Chapter 7

Nutritive Evaluation of Earthworms as Human Food

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Additional information is available at the end of the chapter

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Abstract

Earthworms have been a traditional medicine in China for at least 2300 years. Because of medicine food homology in China, people have been using earthworms as a food for several centuries. Earthworms are rich in protein and various amino acids; the protein content of earthworm meal was 54.6–59.4% on dry weight. Their protein content and amino acid composition are better than those of fish meal, cow milk and soybean meal. The crude fat content of earthworm meal was 7.34%. Earthworm protein is easily hydrolyzed into free amino acids. The hydrolyzed body fluids contain 9.34% protein and 78.73 mg of free amino acids per liter of raw fluid and are rich in vitamins and minerals. By the fast development of molecular biological techniques, more Verm pharmaceuticals and functional components were isolated from earthworms. An antibacterial peptide and a functional earthworm powder were introduced in this paper. In short, based on its nutrient content and functional components, earthworms could be an excellent raw material source as homology of medicine and food for human use, especially as functional food in the future.

Keywords: earthworm, protein, nutrition, functional food and active peptide

1. Introduction

It is a time-honored, traditional custom to use live earthworm as angling bait, fish feed and duck feed. However, only within last 30 years has earthworm culture spread throughout the world as an excellent protein source replacing fish meal and soybean meal on a commercial scale since the potential for use of some earthworms in accelerating waste decomposition and protein production on a large scale was reported. It has been a well-known fact that earthworms can be used in the stabilization of organic wastes. Not only in the laboratory but also in practice has it been demonstrated that *Eisenia fetida*, the species most intensively studied in this respect, grows and reproduces well in many kinds of waste, converting them into live protein-rich feed and dark odorless casts with good physical structure for organic fertilizer.

Locally in China, people have been using earthworms as a food for several centuries. In ancient times, people in Fujian and Guangdong provinces of China had the habit of eating earthworms. Even now, in Taiwan and Henan and Guangdong provinces, some local people prepare special dishes featuring the earthworm as a basic ingredient. Records from Chinese ancient book "On Guo Yi Gong" say that the people who lived in Fujian, who were considered different from other people, considered earthworms to be a delicacy. They cut the earthworms in small pieces and mixed them with meat filling to make their food tastier. Even now, earthworm soup, a traditional delicacy, is still offered in some restaurants of Guangdong province [1].

Earthworms are also consumed by Ye'kuana Amerindians of the Alto Orinoco of Venezuela (Paoletti *et al*., 2002, private communication). In recent years, some countries in Western Europe and the Southern East have produced various earthworm products such as canned earthworms, mushroom-earthworms and earthworm biscuits and bread. In California, a company composed of several earthworm farms in Northern America held an exhibition and competition on earthworm food in 1975. In some African and South American countries, earthworms are commonly eaten. Owing to the high content and good quality of earthworm protein, the high content of vitamin B and other bioactive substances, it is very likely that earthworms could become an important source of animal protein in human nutrition if other sources become limited.

As early as in the 1940s, Lawrence and Miller reported the nutrient composition of earthworms [2]. Now functional molecules have been refined [3, 4]. But these results showed considerable variability due to variations in these wild earthworm species and little attention was paid to earthworms as a potential protein source. Since the 1970s, some people have evaluated the earthworms as animal feeds; it has been well known that the earthworm contains elevated levels of protein and other nutritive compounds and has a high potential value as livestock feed.

However, the most common uses of earthworms are to treat some diseases in traditional Chinese medical practices. "Earth dragon" in Chinese is the name given to the earthworm or "white neck earthworm," recorded early in an ancient medicinal book "Shen-nong-ben-cao-jing." Another famous ancient medicinal book, "Ben-cao-gang-mo," listed 42 entries relating to earthworms in the 42 volumes about insects and described their medicinal use in detail. Now "earth dragons" used as medicine are mainly two species: *Pheretima aspergillum* and *Allolobophora caliginosa trapezoides*. However, besides the two species of earthworm above, the following species are also used as Chinese medical animal materia: *Pheretima carnosa*, *P. medioca*, *P. hupeiensis*, *P. posthuma*, *P. praepinguis*, *P. tschiliensis*, *P. tschiliensis lanzhouensis*, *P. guilleimi*, *P. vulgaris*, *P. peetinifera* and *E. fetida* [5]. Besides the medicinal use of whole earthworms, some pharmaceutical ingredients and special active proteins or peptides have been studied in recent years [6].

In the middle of 1980s, a Japanese scientist extracted an enzyme from earthworms that can dissolve thrombi in experimental conditions. This enzyme preparation has been made into an oral medicine by the pharmaceutical industry for use in the prevention of cardiovascular disease in China, Japan and Korea [7]. With the development of modern biochemical science in recent years, great progress has been achieved in the field of research on isolating some active compounds, including small molecular proteins, peptides and amino acids [8].

The section of proteins and nutritive components of earthworm most cited from the paperof Sun et al. [9] on earthworm as a potential protein resource, which was published in not socommonly available journal Ecology of Food and Nutrition (see [9]).

2. Proteins and nutritive components in earthworms

We selected a common cultivated species *E. fetida*, commercial name "Daping II," as a standard earthworm for nutritive evaluation [9]. Chemical analysis procedures of earthworm were according to standard methods [10]. T tests and Duncan's new multiple range test, cited from Steel and Torrie [11], were applied to separate means when treatment effects were significant. Data in **Tables 1–5** represent mean standard deviation (SD). The results of nutrient composition of the earthworms are summarized in **Table 1**, which also shows some common animal food and feed ingredients. A comparison of the amino acid contents of earthworm meal and casts with that of fishmeal, eggs, cow milk, wheat bran and two kinds of insect is given in **Tables 2** and **3**.

Feed or food	DM	CP	Fat	Ash	Ca	P	ME (kcal/g)
Fresh earthworm (*E. fetida*)	15.7 (±1.47)	11.02 (±0.46)	1.89 (±0.50)	1.4 (±0.01)	0.22 (±0.10)	0.65	
Earthworm meal (*E. fetida*)	90.6 (±2.56)	54.6 (±0.92)	7.34 (±0.60)	21.2 (±0.05)	1.55 (±0.12)	2.75	2.99*
Earthworm casts (*E. fetida*)	82.2 (±1.58)	7.9 (±0.26)	1.1 (±0.43)	34.2 (±0.07)	1.42 (±0.05)	0.28	0.95*
Peruvian fish meal	90.8 (±0.72)	62.0 (±0.28)	9.7 (±0.48)	14.4 (±0.13)	3.91 (±0.04)	2.90	2.90
Chinese fish meal	88.5 (±1.81)	53.9 (±0.09)	9.3 (±0.48)	18.9 (±0.12)	4.59 (±0.09)	2.15	2.35
Cow milk	12.7 (±0.43)	3.5 (±0.29)	3.5 (±0.11)	0.7 (±0.08)	0.12 (±0.04)	0.09	0.65*
Egg	26.3 (±0.34)	12.9 (±0.17)	11.5 (±0.08)	1.0 (±0.01)	0.05 (±0.08)	0.21	1.63**
Soybean meal	88.1 (±1.28)	43 (±0.70)	5.4 (±0.13)	5.9 (±0.08)	0.32 (±0.11)	0.50	2.64
Corn meal	86.5 (±0.49)	8.6 (±0.31)	3.5 (±0.09)	1.4 (±0.02)	0.04 (±0.08)	0.21	3.32
Wheat bran	82.2 (±1.62)	14.2 (±0.10)	2.0 (±0.33)	4.4 (±0.04)	0.14 (±0.21)	1.06	1.78

Data are presented as % (weight/weight on dry matter basis) [9]. DM, dry matter; CP, crude protein; Ca, calcium; P, phosphorus and ME, metabolic energy.

*Calculated figure [12].

**Food energy.

Table 1. Nutrient composition of earthworm (***Eisenia fetida***) and some feed and foods.

Amino acid	Earthworm meal	Earthworm casts	Peruvian fish meal	Chinese fish meal	Hen egg	Raw cow milk	Wheat bran
Thr*	2.72 (±0.09)	0.46 (±0.01)	2.88 (±0.28)	2.22 (±0.24)	2.42 (±0.06)	1.20 (±0.08)	0.45 (±0.038)
Ser	2.71 (±0.08)	0.46 (±0.09)	2.63 (±0.14)	2.01 (±0.24)	3.64 (±0.14)	1.57 (±0.09)	0.74 (±0.036)
Gly	3.12 (±0.24)	0.49 (±0.03)	4.26 (±0.09)	3.26 (±0.25)	1.58 (±0.32)	0.54 (±0.11)	0.84 (±0.033)
Cys	0.42 (±0.10)	0.09 (±0.03)	0.56 (±0.18)	0.42 (±0.22)	1.16 (±0.20)	0.22 (±0.09)	0.33 (±0.028)
Val*	2.39 (±0.27)	0.44 (±0.01)	2.80 (±0.25)	2.29 (±0.37)	3.26 (±0.18)	1.57 (±0.14)	0.67 (±0.014)
Met*	1.01 (±0.42)	0.19 (±0.03)	1.65 (±0.57)	1.64 (±0.33)	1.6 (±0.09)	0.68 (±0.16)	0.15 (±0.003)
Ile*	2.40 (±0.12)	0.38 (±0.026)	2.42 (±0.48)	2.23 (±0.40)	2.99 (±0.011)	1.28 (±0.18)	0.37 (±0.042)
Leu*	3.94 (±0.15)	0.78 (±0.034)	4.28 (±0.22)	3.85 (±0.19)	4.20 (±0.10)	2.58 (±0.15)	0.80 (±0.042)
Tyr	1.73 (±0.08)	0.24 (±0.42)	2.12 (±0.26)	1.63 (±0.12)	1.98 (±0.24)	1.28 (±0.017)	0.52 (±0.042)
Phe*	2.12 (±0.81)	0.31 (0.038)	2.68 (±0.28)	2.10 (±0.45)	2.73 (±0.25)	1.46 (±0.19)	0.48 (±0.034)
Lys*	4.26 (±0.50)	0.68 (±0.033)	4.35 (±0.34)	3.64 (±0.27)	3.32 (±0.22)	2.11 (±0.20)	0.47 (±0.017)
His*	1.36 (±0.24)	0.12 (±0.014)	1.66 (±0.21)	0.90 (±0.32)	1.16 (±0.23)	0.72 (±0.25)	0.35 (±0.002)
Arg	3.27 (±0.33)	0.64 (±0.17)	3.87 (±0.37)	3.02 (±0.51)	2.90 (±0.13)	0.89 (±0.12)	0.95 (±0.027)

Amino acid abbreviations used in **Tables 2–9**: Ala, alanine; Arg, arginine; Asp, aspartic acid; Cys, cysteine; Gln, glutamine; Glu, glutamic acid; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine and Val, valine.

*Essential amino acids for humans.

Table 2. Contents of amino acids in earthworm and common feeds and foods [9] (weight/weight on a dry matter basis).

Amino acid	*Kuru* body (n=2)	*Kuru* gut organs[a] (n=2)	*Motto* body (n=2)	*Motto* smoked (n=2)
Asp	71.3	35.5	62.5	68.1
Thr	34.2	23.2	30.1	32.4
Ser	35.8	18.6	32.2	34.8
Glu	124	66.2	107	109
Pro	26.2	15.9	23.2	23.1
Gly	49.4	28.2	39.1	34.2
Ala	42.9	26.6	37.1	36.9
Val	33.9	21.4	31.5	32.5
Met	17.0	11.2	14.4	16.0
Ile	33.8	25.3	29.6	30.5
Leu	62.0	34.9	55.3	55.4
Tyr	21.4	9.52	20.2	19.9
Phe	28.9	18.3	26.7	27.3
His	18.7	11.1	15.8	14.7
Lys	54.2	32.1	49.7	48.5
Arg	60.5	32.9	55.7	53.5

Amino acid	*Kuru* body (n=2)	*Kuru* gut organs[a] (n=2)	*Motto* body (n=2)	*Motto* smoked (n=2)
Cys	7.17	5.02	5.81	5.86
Trp	8.23	5.54	8.51	9.64
Total protein content	729	421	644	653

a:Parts not eaten.

Source: Paoletti *et al.* [13].

Table 3. Amino acid content of ***motto*** and ***kuru*** ($mg \cdot g^{-1}$ dry weight) [9] (the values reported are the means of two determinations on two different samples).

	E. fetida wild	*E. fetida* cultured	*A. caliginosa*	*P. guillemi*
Protein	59.11±2.96 (39.9±2.56)	59.41±2.23 (44.8±1.49)	49.7±1.71 (31.9±2.95)	50.11±1.36 (31.22±2.37)
Lys	4.17 (3.22)	4.25 (3.77)	3.04 (2.05)	2.86 (1.65)
Met	1.13 (0.81)	0.99 (0.95)	0.82 (0.50)	0.75 (0.40)
Ala	5.22 (2.94)	5.39 (2.75)	3.42 (1.94)	3.10 (1.83)
Arg	4.06 (2.95)	3.89 (3.26)	1.42 (1.02)	3.02 (1.74)
Cys	0.73 (0.47)	0.65 (0.35)	0.50 (0.30)	0.61 (0.44)
Val	2.89 (2.09)	3.29 (2.30)	2.27 (1.25)	2.08 (1.19)
Phe	2.38 (1.60)	2.46 (1.91)	1.70 (1.11)	1.52 (0.89)
Thr	3.40 (2.08)	2.94 (2.66)	2.18 (1.48)	2.01 (1.50)
His	1.56 (0.85)	1.74 (1.22)	0.62 (0.40)	1.04 (0.62)
Ile	2.36 (1.83)	2.60 (2.10)	2.03 (1.54)	1.91 (1.52)

Bracket data in parentheses refer to earthworm meal with gut inclusions.

Table 4. Contents (% weight/weight on dry matter basis) of protein and amino acids in meal of different earthworm species [9].

FAA	Body fluids (g/l)	Fresh bodies (g/kg)	FAA	Body fluids (g/l)	Fresh bodies (g/kg)
Asp	5.9±0.09	9.2±0.28	Ile	4.7±0.11	4.2±0.38
Thr	4.3±0.08	4.7±0.14	Leu	9.4±0.24	5.3±0.36
Ser	3.1±0.33	4.4±0.08	Tyr	3.2±0.22	4.1±0.32
Glu	10.4±0.15	3.3±0.22	Phe	4.2±0.08	4.9±0.12
Gly	2.7±0.42	4.2±0.19	Lys	7.2±0.17	7.2±0.21
Ala	6.0±0.12	9.0±0.26	His	1.8±0.23	2.9±0.15
Cys	/	0.6±0.09	Arg	5.5±0.14	8.0±0.09
Val	5.4±0.10	6.1±0.08	Prs	2.1±0.24	3.3±0.18
Met	3.0±0.09	2.9±0.17			

Table 5. Contents of free amino acids (FAA) in body fluids and fresh body of ***E. fetida*** [9].

Since Lawrence and Miller first reported the protein content of earthworm in 1945, many nutritional evaluations of the earthworm have been published [14–21]. From the literature, it can be concluded that earthworms contain high levels of protein and that this protein is rich in the amino acids, considered essential for food of domesticated animals and humans. However, the protein content varies with the earthworm species and experimental food of earthworms, and the protein contents reported range from 48 to 71% (dry weight basis). *E. fetida* is a species with relatively high protein content of 58–71% dry weight or about 9.7% of its live weight [1, 17]. Generally speaking, on dry weight basis, earthworm protein contents was from 48% to 71%, the species of *E. fetida* was 58-71%. On live weight basis, earthworm serous fluid contained only about 9.7% of crude protein. Our results show that the protein content of *E. fetida* was *ca* 55% dry weight, which is similar to the results of Fosgate and Babb [22], but lower than those reported in Sabine's review [23].

Table 4 summarizes the contents of protein and amino acids of four earthworm species, including three wild earthworm species of *Allolobophora caliginosa*, *Pheretima guillemi* and *E. fetida* besides cultivated *E. fetida*. A comparison of protein and amino acids contents of four earthworm species showed that larger wild species *A. caliginosa* and *P. guillemi* was significantly lower than smaller species *E. fetida* in protein content; but there was no difference of the same species *E. fetida* in between wild and cultivated. It was found that different protein content among earthworm species was perhaps due to variations in gut inclusions. As mentioned above, why large earthworm species, *A. caliginosa* and *P. guillemi*, present lower protein content? It may result from the incomplete excretion of gut contents. If taking longer time for larger species to excrete the gut contents, there may be no significant differences in protein contents between the various species.

The chemical analysis results of earthworm serous fluid, which made of *E. fetida* by adding proteinase was to hydrolyze into a fluid [24], showed in **Table 5**. By this method, 78.73 g/l of free amino acid (FAA) was gotten, and more than 90% of the earthworm protein was hydrolyzed into FAA. The hydrolyzed also contained rich minerals, trace elements and vitamins (**Table 6**).

Few reports are available on the protein content of earthworm fluids because earthworms are usually applied as livestock feed in the form of dry earthworm meal. Zou [25] reported that earthworm body fluids contained a range of 7–9% protein.

Element	mg/l	Element	g/l	Vitamin	mg/l
Mn	3.27±1.01	Ca	0.33±0.046	A	13.46±0.37
Zn	6.90±0.27	Na	0.31±0.038	B_1	54.65±0.80
Cu	1.08±0.30	K	0.99±0.042	B_2	83.06±1.05
Pb	0.30±0.15	Fe	0.33±0.072	E	31.64±0.64
Se	0.30±0.12	Mg	0.11±0.025	C	292.00±2.35

Table 6. Contents of some elements and vitamins in body fluids of ***E. fetida*** [9].

The results from the literature show that the contents of essential elements, such as copper (Cu), manganese (Mn) and zinc (Zn), are between one and six times higher in earthworm meal than in soybean meal and fish meal [1, 25, 26]. A similar trend was found in the present study regarding the mineral element contents of earthworm body fluids, which contained three to six times more of most elements than did fish meal and soybean meal (**Table 5**). Earthworms seem able to tolerate large concentrations of some metals in their tissues, which may be related to specific metal-binding proteins in earthworms [27]. These metal-binding proteins may affect nutrient absorption when earthworms are introduced into feeds and foods; for example, a 90% phosphorus utilization rate from an earthworm-based diet was reported [1].

3. Pharmaceutical and other composition of earthworms

Earthworms contain lumbrofebrine, terrestrolumbrlysin, lumbritin, hypoxanthine and other purines, pyrimidines, choine and guanidine. The fat of earthworm is composed of octade acids, palmitic acids, high-chain unsaturated fatty acids, linear and odd carbon fatty acids, branched fatty acids, phosphatide, cholesterin, etc. The yellow chloragenous cells and organs of *Lumbricus terrestris* contain rich amounts of carbohydrates, lipids, protein, pigments and some alkaline amino acids. The yellow pigment perhaps consists of riboflavin or its analogues [28].

The tissues of *Pheretima* spp. contain large amounts of microelements, Zn 59.1 μg/g, Cu 25.4 μg/g, Fe 1735.5 μg/g, Cr 10.93 μg/g, Mo 0.25 μg/g, Ca 1019.2 μg/g and Mn 1143 μg/g [29]. These of Allolobophora *caliginosa* contain crude protein 57.96%; crude fat 6.53%, crude ash 21.09%, crude fiber 0.36%, and N extract 14.06%. These of Eisenia *fetida* contain crude protein 64.61%; crude fat 12.29%, crude ash10.16%, crude fiber 0.27%, and N extract 12.67% [24].

The blood and body fluids of *L. terrestris* contain small concentrations of glucose (0.01–0.05 μg/ml) [30], considerably lipids, including 35.14% neutral fat, 41.74% glucolipid and 23.12% phosphatide. The C-chain of the fatty acid is between 10 and 22°C. The neutral fat consists mainly of laurel acid, oleate, myristic acid and decanoic acid. The fatty acids of glucolipids are decanoic acid and some short-chain fatty acids. The fatty acids of phosphatide are oleate, decanoic acid, linoleate and behenic acids. The proportion of unsaturated fatty acids is higher than that of neutral fatty acids and saccharides [31]. A P-peptide substance exists in the gut wall of *L. terrestris* [32].

Some active enzymes occur in the yellow chloragenous cells and organs of *L. terrestris* in high concentrations, including catalase, peroxidase, dismutase, β-D-glucosyl enzyme, alkaline phosphatase, esterase, S-amino-γ-ketoglutaric dehydrogenase and porphyrin synthetase. The body fluids of *Eisenia* spp. contained at least 18 proteins with molecular weights between 1000 and 95,000 Da [33].

Kaloustain (1986) reported that earthworm species of *A. caliginosa* contain a protein, which can hydrolyze collagen. Scientists from Japan, China and Korea found and separated enzymes from the earthworm gut and body fluids, which can dissolve fibrin. These enzymes have been developed as innovative medicines to treat cerebral thromboses and myocardial infarction. Wang *et al.* [34] found and separated a kind of acid antibacterial peptide, tetra decapeptide, which has produced a disease-resistant, nutrient earthworm preparation, that can be used in plant and animal production. Six antimicrobial peptides were isolated and purified from earthworm tissue liquid homogenate and coelomic fluid, which contained 5–50 amino acid (AA) residues with the same or similar sequence of Ala-Met-Val-Ser-Gly, and named the antibacterial Verm peptides family (AVPF) according to their structure and antibacterial characteristics [34].

3.1. Earthworm antibacterial properties and polypeptide characterization

Earthworm's active protease and functional peptides have wide development prospect as substitution for fortune green medicine and food. An antibacterial peptide is described as following:

A_{3-4}, a antibacterial peptide from earthworm, was carried out by digestive test with pronase E treatment at 37°C for 4h. After polypeptide was disintegrated, and yellow precipitation in the solution and showed antibacterial activities (**Table 7**).

3.2. Spectrum of earthworm antibacterial peptides

Table 8 showed that the diameter of inhibitory ring of *Erwinia carotovora* is the biggest and inhibitory ring of *Xanthomonas campestris* as well as *E. coli* are second; that of *Staphylococcus aureus's* is the smallest. But A has strong inhibitory effect on *S. aureus*. With the enhancing purity from A, A_3, to A_{3-4-2}, the bacteriostatic activity on various bacteria is depressed except on *X. campestris*. This shows that earthworm antibacterial peptides have wide antibacterial chart, and there are differences among various bacterial strains in their antibacterial efficiency. A has obvious inhibitory effect on *Agrobacterium tumefaciens*, which can cause the formation of plant tumor tissue, but A_3 and A_{3-4-2} have no effect on this bacterium.

3.3. Earthworm antibacterial peptide A_{3-4-2} amino acid composition

After A_{3-4-2} was hydrolyzed in HCl and an analysis showed that the peptide is made of 10 kinds of amino acids, of which Glu included Gln (**Table 9**). The results of alkaline hydrolysis showed that it contained tryptophane. The result suggested that the A_{3-4-2} is composed of 40 amino acid residues belonging to 11 kinds of amino acids

Treatment	The diameter of inhibitory ring (mm)
With pronase E	1.5
Normal saline	0
Without pronase E	6

Table 7. Effect of pronase E on bacteriostatic activity of the A_{3-4}.

The diameter of inhibitory ring (mm)	*E. coli*	*Staphylococcus aureus*	*Xanthomonas campestris*	*Erwinia carotovora*	*Agrobacterium tumefaciens*
Crude extract (A)	4.4	4.3	4.1	4.9	3.8
A_3	4.3	3.5	4.5	3.3	–
$A_{3\text{-}4\text{-}2}$	4.32	3.21	4.43	3.4	–

Table 8. Bacteriostatic activity of worm antibiotics on various bacteria.

Residue kind	Reside numbers	Residue kind	Reside numbers
Asp	4	Ile	/
Thr	1	Leu	4
Ser	1	Tyr	/
Gln	2		
Glu	6	Phe	/
Gly	10	Lys	2
Ala	8	His	/
Cys	1	Trp	1
Val	/	Arg	/
Met	/	Pro	/

Table 9. Amino acid composition of earthworm antibacterial peptide $A_{3\text{-}4\text{-}2}$.

4. The actual nutritional value of earthworm protein

The nutritional value of protein depends upon its specific amino acid composition. Comparing the amino acid contents of *E. fetida* reported in our study with those in other reports [1, 15, 23, 24] and with those in *Eudrilus eugeniae*. *Lumbricus rubellus* [35, 36], *A. caliginosa* and *P. guillemi* [1], there is considerable variability in amino acid contents among the species and even within the same species. Nevertheless, some research suggested that the contents of individual amino acids differ between species by no more than 17% and usually by considerably less [16].

Sabine [23] reviewed the amino acid compositions of earthworm protein reported by five authors, and compared these data with that of two common sources of protein supplements, meat meal and fish meal. Sabine's results showed that valine, leucine and isoleucine were higher in earthworm meal than in fishmeal, but lower than in meat meal. The methionine content of earthworm meal was close to that of meat meal, but 200% of that in fish meal. Arginine, histidine and phenylalanine contents in earthworm meal were close to those in meat meal and slightly higher than in fishmeal. Threonine, cystine and tryptophan in earthworm meal were significantly higher than in fishmeal and meat meal. Therefore, Sabine suggested that since earthworm protein was high in essential amino acids, including the sulfur-containing amino acids, it should be very suitable for animal feed [23].

In our studies, the contents of EAA (on a dry matter basis) in earthworm meal were close to or slightly lower than those in fishmeal and eggs. The methionine content of our earthworm meal was significantly less than that of fish meal, soybean meal and hen egg ($p < 0.05$), which contrasts with data in Sabine's review [23]. The lysine content of our earthworm meal was significantly higher than that of eggs and cow milk ($p < 0.05$).

From the literature cited and the results from our present study, it can be concluded that *E. fetida* (especially when cultured) is relatively high in most essential amino acids, compared to those in other common foods and feeds. Biological value and net protein utilization are the two most important parameters used conventionally to evaluate protein quality of feed materials. Some researchers reported a biological value of 84% and a net protein utilization of 79% in a rat growth assay with *E. fetida* protein. These results were verified in fish and chicken tissues [37–39].

Earthworm protein is easily dissolved by enzymes into free amino acids. This suggests that earthworm protein is easily metabolized by animals. Thus, earthworms seem a promising source of protein supplementation not only for animal feed but also for human food.

5. An earthworm protein powder as a functional food

Modern scientific research indicated earthworms also contain some unknown nutrients, which have important physiological activities or functions for human health and body remediation. In December 2012, the Chinese government listed earthworm protein as a new resource of food.

Recently, a new product of earthworm protein powder has been developed. People use the earthworms as the main material, through hydrolysis extraction, ultrafiltration, nanofiltration and spray drying process, then they got the earthworm protein powder. In the powder, there are lots of water-solubility small molecular proteins.

The earthworm protein powder is a light-yellow powder and there are no visible impurities in it. It tastes sweet with a special flavor. The total protein content according to the provisions of the GB/T5009.5-2003 detection method, the minimum protein content is greater than or equal to 75%. The product standard of the earthworm protein powder is given in **Table 10**.

Materials	Content
Total amino acid, g/100g	≥75
Thr, g/100g	≥2.5
Val, g/100g	≥5.0
Met, g/100g	≥0.8
Phe, g/100g	≥4.0
Ile, g/100g	≥1.0
Leu, g/100g	≥8.0
Lys, g/100g	≥7.0

Materials	Content
Fe, mg/kg	≥200
Zn, mg/kg	≥20
Ca, mg/kg	≥2.0
Total numbers of colony, cfu/g	≤1000
Escherichia coli, cfu/g	≤40
Mould, cfu/g	≤25
Microzyme, cfu/g	≤25
Salmonella spp.	0
Shigella spp.	0
Staphylococcus aureus	0
Hemolytic streptococcus	0

Table 10. Product standard of the earthworm protein powder.

6. The safety of earthworms as a protein source

Due to the assimilation of metals from their environment, earthworms often contain elevated levels of metals, including some heavy metals that could be harmful to animals and human beings [40, 41]. However, in experiments with fish and chicken, no significant increases in heavy metals was found in the carcasses of the trial animals fed on earthworms. Earthworm consumption may pose other possible hazards. For instance, it has been suggested that in natural food chains earthworms might carry some parasitic nematodes of chicken and pigs [1]. However, earthworms have not been found to be significant in the natural distribution of parasitic nematodes in chickens or in pigs [42]. Increasing proportions of earthworms and earthworm casts in fish and chicken diets did not produce any significant changes of the organoleptic qualities of the meat produced, although the earthworm meal itself is reported to have a garlic taste. Continuous monitoring of the organic substrates used in vermiculture, as well as of earthworm composition and contamination, will be necessary to safeguard human health, especially where large-scale commercial earthworm production is considered for human consumption.

7. Conclusions

1. Earthworm (*E. fetida*) meal has a high protein content of 54.6–59.4% of its dry weight. Its protein content and amino acid composition are close to those of Peru fishmeal and eggs and better than those of Chinese fish meal, cow milk and soybean meal. The crude fat content of earthworm meal was 7.34%. The ash and energy content of earthworm meal are the highest of all the materials tested except for the higher energy content of corn meal.

2. Earthworm protein is easily hydrolyzed into free amino acids. Earthworm body fluids contain 9.34% protein and 78.73 mg of free amino acids per liter of raw fluid and are rich in vitamins and minerals, particularly iron (Fe).
3. The protein content of earthworms shows considerable variability between different species and between different experimental treatments in the same species, possibly due to variability in the extent of gut inclusion.
4. Based on its nutrient content, earthworm could be an excellent raw material source as homology of medicine and food for human use, especially as functional food. A new functional food of earthworm protein powder made of earthworm has been developed in China.

Acknowledgements

The section of proteins and nutritive components of earthworm most cited from the paper of earthworm as a potential protein resource, which was published in Ecology of Food and Nutrition (see [9]).

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