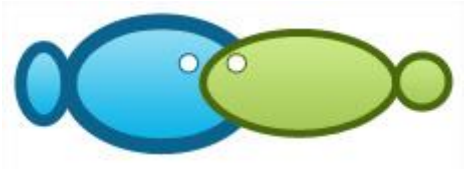

Effects of earthworm (*Perionyx excavates*) inclusion to the growth, feed utilization and lipid composition of common carp (*Cyprinus carpio*)

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Abstract. This experiment was conducted to evaluate the effects of earthworm (*Perionyx excavates*) inclusion in the diet of common carp (*Cyprinus carpio*) on the growth, feed utilization and fatty acid composition. Triplicates of four diets were used: control diet, EW30, EW70, EW100, in which 0, 30, 70 and 100% of fishmeal protein was replaced by earthworm protein, respectively. The experiment was carried out for 8 weeks in a recirculation system that consisted of 12 aquaria (40 L each and 5 fish per aquaria). Fish was fed five times daily with amount of five times higher than the maintenance requirement. Growth of fish was monitored weekly. Fatty acid composition of earthworm, feed and fish carcass were analyzed by gas chromatography to compare with standard peak areas. Results showed that the growth of fish fed on earthworm inclusion was significantly higher than that of the fish administered control feed. Within the earthworm inclusion diets, the EW70 group showed a better growth rate, significantly higher than EW30 and EW100. Results of fatty acid composition showed that the earthworm diet was limited in some poly unsaturated fatty acids (PUFA), such as C20(n-5), C22(n-4) and C24(n-6) fatty acids, which might have negatively affected the lipid utilization, leading to the lower growth rate in EW100. Nevertheless, PUFA concentrations of fish fed on earth worm inclusion diets were significantly higher than that of control.

Key Words: fatty acid composition, replacement, unconventional protein.

Introduction. Aquaculture is one of the fastest growing sectors in food production in the world with an increasing rate above 8% annually from 1950 to 2016 (FAO 2018). In 2016, the total production of aquaculture reached 110.2 million tons, which puts an enormous pressure on the use of fishmeal and fish oil – the utmost important protein and lipid sources in aquafeeds. Thus, sustainable alternatives to these ingredients are searched by nutritionists all over the world.

Earthworm (*Perionyx excavates*) has been considered an excellent ingredient for aquafeeds, because it contains high amounts of crude protein with very good amino acid profiles (Pucher et al 2014; Musyoka et al 2019). In the 80s, earthworm had been tested as a protein source for rainbow trout (Tacon et al 1983). Later, many researchers investigated the nutritive value of several earthworm species (Stafford & Tacon 1984; Nandeesha et al 1988; Paoletti et al 2003). Tuan (2010) pointed out that earthworm (*P. excavates*) contains more than 71% crude protein of dry matter, much higher than many other conventional fishmeal sources. Thus, earthworm has been used more and more for animal and aquatic feeds (Sogbesan et al 2007; Tuan & Hanh 2015). Despite having many advantages, total replacement of fishmeal with earthworm meal in the diets of fish lead to lower growth rates. The study carried out by Stafford & Tacon (1985) showed that a full replacement of fishmeal with earthworm meal led to a negative effect on the growth of rainbow trout (*Oncorhynchus mykiss*). Similarly, Chiu et al (2016) reported that they did not succeed with the total replacement of fishmeal with earthworm meal in the diet for

Penaeus vanamei shrimp. In the same line, Tuan et al (2016) reported that the inclusion of earthworm (*P. excavates*) in the diet could enhance the amino acid composition of feed, and lead to the improvement of common carp (*Cyprius carpio*) growth. However, the total replacement of fishmeal with earthworm did not produce a better growth rate of common carp when compared to the treatment with 70% replacement. Although earthworm was superior in terms of both crude protein and essential amino acids in comparison to fishmeal, the reason for the lower growth rate in the total replacement treatment being still unclear.

Many studies reported that common carp is able to utilize soybean oil independently from fish oil, without compromising growth, and they seem to be able to adjust the function of PUFA on lipid accumulation among different organs to maintain the lipid content in the hepatopancreas (Zhou et al 2008). A change of liver steatosis was found to be adapted to imbalance dietary lipid or essential fatty acid deficiency (Tacon 1996; Montero et al 2001). However, the effects of earthworm meal on lipid utilization, lipid retention and body lipid composition in common carp have been little investigated. Thus, the current study was carried out in order to clarify the effects of earthworm inclusion in the diet on the growth, feed utilization and lipid composition of common carp.

Material and Method

Feed preparation. Earthworm (*P. excavates*) was purchased from a small scale vermiculture farm, where worms were raised solely from cattle manure. The worms were kept in water for several hours for cleaning before being dried by a freeze-drier (Finn-Aqua Lyovac G2, Germany). Afterwards, earthworms and all other feed ingredients were analyzed for chemical composition before formulation. Feeds were formulated with 28% crude protein and 10% crude fat, where 30, 70 and 100% of protein from fishmeal in the control diet was replaced by protein from earthworm powder (respectively for EW30, EW70 and EW100). The ingredients and formulation of feed and chemical composition of earthworm as well as feed were presented in Table 1.

Table 1

Ingredient composition of diets (% of diet dry matter), proximate composition, gross energy content (MJ kg^{-1} dry matter) of the experimental diets

	Control	*EW30*	*EW70*	*EW100*
		Ingredient composition		
Fishmeal	30.3	21.1	9.0	-
Earthworm		8.8	20.2	28.7
Wheat meal	61.7	61.6	61.7	61.7
Sunflower oil	5.0	5.5	6.2	6.7
Mineral*	2.0	2.0	2.0	2.0
Vitamin**	2.0	2.0	2.0	2.0
TiO_2	1.0	1.0	1.0	1.0
		Proximate composition and energy		
Crude protein	27.8	27.7	27.5	27.6
Crude lipid	9.7	9.7	9.7	9.7
Crude ash	8.3	8.4	8.1	8.2
Gross energy	19.4	19.4	19.4	19.4

Note: * - Vitamin premix: retinol palmitate: 500 000 IU kg^{-1}; thiamine: 5 g kg^{-1}; riboflavin: 5 g kg^{-1}; niacin: 25 g kg^{-1}; folic acid: 1 g kg^{-1}; pyridoxine: 5 g kg^{-1}; cyanocobalamine: 5 g kg^{-1}; ascorbic acid: 10 g kg^{-1}; cholecalciferol: 50 000 IU kg^{-1}; a-tocopherol: 2.5 g kg^{-1}; menadione: 2 g kg^{-1}; inositol: 25 g kg^{-1}; pantothenic acid: 10 g kg^{-1}; choline chloride: 100 g kg^{-1}; biotin: 0.25 g kg^{-1}. **Mineral premix (g kg^{-1}): $CaCO_3$: 336; KH_2PO_4: 502; $MgSO_4$+7 H_2O: 162; NaCl: 49.8; Fe(II) gluconate: 10.9; $MnSO_4$+H_2O: 3.12; $ZnSO_4$+7 H_2O: 4.67; $CuSO_4$+5 H_2O: 0.62; KI: 0.16; $CoCl_2$+6 H_2O: 0.08; ammonium molybdate: 0.06; $NaSeO_3$ 0.02.

Experiment set-up. The experiment with triplicates was carried out for 8 weeks in a recirculation system that consisted of 12 aquaria (40 L). Water parameters were

maintained at optimal level for common carp (temperature at 26±1°C, dissolved oxygen above 5 mg L^{-1} and pH between 7–8). The photoperiod was set to 12 h light to 12 h dark. Fish were acclimatized for 2 weeks before being assigned to the experiment. Five common carp were selected randomly to stock each aquarium after weight and length determination. Fish were fed five times daily at 8, 10, 12, 14 and 16 o'clock by auto-feeders with an amount of five times the maintenance requirement of the metabolic body mass (Becker et al 1983). Growth of individual fish was monitored weekly after 24 h of starvation. The experiment procedure followed the protocols approved by the Ethical Committee of the Pibulsongkram Rajabhat University (PSRU-AG-2019-005).

Sample analysis. 5 fish (8 g each) were randomly sampled for initial analyses. At the end of the trial, all fish were sacrificed for determination of HSI (Hepato Somatic Index), ISI (Intestine Somatic Index), and CF (Condition Factor). Fish from each aquarium were autoclaved at 121°C for 15 minutes, ground and then freeze-dried (Finn-Aqua Lyovac G2, Germany). Earthworm powder, feeds, initial and final fish samples were collected for the analysis of chemical composition (AOAC 1990). Extracted lipids from methyl ester extraction by the Soxhlet method were used for fatty acid content analysis (Table 2).

Table 2

Fatty acid composition of earthworm and feed used in the experiment (%)

Fatty acids	*Earthworm*	*Control*	*EW30*	*EW70*	*EW100*
C 6:0	0.35	ud	0.1	0.2	0.6
C 10:0	0.41	ud	0.12	0.24	0.10
C 12:0	0.15	ud	1.00	1.94	3.29
C 13:0	0.23	ud	0.24	0.34	0.58
C 14:0	0.43	1.95	1.13	1.40	1.31
C 15:0	0.36	ud	0.10	0.23	0.33
C 16:0	21.62	11.69	13.20	11.00	11.22
C 17:0	0.31	0.20	0.23	0.34	0.54
C 18:0	0.24	3.19	3.20	4.30	5.87
C 21:0	1.26	0.22	0.25	0.27	0.39
C 22:0	2.24	1.64	0.63	0.82	1.11
C 24:0	ud	0.42	0.21	0.11	0.06
Total SFA	27.59	19.29	20.42	21.22	25.34
C 14:1(n-9)	2.23	ud	0.23	0.32	0.40
C 15:1(n-9)	0.23	ud	0.03	0.71	0.11
C 16:1(n-7)	2.84	1.94	0.42	0.73	0.80
C 18:1(n-7)	6.99	22.11	24.37	23.09	25.34
C 20:1(n-9)	13.43	2.65	0.90	1.96	1.85
C 22:1(n-9)	0.34	3.04	1.61	1.49	1.35
Total MUFA	26.05	29.74	27.57	28.31	29.86
C 18:2(n-6)	39.15	44.11	43.58	41.25	36.27
C 18:3(n-3)	0.64	0.82	0.21	0.42	0.51
C 20:2(n-6)	0.15	0.19	0.50	0.60	0.84
C 20:4(n-6)	0.21	0.19	0.22	0.25	0.26
C 20:5(n-3)	ud	0.20	0.42	0.33	0.25
C 22:4(n-6)	ud	0.18	0.12	0.83	1.22
C 22:6(n-3)	ud	2.47	3.85	2.52	1.34
Total PUFA	40.14	48.16	48.90	46.21	40.69
UD	6.20	2.80	3.12	4.26	4.06

Note: ER30 - fishmeal replacement with 30% earthworm meal; ER70 - fishmeal replacement with 70% earthworm meal; ER100 - total fishmeal replacement with earthworm meal; SFA - Saturated Fatty Acids; MUFA - Mono-Unsaturated Fatty Acids; PUFA - Poly-Unsaturated Fatty Acids; UD - undeterminable.

Fatty acid profile analysis was performed by gas chromatography (ASHMACO, Japan; Model No: ABD20A) using an instrument equipped with a flame ionization detector and a glass column (2m x 3 mm) packed with 1% diethylene glycol succinate on chromosorb-W. The temperature conditions for gas chromatography were: injector at 200°C and detector at 210°C. The temperature of the oven was programmed from 180°C and the carrier gas was nitrogen at a flow rate of 30 mL min^{-1}. Peaks were identified by comparison with authentic standards, quantified by peak area integration and expressed as weight percentage of total methyl esters; the relative weight percentage of each fatty acid was determined from integrated peak areas. The fatty acid composition of some ingredients and feed was presented in Table 2.

Parameters and formulas. The following formulas were used.

Weight Gain (WG, g) = Final Weight (g) – Initial Weight (g) (Han et al 2012)

Specific Growth Rate (SGR) = (ln Final Weight – ln Initial Weight) x 100/days of trial (Han et al 2012)

Condition Factor (CF) = [Fresh Body Weight (g)/Body Length $(cm)^3$] x 100 (Han et al 2012)

Hepato Somatic Index (HSI, %) = [Fresh Weight of Liver (g)/Fresh Body Weight (g)] x 100 (Han et al 2012)

Intestine Somatic Index (ISI) = Length of Intestine (cm)/Standard Body Length (cm) (Han et al 2012)

Feed Conversion Ratio (FCR) = Feed consumption (dry matter, g)/Fresh Body Gain (g) (Han et al 2012)

Protein Productive Value (PPV) = (Final Protein in Fish – Initial Protein in Fish)/Total Protein Consumed (Parazo 1990)

Apparent Net Lipid Utilization (ANLU) = [Final Lipid in Fish (g) – Initial Lipid in Fish (g)]/Total Lipid Consumed (g) (Choi et al 2016)

Apparent Digestibility Coefficient of Crude Protein (P-ADC) = [(Concentration of TiO2 in diet/Concentration of TiO2 in Faeces) x (Crude Protein in Faeces/Crude Protein in Diet)] x 100 (Bureau et al 1999)

Apparent Digestibility Coefficient of Crude Protein (P-ADC) = [(Concentration of TiO2 in diet/Concentration of TiO2 in Faeces) x (Crude Lipid in Faeces/Crude Lipid in Diet)] x 100 (Bureau et al 1999)

Apparent Digestibility Coefficient of Gross Energy (E-ADC) = [(Concentration of TiO_2 in diet/Concentration of TiO2 in Faeces) x (Gross Energy in Faeces/Gross Energy in Diet)] x 100 (Bureau et al 1999)

Data analysis. All data were tested for normal distribution and homogeneity before statistical analysis. Results are presented as mean ±SD (standard deviation). Microsoft Excel and SAS (version 9.0) were used for data analysis. Analysis of Variance (ANOVA) and Tukey post-hoc tests were used to determine any significant differences (at a 95% confidence level).

Results and Discussion. Results of observation showed that, during the experiment, all tested diets sank quickly and were ingested immediately by the fish. Thus, the nutrient loss by leaching was minimal and all administered feed was assumed as feed intake. Fish

did not show any abnormal activity or behavior in any treatments. No mortality was observed during trial and all the morphometric parameters such as CF, HSI and ISI were the same in all treatments (Table 3). The results also pointed out that there were no significant differences in the chemical composition of fish among the feeding groups.

Table 3

Body chemical composition and morphometric parameters of fish (%)

	Control	*EW30*	*EW70*	*EW100*
Water	75.37±0.65	75.59±0.39	76.08±0.54	76.15±0.49
Crude protein	14.26±0.07	14.28±0.18	13.85±0.30	14.09±0.28
Crude lipid	7.91±0.66	8.03±0.59	8.18±0.62	7.97±0.74
Crude ash	2.04±0.03	1.81±0.18	1.80±0.32	1.61±0.27
HSI	1.98±0.11	1.87±0.09	1.83±0.04	1.94±0.06
ISI	6.11±0.23	6.23±0.13	6.16±0.09	6.22±0.34
CF	3.33±0.12	3.24±0.02	3.21±0.13	3.27±0.09

Note: HSI - Hepato-Somatic Index; ISI - Intestine-Somatic Index; CF - Condition Factor.

The growth of fish fed experimental diets was significantly higher than that of the control diet. Fish in EW70 showed the highest SGR (1.97±0.07 % of total fatty acids), followed by fish from EW100 and EW30, with significantly higher values than in control. Most of parameters of feed utilization of EW30 and EW100 were in the same range with those of the control group. However, the results from EW70 were still consistently better in comparison to those of the control group (Table 4). Results of PPV and ANLU in the EW70 group were 76.4±2.2 and 30.7±0.5% of total fatty acids, respectively, higher than those from the control group 65.6±6.2 and 27.6±1.6% of total fatty acids, respectively. However, no significant difference of FCR was observed among treatments. Due to the limited amount of fish feces for analysis, the fecal materials in each treatment were pooled together. Thus, it was not possible to access the statistical difference for digestibility.

The fatty acid analyses of fish carcass showed that total SFA of fish in control group (36.39±1.53%) was not different from that of EW30 and EW100, but significantly higher than EW70 (30.44±2.06% of total fatty acids). The totals MUFA of fish from all treatments decreased inversely proportional to the amount of earthworm inclusion and were significantly lower than that of the control group (Table 5).

Table 4

Growth, feed utilization and feed conversion ratio and apparent digestibility of crude protein, crude lipid and gross energy of the test diets in the experiment

	Control	*EW30*	*EW70*	*EW100*
WG (g)	18.41 ± 0.61^{a}	19.56 ± 0.51^{ab}	21.96 ± 0.08^{b}	20.95 ± 0.49^{ab}
SGR (%)	1.74 ± 0.02^{a}	1.83 ± 0.01^{b}	1.97 ± 0.07^{c}	1.86 ± 0.03^{b}
ANLU (%)	65.6 ± 6.2^{a}	67.3 ± 4.4^{ab}	76.4 ± 2.2^{b}	70.7 ± 2.9^{ab}
PPV (%)	27.6 ± 1.6^{a}	28.3 ± 1.1^{ab}	30.7 ± 0.5^{b}	27.8 ± 0.7^{ab}
FCR	1.46±0.08	1.49±0.06	1.38±0.05	1.41±0.03

Note: values in the same row with different superscript are significantly different ($p<0.05$). WG: Weight Gain; SGR: specific growth rate; ANLU: apparent net lipid utilization; PPV: protein productive value; FCR: feed conversion ratio.

Fish fed on earthworm inclusion diets showed lower concentrations of total MUFA, but significantly higher amounts of PUFA (Table 5). Total MUFA in fish carcass decreased inversely proportional to the earthworm inclusion in the diets. The highest MUFA was achieved by the control feed group, with 48.63±2.16%, whereas the total replacement diet showed the lowest value (42.72±1.83% of total fatty acids). In contrast, fish in the control group had only 9.73±3.1% of total PUFA, while EW70 showed the highest result, with 20.99±3.28%. The results of the analysis also pointed out that the amount of C18:2(n-6) in common carp was highest among all PUFAs. The amount of C18:2 in the control group

was 8.26±2.99%, accounting for nearly 85% of total PUFA. This result was close to that of the initial sample of the fish, but much lower in comparison to other groups. The highest concentration of C18:2 fatty acid was observed in EW70 (18.94±3.05%), followed by EW100 (16.40±2.04%) and EW30 (15.85±6.64% of total fatty acids). Table 5 shows that common carp in the experiment seemed to be richer in n-6 fatty acids than in n-3 ones.

Table 5

Fatty acid composition of fish in the experiment (% of total fatty acid methyl esters)

Fatty acids	*Initial*	*Control*	*EW30*	*EW70*	*EW100*
C 6:0	0.40	0.55±0.35	0.47±0.37	0.27±0.02	0.32±0.16
C 8:0	0.27	0.23±0.14	0.30±0.00	0.10±0.03	0.15±0.00
C 10:0	ud	0.20±0.01	0.20±0.01	0.22±0.02	0.21±0.05
C 12:0	ud	0.07±0.00	0.69±0.47	0.76±0.01	0.98±0.49
C 13:0	ud	ud	0.20±0.15	0.19±0.01	0.96±1.10
C 14:0	5.06	2.57±0.54	2.00 ±0.46	1.73±0.16	1.78±0.19
C 15:0	0.62	0.31±0.07	0.39±0.09	0.37±0.03	0.47±0.12
C 16:0	23.90	23.83±0.74	20.89±2.75	18.44±1.37	20.00±0.82
C 17:0	0.46	0.25±0.05	0.36±0.11	0.36±0.02	0.46±0.14
C 18:0	5.90	7.22±0.57	6.86±1.03	6.90±0.51	7.29±0.27
C 20:0	0.35	0.57±0.52	0.22±0.03	0.20±0.02	0.23±0.04
C 21:0	ud	0.17±0.01	0.31±0.13	0.30±0.04	0.24±0.14
C 22:0	0.57	0.49±0.18	0.47±0.30	0.62±0.07	0.34±0.08
C 24:0	ud	0.04±0.03	0.11±0.04	ud	ud
Total SFA	37.54	36.39±1.53^{a}	33.20±3.84^{ab}	30.44±2.06^{b}	32.79±1.31^{ab}
C 14:1(n-9)	ud	0.06±0.05	0.12±0.03	0.12±0.03	0.16±0.02
C 15:1(n-9)	0.59	0.58±0.38	0.32±0.31	0.17±0.05	0.28±0.05
C 16:1(n-7)	4.28	3.20±0.24	2.38±0.61	2.25±0.19	2.14±0.57
C 17:1(n-7)	0.36	0.25±0.01	0.22±0.02	0.21±0.01	0.23±0.01
C 18:1(n-9)	30.91	38.64±2.08	35.92± 1.37	37.07±1.11	36.64±0.55
C 20:1(n-9)	2.93	1.83±0.70	1.17±0.27	1.11±0.19	1.39±0.25
C 20:1(n-11)	4.63	1.98±1.72	1.79±0.81	0.90±0.78	0.75±1.29
C 22:1(n-9)	4.68	2.09±0.29	1.49±0.70	1.46±0.08	1.13±0.67
Total MUFA	48.36	48.63±2.16^{a}	43.40±2.75^{b}	43.30±0.86^{b}	42.72±1.83^{b}
C 18:2(n-6)	8.46	8.26±2.99	15.85±6.64	18.94±3.05	16.40±2.04
C 18:3(n-3)	0.93	0.24±0.10	0.28±0.07	0.23±0.08	0.22±0.11
C 18:3(n-6)	0.00	0.04±0.07	0.08±0.07	0.09±0.07	0.05±0.09
C 20:2(n-6)	0.34	0.24±0.09	0.65±0.40	0.41±0.02	0.31±0.03
C 20:4(n-6)	ud	0.13±0.01	0.32±0.28	0.35±0.06	0.30±0.21
C 20:5(n-3)	0.37	0.33±0.21	0.45±0.22	0.56±0.07	0.29±0.06
C 22:4(n-6)	ud	0.17±0.12	0.19±0.14	0.18±0.01	0.37±0.06
C 22:6(n-3)	1.02	0.33±0.37	0.19±0.09	0.23±0.11	0.32±0.19
Total PUFA	11.11	9.73±3.10^{a}	18.03±6.57^{b}	20.99±3.28^{b}	18.26±2.20^{b}
UD	3.00	5.25±0.35	5.37±0.12	5.27±0.55	6.23±2.21

Note: SFA - Saturated Fatty Acids; MUFA - Mono-Unsaturated Fatty Acids; PUFA - Poly-Unsaturated Fatty Acids; UD - Un-determinable.

Protein quality and fish growth. As some previous studied determined (Tuan et al 2016) for the same source of earthworm, earthworm meal has higher protein content than fishmeal (71% and 64%, respectively). At a deeper analysis, most of all amino acids of earthworm powder had a similar or higher content than those from fishmeal, earthworm meal being superior than fishmeal in terms of protein content and protein quality (Tuan et al 2016).

The growth rate of fish in this study seemed to be lower compared to that reported by Tuan et al (2016), despite the same feed and ingredients used. However, it is confirmed that total fishmeal replacement with earthworm meal did not produce the highest growth rate of fish. Many studies pointed out that essential amino acids of earthworm, including lysine, cystine+methionine, which is normally the most limited in many conventional ingredients, was comparable or higher than that of fishmeal (Hertrampf & Piedad-Pascual 2012; Tuan et al 2016). The inclusion of earthworm in the diet results in a higher content of almost all essential amino acids (Tuan et al 2016). Although fish in EW100 showed a better growth rate than those from the control diet, it was still lower significantly compared to that of the EW70 group. This result was in line with many previous studies where the total replacement of fishmeal with earthworm meal in diets did not produce the best growth of fish (Tacon et al 1983; Stafford & Tacon 1984; Nandeesha et al 1988). Thus, the cause for the lower growth rate of fish in EW100 does not seem to be the protein quality.

Fatty acid composition of earthworm powder and its effects to the growth of fish. In a previous study, Tuan et al (2016) did not find significant differences of apparent net lipid utilization (ANLU) or apparent digestibility of lipids among treatments with experimental dietary inclusion of earthworm meal. However, in this study, fish in EW70 showed higher values of ANLU and lipid apparent digestibility compared to those of the control groups. The analysis pointed out that different fatty acid composition between earthworm meal and fishmeal led to different fatty acid composition of the feed in the experiment. The results of analysis showed that the total of MUFAs of the feed seemed to be in the same range, ranging from 27.57% of dietary lipid in EW30 to 29.86% in EW100. However, total SFA increased proportionally to the amount of earthworm meal in the diets, while the PUFA decreased proportionally (Table 2). Earthworm contained some SFA such as C6, C10, C12 and C13, fatty acids that were also available in other ingredients in control diet. However, the analysis did not find some important PUFAs such as C20:5, C22:4 and C24:6 fatty acids from earthworm which are necessary to the growth of fish.

So far, not many publications are available on lipid composition of earthworms, especially for *P. excavatus*. The fatty acid composition of earthworm differs from species to species and it could be affected by culture conditions. Lipid composition of earthworm is complex and may vary from species to species. Liu (2006) reported that earthworm (*E. fetida*) was a good source of n-3 PUFA and EPA in particular, but limited in DHA content. Earthworm *Dendrobaena octaedra* contained high amounts of (20:n and 18:n) long-chain unsaturated fatty acids (Holmstrup et al 2007). In another study on earthworms *Andiorrhinus kuru* and *Andiorrhinu motto*, Paoletti et al (2003) pointed out that earthworms contained insufficient amounts of triacyglycerols, which are important as energy sources (stores) for cells. In the current study, earthworm showed to be abundant in C16 SFA (21.62% of total fatty acids), C20:1 MUFA (13.43%), and especially C18:2 PUFA (39.15%). However, the concentration of almost all PUFAs decreased (not proportionally) to the amount of earthworm inclusion in the diets. It could imply that concentrations of PUFA in earthworm were still lower than those from other sources of lipid, especially from fishmeal in the diets. On the other hand, although earthworms were rich in total PUFA, the amount of n-3 was limited, reaching only 0.64% of total fatty acids, much lower than the requirement of carp. Some researchers already reported that common carp will grow best when receiving 1% for the fatty acids C18:2 (n-3) and C18:3 (n-6). In addition, if common carp receive 0.5% of each C20:5(n-3) and C22:6(n-3) in the diet, it will grow better than eating 1% of C18:3(n-3) (Halver 1980; NRC 2011). Furthermore, n6-PUFA was not found. Ljubojevic et al (2012) reported that, in addition to the absolute amount of n-3 and n-6 which can affect fish growth, the ratio of n-3/n-6 is also an important factor in growth. Thus, the deficient and imbalance of fatty acid composition may be the reason for the lower growth rate of fish in EW100. This result was in line with other reports stating that fishmeal contains very high amounts of n-3 and n-6 PUFA (Miles & Chapman 2006; Karalazos et al 2007; Turan et al 2007; De Silva et al 2011), which strongly affect the growth of fish. Similarly, Chiu et al (2016) reported that a mixture of earthworm and fermented soybean could be supplemented to *P. vanamei*

diet, with the condition that fishmeal proportion is above 20% in formulation. A small amount of fishmeal and fish oil added in the diet could result in an improvement of fish growth (Pike 1990; De Silva et al 2011; Župan et al 2016). In fact, the fishmeal used in this study still retained a certain amount of lipid which might have contributed to the fatty acid profile of the EW30 and EW70 diets. In order to enhance the growth of aquatic animals, improvement of earthworm quality, especially long chain fatty acid content could be required. Recently, enrichment technology was demonstrated to elevate essential fatty acid content of earthworm, such as DHA to 8-9 folds, improving the growth of fish and shrimp (Veni 2013; Kumlu et al 2018).

Effects of earthworm to fatty acid of fish carcass. It is impossible to set up the same amount of crude lipid and fatty acid composition of the diets with different proportion of earthworm inclusion. In the experiment, sunflower oil was added to maintain the same level of crude lipid in the diets (9.7%), and ensured equal gross energy (19.4 MJ kg^{-1}) in all diets, which might strongly influence lipid utilization and retention. This means that differences in fatty acid compositions of the fish at the end of the trial cannot be attributed solely to the inclusion of earthworm. However, it could be seen that the used earthworm was not rich in PUFA, leading to the disproportional decline of total PUFA, especially n-3PUFA, in the amount of earthworm inclusion in the diet. Similarly, due to the low content of n-3PUFA, the addition of sunflower oil in the diets (Güçlü et al 2008; Czech et al 2015) did not affect the n-3PUFA of the diets. Thus, PUFA deficiency caused by earthworm inclusion was more visible.

The results of the present research agreed with some reports that the total MUFA in the common carp flesh is usually higher than SFA and PUFA (Yeganeh et al 2012; Župan et al 2016). Total MUFA in the fish from the control group was 48.63±2.16% of total fatty acids, while other groups had lower levels 43.4±2.75, 43.3±0.86 and 42.72±1.83% of total fatty acids for EW30, EW70 and EW100, respectively. In contrast, total PUFA of fish fed control diet was the lowest (9.73±3.10% of total fatty acids). Fish from all other treatments had significantly higher levels of PUFA, ranging from 18.03±6.57% (in EW30) to 20.99±3.28% of total fatty acids (in EW70). However, differences of PUFA in fish carcass did not appear for omega 3 and omega 6, because these fatty acids decreased inversely proportional to the amount of earthworm inclusion in the diets (Table 2). Thus, the increase of PUFA in fish body was attributed to the utilization of C18:2 fatty acid in the diets. This fatty acid had a content of only 8.26±2.99% in the control diet, whereas its content in earthworm inclusion diets was 15.85±6.64, 18.94±3.05 and 16.40±2.04% of total fatty acids for EW30, EW70 and EW100, respectively.

The fatty acid composition of fish could be affected by many factors such as species, sex, maturation, environment and quality of feed. The quality of feed could be the most important parameter (Csengeri 1996; Steffens & Wirth 2007; Mráz 2012; Ljubojević et al 2013). Appearance of ambient food in the pond culture system could contribute to a large proportion of rich lipid source (Mráz & Pickova 2011). However, fish in the experiment received only experimental feed. Many documents reported that common carp are able to elongate and desaturate n-3 and n-6 PUFA from its C18 precursors (Tocher 2003). The increase of fatty acid C18 in the diets could be a reason for the higher concentration of PUFAs in the fish. Although the analysis did not find n-6PUFA in earthworm powder, the carcass of fish fed on earthworm inclusion diets had similar levels of PUFA, meaning that common carp could desaturate this fatty acid in the tissues, confirming the results of Tocher (2003).

Ljubojević et al (2015) pointed out that the lipid source did not show significant effects on the proximate composition of carp fillets. However, it might result in significant changes in fatty acid compositions of fillet. The same authors stated that interactions were observed between the oil source and protein level, and the fatty acid composition of common carp fillet. When the content of α-linolenic acid or linoleic acid in the diets was increased, eicosatrienoic acid 20:3n-3 or eicosadienoic acid 20:2n-6 content in muscle tissue also increased. Ljubojević et al (2015) also stated that carp can be adapted to poor EFA content in the vegetable oil diet by attempting to moderate PUFA deficiencies. In their experiment, increasing levels of docosapentanoic acid (22:5n-3, DPA) and DHA in the

tissues were recognized, suggesting the bioconversion from EPA to DHA, which tended to improve storage and retention of these fatty acids. The study suggests that common carp have high tolerance to diets that differ significantly in fatty acid composition. Common carp was able to retain selectively DHA and bioconvert from LNA to EPA and DHA; EPA to DHA, and LA to ARA. Most of other studies evaluated the capability of fish to convert fatty acids derived from plants to PUFAs in their body. Furthermore, high PUFA in the fillet of carp can still be obtained with a low PUFA in the diet. This could be explained by the capability of the fish in selectively retain it in the tissues (Bell et al 2003). The ratio of n-3/n-6 PUFA of the fish body in the current study was very low, both in control and experimental groups (ranged from 0.05 to 0.1%). This result was much lower than that reported by Yeganeh et al (2012) and Miroslav et al (2011). However, these authors stated that lipid composition of fish could be affected by many other factors, such as season, physiological status and feed quality. Thus, adjustment of lipid composition in the diet could also improve fish quality for human consumption.

Conclusions. From all the results, it could be concluded that earthworm (*P. excavatus*) was rich in protein, but limited in some fatty acids, such as C18:3, 20:5 and 22:6. Thus, the total replacement of fishmeal with earthworm meal in the diet did not show the best growth rate of fish, but was still higher than that of the control group. However, the replacement of fishmeal with earthworm meal could improve the concentration of PUFA in common carp flesh.

Acknowledgements. The study was carried out with the cooperation of Pibulsongkram Rajabat University (Thailand) and Vietnam National University of Agriculture (Vietnam). Authors would like to express special thanks to students, colleagues form both organizations for their support to this study.

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

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Received: 30 May 2020. Accepted: 26 August 2020. Published online: 07 May 2021.
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How to cite this article:
Nguyen Ngoc T., Yaemkong S., Jaipong P., Kotham P., Do Anh M., 2021 Effects of earthworm (*Perionyx excavates*) inclusion to the growth, feed utilization and lipid composition of common carp (*Cyprinus carpio*) AACL Bioflux 14(3):1201-1212.