

An evaluation of fatty acid profiles of two commercial diets on the growth of Indonesian shortfin eel, *Anguilla bicolor*

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Abstract. This study aimed to evaluate the effects of two commercial diets' fatty acid compositions on the growth and survival of *A. bicolor*. Animal test weighing of 52.01 ± 24.21 g were fed two different diets, such as : CEF (diet specially formulated for eels) and CFF (diet for freshwater fish). Myristic acid (4.16% and 4.32%), palmitic acid (22.45% and 33.97%), and stearic acid for the CFF diet (13.15%), comprised the majority of the diet's saturated fatty acids (SFAs). The monounsaturated fatty acids (MUFAs) were mainly palmitoleic acid (4.71% and 5.54%) and oleic acid (17.75% and 19.63%). Polyunsaturated fatty acids (PUFAs) were 37.5% and 14.1%, respectively. Essential fatty acids (EFAs), such as linoleic acid, alpha-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid from the CEF diet were higher (3.73%, 1.25%, 9.22%, and 19.71%, respectively) than the CFF diet of 1.59%, 0.51%, 1.84%, and 7.99%, respectively. For eels fed the CEF and CFF diets, the ratio of n-3 to n-6 fatty acids was 4.14 and 0.66, respectively. Biomass production and fish survival were higher for the CEF diet animals. It can be concluded that the diet's PUFA and especially EFA contents further affect the growth and survival of *A. bicolor*.

1 Introduction

Eel (*A. bicolor*) is a high-value species, and it was cultured for many small farmers, mainly in the Cilacap district, Central Java, Indonesia. These species became the primary target species to compensate for the decline of temperate resources [1], [2]. Seed stocking mainly depends on glass eel sourced from the wild-caught either in the estuary or tidal rivers [3] and further reared into elver as initial stock size before growing in ponds. These animals are grown and fed artificial diets or moist paste with high-protein content as carnivorous species. The feed availability is specially formulated for eel species, though a long cultivation period is still required until marketable size. The slow growth pattern probably was not due to feeding used solely, but the nature of the species might have a significant contribution. As studied by [4], reported that the tropical larval of *A. celebesensis* required 99-122 days to metamorphose from leptocephalus stage into the glass eel. From this stage into elver it typically takes 6 to 8 months, depending on the species [2].

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A study on food and feeding habits of brown-stage eel of *A. bicolor* reported no seasonal variation in the feeding habits and indicated that their feeding habits tend to change as fish grow [5]. Further, they reported that the fish feed mainly on invertebrates during small size and become more piscivorous as they grow [5]. This information will further benefit the provision of high-quality and cost-effectively formulated diets for eel aquaculture.

The primary organic components of fish are lipids and proteins, which are also essential sources of metabolic energy for growth, including reproduction, motility, and migration [6]. Lipids are necessary as cofactors for enzymes, eicosanoid precursors, carriers of fat-soluble vitamins, hormones, and vitamin D [7]. Instead of energy sources and fatty acids, dietary lipids can spare protein energy and limit ammonia production [8]. Fish dietary lipids are absorbed as triacylglycerols aggregating into chylomicron particles and as fatty acids [9].

A study on *A. bicolor* (average weight of 446 g) showed high lipid content, which was reported at 13,26% (65,51% moisture content). Meanwhile, the amount of saturated, monounsaturated, and polyunsaturated fatty was 25,10%, 30,10%, and 17,87%, respectively [10]. Therefore, many studies have been conducted to enrich the lipid content of the diets before these species [11]–[14]. Like all vertebrates, fish have a modest capacity to synthesize n-3 HUFA de novo. Therefore, it must be obtained from the diets [15]. The fatty acids content of *A. bicolor* either from the wild or farm has been reported [10], [12], [16], but the feed used is rarely reported. The current study sought to assess the effects of fatty acid profiles from two different commercial diets on the growth and survival of *A. bicolor*.

2 Material and method

2.1 Animal test and feed

Animal tests were purchased from a local eel collector and further selected for suitable size and health conditions (e.g. bright in color, active, uniform in size, and no sign of deformity) for experimental purposes. Individual weight and length for selected eels were 52.01 ± 24.21 g and 30.61 ± 4.89 cm, respectively. Two commercial diets were provided, one specially formulated for eel culture (Sakai Feed) and another for freshwater fish (Comfeed).

2.2 Experimental design

Animal tests were subjected to culture on four rectangular concrete tanks with 6.9 x 2.7 x 0.6 m dimensions. All the tanks were previously cleaned and allowed to sun-dry for a few days before use. The well water source was pumped into each tank until a certain level was reached, and the water quality was measured for eel culture. 19.3 kg of fish were stocked in each tank, and stocking took place in the morning (around 07.00 am.) to minimize fish stress. The animals were fed two types of commercial feed, namely: A. Commercial diet for eel (CEF) and B. Freshwater fish diet (CFF), and each has two replications. Daily feed allowances were previously provided in a moist paste, placed on a feeding tray at 3% biomass, and divided into two meals daily. Feeding rates were adjusted after the sampling date, and the experiment lasted for four months. Animal sample for growth was monitored monthly by taking 30-40 fish samples from each tank. Water quality parameters such as dissolved oxygen, temperature, pH, nitrate, nitrite, and ammonia were also measured on the same day. Probiotics were added twice a week to control primarily nitrogen compounds in the water. Water exchange was conducted after detecting any indication of bed smell and darkening water colour. Additionally, calcium carbonate ($10\text{--}20$ g/m²) and sea salt ($1\text{--}2$ kg/m³) were added. At the termination of the experiment, the number of fish survivors was counted, and biomass was then calculated.

2.3 Feeds and eel analysis

Proximate and fatty acids composition for both feed and eel were analysed. Proximate compositions (SNI 01-2891-1992 point 5,7, and 8 ; SNI 2354.1 :2010) were analyzed at BRPBATPP Laboratory, while fatty acids (gravimetry method) for both feed and eel were analyzed at Saraswanti Laboratory.

3 Results and discussion

3.1 Proximate and fatty acids composition

From the two diets used during the experiment (Table 1), it was shown that the protein content of CEF diet was slightly lower (37.71%) compared to CFF diet (40.08%), and this protein content is lower than the eel requirement for the optimal growth of 45-47% [17]. Therefore, it is commonly practiced to enrich the protein content of the eel diet (e.g., trash fish, egg, and worm) before being fed to this species [18]. Besides, the sparing effect of lipid on protein requirements has been reported on American eels by reducing the protein content (30%) and increasing their lipid up to 20% [19].

Table 1. Proximate composition of commercially formulated for eel (CEF) and freshwater fish (CFF) diets being used during the experiment.

Proximate composition	CEF	CFF
Crude Protein (%)	37.71	40.08
Crude lipid (%)	7.56	7.53
Carbohydrate (%)	32.02	32.95
Ash (%)	15.33	12.15
Moisture (%)	7.38	7.29
Lipid energy (kcal/100 g)	68.04	67.77
Total energy (kcal/100 g)	346.96	359.89

Lipid in fish has important roles as a source of metabolic energy and essential fatty acids, EFA [20]. Total lipid from the two diets is closely the same 7.56% and 7.53%. In many studies, lipid and fatty acids enhancement in the eel, *A. bicolor* diets have been reported and shown a positive effect on growth after earthworm used [14], [21] and the addition of fish and corn oil [11], [22], [23]. However, fish oil's use to supply EFA has many limitations, such as carcinogen contaminant, stability, cost, undesirable odor, and low recovery of EPA and DHA fatty acid in fish [24]. The lipid content of the eel after fed two different diets ranged from 3,18-5,88% (Table 2), which is lower than the reported 13,26-17,72% [10]. The difference might be caused by size, diet, and culture environment.

Table 2. Proximate composition of eel after fed two different diets of commercially formulated for eel (Eel-CEF) and freshwater fish (Eel-CFF).

Parameters	Eel-CEF	Eel-CFF
Crude Protein (%)	15.08	14.84
Crude lipid (%)	3.18	5.88
Carbohydrate (%)	15.68	3.74
Ash (%)	1.46	2.38
Moisture (%)	64.60	73.16
Lipid energy (kcal/100 g)	28.62	52.92
Total energy (kcal/100 g)	151.66	127.24

The fatty acid content of the commercial feed and eel are presented in Table 3 and Table 4. In the CEF diet, the amount of saturated fatty acids (36.38%) is less than unsaturated fatty acids (63.32%), and conversely, in the CFF diet, 57.63% and 42.37%, respectively. Palmitic acid (C16:0) and myristic acid (C14:0) were the two primary saturated fatty acids (SFA) discovered in the two diets. Further, stearic acid (C18 :0) was also detected in high quantities on CFF diet.

Table 3. Saturated and unsaturated fatty acids content of the two commercial diets used during the experiment.

Fatty acid (% of total FA)	CEF	CFF
<u>Saturated Fatty Acids</u>	36.38	57.63
Caprylic acid, 18 :0	Nd	Nd
Capric acid, 10 :0	Nd	Nd
Lauric acid, 12 :0	0.22	0.06
Myristic acid, 14 :0	4.16	4.32
Pentadecanoid acid, 15 :0	0.74	1.48
Palmitic acid, 16 :0	22.45	33.97
Stearic acid, 18 :0	-	13.15
Arachidic acid, 20 :0	0.36	0.69
Henecosanoid acid, 21 :0	0.09	0.18
Behenic acid, 22 :0	Nd	0.29
Lignoceric acid, 24 :0	0.17	0.54
Cerotic acid, 26 :0	-	-
<u>Unsaturated Fatty acids</u>	63.62	42.37
Myristoleic acid, 14 :1 (n-5)	Nd	Nd
Palmitoleic acid, 16 :1 (n-7)	4.71	5.54
Sapienic acid, 16 :1 (n-10)	-	-
Oleic acid, 18 :1 (n-9)	17.75	19.63
Vaccenic acid, 18 :1 (n-7)	-	-
Linoleic acid, 18 : 2 (n-6)	3.73	1.59
α-Linolenic acid, 18:3 (n-3)	1.25	0.51
Arachidonic acid (ARA), 20 :4 (n-6)	2.91	1.46
Eicosapentaenoid acid (EPA), 20 :5 (n-3)	9.22	1.84
Eruic acid, 22 :1 (n-9)	0.41	0.19
Docosahexaenoid acid (DHA), 22 :6 (n-3)	19.71	7.99

Nd: Not detected

Table 4. Fatty acid composition of eel’s body after being fed from two different diets.

Fatty acid (% of total FA)	Eel- CEF	Eel-CFF
Saturated Fatty Acids	34.66	42.99
Caprylic acid, 18 :0	Nd	Nd
Capric acid, 10 :0	Nd	Nd
Lauric acid, 12 :0	0.13	0.10
Myristic acid, 14 :0	4.28	3.01
Pentadecanoid acid, 15 :0	0.28	0.95
Palmitic acid, 16 :0	24.53	28.86
Stearic acid, 18 :0	4.89	8.51
Arachidic acid, 20 :0	0.15	2.67
Henecosanoid acid, 21 :0	0.02	0.95
Behenic acid, 22 :0	Nd	0.04
Lignoceric acid, 24 :0	Nd	0.05
Cerotic acid, 26 :0	-	-
Unsaturated Fatty acids	65.34	57.01
Myristoleic acid, 14 :1 (n-5)	0.12	Nd
Palmitoleic acid, 16 :1 (n-7)	5.37	6.35
Sapienic acid, 16 :1 (n-10)	-	-
Oleic acid, 18 :1 (n-9)	45.62	30.98
Vaccenic acid, 18 :1 (n-7)	-	-
Linoleic acid, 18 : 2 (n-6)	1.37	6.22
α-Linolenic acid, 18:3 (n-3)	0.27	2.04
Arachidonic acid (ARA), 20 : 4 (n-6)	0.81	2.67
Eicosapentaenoid acid (EPA), 20 :5 (n-3)	1.65	1.13
Eruic acid, 22 :1 (n-9)	0.01	Nd
Docosahexaenoid acid (DHA), 22 :6 (n-3)	7.36	3.15

Nd: Not detected

Palmitoleic acid (C16:1) and oleic acid (C18:1) dominated monounsaturated fatty acids (MUFA), with ranges of 4.71-5.54% and 17.75-19.63%, respectively. Oleic acid was the principal MUFA in many species [25], and our result finding was 45,62% and 30,98% from the two fish groups tested. Both saturated and unsaturated fatty acids mentioned are comparable, as reported by [26], who were studied on *Labeo rohita*, *Cyprinus carpio*, and *Oreochromis mosambicus*.

Polyunsaturated fatty acid (PUFA) was the most abundant fatty acid discovered in the CEF diet. However, it was detected at a lower level in the eel when compared to the CFF diet (Figure 1). Generally, the PUFA content tended to decrease, and monounsaturated fatty acid increased in the fish studied. This suggests that MUFAs are used for metabolic energy, while long chain n-3 fatty acids are required for mainly structural purposes, such as a constituent of membrane phospholipids [25].

Major omega-3 fatty acids found in feed and eel included -linolenic acid (18:3 n-3), EPA (20:5 n-3), and DHA (22:6 n-3), while linoleic acid (18:2 n-6) and ARA (20:4 n-6) were the predominant omega-6 fatty acids. For the last two n-3 HUFA (EPA and DHA) mentioned

which have an important role in human nutrition and health, early development, and prevention of some diseases [25], [27], [28]. In fish, EPA and DHA are necessary for stress resistance and immunity development [7], fluidity, flexibility, membrane permeability, and cholesterol transport within the body [29].

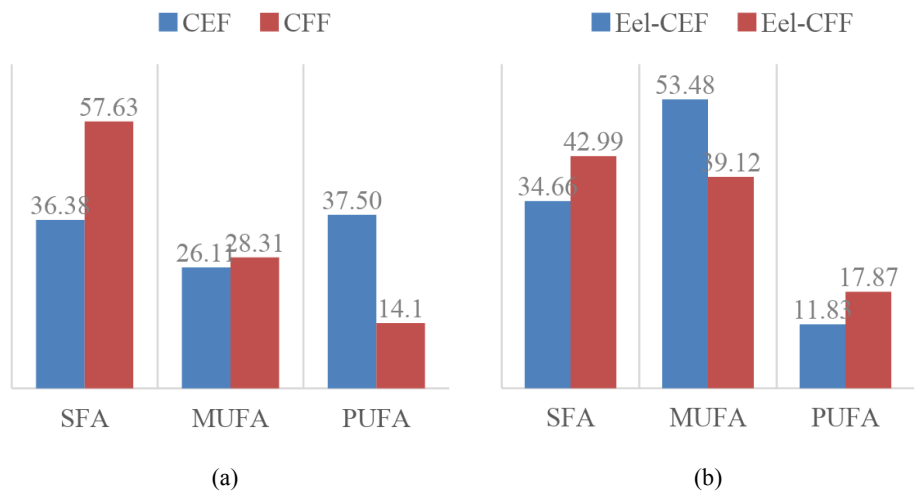


Fig. 1. Saturated, monounsaturated, and polyunsaturated fatty acids (% of total fatty acids) from the feed utilized (a) and eel after being fed with respected diets (b).

Omega-3 and omega-6 fatty acid content from CEF diet was higher compared to CFF diet (Figure 2). Through the desaturation and elongation pathway during fatty acid metabolism, omega-3 series is formed from -linolenic acid (18:3 n-3), whereas omega-6 series is derived from linoleic acid (18:2 n-6)[29]. Both fatty acid series are only synthesized de novo in plants and must be supplied into the diets and considered essential fatty acid (EFA) in fish [30]. EFA can be produced in freshwater fish by converting C18-PUFA (18:3 n-3 and 18:2 n-6) through a sequence of saturation and elongation reactions to C20 or C22 HUFA. [2].

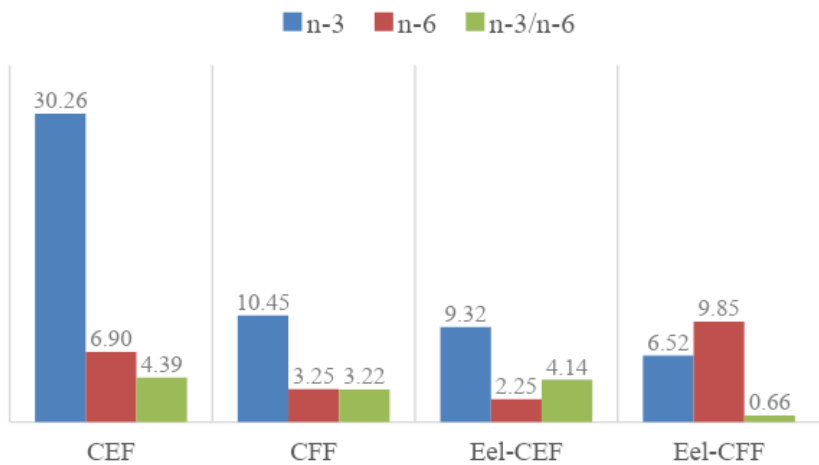


Fig. 2. The amount of n-3, n-6, and the n-3/n-6 fatty acid ratio in the feed and eel groups after feeding two different diets (CEF and CFF).

3.2 Biomass production

After four months culture period, it was found that the animal test-fed CEF diet performed better compared to CFF diet (Table 5). Fish biomass and eel survival after being fed CEF diet increased by around 29,4% and 7% higher than diet CFF.

Table 5. Average biomass and survival of eel after four months culture period and fed two different diets.

Commercial diet	Initial biomass (kg)	Final biomass kg	Survival rate (%)
CEF	19,3	32,1	94,6
CFF	19,3	24,8	88,5

The paired-sample t-test findings demonstrated that the CEF feed produced significant results in supporting eel growth compared to the CFF diet. The growth of eel fish showed a significant difference in the first two months of rearing, with eels receiving the CEF treatment achieving an average weight of 82.9±29.26 g, while those receiving the CFF feed only reached 65.57±32.40 g (Figure 3). At the fourth month of culture, the effect of feed was substantially different ($P<0.05$), with the average weight of eel samples reaching 131.93±33.53 g for the CEF diet and 75.14±35.98 g for the CFF diet. Besides, the fish-fed CEF diet performed better survival (94.6%) than the CFF diet (88.5%).

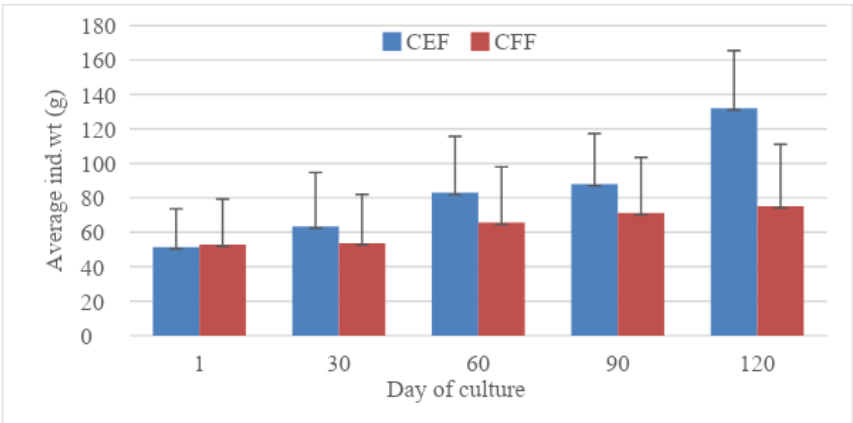


Fig.3. Monthly growth sample of eel after four months culture period and fed two different diets.

As mentioned earlier, the protein content of the diets used was slightly different, but the contribution of lipid energy and total energy was almost the same, with 68.04 vs 67.77 kcal/100 g and 346.96 vs 359.89 kcal/100 g, respectively. The role of fatty acid content, especially essential fatty acids (EFA), in the diet significantly impacts growth and survival and ultimately leads to higher biomass production.

Freshwater fish require C18-PUFA (two or more double bonds) to meet their EFA requirements. In contrast, marine species require long-chain (LC, C20-24) PUFA, such as eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (ARA) [6]. Linoleic acid (18:2 n-6) and linolenic acid (18:3 n-3) must both makeup at least 0.5% of the diet for anguillids, while ARA is also necessary and must be provided at a level of >0.69%

or 0.71% [30]. These fatty acids can be met by the eel's requirements primarily on a CEF diet. Fish fed on a diet with less than 0.5% 18:3 n-3 will experience retarded growth and erosion on the caudal fin [20]. According to a study on *A. japonica*, these two species of C18-PUFA (18:2 n-6 and 18:3 n-3) satisfied the EFA requirement, implying that these species had a complete pathway for the production of LC-PUFA [30]. The animal test's n-3/n-6 fatty acid ratio reveals that fish fed on CEF and CFF had n-3 fatty acid ratios of 4.14 and 0.66, respectively. According to the World Health Organization, these ratios should be at least one [28]. Hence, CEF diet specially formulated for eels showed superiority in fatty acid content and has a pronounced effect on production parameters such as growth and survival.

4 Conclusion

The study concludes that the presence of PUFAs, particularly EFAs, in the diet further influences the growth and survival of *A. bicolor*. The eel-specific diet demonstrated superiority in fatty acid concentration and composition, and it finally generated about 29.4% more biomass than the diet for freshwater fish.

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