

Nutrition Profile of Vermimeal as Organic Supplement for Poultry

Veena G Sonole¹, Dinesh M.S², Sasmitha Sabat³, Radha.D.Kale⁴, Siddaramanna⁴

Research Scholar, Department of Biotechnology, PES Institute of Technology, Bangalore, Karnataka, India¹

Assistant Professor, Department of Biotechnology, PES Institute of Technology, Bangalore, Karnataka, India^{2,3}

Research Advisor, Mount Carmel College (Autonomous), Bangalore, Karnataka, India⁴

Senior Technical officer, Southern Research centre, National Dairy Research Institute, Bangalore, Karnataka, India⁴

ABSTRACT: Earthworm and its applications are being explored to its maximum and have extended towards veterinary sciences. The present study is to evaluate the nutrition profile of earthworm body wall powder for poultry. Proximate analysis has been conducted to reaffirm the digestible high crude protein in vermimeal. Biochemical analysis of novel formulation derived from *E.eugeniae* was analysed using standard analytical techniques.. The results reveal that presence of essential amino acid Leucine has a higher concentration with 1717.83ng/mg of vermimeal. Valine and lysine is also found to be on a higher range with 936.58ng/mg and 638.67ng/mg of vermimeal which is in the dietary allowance range. The results substantiate the fact that vermimeal can be a dietary supplement for poultry. Growth trials have played a vital role in the present study to evaluate the efficacy of vermimeal at field level. The in vivo trials were conducted on broiler breeds to assess the body weight gain and feed conversion ratio. The vermimeal was added at the rate of 2.5% and 5% to the feed additive as per the NRC (1994) guidelines. The experiment was analysed for growth index analyses in both trials on weekly basis. The results showed an increase in body weight. There was a subsequent increase in the body weight gain of 42.83g for 2.5% concentration and 46.65g for 5% in the two treatments when compared to control from first week to sixth week. A significant feed conversion ratio result has also been reported in the present study. The above results give scientific evidence to use vermimeal as feed additive for commercial poultry farming. Since they are from animal origin, the bio molecules satisfy bio safety and environment friendly attributes and constitute the organic animal farming.

KEYWORDS: Earthworms, Vermimeal, Proximate analysis, Nutrient rich, Feed additive, Broiler birds, Organic animal farming.

I. INTRODUCTION

Earthworms have known for its applications in the field of pharmaceutical and agricultural fields. Nevertheless, earthworms have been used for fish bait from more than three decades in different parts of the world. Several studies have been reported on the potentiality of rich protein in earthworms as feed for other animals [1].

Quantification techniques like High performance liquid chromatography (HPLC) have been employed for the analysis of amino acids as in the earlier reports [2]. A detailed study of amino acid profiling has also been reported for *Lumbricus* species [3]. The constituents of food play a major role in development of farming animals. Therefore feed formulation has been given at most importance in dietary requirements. Macromolecules like proteins and amino acids have a positive influence on the growth performance in poultry. The increased weight of the poultry bird is directly influenced by the concentration of additive [4]. Several research works has suggested earthworm meal better than maggot and termite meal grouping them as unconventional feed for aquatic animals [5]. There has been an increase in the growth performance in quails with 5% and 10% inclusion level in the diet [6].

There was no report on application of vermimeal as feed additive to poultry birds in India. The present study successfully produced vermimeal from epigeic earthworms. It was tested for its growth promoting activity on poultry

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birds. This is the first report on analysis and in vivo trials of vermimeal in India with relevant nutritional and statistical data.

II. MATERIALS AND METHODS

2.1 Development of vermimeal.

Adult worms (*Eudrilus eugeniae*) were selected from Zonal Agricultural Research Station (ZRS), GKVK, University of Agricultural sciences, Bengaluru-65. 500g of worms were processed and dried. Dried worms were weighed, subjected to grinding using pestle and mortar to get powder. Powdered earthworm body wall was stored in air tight containers.

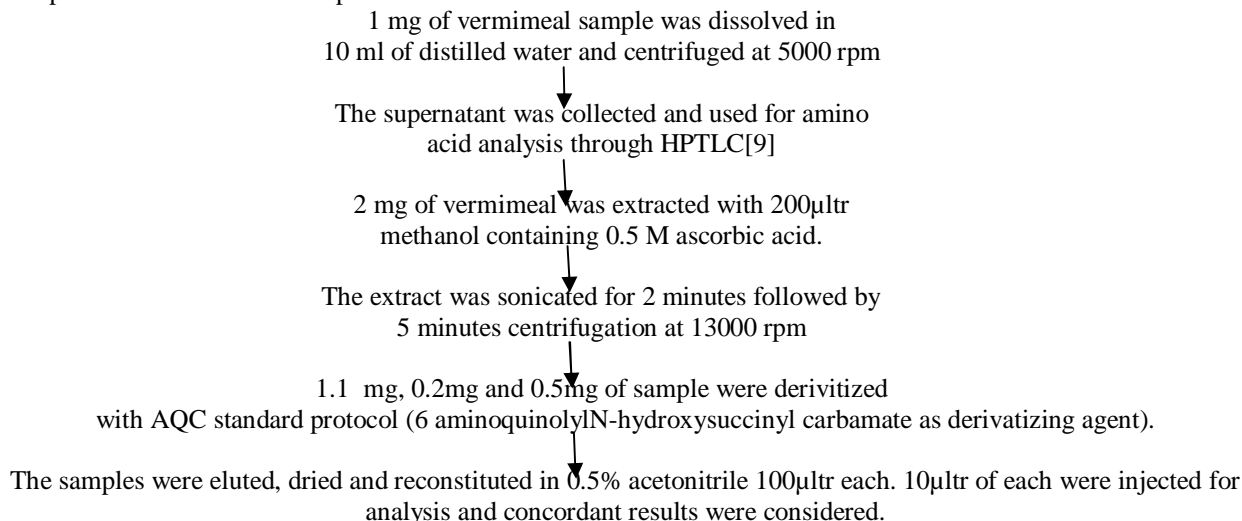
2.2 Proximate analysis of vermimeal

Major nutrients for vermimeal was analysed according to AOAC method [7]. Estimation of crude protein was conducted by kjeldhal nitrogen method. Ether extract, crude fibre and ash were determined chemically [8]. This is required to fix up the dose of additive in the feed

2.3 Amino acid profile of vermimeal.

The novel vermimeal formulation produced from the present study was subjected for nutrient analysis for amino acids having nutritionally essential since they cannot be synthesise in the animal body. The essential amino acids having dietary significance present in the vermimeal formulation was identified and quantified using High performance liquid chromatography (HPTLC). Quantitative Analysis of 22 amino acids was conducted through liquid chromatography, Mass Spectrometry using analytical grade chemical reagents.

2.3a Preparation of vermimeal sample.



2.3b Preparation of standard solutions of essential amino acids: The essential amino acids having dietary importance were prepared in suitable solvents. The solutions of amino acids namely histidine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine and cysteine were prepared at the concentration of 1mg/ml in double distilled water for the analysis. 1 nano gram of standards was injected into C18 column for LCMS. 20 pg of reserpine was injected for instrument sensitivity.

2.4 Field trials on Broiler chicks

2.4a Vermimeal was formulated based on poultry requirement Bureau of Indian Standards (BIS) with an inclusion level of 0% (Control), 2.5% (T1), 5% (T2) as treatment. The experiment was completely randomize design and chicks were weighed individually and randomly allotted to 4 replicate (8 chicks in each replicate)

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Vol. 5, Issue 9, September 2016

2.4b Record of data and statistical analysis: The experimental poultry birds were grown up to 6 weeks. The biomass of birds was recorded in terms of grams at weekly intervals. From the results body weight gain and feed conversion ratio were calculated. The significance of the results were validated using statistical method ANOVA in MS Excel 2010 and results were expressed in $p < 0.05$

III. RESULTS AND DISCUSSION

3.1 Standardization of vermimeal preparation method:

A known quantity of earthworm (500g) was narcotized in a known volume of 100 ml of 2% NaCl solution by keeping for 20 minutes duration. This prevents the external haemorrhage occurred in the case of chemical narcotic usage. 500g of adult worms yielded 50g powdered vermimeal. Since vermimeal is used as additive this has high commercial value at lower quantity. Therefore the present method allowed retaining the earthworm body constituents within it.

3.2 Proximate analysis of vermimeal:

Proximate analysis of feed refers to major constituents in the feed. It constitutes the soluble macronutrients in the feed. Quantitative analysis of macromolecules included crude protein, moisture content, crude ash, and crude fibre and nitrogen free extractives and is presented in table 1. According to NRC requirement, the crude protein for animal feed is 23%. The crude protein in vermimeal was found to be 58% and hence satisfies NRC requirement [10]. The present study also sheds light on the increased crude protein of vermimeal prepared from *Perionyx excavatus* species⁸. However, the results are in par with that of the fish meal[11,12].

Analysis	%
Dry matter basis	79
Crude ash	8.95
Crude fibre	1.19
Crude protein	58.02
Crude fat	4.29
Nitrogen free extract (NFE)	6.55

Table 1: Proximate Analysis of Vermimeal

3.3 Amino acid profile of vermimeal:

3.3a: Using HPTLC method:

Ninhydrin method is one of the popular qualitative methods to study the presence of amino acids in a sample. Vermimeal sample was analysed for the presence of essential amino acids. HP-TLC is advanced and sensitive method when compared to paper and thin layer chromatography. Since the retention factor values of vermimeal were close to each other there was a need to conduct LCMS for accurate quantification of amino acids.

International Journal of Innovative Research in Science, Engineering and Technology

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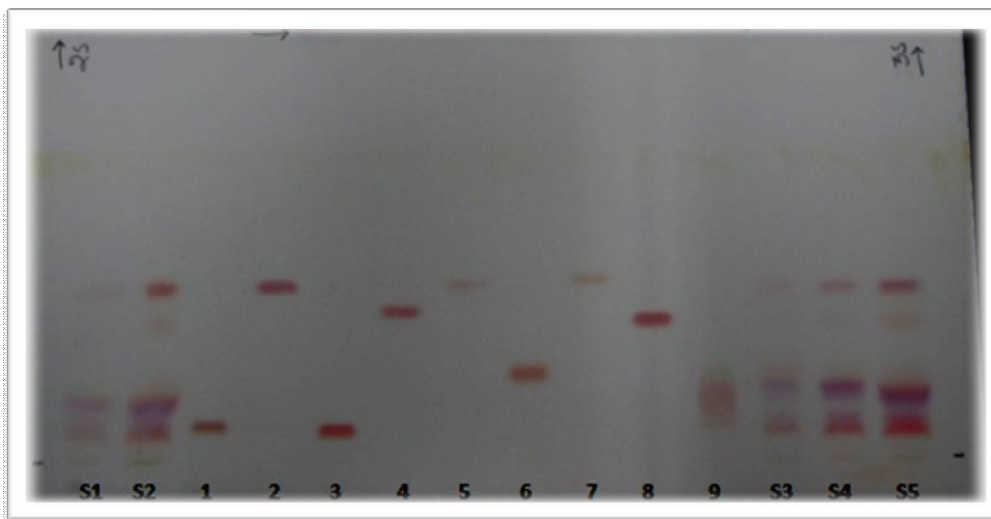


Figure 1: HPTLC screening of standard amino acids (essential amino acid sample loaded 1-histidine, 2-leucine, 3-lysine, 4-methionine, 5-phenylalanine, 6-threonine, 7-tryptophan, 8-valine and 9-cysteine. vermimeal sample (S1-S5) loaded on either side as duplicates)

The standard retention factor value of essential amino acids is tabulated accordingly classified as high, medium and low range (Table 2). Three prominent peaks were recorded from vermimeal sample and compared with the standard values (Fig 1).

Range	Rf value range	Standard Amino acid
Low	0.10 – 0.30	Histidine, lysine, Threonine
Medium	0.30 – 0.50	Cysteine, Methionine, Valine
High	0.50 – 0.55	Leucine, Phenyl alanine, tryptophan

Table 2: Standard table for identification and quantification of essential amino acids based on retention factor values

3.3b: Using LCMS method:

The amount of each amino acid in vermimeal was recorded and tabulated (Table 3) from high to low range from the peaks obtained from Fig 2 and Fig 3. The peaks of standard amino acids were obtained for reference. The sample was compared with standard reference peaks for quantification. Leucine being the highest had 1717.83ng/mg of vermimeal and sarcosine 0.875ng/mg being the lowest. However, glutamic acid, valine and lysine are present on the higher side (1676.27ng/mg, 936.58ng/mg and 638.67ng/mg respectively) (Table 3). The amino acid profile study in Nigerian earthworms is in par with the present study (Gabriel,2010). However, the concentration difference may be due to factors like environmental conditions and substrates used. Thus the presence of amino acids in vermimeal can be a potential dietary supplement for commercially farming animals. The present study also gives a scope of improvement on minimizing the use synthetic amino acids based on NRC requirement.

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(An ISO 3297: 2007 Certified Organization)

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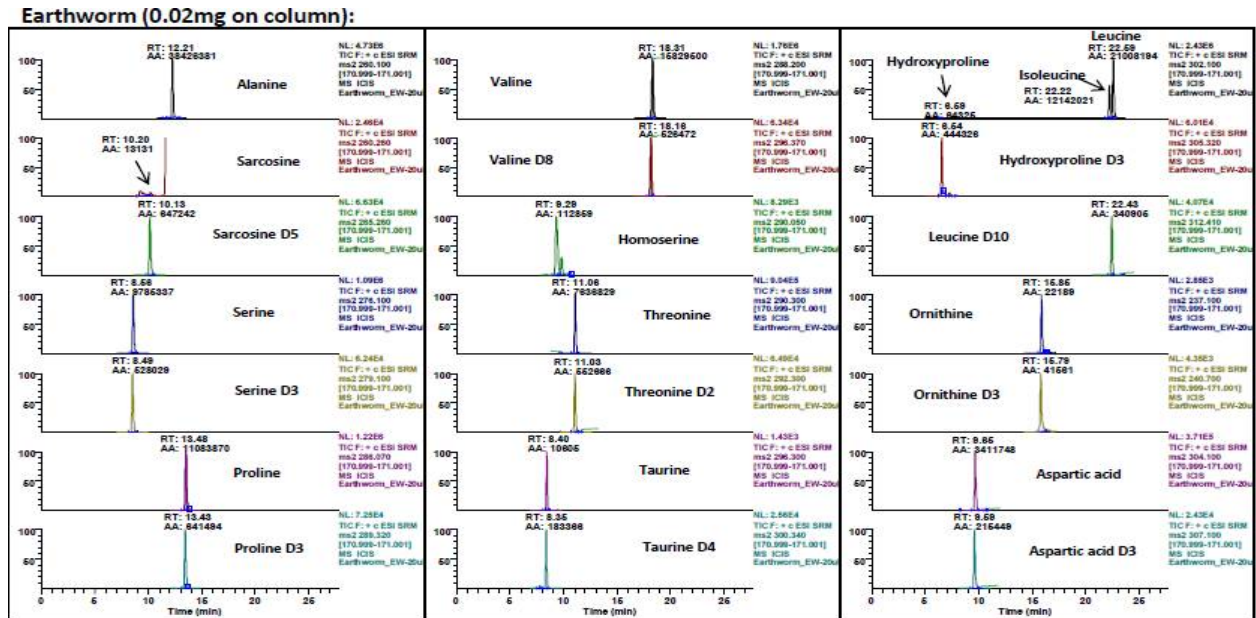


Figure 2: Amino acid peaks in earthworm *Eudrilus eugeniae* body wall powder through LCMS. The Retention time for each amino acid was recorded for quantification.

In figure 2, the following amino acid peaks were obtained: Alanine, Sarcosine, Sarcosine D5, Serine, Serine D3, Proline, Proline D3, Valine, Valine D3, Homoserine, Threonine, Threonine D2, Taurine, Taurine D4, Isoleucine, Hydroxyproline D3, Leucine D10, Ornithine, Ornithine D3, Aspartic acid and Aspartic acid D3.

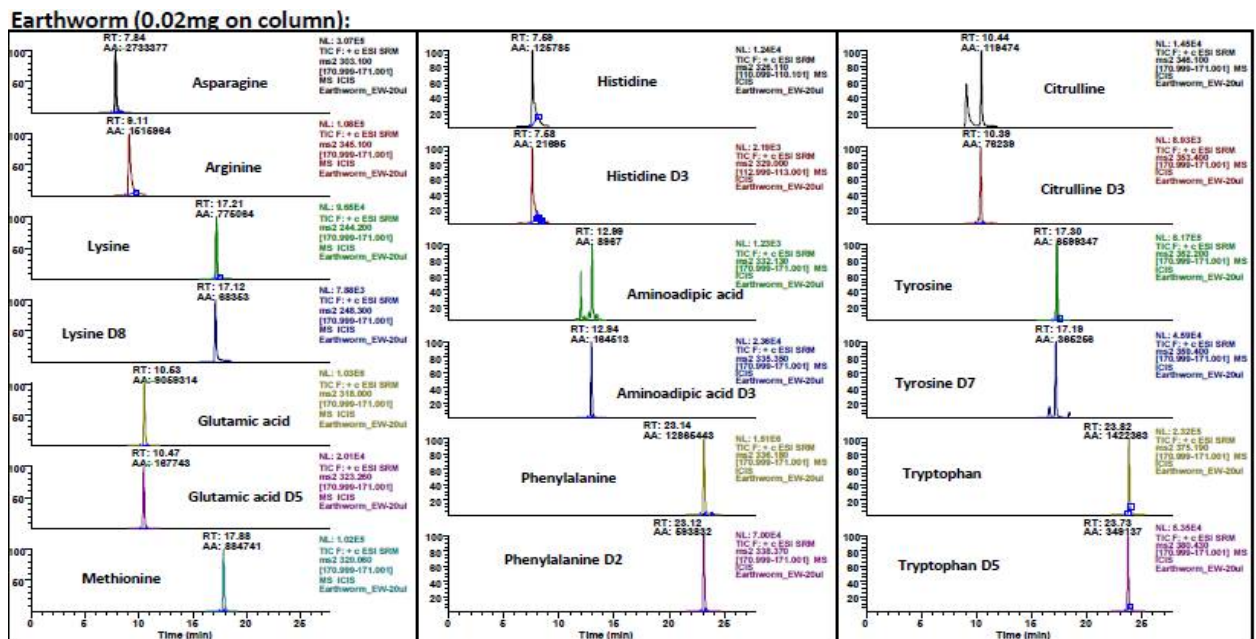


Figure 3: Amino acid peaks in earthworm *Eudrilus eugeniae* body wall powder through LCMS. The Retention time for each amino acid was recorded for quantification.

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Vol. 5, Issue 9, September 2016

In figure 2, the following amino acid peaks were obtained: Asparagine, Arginine, Lysine, Lysine D8, Glutamic acid, Glutamic acid D5, Methionine, Histidine, Histidine D3, Amino adipic acid, Amino adipic acid D3, Phenylalanine, Phenylalanine D2, Citrulline, Citrulline D3, Tyrosine, Tyrosine D7, Tryptophan and Tryptophan D3. The following table was obtained from 20µl of earthworm body wall powder and values are means of duplicates.

Sl no	Amino acid	ng/mg of vermimeal
1	Leucine	1717.83
2	Glutamic acid	1676.275
3	Alanine	1672.78
4	Arginine	954.81
5	Valine	936.585
6	Isoleucine	812.375
7	Serine	768.735
8	Asparagine	685.16
9	Lysine	638.67
10	Proline	635.185
11	Tyrosine	621.41
12	Threonine	616.625
13	Phenylalanine	530.635
14	Aspartic acid	465.415
15	Histidine	315.3
16	Methionine	176.865
17	Tryptophan	115.45
18	Citrulline	35.305
19	Ornithine	28.345
20	Homoserine	11.46
21	Taurine	4.19
22	Hydroxyproline	3.79
23	Amino adipic acid	1.835
24	Sarcosine	0.875

Table 3: Quantification of amino acids in the vermimeal sample (ranging from high to low. The values are means of duplicates.) *Essential amino acids are highlighted.

3.4: Field trials on Broiler chicks:

Several studies on earthworm as meal for fish, carps and quails have demonstrated the improved growth performance [5,6]. The present growth trials substantiate the use of earthworms in poultry sector. Feed formulation for poultry was calculated based on the NRC requirement with inclusion of 2.5% and 5% vermimeal. Since vermimeal is considered as an unconventional feed source, 5% is the maximum amount permissible in poultry diet. The growth performance of vermimeal was conducted on broiler breed for body weight gain and feed conversion ratio. The results are consolidated and presented in the table 4. The statistical analysis of the results reveals that vermimeal significantly enhanced the

International Journal of Innovative Research in Science, Engineering and Technology

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Vol. 5, Issue 9, September 2016

growth of the chicks from 0-6 weeks. The body weight gain was found to be 39.95 ± 0.72 for control and 42.83 ± 0.63 for 2.5% vermimeal inclusion and 46.65 ± 0.57 for 5% vermimeal in poultry feed. The feed conversion ratio was 1.70 ± 0.004 for 5% vermimeal, 1.74 ± 0.006 for 2.5% inclusion level and 1.829 ± 0.01 for control. Based on the in vivo trials, the feed conversion ratio was significantly different with all the two treatments with respect to control. The growth in terms of body weight gain was prominently observed with an increase in the vermimeal in the poultry diet.

	Control (g)	Treatment (g) (2.5% vermimeal)	Treatment (g) (5% vermimeal)
Initial weight	45.03±0.87	45.06±0.83	45.92±0.46
Final weight	1845±32.95 ^a	1972±28.43 ^b	2145±25.92 ^c
Body weight gain	39.95±0.72 ^a	42.83±0.63 ^b	46.65±0.57 ^c
Feed/bird	3363±47.19 ^a	3432±34.55 ^b	3645±38.72
Feed consumption ration(FCR)	1.829±0.01 ^a	1.74±0.006 ^b	1.70±0.004 ^c

Table 4: Growth promotion efficiency of vermimeal resulted from field trials (The values are expressed in Mean±SE)

IV. CONCLUSION

The present study gives a wider scope for the relevance on earthworms as food for commercially farming animals. The work includes standardization for preparation of vermimeal at laboratory scale. This method gives organically prepared product as animal feed additive. The biochemical profiling of vermimeal prepared from *E.eugeniae* for essential amino acids adds on to its nutritional value and satisfy the recommended dietary allowance (RDA) value for poultry which gives scope for its application to fisheries and piggery also. The analytical methods used to characterize the nutri-facts in vermimeal include the advanced instrumentations like HPTLC and LC-MS is of first report in Indian and also international context. The field trials on broiler breed with various inclusion levels was resulted in good biomass yield compare to conventional synthetically produced additives which contributes to novel findings with respect to poultry feed additives in India. Since vermimeal is prepared from biological source, it has a higher bio safety levels with counterparts like synthetic additives. The results from the study gives scientific evidence to take up vermimeal as a potential commercial product as a feed additive for poultry. This study enables to collaborate with industries for further evolving large scale production technology for vermimeal. These results in novel organic feed additive technology with desirable attributes success as higher economic potential and better market value

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