

# AMINO ACID PROFILE OF EARTHWORM AND EARTHWORM MEAL (*Lumbricus rubellus*) FOR ANIMAL FEEDSTUFF

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## ABSTRACT

Earthworm meal (*Lumbricus rubellus*) has become one of natural material that could be used as feed additive. Powdering method of earthworm was done by using formic acid addition. The study was carried out (1) to evaluate the essential amino acid profile of earthworm and earthworm meal, (2) to calculate the value of essential amino acid index (EAAI) of both materials. A modified EAAI equation was developed from the essential amino acid profile of earthworm and earthworm meal. The result showed that essential amino acid of earthworm was dominated by histidine (0.63% of dry matter basis), meanwhile the earthworm meal was dominated by isoleucine (1.98% of dry matter basis). The non essential amino acid of earthworm and earthworm meal was dominated by glutamic acid (1.52% and 3.60% of dry matter basis respectively). The value of essential amino acid index obtained from earthworm meal was higher (58.67%) than those from earthworm (21.23%). It is concluded that powdering method of earthworm by using formic acid addition had higher amino acid balance than earthworm.

*Keywords: amino acid, earthworm meal, feed additive L. rubellus*

## INTRODUCTION

Fish meal is the conventional source of animal protein in poultry feed and it has been valued for its balanced amino acids, vitamin content, palatability and growth factors (Tacon, 1993). Because of the increasing cost of high quality fish meal required for poultry feed and due to declining stocks of fish from capture fishery and competition for feed in animal husbandry, there is now need to search for alternative sources of animal protein for poultry feeds especially in developing countries (Sales and Janssens, 2006). These alternatives must be able to supply adequate indispensable and dispensable amino acid requirements of the fish or sufficient amino nitrogen to enable their synthesis.

Many scientists have reported the possible use of some alternative animal protein feedstuffs to fish meal such as earthworm meal (Hilton, 1983; Yaqub, 1991; Edwards, 1998; Sogbesan and Ugwumba, 2006; Sogbesan and Madu, 2008). As a feed supplement, it has been found that earthworm is equal to or surpass fish meal and meat meal as an animal protein source for poultry.

The biological value has positive correlation with amino acid balance (Rama Rao *et al.*, 1964). Meanwhile the amino acid balance could be calculated by Essential Amino Acid Index (EAAI) (Oser, 1951 *cit.* Mitchell and Block, 1946). The increase of EAAI value will enhance nutrient absorption (Raghunath and Narasinga Rao, 1984). There were some treatments that could increase the EAAI value in physically or mechanically way, one of that was powdering method.

Previous study showed that earthworm meal of *Lumbricus rubellus* had 65.63% crude protein content (Damayanti *et al.*, 2008), earthworm meal of *Lumbricus terrestris* contained 32.60% protein (Julendra, 2003), and earthworm meal of *Perionyx excavatus* contained 57.2% crude protein and had complete amino acid (Tram *et al.*, 2005) Although the special quality of earthworm meal has been known well as potential fish meal replacement as protein source (Yaqub, 1991), but it's still necessary to study about the essential amino acid composition in earthworm meal.

The experiments were carried out (1) to evaluate the profile of essential amino acid composition of earthworm and earthworm meals,

(2) to the value of essential amino acid index (EAAI) of both materials.

## MATERIALS AND METHODS

### Earthworm Meal Preparation

Based on United States patent US005128148A (Ishii *et al.*, 1992), disclosed processing for the production of dried earthworm powder which comprises the steps of leaving a species of 1 kg earthworms *Lumbricus rubellus*, *Allolobophora caliginosa* or *Pheretima communissima* in 2 – 4 litres fresh water or slightly acidic aqueous solution such as malate acid, citrate acid, phosphate acid, tartate acid, lactic acid and succinate acid with comparison of pH average 5.2 – 6.2, kept in 8 – 15°C temperature for 2.5 – 18 hours until the alimentary canal thereof is freed of soil, wet-grinding the earthworm, and freeze-drying and then vacuum drying the resulting suspension under a vacuum of 10 mmHg or below for 10 to 100 hours while raising the temperature gradually from -60°C to 80°C. This steps would produced dried earthworm powder.

Based on Edwards (1985) method, the earthworm washed using water to separate manure from outside skin and fecal (fecal mud) then kept in cold water (14°C) for 24 hours. Formic acid (80%) was added around 3% of earthworm body weight. The earthworm was grinded and dried using oven 50°C for 12 hours and sheaved to achieve homogenize powder.

In this study, earthworm meal (*L. rubellus*) was produced by using modified method from Edwards (1985). Earthworm sample was separated from media then washed using water and kept in the refrigerator (4°C) for 12 hours. Formic acid (3% v/w) was added into earthworm and dried by using oven 50°C for 10-12 hours. Dried sample was grinded and sheaved become to particle size  $\pm 40$  mesh. Nutrient composition proximately of earthworm meal was summarized on Table 1.

### Analyzing Amino Acid

Amino acid of earthworm meal was analyzed by using High Performance Liquid Chromatography (HPLC). Chloride acid 6N (10 ml) was added into 2 gram of earthworm meal and kept in highly temperature of disclosed reaction cylinder. Sample was hydrolysed by autoclave (110°C) for 20 hours then preserved in well-closed state at room temperature. After that, sample was evaporated in 50°C temperature then

Table 1. Nutrient Composition of Earthworm Meal

Composition	Earthworm meal
Moisture	9.03
Crude Protein	63.06
Ether Extract	18.5
Crude Fiber	0.19
Nitrogen Free Extract	12.41
Organic Matter	94.16
Ash	5.81

\* Dry matter basis (%)

neutralized by NaOH 6N addition. The sample was filtered by 0.45 µm millex filter paper using vacuum filter. Before the sample was injected to the HPLC, 275 µL OPA solution (0,01 g phetaldialdehyde, 9 ml methanol, 40 ml buffer borax pH 9.1, 100 µL 2-mercaptoethanol were added into 25 µL sample, vortex and being reacted for 5 minutes. The HPLC operation was C18 column, move phase A: Sodium acetate pH 5.0; B : 80 MeOH / 15 Buffer acetate / 5 THF (v/v/v), rate flow 1.5 ml/minute, detector : fluorescence  $\lambda$  excitation 340 nm;  $\lambda$  emission 450 nm.

### Observed Variables

Variables measured were essential amino acid composition in earthworm and earthworm meals and also essential amino acid index in both materials. Amino acid composition was analyzed by HPLC. EAAI value was calculated by Oser equation (1951) *cit.* Mitchell and Block (1946) with mathematical model:

$$\log EAAI = \frac{1}{10} [\log \frac{100 \times A1}{At1} + \log \frac{100 \times A2}{At2} + \dots + \log \frac{100 \times An}{Atn}]$$

Note:

A1: first, ... (1,2,3,4,.....n) essential amino acid rate from experiment sample

At1: first, ... (1,2,3,4,.....n) essential amino acid rate from standard (whole egg)

## RESULTS AND DISCUSSION

Earthworm meal *L. rubellus* had high protein content and it was very important as nutrient source for animal. Protein content of earthworm meal was equal to fish meal (55-60% crude

protein) as protein source for poultry feed. Earthworm meal had also containing bioactive 'lumbricin' in which able to inhibit pathogenic bacteria (Cho *et al.*, 1998). Sofyan *et al.* (2008) reported that earthworm meal with or without chitosan capable to reduce *E. coli* colonization and improve protein retention in broiler chicken.

Formic acid addition (3%) into the earthworm meal could increase the crude protein rate and produced low pH. The formic acid addition into earthworm before entering the grinding process was estimated could protect the earthworm meal from decomposer microbes, therefore it capable to maintain the highly protein content and also bioactive compound.

Earthworm *L. rubellus* contained 'lumbricin I' which had antibacterial activity, included in peptide group which contained 62 amino acids (Salzet *et al.*, 2006). Amino acid utilized as proteins are primary constituents of structural and protective tissues, including skin, feathers, bone, ligaments, as well as muscles and organs. Amino acids are used as poultry feed supplement for optimum performance and also used in livestock health care (NOSB, 2001). As feed supplement amino acids form protein. Of the 22 amino acids found in body proteins, the National Research Council lists 13 as essential in poultry diets, and these must be consumed in feed. These 13 are:

arginine, glycine, histidine, isoleucine, leucine, lysine, methionine, cystine, phenylalanine, proline, threonine, tryptophan, and valine (NRC 1994). Five of them that are deemed critical in poultry rations are methionine, cystine, lysine, tryptophan, and arginine (North, 1990).

Amino acid analysis with HPLC showed that earthworm and earthworm meal *L. rubellus* had essential and non essential amino acid content (Table 2). The highest essential amino acid of earthworm was dominated by histidine (0.63% of dry matter basis) and earthworm meal was dominated by isoleucine (1.98% of dry matter basis). Histidine was essential for protein synthesis, involved in carnitine and haemoglobin synthesis. It was effective in allergic disease and tension of the autonomic nervous system, meanwhile isoleucine was involved in protein synthesis, energy production and muscle building.

Tram *et al.* (2005) reported that the highest essential amino acid composition of *P. excavatus* was leucine (3.47% and 0.76% of dry matter basis respectively). The non essential amino acid of earthworm and earthworm meal was dominated by glutamic acid (1.52% and 3.60% of dry matter basis respectively). This result also similar to previous experiments reported by Tram *et al.* (2005) that the highest non essential amino acid composition of *Perionyx excavatus* was glutamic

Table 2. Amino acid composition of earthworm and earthworm meal

Amino Acid*	Earthworm <i>L. rubellus</i>	Earthworm meal <i>L. rubellus</i>	Earthworm meal <i>P. excavatus</i> <sup>1)</sup>	<i>Promin earthworm flour</i> <sup>2)</sup>
<u>Essential</u>				
Phenylalanine	0.45	1.03	-	-
Valine	0.52	1.3	-	-
Methionine	0.35	1.72	1.2	0.22
Isoleucine	0.47	1.98	2.74	0.47
Threonine	0.45	1.03	1.25	0.52
Histidine	0.63	1.91	2.87	0.26
Arginine	0.56	1.24	0.4	0.65
Lysine	0.51	1.43	0.13	0.76
Leucine	0.54	1.16	3.47	0.76
Cysteine	0.31	0.39	-	-
Tyrosine	0.43	0.82	-	-
<u>Non Essential</u>				
Aspartic acid	0.98	2.38	3.18	1.16
Glutamic acid	1.52	3.6	7.13	1.42
Serine	0.54	0.7	2.45	0.5
Glycine	0.35	0.55	-	-
Alanine	0.32	0.99	-	-
Proline	0.54	0.71	1.81	0.53

\* Dry matter basis (%); 1) Tram *et al.*, 2005

acid (7.13% and 1.42% of dry matter basis). Glutamic acid was involved in protein synthesis and as source of energy for cells lining the intestine. Facilitates immune function and improve anti-inflammatory effects, aids in preventing and healing of peptic ulcer and ulcerative colitis.

The high essential amino acid composition of earthworm meal would produce great result when added in animal feedstuff ratio. By protein content rich with amino acids, the earthworm meal presents around 98% of absorption by animal organism due the balance between vitamins and amino acids (Promin, 2006).

According to Table 2, the amino acid contents of earthworm meal were varied depending on species and food source. However, earthworms have been found to accumulate and concentrate methionine and lysine as limiting amino acid found in ecosystem in proportions greater than for other amino acids (Pokarzhevskii, et al., 1997). Earthworm meal contains all the essential amino acids required in feedstuff. A shortage of the limiting amino acid will constrain animal growth, reduce feed efficiency, and in extreme cases cause a nutritional deficiency. NRC requirements for amino acids and protein are designed to support maximum growth and production. The recommended levels for methionine in poultry depend on species, stage, and level of feed consumption. For chickens, recommendations for layers range from 0.25% to 0.38% and for broilers 0.32 - 0.50% (NRC, 1994).

Amino acid composition both essential and non essential of earthworm meal were higher than those of the earthworm, except cysteine composition which is almost similar (0.31% of dry matter basis of earthworm and 0.39% of dry matter basis of earthworm meal). Powdering method could increase the amino acid composition because this method used heating process.

Quality of powdering method could be performed by amino acid balance which indicated by essential amino acids index (EAAI) value (Mitchell and Block, 1946). Highly EAAI value on earthworm meal showed that earthworm meal had higher amino acid balance than earthworm (Table 3).

Besides powdering method by formic acid (3%) addition, the amino acid balance (EAAI) could be increased by another physical and mechanical treatment such as fermentation and also pelleting (Mitchell and Block, 1946). This

EAAI value means that essential amino acid more balance and it could increase the biological value of feed material, absorption and also supporting the poultry growth (Rama Rao *et al.*, 1964; Raghunath and Narasinga Rao, 1984).

Tabel 3. Essential Amino Acid Index (EAAI)

Material	EAAI Value (%)
Earthworm <i>L. rubellus</i>	21.23
Earthworm meal <i>L. rubellus</i>	58.67
Whole egg (standard)	100

## CONCLUSION

Amino acid composition both essential and non essential of earthworm meal were higher than those of earthworm. The value of essential amino acid index obtained from earthworm meal process was higher (58.67%) than earthworm (21.23%). Powdering method of earthworm by using formic acid had higher amino acid balance than earthworm.

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