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Evaluation of the Fatty Acid Composition of Earthworm *Eisenia andrei* Meal as an Alternative Lipid Source for Fish Feed

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Abstract This study propose to evaluate the fatty acid composition of the earthworm *Eisenia andrei* as an alternative lipid source for fish feed. **Methods:** The analytical composition of moisture, protein, lipids, ash and non-nitrogenous extract of earthworm meal *Eisenia andrei* and fishmeal was initially determined. Subsequently, the fatty acid contents of the lipids of these two ingredients were determined by gas chromatography. **Results:** Significant differences were found in the content of group ω-6 Polyunsaturated Fatty Acids (PUFA), with higher value (19.85 %) in the earthworm flour vs. 13,29% in fish meal, specifically the group of C14 PUFA, C16:2 n-4; C18: 2 n-6; C18: 3 n-6; C20:2 n-6; C20:3 n-6 and n-6 C20:4. The content of ω-3 fatty acids, mainly C20:5 n-3 and n-3 C22:6 is also important in the earthworn flour. **Conclusions:** the flour of *E. Andrei* could be recommended as a source of protein and lipid in fish feed because of its high protein and PUFA content.

Keywords: *earthworm, biochemical composition, fish feed, PUFA, nutritional value*

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1. Introduction

According to Food and Agriculture Organization FAO [1], worldwide aquaculture production in 2016 reached 80 million tons of edible fish, 30.1 million tons of aquatic plants and 37.900 tons of non-food products. Production intended for consumption amounted to 54.1 million tons of finfish, 17.1 million tons of mollusks, 7.9 million tons of crustaceans and 938.500 tons of other aquatic animals.

In intensive aquaculture, a diet of adequate nutritional quality that allows for better growth of fish is the main factor affecting production [2]. In intensive aquaculture, a diet of adequate nutritional quality that allows better fish growth is the main factor affecting production. Fish meal due to its high protein content and high biological value is currently the ingredient of choice used in the aquafeed. However, being an imported raw material in many countries generates high operational costs, ranging between 40 and 50%, and even up to a 70% [3]. In addition, its use in fish feed contributes to the depletion of natural fish stocks. This leads to the need to assess potential alternative raw materials to sustain the feeding of aquatic organisms under controlled farming systems.

Nowadays many investors in the world are interested in vermiculture as agro-industrial practice for the recycling of organic waste. This practice has many economic, ecological and environmental benefits. The use of earthworms for the recycling of organic matter is linked to their short life cycles, their high reproduction rate and their wide tolerance to temperature and humidity [4]. *Eisenia foetida* (Savigny, 1826) and *E. Andrei* (Bouché, 1972), two related earthworm species, are widely used in the recycling of organic waste and can also be used as protein source in fish feed [5,6].

Eisenia foetida is one of the most recommended earthworms in fish diet, due to the high protein content (61% to 82 %), essential fatty acids, minerals [7,8]. This species is used as a biotechnological resource and as unconventional source of low cost protein. These nutritionnal characteristics show that earthworms can serve as raw material to supply the levels of amino acids and some fatty acids for the nutrition of aquatic organisms [9].

However, little information on the nutritional value of *E. andrei* are available. Isea et al. [10], reported that

E. andrei flour had high protein digestibility and could partly replace fishmeal in rainbow trout feed. The objective of this study is to determine the fatty acid composition of *Eseinia* in order to have sufficient data on the nutritional value of this species used as an ingredient in fish feed.

2. Materials and Methods

The matrices for analyses of *Eisenia andrei* were crude earthworms from cultivation systems and worm flour manufactured in the Laboratory of Food Sciences (LFS) of Department of Food Sciences of Faculty of Pharmacy and Bioanalysis (FPB) at Universidad de Los Andes, Mérida, Venezuela (ULA). The proximate composition was only evaluated to the flour, while the fatty acid profiles were determined in both matrices.

2.1. Culture System of Earthworms

The culture of earthworms to obtain meat and raw material for the manufacture of flour was made in a greenhouse located in the garden of medicinal plants of Herbarium "Luis Ruiz Teran" of FPB. This culture system consisted in a mixture of organic peat and organic waste from the Processing Pilot Plant of FPB, carrot (*Daucus carota* L.) and guava (*Psidium guajava* L.) and incorporating wheat bran, *Triticum aestivum* (L.), obtained from the local market of Mérida, Venezuela.

The worms were fed with a composting of the organic waste, liquefied in an industrial Blender brand F.B: Lehmann Maschinenfabrik GMBH, Aalen-Wiirt, Germany, N-0224, type FDA, at Pilot Plant of FPB. This composting was left to ferment for one week in plastic containers of 7 L, controlling pH and temperature. The composted material was combined with wheat bran according to recommendation of Isea [5], in order to nurture and at the same time promote the texture of the mixture. Feeding frequency was once a week at the beginning of the culture and then increased to twice per week as the earthworms population raised.

The crop was installed using Premium multipurpose plastic containers of 68 L capacity with dimensions 47 cm long and 38 cm in height. These containers were drilled at the bottom to facilitate drainage and water leakage. They were also covered inside with black mesh to avoid the escape of earthworms and better handling at the time of the extraction of the substrate with the worms. After conditioning the containers, they were filled with organic peat and solid decaying wood materials, in layers of approximately 30 cm thick by container. Then, water was supplied for the first irrigation to field capacity until the substrate was thoroughly wet without producing much drainage of fluid.

Two hundred individuals of *E. andrei* were subsequently distributed in each container and the first feeding was supplied based on 500 g of the organic waste mixture. During the course of cultivation, moisture, pH and temperature were controlled. Daily moisture from the packaging was verified and running water was added with a garden sprinkler to keep the substrate to field capacity. The temperature was maintained between 21-22°C at the bottom and 24-25 °C in the upper part of the substrate during the 3 months of cultivation. Litmus paper was used to measure substrate pH and was maintained in the range 6-7. Under these conditions of pH, temperature and humidity the cultivation of earthworms was considered optimal.

After three months of cultivation, adult earthworms with average length and weight of 7.0 cm and 0.49 g respectively, were manually removed. To do this, it was necessary to move the substrate containing the worms to a larger container for easier separation. Then the worms were washed with tap water and blot dried.

2.2. Processing of Earthworms and Production of Flour

The washed earthworms were then kept for 24 h in a plastic container with water and aeration at room temperature [11], using a DOMOSA 2.0 HP air compressor, in order for the worms to completely evacuate the contents of their digestive tract. Then the worms were washed again and scalded at 100 °C for 5 min (slaughter of earthworms), according to Stafford and Tacon [12]. After slaughter, the worms were washed with water to remove residues, placed on absorbent paper to discard excess water and then placed in labeled plastic bags for their transportation to the Laboratory of Unit Operations of the Faculty of Engineering of ULA. They were then distributed in perforated trays in layers of less than one (1) cm in height to be then transferred to dry in a ventilated oven at 60 °C for 4 h. At higher temperatures the proteins could be denatured. Finally, the dried worms were separated in trays and milled using an Oster classic blender. The flour was placed in labeled plastic bags and kept under refrigeration at 2 °C prior to its use [10].

2.3. Proximate Composition

The content (%) of moisture, ash, crude protein and crude fat were evaluated according to AOAC [13]. Non-nitrogenous extracts [NNE] were obtained by differences in weight.

2.4. Analysis of Fatty Acids

Fatty acids analysis of raw meat and flour of *E. andrei* was carried out at INRA, Saint Pée sur Nivelle, France. It consisted in a lipid transesterification by action of methanol in presence of a catalyst, boron trifluoride (BF_3), under heat, allowing the formation of methyl esters. An initial extraction of total lipids was performed by the method of Folch et al. [14], after a saponification, making the calculation to obtain approximately 100 mg of total lipids in 0,5-1 mL of dichloromethane. Alcoholic potash 0.5 M was added at a rate of 1.5 mL per 25 mg of lipid, and the mixture was placed within amber jars and moved inside a closed metal container to the stove at 103 °C for 10 min. It was left to cool at room temperature for about 5 min and was placed in an ice bath to stop the reaction. Then, methylation followed by adding BF_3 methanol 14 % (1 mL for 10 mg of lipids), and the jars were placed again inside the closed metal container and taken to the stove at 103 °C for 30-40 min; the jars were stirred from time to

time being careful because it can cause an explosive effect. The jars were let to cool and placed in an ice bath, transferring the content to centrifuge tubes, and adding the same volume of water as that of BF_3. Then, 5 mL of hexane were added, and after agitation, centrifuged at 3000 rpm for 5 min. The content was transferred to a separating funnel and residual volume of the pipe was taken with 2 mL of hexane, to retrieve all methylated lipid. It was left to decant for 5-10 min and the lower phase was removed. The upper hexanic phase was recovered and it was filtered with sodium sulphate anhydrous in a jar, cleaning the funnel with a 1 mL of hexane and the bottle with two 2 mL of this reagent. The recovered lipid was kept under refrigeration at 4 °C. The final volume of hexane should be such that allowed a final concentration of 10 mg/mL. The methylated fatty acids of both samples were later analyzed using a CPG 3800 gas chromatographer equipped with a carousel for automatic injection of the samples.

3. Results and Discussion

The moisture content of the flour of *E. andrei* (7.72 ± 0.27 %) presented a value within the quality levels established for flours, whose magnitude must not exceed 10% (Table 1). In contrast, Velasquez et al. [15] and Vielma [16], report a much lower value for the flour of *E. foetida*. With respect to the protein content, the flour of *E. andrei* was higher than that reported for *E. foetida* (Núñez, 1995 [17] with 57.7%; and Tacon et al. [18] with 58.8 %), becoming a possible alternative for the formulation of feed for fish due to its greater contribution of protein. The fat content did not present significant differences with respect to the values reported by Núñez [17] and Vielma [16] for *E. foetida*.

Table 1. Proximate composition of the flour of *E. andrei*

Composition					
Moisture	**Protein**	**Total lipids**	**Ash**	**NNE***	**Unit**
7.72 ± 0.27	61.51 ± 0.37	10.65 ± 0.01	4.76 ± 0.06	15.36	% DW

* Non nitrogenous extracts.

The high digestibility of earthworm flour has been associated to its composition, preliminarily determined by different authors. According to Tacon et al. [18] the earthworm *E. foetida* has a crude protein content between 50 to 67% and a good profile of essential amino acids, with the possible exception of Lysine as the first limiting amino acid and sulfurous amino acids (methionine and cysteine). Ferruzzi [19] reported values ranging between 65 to 82%. However, ZhenJun [20] this flour has a protein content in the range 54.6 – 71.0% on a dry basis. Chemical analysis revealed that the earthworm has 61.8, 11.3 and 8.7 % protein, fat and ash contents, respectively, and the levels of heavy metals are low and similar to those found in tuna from sea [21]. The earthworm flour, besides of containing essential amino acids [22,23,24] and minerals [25], it also has essential fatty acids for human nutrition.

The content of neutral lipids was higher in the fresh meat of *E. Andrei* (57.8%) compared with the earthworm flour (38.3%), which may be due to the technological process of drying (60 °C for 9 hours), which could result in a loss of volatile fatty acids that are part of this lipid fraction (Table 2). The opposite was observed in the content of polar lipids, since the earthworm flour showed the highest value (61.7%), for being a dry matrix where the components were concentrated as it lost moisture.

Table 2. Composition of neutral. polar lípids and fatty acids in the fresh meat and flour of the earthworm *E. andrei*.

	Lipid name	Fresh meat	Earthworm Flour	Unit
Neutral lipids		57.8 ± 0.2	38.3 ± 0.1	% DW
Polar lipids		42.2 ± 0.1	61.7 ± 0.1	% DW
SFA				
C10:0	Capric	0.86 ± 0.1	1.24 ± 0.1	% DW
C11:0	Undecilic	0.28 ± 0.1	0.36 ± 0.1	% DW
C12:0	Lauric	23.96 ± 0.3	9.96 ± 0.1	% DW
C14:0	Myristic	5.09 ± 0.2	3.11 ± 0.1	% DW
C15:0	Pentadecilic	1.39 ± 0.1	0.71 ± 0.1	% DW
C16:0	Palmitic	5.19 ± 0.1	4.73 ± 0.2	% DW
C17:0	Margaric	0.51 ± 0.1	1.45 ± 0.1	% DW
C18:0	Stearic	2.43 ± 0.1	4.49 ± 0.1	% DW
C20:0	Arachidonic	0.06 ± 0.1	0.07 ± 0.1	% DW
Total		39.77	26.12	% DW
MIFA				
11:1	Undecenoic	0.93 ± 0.1	2.59 ± 0.1	% DW
14:1 n-5	Myristoleic	0.23 ± 0.1	0.06 ± 0.1	% DW
15:1 n-6	Pentadecanoic	0.24 ± 0.1	0.10 ± 0.1	% DW
16:1 n-7	Palmitoleic	3.42 ± 0.2	3.77 ± 0.1	% DW
17:1 n-8	Heptadecanoic	0.14 ± 0.1	0.35 ± 0.1	% DW
18:1	Oleic	5.36 ± 0.3	7.84 ± 0.1	% DW
20:1 n-11	Gadoleic	1.02 ± 0.2	2.60 ± 0.1	% DW
22:1	Cetoleic	-	0.08	% DW
Total		11.34	17.39	% DW

SFA: Saturated fatty acids; MIFA: mono-unsaturated fatty acids.

With regard to fatty acids, 10 saturated fatty acids were detected with a higher proportion in the fresh earthworm (39.77%). The highest percentage was associated to lauric (23.96%), palmitic (5.19 %), myristic (5.09 %) and pentadecilic (1.39%) fatty acids (Table 2). It was remarkable the very high proportion (23.96%) of Lauric fatty acid (C12:0) in the fresh earthworm in comparison with the earthworm flour (9.96%). The loss of this fatty acid could be due to volatilization during the drying up of the flour. In general, the highest concentrations of monounsaturated fatty acids were detected in the flour, with predomination of oleic (7.84%), palmitoleic (3.77 %), gadoleic (2.60 %) and heptadecenoic (0.35%). The presence of the cetoleic fatty acid was also evidenced in the flour at a very low percentage (0.08 %), but it was not evidenced in the fresh earthworm.

Both groups, n-6 and n-3 polyunsaturated fatty acids were found in greater proportion (19.85% and 6.13%, respectively) in the earthworm flour, specifically the group formed by the PUFA C14, C16:2 n-4; C18: 2 n-6; C18: 3 n-6; C20:2 n-6; C20:3 n-6 and C20:4 n-6 (Table 3). Likewise, the n-3 fatty acids group showed slightly higher percentages than those present in the fresh worm. The latter being the most important for the feeding of trout, particularly the C20:5 n-3 and n-3 C22:6 [26]. These

findings indicate that the *E. andrei* flour has a high nutritional potential. In this sense, the fatty acids composition in the earthworm *E. foetida*, was studied by Tacon et al. [18], Velasquez et al. [15] and Salazar and Rojas [27] who also report the presence of linoleic and linolenic acids in this species.

Table 3. Composition of poly-unsaturated fatty acids (PUFA. n-6 y n-3) in the fresh meat and flour of the earthworm *E. andrei*.

	Lipid name	Fresh meat	Earthworm flour	Unit
AGPI (n-6)				
12 PUFA	-	0.18 ± 0.1	0.05 ± 0.1	% DW
14 PUFA	-	4.98 ± 0.2	7.31 ± 0.1	% DW
16:2 n-4	-	0.04 ± 0.1	0.76 ± 0.1	% DW
16:3 n-4	-	0.76 ± 0.1	0.23 ± 0.1	% DW
16:4 n-1	-	0.44 ± 0.1	0.09 ± 0.1	% DW
18:2 n-6	Linoleic	3.25 ± 0.2	5.47 ± 0.1	% DW
18:3 n-6	Gamma linolenic	0.07 ± 0.1	0.14 ± 0.1	% DW
20:2 n-6	-	1.03 ± 0.1	1.16 ± 0.1	% DW
20:3 n-6	Homo-gamma-linolenic	0.64 ± 0.1	0.90 ± 0.1	% DW
20:4 n-6	Eicosatetraenoic	1.90 ± 0.1	3.74 ± 0.2	% DW
Total		13.29	19.85	% DW
AGPI (n-3)				
18:3 n-3	α-Linolenic	0.33 ± 0.1	0.62 ± 0.1	% DW
18:4 n-3	Stearidonic	0.03 ± 0.1	0.05 ± 0.1	% DW
20:3 n-3	Dihomo γ linoleic	0.08 ± 0.1	0.15 ± 0.1	% DW
20:4 n-3	-	0.14 ± 0.1	0.35 ± 0.1	% DW
20:5 n-3	Eicosapentaenoic	2.56 ± 0.1	4.80 ± 0.1	% DW
22:5 n-3	Docosapentaenoic	-	0.16 ± 0.1	% DW
22:6 n-3	Docosahexaenoic	-	-	% DW
Total		**3.14**	**6.13**	**% DW**

The fresh meat and flour of *E. andrei* have contents of linolenic acid (C18:2 n-6) of 3.25% and 5.47%, respectively, exceeding the contents reported in fishmeal from sardine (1.49%), anchovy (1.68%) and herring, *Clupea harengus* (1.48 %). However, fishmeal has higher values of the n-3 fatty acids group ([28].

The flour of *E. andrei* presented a total of 26.12% saturated fatty acids (Table 2), a value that approximates the one registered by Velasquez et al. (1986b) from *E. foetida* (20.67%) (Table 4). In the other hand, Vielma et al. [16] registered the lowest value of saturated fatty acids (5.11%), while Salazar and Rojas [27] obtained the highest concentration of saturated fatty acids in *E. foetida* flour, which is normal in terrestrial organisms.

The flour of *E. andrei* also had a higher concentration of lauric, myristic, palmitic and margaric saturated fatty acids (Table 2), when compared with the values registered in the lyophilized flour of *E. foetida* [16] (Table 4). In this sense, Velasquez et al. [15] found lower concentrations of the saturated fatty acids capric, undeciclic, lauric, myristic and palmitic and a higher concentration of pentadecilic in *E. foetia* flour. However, Vielma et al. [16] and Velasquez et al. [15] found higher concentrations of stearic fatty acid. Evaluated by Salazar and Red [27], the flour of *E. foetida* showed higher concentrations of lauric, myristic, palmitic and stearic saturated fatty acids.

Table 4. Comparison of fatty acids from earthworm flours (EF) from *E. foetida*. reported by three authors

	Lipid name	EFa	EFb	EFc	Unit
SFA					
C6:0	Caproic	-	1.74	-	% DW
C10:0	Capric	-	0.49	-	% DW
C11:0	Undecilic	-	0.61	-	% DW
C12:0	Lauric	1.58	2.10	14.50	% DW
C13:0	Tridecilic	0.37	0.67	-	% DW
C14:0	Myristic	0.55	1.04	14.50	% DW
C15:0	Pentadecílic	-	3.33	-	% DW
C16:0	Palmitic	0.72	3.95	13.70	% DW
C17:0	Margaric	0.45	-	-	% DW
C18:0	Esteáric	1.44	6.74	34.40	% DW
Total		5.11	20.67	77.1	% DW
MIFA					
C11:1	Undecenoic	-	-	-	% DW
14:1 n-5	Myristoleic	-	-	0.90	% DW
16:1 n-7	Palmitoleic	-	4.07	0.90	% DW
18:1	Oleic	0.98	9.18	4.70	% DW
C18:1	Elaidic	0.84	-	-	% DW
C20:1	Gadoleic	0.94	24.52	5.30	% DW
22:1	Cetoleic	-	6.54	-	% DW
Total		2.76	44.31	11.80	% DW
PUFA					
C18:2 n-6	Linoleic	0.93	4.07	4.60	% DW
C18:3 n-3	α-Linolenic	-	-	6.20	% DW
C20:5	Eicosapentaenoic	-	12.54	-	% DW
C22:2	Docosadienoic	-	1.15	-	% DW
C25:2	Docosapentaenoic	-	0.21	-	% DW
Total		0.93	17.97	10.80	% DW

EFa: after Vielma et al. (2003); EFb: after Velásquez et al. (1986b); EFc: after Salazar and Rojas (1992).

The lyophilized flour of *E. foetida* evaluated by Vielma et al. [16] presented lower concentrations of the monounsaturated fatty acids oleic and gadoleic (Table 4) compared with the flour of *E. andrei* (Table 2). These results may be associated with the type of process in the manufacture of the flours and the species used. Furthermore, Velasquez et al. [15] report higher concentrations of palmitoleic, oleic, gadoleic and cetoleic fatty acids, while Salazar and Red [27] obtained lower concentrations of palmitoleic and oleic fatty acids, but greater concentration of the gadoleic in contrast to the lyophilized flour of *E. andrei*.

The flours of *E. foetida*, evaluated by Vielma et al. [16], Velásquez et al. [16] and Salazar and Rojas [27], presented a lower percentage of linoleic polyunsaturated fatty acid (Table 4), when compared to flour of *E. andrei* (Table 2). Velásquez et al. [15] obtained higher concentrations of eicosapentaenoic and docosapentanoico fatty acids.; and Salazar and Red [27] indicate a higher proportion of the essential fatty acid alpha-linolenic. However, the total content of PUFAs detected in the flour of *E. andrei* was 25.98%, which lies well above the values obtained in earthworms by other authors.

4. Conclusions

Worm meat showed a higher concentration of saturated fatty acids (39.77 %) in comparison with the earthworm flour (26.12%). The flour presented a higher concentration in monounsaturated and polyunsaturated fatty acids. The differences in the concentration of fatty acids between the fresh meat and flour of *E. andrei* might be that some fatty acids are concentrated in the flour, while others are volatilized by the drying up process. Thus, it is recommended to perform future studies, evaluating the optimal temperature of the drying process, which allows preserving most of the fatty acids content and maintaining the quality of the flour. According to the obtained results it is suggested that the flour of *E. andrei* can be used as a source of fatty acids to feed different species of fish and crustaceans.

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References

[1] FAO., The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO. Jan. 2018.

[2] Türker, A., Yigit, M., Ergun, S., Karaali, B., Erteken, A., Potential of poultry by-product meal as a substitute for fish meal in diets for Black Sea Turbot *Scophthalmus maeoticus* growth and nutrient utilization in winter. *Isr. J Aquacult-Bamid*, 57(1). 49-61. Feb. 2005.

[3] Abimorad, E. G., Carneiro, D. J., Métodos de coleta de fezes e determinação dos coeficientes de digestibilidade da fração protéicae da energía de alimentos para o pacu, *Piaractus mesopotamicus* (Holmberg, 1887). *Rev. Bras. Zootec*, 33. 1101-1109. Sep. 2004.

[4] Domínguez, J. Edwards, C. A., Biology and ecology of earthworm species used for vermicomposting. Chapter 3:27-40. In: vermiculture technology. Earthworms, organic wastes, and environmental management. Edwards, C. A.; Arancon, N. Q. and Sherman, R. (Eds.). CRC Press. Taylor & Francis Group. Boca Raton, FL. 587 p. Dec. 2011a.

[5] Isea, L. F., Efecto de diferentes formulaciones alimenticias a base de materias primas no convencionales de origen animal y vegetal usadas en la alimentación de trucha arco iris *Oncorhynchus mykiss* (Walbaum, 1792). Tesis Doctorado. Universidad de Los Andes-Venezuela. 334 p. Jan. 2008.

[6] Domínguez, J., Pérez-Losada, M., E*isenia fetida* (SAVIGNY, 1826) Y *Eisenia andrei* BOUCHÉ, 1972 son dos especies diferentes de lombrices de tierra. Acta *Zool Mex* (n.s.) Número Especial, 2. 221-231. Jan. 2010.

[7] Mohanta, K.N., Subramanian, S., Korikanthimath, V. S., González-Redondo, P., (Reviewing Editor). Potential of earthworm (*Eisenia foetida*) as dietary protein source for rohu (*Labeo rohita*) advanced Fry. *Cogent Food Agric*, 2:1. Feb. 2016.

[8] Villegas-Cornelio, M.L., Laines-Canepa, J. R., Vermicompostaje: I avances y estrategias en el tratamiento de residuos sólidos orgánicos. *Rev. Mexicana Cienc Agric*, 8 (2). 393-406. Mar.2017.

[9] Musyoka, S.N., Liti, D.M., Ogello. E., Waidbacher. H., Utilization of the earthworm, *Eisenia fétida* (Savigny, 1826) as an alternative protein source in fish feeds processing: A review. *Aquac Res*, 50. 2301- Apr. 2019.

[10] Isea, L.F., Blé, M.C., Medina, G.A., Aguirre, P., Bianchi, PG., Kaushik, S., Study of the apparent digestibility of earthworm *(Eisenia andrei)* flour in the feeding of the rainbow trout *(Onchorinchus mykiss). Rev. Chil Nutr*, 35(1). 62-68. Mar. 2008.

[11] Velásquez, L., Herrera, C., Ibáñez, I., Harina de lombriz. I Parte: Obtención, composición química, valor nutricional y calidad bacteriológica. *Alimentos*, 11(1). 15-21. 1986a.

[12] Stafford, E. A., Tacon, A.G., The nutritional evaluation of dried earthworm meal (*Eisenia foetida*, Savigny, 1826) included at low levels in production diets for rainbow trout,*Salmo gairdneri* Richardson. *Aquac Res*, 213-222. Jul. 1985.

[13] AOAC., International. *Official methods of analysis of AOAC International*. 17th edition. 2nd revision. Gaithersburg, MD, USA, Association of Analytical Communities. 2003.

[14] Folch, J., Lees, M., Stanley, G. H., A simple method for isolation and purification of total lipids from animal tissues. *J Biol Chem*, 226. 497-509. May. 1957.

[15] Velásquez, L., Herrera, C., Ibáñez, I., Harina de lombriz. II Parte: Composición de ácidos grasos, factores antinutricionales y tratamiento térmico para control bacterial. *Alimentos*, 11(4). 9-13. Jan.1986b.

[16] Vielma, R., Ovalles-Durán, J.F., León-Leal, A., Medina, A., Valor nutritivo de la harina de lombriz (*Eisenia foetida*) como fuente de aminoácidos y su estimación cuantitativa mediante cromatografía en fase reversa (HPLC) y derivatización precolumna con o-ftalaldehído (OPA), *Ars. Pharmaceutica*, 44 (1). 43-58. Mar. 2003.

[17] Núñez, M., Utilización de la harina de lombriz de tierra (*Eisenia foetida*) como sustituto de la harina de pescado en la alimentación de post-larvas de *Macrobrachium rosenbergii*. Tesis de pregrado, Facultad de Pesquería, Universidad Agraria La Molina, Lima, Perú, 84 pp.

[18] Tacon, A.G., Stafford, E.A., Edwards, C.A., A preliminary investigation of the nutritive value of three terrestrial lumbricid worms for rainbow trout. *Aquaculture*, 35. 187-199.. Oct. 1983.

[19] Ferruzzi, C., Manual de lombricultura. Ed. 3ra. ed. Mundi, Prensa, Madrid, España. 138p. ISBN 13: 9788471141613. Jan. 1994.

[20] Zhen Jun, S., Nutritive value of earthworms. Ecological implications of minilivestock: potential of insects, rodents, frogs and snails: 491-503. College of resources and Environmental sciences, China agricultural Universit12y, 100 094 Beijing, China. Science Publisher, Inc.; Enfield; USA. ISBN: 1-57808-339-7. 2005. Oct. 2017.

[21] Medina, A. L., Cova, J. A., Vielma, R. A., Pujic, P., Carlos, M. P., Torres, J. V., Immunological and chemical analysis of proteins from *Eisenia foetida* earthworm. *Food Agric Immunol*, 15 (3/4). 255-263. Sep. 2003.

[22] Vielma, R. A., Estudio físico-químico y funcional de las proteínas de la lombriz *Eisenia foetida*. Tesis Doctoral. Programa Ciencias Aplicadas. Facultad de Ingeniería. Universidad de Los Andes. 275 pp. Mar. 2004.

[23] Dedeke, G., Owa, S. O., Olurin, K. B., Amino acid profile of four earthworms species from Nigeria. *Agric. Biol J N Am*, 1(2). 97-102. Jan. 2010.

[24] Fadaee, R., A review on earthworm *Esienia foetida* and its applications. *Biol Res*, 3(5). 2500-2506. 2012.

[25] Gunya, B., Masika, PJ., Hugo, A., Muchenje, V., Nutrient Composition and Fatty Acid Profiles of Oven-dried and Freeze-dried Earthworm *Eisenia foetida*. *J Food Nutr Res*, 4(6). 343-348. Ago. 2016.

[26] Castro González M., Maafs-Rodríguez, A.G., Galindo-Gómez, C., Perfil de ácidos grasos de diversas especies de pescados consumidos en México. *Rev Biol Trop*, 61(4). 1981-1998. Oct/ Dec. 2013.

[27] Salazar, E., Rojas, C., Curso fundamental de lombricultura. Aspectos Generales-Teoría. Asociación Colombiana de lombricultores, Asolombriz. Grupo CorpoAndes, Mérida. Venezuela. 88 pp. Mar. 1992

[28] Öksüz, A., Özyılmaz A., Changes in Fatty Acid Compositions of Black Sea Anchovy (*Engraulis encrasicolus* L. 1758) During Catching Season. *Turk J Fish Aquat Sci*, 10. 381-385. Apr. 2010.