Available online at www.worldscientificnews.com

World Scientific News

An International Scientific Journal

WSN 152 (2021) 39-54 EISSN 2392-2192

Organic permaculture: cultivation of *Eisenia andrei* earthworms for use as feed for aquatic organisms

Selivanov Yevhen, Marenkov Oleh*

Department of General Biology and Water Bioresources, Faculty of Biology and Ecology,
Oles Honchar Dnipro National University, P.M.B. 49050, Dnipro, Ukraine

*E-mail address: DNUbiology2020@gmail.com , gidrobions@gmail.com

ABSTRACT

This paper examines the biological features and taxonomic status of the red wiggler worm *Eisenia andrei* as presented in the relevant literature. We evaluate the economic feasibility of cultivating this species as feed for aquaculture purposes and discuss conventional cultivation methods.

***Keywords*:** *Eisenia andrei*, *Eisenia fetida*, aquaculture, vermiculture, feed

1. INTRODUCTION

The modern approach in organic agriculture development relies on the scientific design of the production environment, as well as farming optimization based on natural interconnections between ecosystems. This approach is called "permaculture" (a portmanteau of "permanent agriculture"). The aim of permaculture development is focused on the creation of self-sustaining closed biosystems for agricultural production (fishery, aquaculture, animal husbandry, etc.) while utilizing traditional agricultural methods along with modern advances in science and technology. The development of permaculture includes the development of aquaculture as one of its key areas.

Aquaculture is a controlled process of farming aquatic organisms, including fish, mollusks, crustaceans, and aquatic plants [1]. In terms of production, aquaculture has

(Received 21 November 2020; Accepted 05 December 2020; Date of Publication 06 December 2020)

surpassed capture fisheries and is growing faster than any other branch of the food industry; in 2018, about 46% of the global aquatic animal production was aquaculture-sourced [1, 2]. Despite this, Ukrainian domestic market is dominated by the imported fish products – in 2016, the total value of fishery commodities imported to Ukraine amounted to $466 mil; in 2017, this value reached $527 mil; in 2018, it saw a further increase to $636 mil [1].

This growing reliance on imports is partly due to the decreased catches suffered by the Ukrainian ocean fishing fleet after complications in March 2014, when Ukraine effectively lost control of its fishing vessels that were under a freight contract in New Zealand at the time [3, 4]. Development of local aquaculture can reduce Ukrainian dependence on the global fish market, ensuring a stable increase in food resources.

According to the Law of Ukraine "On Aquaculture," the government of Ukraine intends to support the field of aquaculture by stimulating local production of aquaculture-oriented feed [5]. Live feeds are commonly considered to be of particularly high quality; they are abundant in proteins, fats, essential amino acids, vitamins, and enzymes [6]. Mass capture of live feed in wild fisheries is not sufficient to satisfy the current demand; controlled farming appears to be the most promising way to guarantee mass production of live feed [6]. In choosing an optimal feed source among alternatives, preference should be given to species exhibiting high fertility, fast growth rate, high nutritional value, tolerance to adverse environmental conditions and crowding [6]. These are the characteristics of *Eisenia andrei*, commonly known as the red wiggler or red tiger worm. Compared to related species, this worm is characterized by high fertility, tolerance to noise and vibration [6-8].

Vermiculture (cultivation of worms) has become widespread due to the growing need to dispose of agricultural and household organic waste [7, 9]. Worm cultures optimized in accordance with the principles of vermiculture can be used to process this waste into vermicompost (also called worm castings or vermicast), which is composed of worm excreta and is a valuable fertilizer. Excess worm biomass, meanwhile, can be used as feed in aquaculture [6, 7, 9, 10].

In order to attain maximum yield from *Eisenia andrei* culture, optimal conditions for their growth, breeding, and metabolism must be ensured. Thus, detailed knowledge of the biological and physiological characteristics of this species is crucial for their effective cultivation.

2. BIOLOGICAL FEATURES OF *E. ANDREI*

The body of *E. andrei* earthworm is covered with a thin elastic cuticle that is dark red in color [9-12]. Beneath the cuticle lies the epidermis, which is rich in glandular cells that produce mucus. Secretion of this mucus is of utmost importance to the worm's well-being, as it reduces friction between the cuticle and surrounding soil, protects the outer body surface from damage during contact with sharp bodies, aids in skin respiration. Prolonged exposure to air causes the mucus to dry out, which is followed by the worm's death [10].

Underneath the worm's epidermis lie the ring muscles, and below them – the longitudinal muscles. Together they form the body wall. The muscular wall of the intestine, located deeper in the worm's body, is somewhat similar. Muscles that comprise the body wall allow the worm to move, while peristaltic contractions of the intestinal wall muscles push food through the intestine. The space between the body wall and the intestinal wall is called the secondary body cavity, or coelom. The secondary body cavity is filled with fluid which acts as a hydrostatic skeleton, serving as support during digging and crawling. As the ring muscles contract, the fluid pressure in the secondary body cavity rises and lengthens the worm's body, while with the contraction of the longitudinal muscles, the worm's body thickens [10].

The worm's body is divided into segments along its entire length; this segmentation of the body is called metamerism. These segments are separated by internal partitions passing through the secondary body cavity – septa. The nervous, circulatory, and excretory systems are segmented, but some organ systems (e.g., the digestive system) penetrate the septa and extend through the whole body of the worm [10].

Due to the body metamerism of *E. andrei*, its muscles can work in a coordinated fashion to lengthen or thicken certain parts of the body; this facilitates burrowing into the substrate [10].

A closed circulatory system mediates between the intestine and the body wall of *E. andrei* by transporting nutrients, dissolved gases, and metabolic waste [10].

2. 1. Nutrition

Worms need oxygen, carbon, nitrogen, phosphorus, and cellulose to grow and reproduce [13]. They get these substances from leaf litter, soil, and microbes. To feed, a worm sucks semi-liquid matter into its mouth; the particle size of this matter reaches up to 1 mm [6]. From the mouth, food flows into the pharynx; it is this organ that ensures the inflow of food into the mouth. From the pharynx, food enters the esophagus, from where it proceeds to the stomach, which consists of two parts – the thin-walled crop, where food is stored, and the subsequent muscular stomach with thick muscular walls, where food is ground up. Next lies the long and straight intestine, where ground food is digested. This intestine ends with an anal opening at the posterior end of the body [10, 14]. As organic residues pass through the worm's digestive tract, they are being converted into worm castings – an organic material rich in minerals, which is similar in appearance to well-textured soil [10].

On cursory observation, it may appear as if the worms are feeding at a constant rate and indiscriminately. In fact, as the worms feed, they are able to choose the part that contains most organic matter from the total soil mass [15, 16]. Earthworms prefer nutritious food sources such as fallen leaves or manure, but will still consume some of the mineral soil (mineral particles likely perform a grinding function in worms' digestive systems) [15, 17-19].

While *E. andrei* worms cannot produce cellulolytic enzymes, decomposition of cellulose in their intestines still occurs under the influence of fungal enzymes (from unicellular fungi that are digested together with cellulose) and symbiotic cellulolytic bacteria [20]. Intestinal microflora of *E. andrei* contains three times more symbiotic microorganisms than the substrate [20].

The rate at which the intestinal tract fills up is not significantly affected by temperature changes, but the duration of food retention in the intestine is almost halved as temperature decreases from 20 °C to 10 °C (from 2.9 hours to 5.5 hours), which corresponds to a twofold decrease in feeding speed [15]. The duration of soil retention in the intestinal tract also depends on whether the worm is feeding or making new burrows [17, 21].

Over the course of a day, one worm can eat an amount of food approximately equal to its weight [10]. Worms prefer food that's been colonized and partially fermented by microbes. They avoid food that contains tannins, polyhydric phenols, and water-soluble polyphenols (oak leaves are one example of an unpalatable substrate) [22-24]. Leaves with a high concentration of tannins are less palatable for worms [25-27] because tannins reduce the availability of soluble proteins and polysaccharides, as well as the activity of digestive enzymes [22, 28].

In soils contaminated with sodium trichloroacetate, chlorobenzenes, polychlorobiphenyls or heavy metals these substances accumulate in the worms' bodies, which inhibits their growth and reproduction, and increases mortality [10]. *Eisenia andrei* worms are epigeic, which means they live and feed in the upper layer of soil and on its surface.

After sunset, they come to the surface to search for food and mates [10].

2. 2. Reproduction

Eisenia andrei is a hermaphroditic species. An individual specimen reaches sexual maturity 21 to 30 days after it emerges from its cocoon [29]. There is a direct relationship between the biomass of an individual *E. andrei* specimen and its clitellum development; to become sexually mature, a worm must weigh at least 0.4 g [22].

Mating occurs under the soil surface, and the laying of cocoons begins 48 hours after mating. If the temperature is favorable (see chapter 2. 3. 1.), laying of cocoons may continue throughout the year [9, 29]. On average, mature *E. andrei* lays one cocoon every two to three days. The incubation period lasts from 18 to 26 days; out of all the cocoons, approximately 72% to 82% are viable. The number of newborn worms emerging from each viable cocoon varies between 2.5 and 3.8 depending on the temperature [29].

Experimental results obtained by J. Domínguez et al. at the Spanish University of Vigo in 1997 indicate that the mating process of *E. andrei* is not required for the formation of viable cocoons [22]. The rate of sexual maturation in worms is dependent on population density. The amount of time required to form clitellum varies between worms in dense populations and single individuals [29].

Compared to closely related worm species, such as *E. fetida*, *E. andrei* has higher fertility and reaches sexual maturity faster [10, 30-32]. The rate at which worms lay cocoons, grow, and reach puberty is closely related to environmental conditions [29].

2. 3. Sensitivity to environmental conditions

2. 3. 1. Temperature

The optimal growth temperature for *E. andrei* is 25 °C. Temperatures below 10 °C usually lead to a decrease in feeding activity; below 4 °C cocoon laying and development of young worms stop completely. In response to freezing temperatures worms migrate to deeper substrate layers, where they try to overwinter [29].

The adverse effects of high temperatures (above 30 °C) on worms are not completely direct. High temperatures promote microbial growth in the substrate, and microbes usually consume available oxygen, which negatively affects the worm population [29]. It should be noted that *E. andrei* possesses some resistance to fermentation processes: in the mixed cultivation of *E. andrei* with closely related *E. fetida* in household waste all *E. fetida* specimens died within 30 days, while *E. andrei* specimens overcame a critical period of active fermentation with relatively low mortality [30]. Under controlled conditions, the average worm lifespan is 594 days at 18 °C and 589 days at 28 °C, with a maximum lifespan of four and a half to five years. In nature they usually live for one to two years [10, 29].

2. 3. 2. Illumination

Eisenia andrei lacks eyes, but it can sense light due to its photosensitive skin cells [10, 33]. Worms are very sensitive to daylight and artificial light (except for red light); if exposed to sunlight, they die within minutes [10].

2. 3. 3. Moisture

The moisture content of the substrate undoubtedly affects the growth rate of earthworms. *Eisenia andrei* can survive at 60% substrate moisture, but faster growth occurs at moisture levels ranging from 80% to 90% [29, 34, 35].

2. 3. 4. pH

Eisenia andrei worms are relatively tolerant to pH changes and can live in a substrate with a pH index of 5 to 9, but in the presence of a pH gradient they move towards a more acidic material, preferring a pH of 5.0 [29].

2. 3. 5. Aeration

Worms don't have specialized respiratory organs; oxygen and carbon dioxide diffuse through their cuticle [29]. Consequently, they are very sensitive to anaerobic conditions. In rainy weather, when the soil or substrate becomes soaked with water that contains a high amount of carbon dioxide (from the decomposition of organic matter), the worms experience a lack of oxygen, which causes them to come to the surface [10, 36].

2. 3. 6. Ammonia

Earthworms are very vulnerable to ammonia and cannot survive in organic substrates that contain high levels of this compound (for example, in fresh poultry waste). For optimal worm activity, ammonia levels should not exceed 1 mg per 100 mg of a substrate [29]. Reducing ammonia levels in the substrate is possible by pre-composting it or flushing it with water.

3. TAXONOMIC STATUS OF *E. ANDREI*

Earthworm taxonomy is poorly developed due to the low number of significant morphological differences between different species; there is confusion regarding the taxonomic status of some species [12].

Eisenia andrei worms have long been considered a subspecies of *Eisenia fetida* (this species is also known as *Eisenia foetida* due to the erroneous "correction" of the original name; the currently accepted name is *Eisenia fetida*) [11, 30, 37]. *E. fetida* and *E. andrei* both have an average length of 60-120 mm, a width of 3-6 mm, and a number of segments that ranges from 80 to 120. In both species, 6 to 8 of these segments are covered by the clitellum; tubercula pubertatis extends along the ventral border of the clitellum over three segments; both species lay cocoons 2.4 to 5.2 mm long and 2.3 to 4.4 mm wide [9, 32].

The only obvious difference between *E. fetida* and *E. andrei* is pigmentation [11, 30, 32]. French scientist Francis André was the first to note this difference in 1963, which prompted him to divide *Eisenia fetida* into two forms: *Eisenia foetida typica* (possesses characteristic striped pigmentation) and *Eisenia foetida unicolor* (has a uniform dark red color) [11, 12, 38]. French earthworm expert Marcel Bouché pointed to the low systematic value of the term "unicolor" since samples stored in preservative liquids for extended periods of time tend to lose their natural color [30, 32, 39]. In 1972 M. Bouché changed the names proposed by F. André, renaming *Eisenia foetida typica* to *Eisenia foetida foetida*, while *Eisenia foetida unicolor* was renamed to *Eisenia foetida andrei* [30, 32, 40].

In 1980 French scientists from the University of Montpellier (P. Roch, P. Valembois, M. Lassegues) found important biochemical differences between *Eisenia foetida foetida* and *Eisenia foetida andrei*. They suggested a hypothesis by which *E. f. andrei* originated from *E. f. foetida* through the loss of some alleles [31, 41, 42].

In 1982 American biologist John Jaenike demonstrated genetic divergence between the striped and monochromatic forms of *E. fetida* using starch gel electrophoresis [11, 30, 42]. Jaenike found that at least three loci between *Eisenia foetida foetida* and *Eisenia foetida andrei* lacked common alleles. These experimental results seem to indicated complete reproductive isolation between *E. f. foetida* and *E. f. andrei* [30, 32, 42].

According to the biological definition of species proposed by Ernst Mayr in 1942, "species are groups of interbreeding natural populations that are reproductively isolated from other such groups" [43]. Thus, in his research Jaenike confirmed that the "forms" *Eisenia foetida foetida* and *Eisenia foetida andrei* are, in fact, separate species (according to the definition proposed by E. Mayr). Jaenike suggested renaming *Eisenia foetida foetida* to *Eisenia foetida*, while *Eisenia foetida andrei* was changed to *Eisenia andrei* [30, 42]. Later the name *Eisenia foetida* was changed to *Eisenia fetida*, thus canceling the erroneous "correction" of the original name [11, 30, 37, 44].

Despite Jaenike's work, in numerous publications species *Eisenia fetida* and *Eisenia andrei* are grouped under the mutual name *Eisenia fetida* or *Eisenia foetida* [12, 30, 31, 32]. The situation is complicated by the fact that both species often live in mixed colonies in manure and compost heaps [31].

According to the modern biological taxonomy, *E. andrei* belongs to the family Lumbricidae of the class Clitellata [45, 46]. In vermiculture *E. andrei* is usually considered superior due to its quicker growth, sexual maturation and cocoon laying when compared with *E. fetida* [30, 31, 32].

3. 1. Hybridization between *E. andrei* and *E. fetida*

Early reports of hybridization between *Eisenia fetida* and *Eisenia andrei* are somewhat inconsistent with one another. According to F. André's report in 1963, he managed to surgically obtain chimeric worms which combined male gonads of *E. fetida* with female gonads of *E. andrei*, as well as chimeras in which female gonads of *E. fetida* were combined with male gonads of *E. andrei* [30, 38]. André found that although fertilization of female *E. andrei* gametes with male *E. fetida* gametes doesn't produce viable offspring, reverse procedure (fertilization of female *E. fetida* gametes with male *E. andrei* gametes) yields hybrid offspring. In his report F. André states that these hybrid offspring had a striped pigmentation which was intermediate between their parents; in addition, they laid cocoons, which were completely sterile [31, 38].

The results of these crossbreeding experiments reported by André are in conflict with several later studies. In 2001 Thomas McElroy and Walter Diehl of the Florida International

University were unable to obtain any viable hybrids from the cross between *E fetida* and *E. andrei*, confirming John Jaenike's hypothesis regarding reproductive isolation between these species [31, 47]. The same result was obtained by Jorge Domínguez et al. in 2004 – *E. fetida* and *E. andrei* were found to lack any effective mechanisms to prevent interspecies mating; the mechanism of reproductive isolation between them was found to be post-copulative (cocoons are formed, but are sterile) [31]. Taking into account the energy and time spent on the formation of sterile cocoons, it seems reasonable to assume that interspecific mating adversely affects the growth of the worm population; however, in a 1996 study Carmen Elvira et al. found no negative trends in the growth and reproduction of *E. fetida* and *E. andrei* in mixed populations. Moreover, C. Elvira et al. reported that the growth of *E. andrei* was more intense in the mixed culture rather than in isolation [30].

In 2018 Barbara Plytycz et al. reported a successful cross between *E. andrei* and *E. fetida*, which produced a hybrid derived from *E. andrei* egg cell. This hybrid was shown to possess a DNA sequence specific to *E. andrei* (denoted here as A) along with a DNA sequence specific to *E. fetida* (denoted here as F), as well as a maternally-derived mitochondrial DNA of *E. andrei* (denoted here as a). A cross between this hybrid (aAF) and *E. andrei* specimen (aAA) produced offspring (aAA, aAF), proving the aAF hybrid to be fertile [48, 49].

Additionally, B. Plytycz et al. performed a cross between the aAF hybrid and *E. fetida*, which resulted in fertile offspring (aAF) and a sterile hybrid derived from *E. fetida* egg cell (fFA). This second type of hybrid possessed a combination of species-specific DNA sequences from *E. andrei* and *E. fetida* (A and F, respectively), as well as a maternally-derived mitochondrial DNA of *E. fetida* (f) [48].

Hybrids derived from *E. andrei* egg cells (aAF) were fertile and relatively common, while the hybrids derived from *E. fetida* egg cells (fFA) were sterile and rare among the progeny. Based on these results, B. Plytycz et al. concluded that hybridization between *E. andrei* and *E. fetida* is asymmetrical, with hybrids derived preferentially from *E. andrei* egg cells [48]. Both types of hybrids exhibited a striped pigmentation pattern in their posterior body segments [48]. A cross between two hybrids produced sterile cocoons regardless of the hybrid type [49].

The apparent confusion among the reports regarding hybridization between *E. fetida* and *E. andrei* may be due to the difficulty of differentiation between these two species, confusion between two possible types of hybrids, or flawed research methodology. Further research on this topic may be required.

4. METHODS OF *E. ANDREI* CULTIVATION

Eisenia andrei is one of the most popular species in vermiculture, mainly due to its ubiquitous distribution, natural aptitude for colonizing organic substrates, short life cycle, and ability to survive in a wide range of temperature and moisture conditions [29].

Despite the relative endurance of *E. andrei* it processes substrate most efficiently in a relatively narrow range of environmental conditions (see chapter 2. 3). If there are significant deviations from these conditions, worms will migrate to more favorable areas of the substrate, leave the substrate altogether or die. This behavior in worms significantly hinders substrate processing and biomass accretion [29]. Therefore, in order to ensure maximum productivity of a worm culture, it is necessary to maintain conditions that meet the biological needs of worms and allow them to reach peak growth and reproduction. Small-scale vermiculture utilizes simple and affordable designs, such as plastic or wooden boxes perforated for aeration and drainage, equipped with lids to exclude light. Wooden boxes better absorb moisture and retain heat, while plastic boxes are resistant to mold and rot [10]. Several small boxes are usually preferred over a single large box since smaller boxes can be transported more easily and allow for a combination of outdoor cultivation with indoor cultivation. A standard box for growing worms has a height of 30 cm, a width of 60 cm, and a length of 90 cm; a box of these dimensions allows to process up to 5 kg of organic waste per week. Holes with a diameter of 6.5 to 12.5 mm are drilled in the sides and in the bottom of the box to ensure sufficient water drainage and air circulation. The box is placed on bricks or wooden supports, then a vessel for leachate collection is placed underneath the box [10].

Worm cultivation can be carried out both indoors and outdoors [6, 10]. Ukrainian climate conditions are not particularly conductive to outdoor cultivation of *E. andrei* due to the lack of effective temperature control methods. These worms grow most efficiently at 25 °C; at low temperatures their growth, sexual development, and laying of cocoons stop (see chapter 2. 3. 1.). This problem is less pronounced in the mild winters of Zakarpatska Oblast, where worm substrate can be sufficiently protected from frost by a layer of straw about 40 cm high [10]. In regions with a more continental climate, outdoor cultivation requires a more substantial protection from the cold. The substrate may be covered with straw, manure and plastic film for insulation. It's advisable to leave an empty space 10 to 15 cm wide between the surface of the substrate and the plastic film to create a greenhouse effect [10]. The top 5 cm of the substrate may freeze – this is acceptable since the frozen layer aids in insulation (there's even a method to purposefully create an insulating ice coating on the substrate's surface by spraying it with a fine water mist) [10, 50]. It's usually best to avoid moisturizing the substrate throughout the winter. Worms must only be fed during the periods of relatively high temperature and without disturbing the deep substrate layers, so as to prevent heat loss. Feeding in freezing temperatures may be lethal for the worms [10].

Cultivation of *E. andrei* in a heated room can go on year-round and yields twice the amount of worms when compared to outdoor cultivation [6, 8]. However, significant funds are required to heat such a room throughout the winter [8]. If worms are bred in a small, portable container, it is advisable to keep the worm culture outdoors during the warm season, then move it into a heated room once the cold season begins. This allows for a combination of outdoor cultivation with indoor cultivation and reduces heating expenses during the warm season [10].

4. 1. Feeding

Worm feed consists of the starting (base) substrate and organic waste; the latter is added to enrich the base substrate with additional organic matter [6, 10]. Finely chopped cardboard or paper, sawdust mixed with straw, fallen leaves, and composted manure can all be used as a base substrate for a worm culture [10].

Before worms are introduced to the base substrate, it must be properly prepared. First, the substrate is soaked in water for a period ranging from 2 to 24 hours, depending on substrate composition. Next, the water is drained, excess moisture squeezed out. After that, the substrate is loosened to increase aeration. Base substrate should contain at least 20% cellulose, which aids in aeration. Some sand or soil is added to provide the worms' digestive systems with abrasive material [6, 10].

An important role in vermiculture is played by compost, which can be used either as a nutritional supplement for the worms or as a base substrate. Composted herbivore manure is high in nitrogen and contains microbes beneficial for worm digestion [10]. A mixture of manure from several animals is used to supplement the worms' diet: 10% rabbit manure, 15% horse manure, 35% cow manure, 10% sheep manure, 30% pig manure [6]. Horse manure contains a considerable amount of cellulose; cattle manure contains fungi that are readily eaten by worms; rabbit manure does not require fermentation; swine manure should be mixed with straw to reduce protein and moisture content [6, 10, 22]. Under no circumstance should worms be introduced to uncomposted manure (except for rabbit manure) since it may heat up to 60-70 °C during the fermentation, killing the worms.

A high-quality compost that's ready to be colonized by worms has the appearance of a moist, homogeneous mass; its temperature should be about 19 to 22 °C, pH about 7, with relative moisture content of 82.5% [10, 51].

Since *E. andrei* are epigeic worms (they live and feed in the topsoil) the substrate must not be piled up higher than 30 cm lest it compress under its own weight, which may lead to anaerobic conditions [10]. Once the substrate is ready, worms are introduced to its surface. Their initial number depends on the amount of substrate to be processed. After the worms settle, optimum temperature, pH, and moisture levels must be maintained so that the worms may grow and reproduce most efficiently. Under optimal conditions, worms eat an amount of food roughly equal in weight to their own body weight daily [6, 10].

It is recommended to document the cultivation process in a journal, especially when it comes to feeding dates, sampling dates, substrate preparation dates, and the number of adult worms harvested from a culture [10]. This allows for better planning of daily operations and the whole cultivation process. Additionally, it leaves a log of previous operations, which provides ground for retrospection and future improvement.

4. 2. Overpopulation

It's best to avoid an overabundance of worms in the substrate. Excessive population density leads to a decrease in cocoon laying and retards growth and maturation of some specimens even under ideal physicochemical conditions [10, 29]. As the worm population grows, competition for food and territory begins to arise; worm castings accumulate, which have a toxic effect on worms [10].

If desired, the worm population can be kept ever-growing by separating some worms from the main culture and introducing them into another substrate. Worm separation can be carried out by a variety of methods: the substrate may be sifted through a sieve, vibrating devices may be utilized to force the worms out of the substrate, worms may be attracted to the surface with food, and so forth [10].

The last method mentioned (attracting worms with food) is of particular interest to vermiculture because it requires no specialized equipment and can be used to separate adult worms from the general culture. Adult worms that haven't been fed for some time react fairly quickly when a nutritious substrate is placed adjacently to their culture (about 50% of them migrate into the new substrate within a few hours), while the smaller worms and cocoons remain in place. Since worms reach peak biomass in adulthood, adult worms are most suitable to use as fish feed. Quality worms have a dense, smooth, and moist body; they actively react to the touch [10].

5. ECONOMIC FEASIBILITY OF *E. ANDREI* CULTIVATION AS FEED FOR AQUACULTURE

From an economic standpoint, the feed cost (30% to 50% of total production costs) is one of the main challenges in the development of modern aquaculture [52, 53]. Protein is the most important nutrient for all fish, regardless of species. Today, fish meal is the main protein source in fish feed; however, fish meal is becoming increasingly expensive due to strong global demand, prompting aquaculture keepers to look for alternate protein sources [3, 6, 52, 53, 54].

Eisenia andrei earthworms appear to be a promising alternative to fish meal. By dry weight, the body of an adult *E. andrei* consists of 70.95% protein, the rest is ash (6.17%), carbohydrates (10.25%), and lipids (12.63%) [9].

It should be noted that the main value of any dietary protein lies in its content of essential amino acids, which are required for the growth and regeneration of various animal tissues. Protein degradation and amino acid catabolism are continuous processes that occur regardless of the animal's age or physiological condition; essential amino acids must be supplied with food in order to compensate for the catabolic processes in the animal's body and support anabolic processes [53]. The presence of essential amino acids in sufficient quantities and variety largely depends on the fish's diet; the relative contribution of dietary essential amino acids to the body's total amino acid supply is much greater in fish than in mammals, which highlights the importance of essential amino acids in fish feeding [52, 55].

Earthworms can be used as a live feed or processed into earthworm meal, which is easier to store. To prepare earthworm meal, worms are kept in running water for one to two days to clean them; they are subsequently frozen, dried, and ground [10].

By the mass fraction of dry matter, *E. andrei* earthworm meal is high in glutamic acid, aspartic acid, arginine, leucine, and lysine (4% to 10%); methionine content is much lower (<1.5%), which is similar to the amino acid composition of fish meal [53]. Thus, when it comes to amino acids, *E. andrei* earthworm meal can meet the dietary needs of fish as adequately as the commonly used fish meal.

Feeding fish with earthworm meal instead of fish meal leads to more intensive (20% to 30%) growth for a number of fish, such as tilapia [56], trout [57], carp [58], and others [9, 59-63]. Earthworm meal is cheaper than fish meal because the nutritious substrates and organic wastes fed to worms are usually free [53].

We also must not forget that *E. andrei* process organic waste into worm castings, which are an excellent fertilizer. Worm casting fertilization in agriculture is three to four times cheaper than manuring, and it increases crop quantity and quality [10]. Widespread use of vermiculture in combination with other environmentally friendly agricultural techniques will lead to a wider supply of food products being produced without the use of agrochemicals, and to a general environmental improvement [10].

Thus, when growing worms for aquaculture needs, it is advisable to make full use of the worms' ability to process waste into castings in order to obtain additional profits that offset the maintenance costs. Nevertheless, nutritious biomass production still remains a top priority in such an arrangement. This affects the production process since there's a tangible difference between culturing methods focused on worm biomass production and those focused on production of worm castings. If worm biomass is a priority, the preferred substrate would consist of fermented waste with a porous structure and a high moisture-holding capacity:

leaves, grass, hay, chaff, deciduous tree sawdust (except oak), cut corn cobs, and similar substrates. In these kinds of substrates, earthworm population density should be approximately 1200 to 2500 individuals per square meter [10]. To increase their biomass, worms are fed composted manure mixed with organic waste that imparts porosity and looseness onto the substrate. One month before the worms are harvested, various nutritious mixtures are added to the main feed. For example, to grow small quantities of large and thick worms, the following feed additive is used: five parts chicken starter feed, two parts wheat or rice bran, two parts alfalfa granules, one part whole grain wheat flour, one part agricultural calcium carbonate, one part powdered milk. A handful of this mixture is mixed into feed during each feeding; worms grown with this additive have a healthy appearance and are attractive to fish [10].

When designing a worm farm, the location of the worm culture and the required amount of substrate must be decided in advance. Worm culture should be located close to the substrate source and areas of its fermentation; it also requires a water supply. In temperate climates, outdoor cultivation is highly productive only during the warm season. In winter worm activity decreases, culture maintenance becomes more difficult. As a consequence, worm farms in temperate climates usually incorporate a heated room with an optimal round-the-clock temperature, where the culture is transferred at the onset of the cold season [10]. This need for heating in order to maintain the optimum cultivation temperature makes *E. andrei* cultivation during the cold season somewhat expensive [8].

A medium-scale worm farm consisting of 350 to 400 worm beds (each bed approximately one by two meters large) can be maintained by a single person working 8 hours a day (40 hours a week) [10]. A year and a half after its establishment, such a farm would be producing about 0.4 tons of worm biomass annually [10].

Commercial worm farms tend to mechanize labor-intensive processes as much as possible. It pays to have a tractor for transportation of substrate and finished products, an excavator with a bucket, and several vibrating screens of various designs with mesh size ranging up to 5 mm (to separate the worms from the substrate). A pH meter or litmus paper is required to determine the acidity or basicity of the substrate, and soil thermometers about 60 cm long are needed to measure substrate temperature. Among other equipment required to efficiently manage a worm farm are plastic bags, rakes, pitchforks, shovels, 20 m long hose made of synthetic material, as well as a wheelbarrow [10]. These tools are commonly used in farming; therefore, it would seem practical to create a worm farm on the basis of an existing farm. Farms are known to produce a significant amount of organic waste that could be processed by worms; on the other hand, worms create castings, which can be used to fertilize farm crops. Thus, collaboration between vermiculture and agriculture benefits both of these industries.

6. CONCLUSIONS

In order to promote the development of aquaculture in Ukraine, large quantities of cheap, high-quality fish feed are required. The currently used fish meal is becoming increasingly expensive; there's a growing demand for an alternate feed source.

Eisenia andrei earthworms appear to be an excellent alternative to fish meal. The earthworm flour produced from *E. andrei* has an amino acid profile very similar to that of fish meal; furthermore, *E. andrei* flour is substantially less expensive because these worms can be grown in organic waste or other cheap substrates.

When compared to closely related *Eisenia fetida*, *Eisenia andrei* is characterized by quicker growth, sexual maturation, and cocoon laying. As long as adequate temperature, moisture content, aeration, pH and nutrition are maintained, *E. andrei* can reproduce abundantly. While the production of nutritious earthworm biomass is the main priority for aquaculture-oriented worm farms, some attention should also be given to the worm casting, which are produced as a byproduct of worm farming. Worm castings can be used as a fertilizer, thus benefitting agriculture. At the same time, organic waste produced by agriculture may be used to feed the worms. In this manner waste from one industry can be processed by the other, creating a mutually beneficial arrangement.

References

[1] M. Barange, A. Egger, J. Gee, J. Geehan, G. Laurenti, P. Maudoux, S. Montanaro, F. Perfetto, B. Senfter, R. Sfeir, S. Vannuccini, X. Zhou, C. Benkabbour, M. Guyonnet, K. Sullivan, FAO Yearbook. Fishery and Aquaculture Statistics 2018. FAO Fisheries and Aquaculture Department (2020) Access mode: http://www.fao.org/3/cb1213t/cb1213t.pdf

[2] M. Halwart, FAN coming of age! *FAO Aquaculture Newsletter* 60 (2019) 2-3.

[3] V. Bekh, National Aquaculture Sector Overview: Ukraine. FAO Fisheries and Aquaculture Department Access mode: www.fao.org/fishery/countrysector/naso_ukraine

[4] Океанічний риболовецький флот приніс Україні в 2015 р. близько 19 млн гривень чистого прибутку. Державне Агентство Рибного Господарства України (2016) Режим доступу: www.darg.gov.ua/_okeanichnij_ribolovecjkij_0_0_0_2464_1.html

[5] Закон України – Про аквакультуру. *Відомості Верховної Ради (ВВР)* 43 (2013) 616 с.

[6] О. В. Федоненко, Т. С. Шарамок, О. М. Маренков, Основи аквакультури: культивування мікроводоростей та безхребетних. Дніпропетровськ (2014) 44 с.

[7] Е. В. Просянников, Н. Ю. Купцова, К. А. Трувелер, Эколого-продукционная разнокачественность дождевых компостных червей по отношению к разным субстратам. *Агрохимический вестник* 1 (2007) 22-24.

[8] Е. М. Романова, М. Э. Мухитова, Е. В. Титова, Общие и отличительные черты микробиоценоза промышленной вермикультуры Eisenia fetida andrei (Bouché, 1972) и ее природного аналога Eisenia fetida (Savigny, 1826). *Вестник УГСА* 4(16) (2011) 64-70

[9] M. Rodrigues, W. M. Carlesso, D. Kuhn, T. Altmayer, M. C. Martini, C. D. Tamiosso, C. A. Mallmann, C. F. Volken De Souza, E. M. Ethur, L. Hoehne, Enzymatic hydrolysis of the Eisenia andrei earthworm: Characterization and evaluation of its properties. *Biocatalysis and Biotransformation* 35(2) (2017) 110-119

[10] Б. М. Шарга, В. І. Ніколайчук, І. М. Мага, Вермікультура: методичні рекомендації для студентів з курсу "Ґрунтознавство". Ужгород (2006) 126 с.

[11] R. W. Sims, B. M. Gerard, Earthworms: Keys and Notes for the Identification and Study of the Species. *Synopses of the British Fauna* 31 (1985) 171 c.

[12] J. E. Kammenga, D. J. Spurgeon, C. Svendsen, J. M. Weeks, Explaining density-dependent regulation in earthworm populations using life-history analysis. *OIKOS* 100(1) (2003) 89-95.

[13] B. Sivasankari, A study on life cycle of earthworm Eisenia foetida. *International Research Journal of Natural and Applied Sciences* 3(5) (2016) 83-93.

[14] В.А. Догель, Зоология беспозвоночных: учебник для университетов Москва: *Высшая школа* (1981) 650 с.

[15] T. Jager, R. Fleuren, W. Roelofs, A. de Groot, Feeding activity of the earthworm Eisenia andrei in artificial soil. *Soil Biology & Biochemistry* 35(2) (2003) 313–322.

[16] P. J. Bolton, J. Phillipson, Burrowing, feeding, egestion and energy budgets of Allolobophora rosea (Savigny) (Lumbricidae). *Oecologia* 23(3) (1976) 225-245.

[17] K.P. Barley, The influence of earthworms on soil fertility II. Consumption of soil and OM by the earthworm Allolobophora caliginosa. *Australian Journal of Agricultural Research* 10(2) (1958) 179-185.

[18] N. B. Hendriksen, Gut load and food-retention time in the earthworms *Lumbricus festivus* and *L. castaneus*: a field study. *Biology and Fertility of Soils* 11 (1991) 170-173

[19] O. P. Schulmann, A. V. Tiunov, Leaf litter fragmentation by the earthworm Lumbricus terrestris. *Pedobiologia* 43 (1999) 453-458

[20] Н. С. Паников, А. Ю. Горбенко, Д. Г. Звягинцев, Количественная оценка влияния мезофауны на скорость разложения растительного опада. *Вестн. МГУ, Сер. 17 Почвоведение* 3 (1985) 37-45

[21] J.N. Parle, Microorganisms in the intestines of earthworms. *Journal of General Microbiology* 31 (1963) 1–11

[22] J. Domínguez, M. J. I. Briones, S. Mato, Effect of the diet on growth and reproduction of Eisenia andrei (Oligochaeta, Lumbricidae). *Pedobiologia* 41 (1997) 566-576

[23] B. R. Brown, C. W. Love, W. R. C. Handley, Protein-fixing constituents of plants: Part III. *Report on Forest Research* (1963) 90-93

[24] C. A. Edwards, J. R. Lofty, Biology of Earthworms. London: Chapman and Hall (1977) 333 p.

[25] J. E. Satchell, D. G. Lowe, Selection of leaf litter by Lumbricus terrestris. In: Progress in Soil Biology. Amsterdam: North Holland (1967) 102-119

[26] G. J. F. Pugh, Terrestrial fungi. In: Biology of Litter Decomposition. London: *Academic Press* (1974) 329-336

[27] C. A. Edwards, G. W. Heath, Studies in leaf litter breakdown. III. The influence of leaf age. *Pedobiologia* 15(5) (1975) 348-354

[28] Swain T., Tannins and lignins. In: Herbivores – Their Interaction with Secondary Plant Metabolites. London: *Academic Press* (1979) 657-682.

[29] J. Domínguez, C. A. Edwards, Biology and Ecology of Earthworm Species Used for Vermicomposting. In: Vermiculture Technology: Earthworms, Organic Wastes, and Environmental Management. New York: *CRC Press* (2010) 27-40

[30] C. Elvira, J. Domínguez, M. J. I. Briones, Growth and reproduction of Eisenia andrei and E. fetida (Oligochaeta, Lumbricidae) in different organic residues. *Pedobiologia* 40(4) (1996) 377-384

[31] J. Domínguez, A. Velando, A. Ferreiro, Are Eisenia fetida (Savigny, 1826) and Eisenia andrei Bouché (1972) (Oligochaeta, Lumbricidae) different biological species? *Pedobiologia* 49(1) (2005) 81-87

[32] A. J. Reinecke, S. A. Viljoen, A comparison of the biology of Eisenia fetida and Eisenia andrei (Oligochaeta). *Biology and Fertility of Soils* 11 (1991) 295-300

[33] C. Starr, Biology: Concepts and Applications. Belmont: *Wadsworth* (1991) 950 p.

[34] C. A. Edwards, Breakdown of animal, vegetable and industrial organic wastes by earthworms. In: Earthworms in Waste and Environmental Management. Hague: *SPB Academic Publishing* (1988) 21-31

[35] J. Domínguez, C. A. Edwards Effects of stocking rate and moisture content on the growth and maturation of Eisenia andrei (Oligochaeta) in pig manure. *Soil Biology and Biochemistry* 29(3-4) (1997) 743-746

[36] C.A. Edwards, P. J. Bohlen. The Biology and Ecology of Earthworms. London: Chapman & Hall (1996) 426 p.

[37] E. G. Easton, A guide to the valid names of Lumbricidae (Oligochaeta). In: Earthworm Ecology from Darwin to Vermiculture. London: Chapman & Hall (1983) 475-485

[38] F. André, Contribution à l'analyse expérimentale de la reproduction des lombriciens. *Bulletin biologique de la France et de la Belgique* 97 (1963) 1–101.

[39] A. Kruger, Vers la terre et vers de terre. Mode d'accès: www.franceculture.fr/emissions/ne-parle-pas-la-bouche-pleine/vers-la-terre-et-vers-de-terre-0

[40] M. B. Bouché, Lombriciens de France: Écologie et Systématique. *Annales de Zoologie Ecologie Animale* 72(2) (1972) 671 pp.

[41] P. Roch, P. Valembois, M. Lassegues, Biochemical particulars of the antibacterial factor of the two subspecies Eisenia fetida fetida and Eisenia fetida andrei. *American Society of Zoologists* 20 (1980) 790-794.

[42] J. Jaenike, *Eisenia foetida* is two biological species. *Megadrilogica* 4 (1982) 6-8

[43] E. Mayr, Systematics and the Origin of Species from the Viewpoint of a Zoologist. New York: Columbia University Press (1949) 372 p.

[44] R. W. Sims, The scientific names of earthworms. In: Earthworm Ecology from Darwin to Vermiculture. London: Chapman & Hall (1983) 467-474

[45] ITIS Standard Report Page: Eisenia andrei. Integrated Taxonomic Information System on-line database (Retrieved 30. 11. 2020) Access mode:www.itis.gov/servlet/SingleRpt/ SingleRpt?search_topic=TSN&search_value=976613#null

[46] C. L. Schoch, S. Ciufo, M. Domrachev, C. L. Hotton, S. Kannan, R. Khovanskaya, D. Leipe, R. Mcveigh, K. O'Neill, B. Robbertse, S. Sharma, V. Soussov, J. P. Sullivan, L. Sun, S. Turner, I. Karsch-Mizrachi, NCBI Taxonomy: a comprehensive update on curation, resources and tools. Database (Oxford) (2020) Access mode: www.ncbi.nlm.nih.gov/Taxonomy/ Browser/wwwtax.cgi?id=168636

[47] T. C. McElroy, W. J. Diehl, Heterosis in two closely related species of earthworm (Eisenia fetida and E. andrei). *Heredity* 87 (2001) 598-608

[48] B. Plytycz, J. Bigaj, T. Panz, P. Grzmil, Asymmetrical hybridization and gene flow between Eisenia andrei and E. fetida lumbricid earthworms. *PLoS ONE* 13(9) (2018) 1-16

[49] B. Plytycz, J. Bigaj, A. Osikowski, S. Hofman, A. Falniowski, T. Panz, P. Grzmil, F. Vandenbulcke, The existence of fertile hybrids of closely related model earthworm species, Eisenia andrei and E. fetida. *PLoS ONE* 13(1) (2018) 1-18

[50] R. Sherman-Huntoon, Latest developments in mid-to-large-scale vermicomposting. *BioCycle Magazine* 41(11) (2000) 51-54

[51] J. Temple, Worm compost. London: Soil Association (1979) 18 p.

[52] S. J. Kaushik, Use of alternative protein source for the intensive rearing of carnivorous fishes. *Mediterranean Aquaculture* (1990) 125-138

[53] J. Ovalles, A. Medina, E. Márquez, J. Rochette, M. Morillo, J. Luna, Quantitative determination of amino acids in earthworm meal (Eisenia andrei) by a Surveyor HPLC system in conjunction with pre-column 6-aminoquinolyl-Nhydroxysuccinimidylcarbamate derivatization. *Ars Pharmaceutica* 55(3) (2014) 35-44

[54] S. Vannuccini, F. Dent, Fish trade and products. In: FAO The State of World Fisheries and Aquaculture – 2020 [Cited 27 Nov. 2020]. Access mode: http://www.fao.org/publications/ sofia/2020/en/

[55] B. Fauconneau, Protein synthesis and protein deposition in fish. In: Nutrition and Feeding in Fish. London: Academic Press (1985) 169-176

[56] A. N. Sayed, Evaluation of poultry by-product and earthworm meals as protein sources for Tilapia fish. *Assiut Veterinary Medical Journal* 40(79) (1999) 133-149

[57] E. A. Stafford, A. G. J. Tacon, The nutritional evaluation of dried earthworm meal (Eisenia foetida, 1826) included at low levels in production diets for rainbow trout Salmo gairdneri Richardson. *Aquaculture Research* 16(3) (1985) 213-222

[58] M. C. Nandeesha, G. K. Srikanth, N. Basavaraja, P. Keshavanath, T. J. Varghese, K. Bano, A. K. Ray, R. D. Kale, Influence of earthworm meal on the growth and flesh quality of common carp. *Biological Wastes* 26(3) (1988) 189-198

[59] P. I. Mombach, D. Pianesso , T. J. Adorian, J. Uczay, R. Lazzari, Farinha de minhoca em dietas para juvenis de jundiá. *Pesquisa Agropecuária Tropical* 44(2) (2014) 151-157

[60] M. S. Morillo, T. B. Visbal, D. Altuve, F. D. Ovalles, A. L. G. Medina, Valoracio´n de dietas para alevines de Colossoma macropomum utilizando como fuentes proteicas harinas: de lombriz (Eisenia foetida), soya (Glycine max) y caraotas (Phaseolus vulgaris). *Revista chilena de nutrición* 40(2) (2013) 147-154

[61] N. Kolesnyk, M. Simon, O. Marenkov, O. Nesterenko, Cultivation of dipterous (Diptera Linnaeus, 1758) insects, such as fruit flies, synanthropic flies larvae and chironomids larvae for fish feeding. *Fisheries Science of Ukraine* 1(51) (2020) 53-78. https://doi.org/10.15407/fsu2020.01.053

[62] O. Marenkov, Biotechnological bases of organization of industrial crayfish farm in Ukraine. *World News of Natural Sciences* 28 (2020) 1-12.

[63] N. Kolesnyk, M. Simon, O. Marenkov, T. Sharamok, Red Californian earthworm (Eisenia foetida andrei) as a valuable food item in fish farming. *Fisheries Science of Ukraine* 4(46) (2018) 26-48. DOI: 10.15407/fsu2018.04.026