Effect of enriching sea worms (*Nereis virens*) with DHA on the growth performance and body composition of cobia (*Rachycentron canadum*)

[1]Seto Windarto, [1]Tita Elfitasari, [2]I Made B. Dirgantara, [1]Vivi E. Herawati

[1] Department of Aquaculture, Faculty of Fisheries and Marine Science, Diponegoro University, Semarang, Indonesia; [2] Department of Management, Faculty of Economics and Business, Diponegoro University, Semarang, Indonesia. Corresponding author: V. E. Herawati, viviendar23@gmail.com

Abstract. Sea worms (*Nereis virens*) are one of the natural foods that can increase shrimp and fish egg production. Sea worms are distributed and found in the marine environment in Indonesia, making them a natural feed for cobia (*Rachycentron canadum*) fry. Selco DHA is a commercial product commonly used as an enrichment ingredient in natural feeds to increase the nutritional quality. This study aimed to determine the growth and survival of cobia fed with Selco DHA enriched sea worms, and to determine the best dosage of *N. virens* enriched with DHA Selco. This research used a completely randomized design (CRD), with 4 treatment levels and 3 replications. The sea worms were enriched with DHA Selco for 2 hours, with different doses, such as 0 g L^{-1} (A); 0.6 g L^{-1} (B); 0.9 g L^{-1} (C); and 1.2 g L^{-1} (D). 30-day-old cobia fry with initial weight and length of 0.38 g and 4.2 cm, respectively, were used in this study. Fish were reared in 45x45x100 cm hapa placed in 6x2 m concrete tanks. The rearing period was 30 days with a stocking density of 50 fish per hapa. The feeding frequency was 3 times a day, using the satiety method. The results showed that adding DHA Selco significantly affected ($p<0.05$) total feed consumption (TFC), feed utilization efficiency (FUE), relative growth rate (RGR), absolute length, biomass weight, protein efficiency ratio (PER), and survival rate (SR). The best results were found in treatment D, resulting in a TFC of 49.69%, a FUE of 83.78%, a RGR of 17.92% day^{-1}, a final length of 5.1 cm, a final weight of 2.37 g, a PER of 3.59% and a SR of 88.33%. Cobia contained 7.05% methionine and 5.5% glutamic acid. Water quality during rearing was in optimal conditions.

Key Words: enlargement, fatty acid, marine worms, nutrient, *R. canadum*.

Introduction. Cobia (*Rachycentron canadum*) can grow quickly, reaching 4-6 kg in one year (Chou et al 2001). According to Syuhriatin (2020), the growth rate of fish is strongly influenced by the type and quality of feed. If the feed has good quality and is supplied in sufficient quantity, together with favorable environmental parameters, it will produce a good growth rate. Sea worm (*Nereis virens*) is a natural feed for cobia, member of the invertebrate family Nereidae, classis Polychaeta. It lives in the estuarine ecosystem as a benthic organism. Sea worms are abundant and easy to find along the marine environment in Indonesia, making them a natural food for cobia fry (Wibowo et al 2019).

The nutritional composition of seaworm flour includes 56.29% crude protein, 11.32% fat, 14.34% ash, 1.19% crude fiber, and 5.63% water (Asnawi et al 2018). DHA selco is a source of essential fatty acids. It is sold commercially. The content of DHA Selco includes 67% fat, 0.2% phosphorus, vitamin A (1500000 IU kg^{-1}), vitamin D3 (150000 IU kg^{-1}), vitamin E (3600 mg kg^{-1}), vitamin C (800 mg kg^{-1}) and antioxidants (Laksamana et al 2011).

Several enrichment studies using DHA Selco have been applied to natural foods such as *Artemia* sp. tested on *Carassius auratus* (Muliani et al 2016). Enrichment with DHA Selco has also been done on rotifers, tested then on *Litopenaues vannamei* shrimp (Wahyudin 2005). This study aimed to determine the optimal enrichment with DHA Selco of sea worms, which was expected to increase fatty acid levels and the survival and growth of cobia fed with enriched sea worms.

Material and Method

Preparation of the study. This study was conducted in Lampung Cultivation Center (BBL), Indonesia. The tools used during this study were hapa as a fry cultivation container, refractometer, COD meter, and pH. The materials used were sea worms, DHA Selco, and cobia fry aged 30 days with an initial biomass weight of 0.38 g and an initial total length of 4.2 cm. Sea worms and cobia were obtained from the Lampung Cultivation Center. The cobia fish were reared for 30 days with a 50 fish per hapa density. The size of the hapa was 80x80x100 cm. This study used a completely randomized design (CRD), with 4 treatments and 3 replications. The treatments were the following: A - sea worm without enrichment of DHA Selco; B - 0.5 g L^{-1} DHA Selco; C - 1 g L^{-1} DHA Selco; D - 1.5 g L^{-1} DHA Selco. Enrichment of sea worms (*Nereis* sp.) was done by mixing DHA Selco with egg yolk and yeast, which was added to the sea worm rearing container. Sea worms absorb it as food because sea worms have a feeler feeder way of eating (Putri et al 2021). The soaking time for sea worms was 2 hours.

Observed parameters. Measurements were conducted weekly to observe the growth performance, which consists of total feed consumption (TFC), feed utilization efficiency (FUE), relative growth rate (RGR), absolute length and weight growth, protein efficiency ratio (PER), and survival rate (SR).

Total feed consumption (TFC). TFC was calculated using the formula (Tacon 1993):

$$TFC=C-S$$

Where: TFC - total feed consumption (g); C - administered feed (g); S - remaining feed (g).

Feed utilization efficiency (FUE). FUE was calculated using the formula (Tacon 1993):

$$FUE = (W_t-W_0)/F \times 100$$

Where: FUE - feed utilization efficiency (%); W_t - weight of fish at the end of the rearing period (g); W_0 - fish weight at the beginning of the rearing period (g); F - weight of feed administered (g).

Relative growth rate (RGR). RGR (or daily weight gain) was calculated using the formula (Tacon 1987):

$$RGR = (W_t-W_0)/(W_0 \times t) \times 100$$

Where: RGR - relative growth rate (% day^{-1}); W_0 - biomass at the end of the study (g); W_t - biomass at the beginning of the study (g); t - length of rearing (days).

Absolute length growth. Length measurements on cobia were carried out at the beginning and end of the study. Absolute length growth was calculated using the formula (Tacon 1993):

$$L = L_0 - L_t$$

Where: L - absolute length (cm); L_t - length of fish at the end of the study (cm); L_0 - fish length at the beginning of the study (cm).

Absolute weight. The growth of fish biomass represents the difference between fish biomass at the end of maintenance and fish biomass at the beginning of maintenance (Tacon 1993).

$$W = W_t – W_0$$

Where: W - weight gain (g); W_t - weight of fish at the end of the study (g); W_0 - fish weight at the beginning of the study (g).

Protein efficiency ratio (PER). PER was calculated using the following formula (Tacon 1987):

$PER = (W_t-W_0)/P_i \times 100$

Where: PER - protein efficiency ratio (%); W_t - biomass at the end of the study (g); W_0 - biomass at the beginning of the study (g); P_i - weight of feed consumed x % protein in feed (g).

Survival rate (SR). The SR was calculated using the formula (Tacon 1993):

$SR = N_t/N_0 \times 100$

Where: SR - survival rate (%); N_t - number of fish at the end of rearing; N_0 - number of fish at the beginning of the study.

Water quality measurements. Temperature, pH, salinity, and dissolved oxygen (DO) were measured using a Water Quality Checker (WQC) to determine water quality. Ammonia was tested at the Environmental Quality Seawater Aquaculture Center, Lampung, Indonesia. Temperature, salinity, and pH measurements were carried out twice a day, oxygen was measured once a day, and ammonia was measured once a week.

Proximate analysis. The protein, fat, ash, carbohydrate, fiber, and water content of shrimp samples were determined via proximate analysis (AOAC 2005). The Kjeldahl method was used to analyze the protein content, whereas the Soxhlet method was used to analyze the fat content. The water and ash content was assessed using gravimetric principles. The carbohydrate content was calculated mathematically after the other proximate analysis results were obtained.

Amino acid analysis. The amino acid profile was evaluated using a HPLC type 1100 instrument fitted with a Eurosphere 100-5 C18, 2504.6 mm column, and a column P/N 1115Y535 as the starting column P/N. 0.01 M acetate buffer at pH 5.9 and 0.01 M MeOH acetate buffer at pH 5.9, THF>80:15:5 0.01 M acetate buffer in MeOH constituted the wastes. Fluorescence was used at extra 340 mm and Em 450 nm. Approximately 2.5 g of sample were placed in a closed glass recipient, and 15 mL of 6 M HCl was added. Before being neutralized with 6 M NaOH and cooled to room temperature, the mixture was homogenized by vortexing and hydrolyzed at 110°C for 12 h in an autoclave. After adding 2.5 mL of 40% lead acetate and 1 mL of 15% oxalic acid to the mixture, 3 mL were filtered using a 0.45 m Millex-HV filter (Merck). 25 mL of the filtered mixture and 475 mL of the OPA anhydrase solution were mixed and incubated for 3 min before injection into the HPLC. The HPLC system was injected with 30 mL of the final combination (AOAC 2005).

Fatty acid analysis. The fatty acid profile was measured using a QP-2010 Gas Chromatograph - Mass Spectrophotometer (GCMS) (Shimadzu) with a 50 m, 0.22 mm wall coat open tubular CP-SIL-88 column (Agilent, USA). Analyses were undertaken at column temperatures ranging from 120 to 200°C. The approach adopted was *in situ* transesterification. 100 mg of the sample was homogenized with 4 mL of water. 104 mg of the homogenate was transferred to a test tube. There was an addition of 100 mL of methylene chloride and 1 mL of 0.5 M NaOH in methanol. After adding nitrogen, the tubes were firmly sealed. They were kept at 90°C for 10 min. After the test tubes were cooled, 1 mL of 14% BF3 in methanol was added. After adding nitrogen, it was kept at the same temperature for 10 min. Once the test tubes reached room temperature, 1 mL of water and 200-500 mL of hexane were added, and 1 min of stirring separated the methyl esters from the fatty acids. After centrifugation, the sample's top layer was prepared for GC analysis (AOAC 2005).

Statistical analysis. Analysis of variance was used to evaluate the collected data (ANOVA). Normality, homogeneity, and additivity tests were performed to evaluate whether the data were typical, homogenous, and additive. If significant differences were discovered ($p<0.05$), Duncan's Multiple Area Test was performed to identify the groups with significant differences. The water quality data were descriptively examined.

Results. The TFC, FUE, RGR, length and weight growth, PER and SR obtained after 30 days of rearing cobia are presented in Table 1.

Table 1

Growth performance of cobia (*Rachycentron canadum*) during 30 days of rearing

Variable	*Treatments*			
	A	*B*	*C*	*D*
TFC (g)	43.68±0.08^{a}	46.33±0.95^{b}	48.00±1.00^{bc}	49.69±0.15^{c}
FUE (%)	67.07±2.51^{a}	72.08±1.80^{ab}	72.76±1.90^{bc}	83.78±1.25^{d}
RGR (% day^{-1})	13.45±0.43^{a}	14.39±0.38^{ab}	14.82±0.66^{bc}	17.92±0.13^{d}
Length (cm)	4.64±0.15^{a}	4.74±0.15^{ab}	4.77±0.12^{bc}	5.10±0.18^{d}
Weight (gr)	1.98±0.05^{a}	2.04±0.03^{ab}	2.07±0.04^{abc}	2.37±0.02^{d}
PER (%)	2.98±0.08^{a}	3.19±0.03^{bc}	3.21±0.05^{abc}	3.59±0.08^{d}
SR (%)	73.33±1.15^{a}	74.00±2.00^{ab}	75.33±1.15^{abc}	88.33±2.52^{d}

Note: A - sea worm without enrichment of DHA Selco; B - 0.5 g L^{-1} DHA Selco; C - 1 g L^{-1} DHA Selco; D - 1.5 g L^{-1} DHA Selco; different superscripts indicate significant differences ($p<0.05$).

The enrichment of sea worms with DHA Selco as a feed for cobia with a rearing period of 30 days has a significant effect ($p<0.05$) on TFC, FUE, RGR, absolute length, weight and SR. The best results were observed in treatment D, with a TFC of 49.69%, FUE of 83.78%, RGR of 17.92% day^{-1}, the total length of 5.1 cm, weight of 2.37 g, PER of 3.59% and SR of 88.33%. Treatment A produced the poorest results: 43.68 g TFC, 67.07% FUE, 13.45% day^{-1} RGR, 4.64 cm total length, 1.98 g weight, 2.98% PER and 73.33% SR. Treatment D had the best nutritional content of the experimental diet: 56.75% protein and 12.52% fat. The lowest nutritional content was in treatment A: 45.26% protein and 9.04% fat. A proximate analysis of the experimental diet is presented in Table 2.

Table 2

Proximate analysis of experimental diets

Dry weight content	*Treatments*			
	A	*B*	*C*	*D*
Protein (%)	45.26±0.03	50.07±0.02	53.65±0.09	56.75±0.08
Carbohydrate (%)	25.55±0.03	22.25±0.02	18.07±0.08	15.2±0.09
Crude fat (%)	9.04±0.05	10.4±0.03	11.57±0.09	12.52±0.07
Ash (%)	13.52±0.06	12.08±0.03	10.93±0.08	11.28±0.02
Crude fiber (%)	6.63±0.04	5.2±0.07	5.78±0.02	4.25±0.08

Note: A - sea worm without enrichment of DHA Selco; B - 0.5 g L^{-1} DHA Selco; C - 1 g L^{-1} DHA Selco; D - 1.5 g L^{-1} DHA Selco; different superscripts indicate significant differences ($p<0.05$).

Based on the proximate analysis of cobia, treatment D produced the best results (62.55% protein and 14.24% fat), while the poorest results were found in treatment A (50.2% protein and 11.1% fat) (Table 3).

Based on the fatty acid profile of the experimental diet, the best treatment was treatment D, resulting in 6.7% EPA and 5.45% palmitate. The treatment with the poorest results was A, resulting in 3.4% EPA and 3.14% palmitate.

Table 3

Proximate analysis of cobia (*Rachycentron canadum*) after 30 days of rearing

Dry weight content	*Treatments*			
	A (0%)	*B (20%)*	*C (25%)*	*D (30%)*
Protein (%)	50.2±0.05	53.67±0.05	58.65±0.03	62.55±0.05
Carbohydrate (%)	12.55±0.04	10.25±0.08	13.07±0.03	10.48±0.04
Crude fat (%)	11.1±0.04	12.4±0.01	13.57±0.05	14.24±0.02
Ash (%)	20.52±0.02	18.08±0.04	8.93±0.01	8.28±0.05
Crude fiber (%)	5.63±0.06	5.6±0.04	5.78±0.04	4.45±0.01

Note: A - sea worm without enrichment of DHA Selco; B - 0.5 g L^{-1} DHA Selco; C - 1 g L^{-1} DHA Selco; D - 1.5 g L^{-1} DHA Selco; different superscripts indicate significant differences ($p<0.05$).

Fatty acid profile. The fatty acid profile of the test feed is presented in Table 4.

Table 4

The fatty acid profile of experimental diets

Amino acid (%)	*Treatments*			
	A	*B*	*C*	*D*
C14:0 (Myristic)	2.52±0.09^a	3.29±0.06^b	3.41±0.02^b	4.3±0.05^b
C15:0 (Pentadecanoic)	1.76±0.08^a	2.18±0.03^a	3.17±0.04^b	3.15±0.07^b
C16:0 (Palmitic)	3.14±0.07^a	4.29±0.09^b	4.67±0.08^b	5.45±0.05^b
C18:0 (Stearic)	2.71±0.05^a	2.65±0.01^b	3.52±0.03^b	3.91±0.01^b
C18:1 n-9 (Oleic/ω9)	3.07±0.03^a	3.95±0.03^a	4.09±0.08^b	5.2±0.02^b
C18:2 n-6 (Linoleic/ω6)	3.83±0.09^a	4.46±0.07^b	4.49±0.07^b	5.6±0.08^b
C18:3 n-3 (Linolenic/ω3)	3.54±0.02^a	4.76±0.08^b	4.89±0.03^b	5.54±0.03^b
C20:0 (Arachidic)	2.3±0.04^a	3.23±0.02^a	3.53±0.04^a	2.98±0.09^a
C20:4 n-6 (Arachidonic)	2.71±0.03^a	3.15±0.03^b	3.75±0.09^b	4.39±0.02^b
C20:5 n-3 (EPA)	3.2±0.08^a	4.24±0.07^b	4.75±0.02^b	6.7±0.04 ^{b}
C22:6 n-3 (DHA)	1.23±0.05^a	4.07±0.04^b	4.65±0.01^b	5.67±0.02^b

Note: A - sea worm without enrichment of DHA Selco; B - 0.5 g L^{-1} DHA Selco; C - 1 g L^{-1} DHA Selco; D - 1.5 g L^{-1} DHA Selco; different superscripts indicate significant differences ($p<0.05$).

Based on the fatty acid profile of cobia, the best treatment was D, resulting in 8.9% EPA and 5.95% palmitate. The poorest results were in treatment A, resulting in 4.98% EPA and 3.4% palmitate. The fatty acid profile of cobia during 30 days of rearing is presented in Table 5.

Table 5

Fatty acid profile of cobia (*Rachycentron canadum*) fish during 30 days of rearing

Amino acid (%)	*Treatments*			
	A	*B*	*C*	*D*
C14:0 (Myristic)	3.15±0.08^a	3.8±0.05^a	4.4±0.03^b	4.85±0.03^b
C15:0 (Pentadecanoic)	2.25±0.05^a	2.3±0.02^a	2.98±0.06^b	3.3±0.07^b
C16:0 (Palmitic)	3.4±0.03^a	4.6±0.01^b	4.88±0.02^b	5.95±0.04^b
C18:0 (Stearic)	2.08±0.02^a	2.9±0.06^a	3.6±0.08^b	4.2±0.03^b
C18:1 n-9 (Oleic/ω9)	3.35±0.04^a	4.1±0.02^b	4.25±0.09^b	6.15±0.07^b
C18:2 n-6 (Linoleic/ω6)	3.9±0.01^a	4.65±0.08^b	4.95±0.03^b	5.9±0.09^b
C18:3 n-3 (Linolenic/ω3)	3.54±0.03^a	4.76±0.09^b	4.89±0.08^b	5.32±0.05^b
C20:0 (Arachidic)	2.45±0.02^a	3.5±0.03^b	3.98±0.09^b	4.2±0.03^b
C20:4 n-6 (Arachidonic)	2.95±0.06^a	3.23±0.02^a	3.9±0.04^b	3.6±0.08^b
C20:5 n-3 (EPA)	4.98±0.07^a	6.77±0.08^b	7.56±0.08^b	8.9±0.09^b
C22:6 n-3 (DHA)	1.6±0.02^a	4.05±0.09^b	4.8±0.04^b	6.88±0.08^b

Note: A - sea worm without enrichment of DHA Selco; B - 0.5 g L^{-1} DHA Selco; C - 1 g L^{-1} DHA Selco; D - 1.5 g L^{-1} DHA Selco; different superscripts indicate significant differences ($p<0.05$).

Amino acid profile. The amino acid profile of the experimental diets is presented in Table 6. Based on the amino acid profile of the experimental diet, the best treatment results were observed in treatment D, resulting in 7.05% methionine and 5.5% glutamic acid. The poorest amino acid profile was in treatment A feed, resulting in 4.8% methionine and 2.19% glutamic acid.

Table 6

The amino acid profile of the experimental diet

Amino acids profile (%)	*Treatments*			
	A	*B*	*C*	*D*
Aspartic acid	$2±0.05^a$	$2.95±0.03^a$	$3.17±0.05^b$	$3.98±0.02^b$
Proline	$1.04±0.03^a$	$2.5±0.02^a$	$2.9±0.03^b$	$3.15±0.04^b$
Serine	$0.82±0.04^a$	$1.75±0.07^b$	$2.25±0.02^b$	$2.88±0.06^b$
Glutamic acid	$2.19±0.05^a$	$4.29±0.02^b$	$4.85±0.07^b$	$5.5±0.04^b$
Glycine	$1.09±0.02^a$	$2.35±0.04^a$	$2.88±0.04^b$	$3.45±0.05^b$
Histidine	$0.86±0.01^a$	$1.45±0.02^b$	$1.9±0.02^b$	$2.2±0.02^b$
Arginine	$2.61±0.05^a$	$2.75±0.06^a$	$3.25±0.01^b$	$3.98±0.09^b$
Threonine	$2.05±0.08^a$	$2.3±0.02^a$	$2.7±0.08^b$	$3.2±0.02^b$
Alanine	$3.23±0.09^a$	$3.5±0.09^a$	$3.68±0.06^a$	$4.05±0.04^b$
Valine	$0.9±0.02^a$	$1.6±0.07^a$	$2.37±0.02^b$	$2.85±0.05^b$
Methionine	$4.8±0.04^a$	$6.2±0.04^b$	$6.75±0.05^b$	$7.05±0.03^b$
Lysine	$3.9±0.03^a$	$5.25±0.02^b$	$5.9±0.06^b$	$5.55 ± 0.02^b$
Isoleucine	$3.24±0.03^a$	$3.8±0.01^a$	$4.15±0.03^b$	$4.55±0.01^b$
Phenylalanine	$1.04±0.09^a$	$1.8±0.09^a$	$2.05±0.01^b$	$2.44±0.09^b$

Note: A - sea worm without enrichment of DHA Selco; B - 0.5 g L^{-1} DHA Selco; C - 1 g L^{-1} DHA Selco; D - 1.5 g L^{-1} DHA Selco; different superscripts indicate significant differences ($p<0.05$).

Based on the amino acid profile of cobia, the best treatment was treatment D, with 8.95% methionine and 5.5% glutamic acid. Treatment A resulted in 4.98% methionine and 3.15% glutamic acid in cobia. The amino acid profile of cobia after 30 days of rearing is presented in Table 7.

Table 7

The amino acid profile of cobia fish (*Rachycentron canadum*) during 30 days of rearing

Amino acids profile (%)	*Treatments*			
	A	*B*	*C*	*D*
Aspartic acid	$1.78±0.03^a$	$3.08±0.02^b$	$3.56±0.04^b$	$4.1±0.04^b$
Serine	$1.45±0.04^a$	$2.77±0.01^b$	$2.35±0.02^a$	$2.7±0.06^b$
Glutamic acid	$3.15±0.02^a$	$4.18±0.05^b$	$4.75±0.01^b$	$6.5±0.07^b$
Glycine	$2.2±0.01^a$	$3.08±0.06^b$	$3.17±0.06^b$	$3.35±0.05^b$
Histidine	$1.2±0.07^a$	$2.4±0.08^b$	$2.9±0.08^b$	$3.15±0.03^b$
Arginine	$1.29±0.08^a$	$1.76±0.02^a$	$2.06±0.03^b$	$2.4±0.06^b$
Threonine	$2.8±0.03^a$	$2.99±0.04^a$	$3.4±0.02^b$	$2.1±0.03^b$
Alanine	$2.3±0.09^a$	$2.87±0.01^a$	$2.95±0.05^a$	$3.35±0.07^b$
Proline	$3.3±0.02^a$	$3.75±0.06^b$	$3.9±0.08^b$	$4.18±0.09^b$
Valine	$1.2±0.06^a$	$1.95±0.05^a$	$2.4±0.02^b$	$3.07±0.02^b$
Methionine	$3.75±0.02^a$	$5.6±0.03^b$	$6.95±0.01^b$	$8.95±0.01^b$
Lysine	$5.4±0.09^a$	$6.26±0.06^a$	$6.83±0.08^a$	$7.75±0.04^b$
Isoleucine	$3.3±0.05^a$	$4.18±0.03^b$	$4.56±0.02^b$	$4.98±0.02^b$
Leucine	$1.04±0.02^a$	$1.8±0.08^a$	$2.05±0.04^b$	$2.44±0.08^b$
Phenylalanine	$2.2±0.06^a$	$3.07±0.03^b$	$3.47±0.08^b$	$4.05±0.03^b$
Tryptophan	$1.35±0.01^a$	$2.88±0.01^b$	$3.15±0.02^b$	$3.88±0.02^b$

Note: A - sea worm without enrichment of DHA Selco; B - 0.5 g L^{-1} DHA Selco; C - 1 g L^{-1} DHA Selco; D - 1.5 g L^{-1} DHA Selco; different superscripts indicate significant differences ($p<0.05$).

Water quality. Water quality measurements are presented in Table 8.

Table 8

Water quality in cobia (*Rachycentron canadum*) fry-rearing media

Parameter	*Result*	*Reference values*
Temperature (°C)	28.4-28.6	27-30
pH	7.57	7-8.5
Salinity	32-33	30-34
DO (mg L^{-1})	5.12-5.33	>4

Note: DO - dissolved oxygen; reference values are based on the Decision of the Minister of Environment of Indonesia No. 51 of 2004.

Discussion. The results showed that adding DHA Selco significantly affected ($p<0.05$) TFC, FUE, RGR, absolute length, weight, PER, and SR of cobia fry. The increase in length and weight of fry indicates good feed consumption and utilization of seaworm protein. The RGR of cobia is directly proportional to the total length growth and weight growth. This is because of good feed efficiency. In addition, the enrichment of *N. virens* with DHA at a dose of 1.2 g L^{-1} has the highest effect on FUE and the amount of feed consumed. The fatty acid content increases appetite and feed utilization in cobia. A higher feed efficiency produces a better growth rate. According to Hasyim et al (2018), a diet with higher fatty acids makes fish respond better than a diet with a low content of fatty acids. Enriching DHA in sea worms can increase the nutritional content of cobia fed with sea worms and may also act as an attractant.

Attractants in feed will increase appetite and the amount of feed consumption in fish. The results of this study are supported by the research of Izal et al (2019), who note that attractants may be additional ingredients to stimulate the appetite in fish. The attractant contains DHA and EPA (ω-3 PUFA fatty acids), which can affect the absorption process of feed in marine fish. The DHA chain improves the absorption of protein and other nutrients in fish. According to Pangkey (2011), fatty acids are instrumental in the natural growth and development of fish. Marine fish have a different enzymatic system compared to freshwater fish, requiring long-chain n-3 and n-6 fatty acids from the diet for optimal growth. The essential fatty acids are EPA and DHA. EPA and DHA requirements for cobia fry range from 0.9 to 2.1% (Trushenski et al 2012). The fatty acid content in seaworms is influenced by the composition of feed nutrients (Wibowo et al 2020). EPA in the diet functions as an essential component of phospholipids in membranes and nervous tissue. It plays a role in survival. Khudyi et al (2017) note that EPA plays a role in the survival and fish growth. EPA is an essential component of phospholipids in membranes and nervous tissue. At first feedings, larvae have a very high neurosomatic index, so they need a high n-3 HUFA content in the feed to avoid nerve formation abnormalities (Limsuwatthanathamrong et al 2012).

The nutritional content of the feed also influences growth. The experimental diets meet the protein and fatty acids requirements of cobia. According to Fraser & Davies (2009), the required protein content for cobia fry is 45-55% and fat is 7-15%. This is reinforced by Herawati et al (2020), who note that the nutritional balance of protein and fat in seaworm feed will determine sea worm quality. The high protein content in sea worms and a well-balanced absorption process will affect the growth of cobia. The high protein absorption process of sea worms will also produce good growth in cobia. This is supported by Herawati et al (2017), who stated that the feed consumed by fish will be used first for basic metabolism, movement, production of sexual organs, and replacement of damaged cells. The remaining energy from the diet will be used after meeting metabolism needs.

Dietary methionine improves the balance and utilization of other amino acids. Methionine has an important part in protein synthesis and physiological functions. Bhagavan (1992) stated that the fish body requires methionine for the formation of nucleic acids and tissues and protein synthesis. Methionine works with vitamin B12 and folic acid to help the body regulate excessive protein supply in high-protein diets. Moreover, methionine is required by the body to initiate protein synthesis, which can affect muscle growth (Belghit et al 2014). Feed with a high methionine content increases growth and immune response (Rolland et al 2015). A lack of methionine can decrease growth and survival in cobia fry (Setianingsih et al 2019).

Survival is mainly influenced by feed and water quality. The diet influences the survival of cobia. The addition of DHA to sea worms had a significant effect ($p<0.05$) on the survival of cobia. The highest SR in cobia was found in treatment D, with 88.33%. Cobia is a cannibalistic fish, especially when food is scarce, grading and sorting being important processes in the aquaculture of this species (Nguyen et al 2019).

According to Setianingsih et al (2019), the condition of each water parameter will affect the growth and health of fish. The water quality is influenced by the running water system, aeration, and other factors. According to Nazar et al (2012), the environmental conditions required by cobia are a DO higher than 5 mg L^{-1}, a pH between 7.8-8.4, and a salinity between 25-35 ppt. In this study, the water quality allowed the growth of cobia.

Conclusions. Enrichment of sea worms with DHA Selco improved the growth and survival of cobia. It improved TFC, FUE, RGR, and PER. The increasingly higher dose of DHA Selco enrichment provided increasingly better feed consumption and utilization.

Acknowledgements. This work was supported by Universitas Diponegoro (Selain APBN) 2023 Number: 569-03/UN7.D2/PP/V/2023.

Conflict of Interest. The authors declare that there is no conflict of interest.

References

Asnawi, Yusnaini, Idris M., 2018 [Effect of different substrates on marine worm biomass growth (*Nereis* sp.)]. Media Akuatika 3(2):670-679. [In Indonesia].

Belghit I., Skiba-Cassy S., Geurden I., Dias K., Surget A., Kaushik S., Panserat S., Seiliez I., 2014 Dietary methionine availability affects the main factors involved in muscle protein turnover in rainbow trout (*Oncorhynchus mykiss*). British Journal of Nutrition 112:493-503.

Bhagavan N. V., 1992 Medical biochemistry. Jones and Bartlett, 980 p.

Chou R. L., Su M. S., Chen H. Y., 2001 Optimal dietary protein and lipid levels for juvenile cobia (*Rachycentron canadum*). Aquaculture 193(1-2):81-89.

Fraser T. W. K., Davies S. J., 2009 Nutritional requirement of cobia, *Rachycentron canadum* (Linnaeus): a review. Aquaculture Research 40(11):1219-1234.

Hasyim R. A. Y., Arief M., Rahardja B. S., 2018 [Addition of attractant in pasta feed against feed consumption, protein retention and fat retention of Asian swamp eel (*Monopterus albus*) cultured using recirculation system]. Journal of Aquaculture and Fish Health 7(1):1-9. [In Indonesian].

Herawati V. E., Hutabarat J., Karnaradjasa O., 2017 [Growth performance and survival of catfish (*Clarias gariepenus*) larvae by feeding *Tubifex* sp. mass cultured using industrial waste fermentation]. Aquaculture Engineering and Technology Journal 6(1):675-682. [In Indonesian].

Herawati V. E., Pinandoyo., Windarto S., Rismaningsih N., Darmanto Y. S., Prayitno S. B., Radjasa O. K., 2020 Nutritional value and growth performances of sea worms (*Nereis* sp.) fed with *Hermetia illucens* maggot flour and grated coconut (*Cocos nucifera*) as natural feed. Biodiversity 21(11):5431-5437.

Izal, Putra W. K. A., Yulianto T., 2019 [The effect of different treatment of attractants on the feed consumption in sea bass *Lates calcarifer*]. Intek Aquaculture 3(1):25-33. [In Indonesian].

Khudyi O., Khuda L., Kushniryk O., Prusinska M., Kolman R., Marchenko M., 2017 An effectiveness of *Artemia nauplii* enrichment with polyunsaturated fatty acids using a supplement easy DHA Selco. Acta Biologica Universitatis Daugavpiliensis 17(2):169-183.

Laksamana S. S., Ajith K. T. T., Pandi T. M., Devi K. N., Balasubramanian T., 2011 Comparison of A1 DHA and micro algae on biochemical signatures of enriched *Artemia salina*. Annals of Biological Research 2(4):110-121.
Limsuwatthanathamrong M., Sooksai S., Chunhabundit S., Noitung S., Ngamrojanavanich N., Petsom A., 2012 Fatty acid profile and lipid composition of farm-raised and wild-caught sandworms, *Perinereis nuntia*, the diet for marine shrimp broodstock. Asian Journal of Animal Sciences 6(2):65-75.
Muliani M., Ayuzar E., Rizal M., 2016 [Enrichment of *Artemia* sp in larviculture of comet fish (*Carassius auratus*)]. Terubuk Fisheries Berkala 44(1):17-32. [In Indonesian].
Nazar A. K., Jayakumar R., Tamilmani G., Sakthivel M., Kalidas C., Kumar R. P., Anbarasu M., Sirajudeen S., Balamurugan V., Jayasingh M., Gopakumar G., 2012 Larviculture and seed production of the silver pompano, *Trachinotus blochii* (Lacepede, 1801) for the first time in India. Indian Journal fo Fisheries 59(3):83-87.
Nguyen M. V., Espe M., Conceicao L. E. C., Le H. M., Yufera M., Engrola S. A. D., Jordal A. E. O., Ronnestad I., 2019 The role dietary methionine concentrations on growth, metabolism, and N-retention in cobia (*Rachycentron canadum*) at elevated temperatures. Aquaculture Nutrition 25(2):495-507.
Pangkey H., 2011 [Essential fatty acid requirements in marine fish]. Journal of Tropical Fisheries and Oceanography 7(2):93-102. [In Indonesian].
Putri F. S., Hasan Z., Haetami K., 2021 [Effect of probiotic bacterium addition on pellet enriched with kaliandra (*Calliandra calothyrsus*) on the growth of Nile tilapia seed (*Oreochromis niloticus*)]. Journal of Fisheries and Marine 3(4):253-291. [In Indonesian].
Rolland M., Dalsgaard J., Holm J., Gómez-Requeni P., Skov P. V., 2015 Dietary methionine level affects growth performance and hepatic gene expression of GH–IGF system and protein turnover regulators in rainbow trout (*Oncorhynchus mykiss*) fed plant protein-based diets. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 181:33-41.
Setianingsih L., Santoso L., Saputra S., 2019 [Effects of diets with different protein level for cobia (*Rachycentron canadum*) growth in controlled tank]. Aquaculture Engineering and Technology Journal 7(2):845-858. [In Indonesian].
Syuhriatin, 2020 [Analysis of growth of Nile fish (*Oreochromis niloticus*) on different feeding (Case study: Sigerongan village Kecamatan Lingsar, West Lombok district)]. Media Bina Ilmiah 14(6):2745-2748. [In Indonesian].
Tacon A. E. J., 1987 The nutrition and feeding of farmed fish and shrimp - A training manual. FAO, Brazil, pp. 106-109.
Tacon A. E. J., 1993 Feed ingredients for warmwater fish: Fish meal and other processed feedstuffs. FAO Fisheries Circular No. 856, 64 p.
Trushenski J., Schwarz M., Bergman A., Rombenso A., Delbos B., 2012 DHA is essential, EPA appears largely expendable, in meeting the n-3 long-chain polyunsaturated fatty acid requirements of juvenile cobia (*Rachycentron canadum*). Aquaculture 326-329: 81-89.
Wahyudin, 2005 [Effect of Rotifera enriched with several types of fat sources on the survival of *Litopenaeus vannamei* shrimp larvae]. Thesis, Faculty of Fisheries and Marine Science, Library of IPB University, Bogor, Indonesia, 41 p. [In Indonesian].
Wibowo E. S., Palupi E. S., Puspitasari I. A. R., Atang A., 2019 [Survival rate and growth of polychaeta *Nereis* sp. from the ponds of the Jeruklegi Cilacap Village area with different salinity and feed types]. DEPIK 8(2):67-75. [In Indonesian].
Wibowo E. S., Palupi E. S., Sari I. G. A. A. R. P., Atang, Hana, 2020 [Biological and environmental aspects of polychaeta *Nereis* sp. in the aquaculture area of Jeruklegi Village, Cilacap Regency: Potential as natural shrimp feed]. Pancasakti Science Education Journal 3(1):18-24. [In Indonesian].
*** AOAC, 2005 Official methods of analysis. 18th Edition. Association of Official Analytical Chemists, Washington.

Received: 28 August 2023. Accepted: 22 September 2023. Published online: 24 December 2023.
Authors:
Seto Windarto, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof. Jacub Rais, Tembalang, 50275 Semarang, Central Java, Indonesia, e-mail: seto.windarto@live.undip.ac.id
Tita Elfitasari, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof. Jacub Rais, Tembalang, 50275 Semarang, Central Java, Indonesia, e-mail: t.elfitasari@gmail.com
I Made B. Dirgantara, Department of Management, Faculty of Economics and Business, Diponegoro University, Semarang, Indonesia, e-mail: imadebdirgantara@lecturer.undip.ac.id
Vivi Endar Herawati, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof. Jacub Rais, Tembalang, 50275 Semarang, Central Java, Indonesia, e-mail: viviendar23@gmail.com

How to cite this article:
Windarto S., Elfitasari T., Dirgantara I. M. B., Herawati V. E., 2023 Effect of enriching sea worms (*Nereis virens*) with DHA on the growth performance and body composition of cobia (*Rachycentron canadum*). AACL Bioflux 16(6):3275-3284.