∑Poly Unsaturated Fatty Acid (PUFA) | 16.725 | 16.010 | 16.396 | 16.799 | 16.208 |
| ∑Unsaturated Fatty Acid (UFA) | 61.877 | 61.190 | 61.889 | 62.450 | 61.147 |
| SFA/UFA | 0.597 | 0.615 | 0.600 | 0.582 | 0.617 |
| UFA/SFA | 1.673 | 1.625 | 1.664 | 1.715 | 1.619 |
| ∑n3 | 6.442 | 4.812 | 5.816 | 5.356 | 5.406 |
| ∑n6 | 10.283 | 11.198 | 10.580 | 11.443 | 10.802 |
| n3/n6 | 0.626 | 0.429 | 0.549 | 0.468 | 0.500 |
| n6/n3 | 1.596 | 2.327 | 1.819 | 2.136 | 1.998 |
| EPA | $1.243^{a}$ | $1.014^{c}$ | $1.194^{ab}$ | $1.101^{abc}$ | $1.073^{bc}$ |
| DHA | $4.599^{a}$ | $3.257^{b}$ | $4.04^{5ab}$ | $3.655^{ab}$ | $3.790^{ab}$ |
| EPA/DHA | 0.270 | 0.311 | 0.295 | 0.301 | 0.283 |

P0 = commercial feed and earthworm (100%: 0%). P1 = commercial feed and earthworm (75%: 25%). P2: commercial feed and earthworm (50%: 50%). P3 = commercial feed and earthworms (25%: 75%). P4 = commercial feed and earthworm (0%: 100%)

## 4. Discussion

Figure 1 is in accordance with the results of Litzow *et al.* [10] who state the fat content in fish feed should be about 15%.Fat content in eel meat is highly correlated with the content of essential fatty acids. Moreover, Kandemir and Polat [11] stated that the content of fatty acids in aquatic organisms can be influenced by the living condition, either wild in nature or in captivity. There was lack of linolenic acid found in feed formula although the fatty acid was found in the earthworms.

The fatty acid profile of eel meat as shown Table 2 is in accordance with Oku *et al.* [12] who reported fatty acid content of Japanese eel (*Anguilla japonica*) fresh meat consisted mostly of monounsaturated fatty acids (MUFA), while unsaturated fatty acids (PUFA) appeared in low amount. Variation of fatty acids in aquatic organisms can be influenced by seasons, geographical location, and environment salinity [13].

Different doses of earthworms in eel feed formula could increase EPA and DHA contents in eel meat. The content of the omega-3 fatty acids EPA and DHA was affected by the presence or absence of earthworms in the feed formulation. According to Robin *et al.* [14] stated that when feed isrich in omega-3 fatty, Then the fish meat composition would beinfluenced. This is in accordance with the results of Huang *et al.* [15] stating that fatty acids contained in fish meat is derived from the fatty acids consumed by the fish.

Omega 3 and omega 6 fatty acids arepolyunsaturated fatty acids (PUFA). Omega 6 in eel meat showed a higher percentage compared with omega 3 [1]. Extremely high Omega 6 can negatively affect the body. The number and ratio of omega 3 and omega 6 fatty acids are important to be considered in formulating fish feed. A good ratio of omega 3: omega 6 is 10:1, which means there should be higher omega 3 content compared with omega 6 [3]. The best ratio was found in treatment P1 (75 %: 25 %) that was 1: 1.8. The composition of fatty acids in feed formulation can affect the ratio of omega 3 and omega 6 [16].

Fatty acids n-3 and n-6 are required in fat biosynthesis, so that in the event of a shortage or excess of one of the fatty acids,it will inhibit the rate of biosynthesis of other fatty acids and eventually it will

affect the composition of fatty acids in fish. an imbalance ratio of omega 3 and omega 6 can lead to competition inutilizing enzymes in fat metabolism, which can affectgrowth. As a conclusion, the use of earthworms in eel feed did not significantly affect fatty acid profile in eel meat.

## 5. Conclutions

Additions of earthworm in eel feed formula increase EPA and DHA contents in eel meets. The best EPA and DHA ratio of commercial feed and earthworm ws oin treatment P1 (75 %: 25%).

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