Australian Government

Rural Industries Research and Development Corporation

EARTHWORMS

Technology information to enable the development of earthworm production

A report for the Rural Industries Research and Development Corporation

by R.A. Dynes

RIRDC Publication No 03/085
RIRDC Project No CSA-6A

ISBN 0 642 58652 7
ISSN 1440-6845

Technology information to enable the development of earthworm production
Publication No. 03/085
Project NoCSA-6A

Researcher Contact Details
Dr Robyn Dynes
CSIRO Livestock Industries Private Bag 5 Wembley
WA 6913

Phone: 08 9333 6000
Fax: 08 9387 8991
Email: Robyn.Dynes@csiro.au

In submitting this report, the researcher has agreed to RIRDC publishing this material in its edited form.

RIRDC Contact Details
Rural Industries Research and Development Corporation
Level 1, AMA House
42 Macquarie Street
BARTON ACT 2600
PO Box 4776
KINGSTON ACT 2604

Phone: 02 6272 4539
Fax: 02 6272 5877
Email: rirdc@rirdc.gov.au.
Website: http://www.rirdc.gov.au

Published in September 2003
Printed on environmentally friendly paper by Union Offset Printing, Canberra

Foreword

Earthworm production systems involving waste management are evolving and are in the growth phase where emphasis is upon marketing, inoculum and vermicast. As the industry matures there will be a need to change the marketing emphasis from “the worm as an inoculum” to “the worm as a value-added marketable product”. Very large quantities of worm products will be available if production systems are designed and managed appropriately.

The integration of waste utilisation with the production of high quality commercial products is an attractive concept and provides real opportunities to the earthworm industry and agricultural processing industries which face increasing waste management issues. This would be a major and sustainable contribution to both urban and rural environmental management. There is thus considerable scope to develop technologies to assist the development of the earthworm industry.

While Australian rural industries are typically based more on an individualistic rather than on active cooperation and coordination approaches, there are a number of instances where a particular industry or section of an industry has adopted a successful collaborative approach to the export or domestic marketing of their produce.

This project aimed to deliver technologies to underpin the development of an earthworm industry which has a range of products including ‘value-added’ worm meal products.

This project is part of the RIRDC New Animal Products program, and was funded from RIRDC Core Funds which are provided by the Australian Government.

This report, a new addition to RIRDC’s diverse range of over 900 research publications, forms part of our New Animal Products R&D program, which aims to accelerate the development of viable new animal industries

Most of our publications are available for viewing, downloading or purchasing online through our website:

- downloads at www.rirdc.gov.au/reports/Index.htm
- purchases at www.rirdc.gov.au/eshop

Simon Hearn
Managing Director
Rural Industries Research and Development Corporation

Acknowledgements

Significant in-kind support and technical advice for this project was received from AET and Mr Nick Ludkins. Dr S.K. Baker and Dr D.B. Purser were major contributors to the development of this project and the initial experimental work.

The technical support from Mr Kevin Smith (Chittering Worm Farm) is acknowledged. The technical expertise of Ms N. van Aurich and Ms S.Martin was critical to the success of the project. In addition, Ms B.Hostalek and Ms L.Hulm contributed significantly to the conduct of the work. A valuable collaboration was developed with Animal Sciences Group of The University of Western Australia: Dr I.Williams, Dr J.Milton and Ms T.Hewson.

Contents

Executive Summary

Earthworms are recognised as an effective and environmentally sound method of increasing the rate of composting of organic matter. However the industry developing around the use of earthworms for composting is still evolving and in the growth phase with emphasis is upon marketing, inoculum and vermicast. As the industry matures there will be a need to change marketing emphasis from "the worm as an inoculum" to "the worm as a value-added marketable product". Very large quantities of worm products will be available if production systems are designed and managed appropriately.

Earthworm meal is a potentially valuable product for use in intensive animal industries. Worm meal is high in protein and the amino acid composition is very similar to that of fishmeal, an alternative to meat meal in this age of Mad Cow Disease. Similarly the composition of the lipid component is similar to some fish oils; it is relatively high in φ3 polyunsaturated lipids. They are therefore high quality products that could attract premium prices if cost effective methods can be implemented to produce the worm meal.

Project objectives

- Define and optimise the biological parameters of production systems which include efficiency of feed conversion growth rate, composition of the biomass and reproductive efficiency.
- Identify opportunities to manipulate the production system for specified products including markets for high added-value products.
- With a commercial collaborator, identify costs associated with production and processing in earthworm production systems.

Major outcomes

- Worm meal was successfully integrated into the diet of Marron in a research-scale study, replacing fish meal as the protein source. The potential value of the individual fatty acid components of the worm meal was not investigated and further research is required.
- Abattoir paunch waste was found to be an excellent feed source for earthworm production. Industry collaborative research between the meat processing and waste management industries is recommended to develop sustainable and cost effective use of earthworm composting for paunch disposal.
- The protein content of worm meal was increased by 20% by the inclusion of increased protein in the diet. Growth and reproduction rates were doubled by week 12 of protein supplementation.
- The fatty acid composition of worm meal can be managed by manipulating the composition of the organic matter fed to the earthworms.
- Nutrient content of vermicast changes with nutrient density of the diet
- Productivity is partly driven by sensitivity of earthworms to the external environment. This sensitivity increases the risks associated with the use earthworms in waste management and international standards may not be appropriate for Australian conditions.
- A significant issue that was not resolved in this study was the development of a commercial scale method for processing earthworms into worm meals. This was due to the financial failure of two earthworm production companies, which had undertaken to do the engineering research in the project.

1. Introduction

Our highly urbanised and industrialised society is producing large quantities of biological waste and the costs associated with disposing of this waste continue to rise. There is increasing demand for disposal mechanisms to be environmentally compatible and sustainable. Earthworms in dense culture and in large quantities can physically handle most biological waste and potentially at a fraction of the cost of conventional methods of waste management (e.g. landfill). The earthworm "vermiculture" industry has grown considerably in recent years, particularly in relation to its role in waste management and the production of worms for this purpose has required the development of appropriate production systems.

Long-term viability of a vermiculture industry will depend upon full exploitation and marketing of all of the outputs of a production system. At present the industry is in a growth phase and is largely reliant upon the sale of inoculum and vermicast. Greater stability of the industry through increased diversity and increased profitability will be achieved if markets are developed and established for value-added products from the worms themselves, as meal or lipid extracts. This project aims to "add value" to meal and oil products by developing methods for efficient production of worm products together with methods of modifying both the composition and quantity of meals and oils produced by processing earthworms.

Earthworm meal has an amino acid composition very similar to that of fishmeal and potentially superior to meat meal. Similarly the composition of the lipid component is similar to some fish oils; it is relatively high in φ3 polyunsaturated lipids. Therefore they are high quality products that have the potential to receive premium prices. Earthworm production systems have not been developed where the profitability of the enterprise is determined by the sale of the worm based on its value as a source of φ3 oil, protein meal, or for the value of the castings as a by-product of an oil and meal production system.

There is an increasing need for high quality protein sources for use in intensive industries. Fish meal is unlikely to be able to fill this gap, if anything, ethical issues of extensive fish harvesting are likely to lead to a decline in the use of fish meal in animal rations. The lipid content of earthworms can vary between 1 and 20% of the dry matter and it also appears that the amino acid composition varies with worm diet. There is considerable scope to identify or select worms that will maximise these desirable components or to develop feeding regimes to achieve the desired outcome. The integration of waste utilisation with the production of high quality commercial products is an attractive concept.

1.1 Review of Literature

Databases searched: CABI and Current Contents

An abundance of literature has been published on earthworms, however this review will focus on issues relevant to the aims of this project, which is firstly to optimise (and /or maximise) the efficiency of conversion of organic material into saleable worm products and secondly to optimise the composition of earthworm products.

While awareness of the value of earthworms to farming systems through soil amelioration is increasing, the greatest increase in awareness is in the waste management industry of the value of earthworms in environmental and waste management. Long-term research studies have shown species like *Eisenia fetida* (foetida), *E. andrei* or *Perionyx excavatus* are most suited to waste management systems that involve composting earthworms.

In waste management systems the principle objective is to process waste, while in worm "value adding" production systems waste is feedstock for the production system. There is a need to understand the competing interests of these two objectives, and the technology needed if worm products, as such, are to be managed effectively. The products from seed stock include vermicompost, worms for sale as seed stock, inoculum containing cocoons and worm meal.

Organic wastes from livestock, plant and vegetable industries are becoming increasingly difficult and expensive to dispose of using conventional technology. Waste management using earthworms is an increasingly attractive option with earthworms being commercially produced on a large scale using organic waste. To achieve this organic wastes from cattle and pigs may require solid separation from slurry, and poultry waste requires composting, washing or ageing to remove inorganic salts and ammonia. However horse manure, paper waste, paper pulp solids, brewery waste and spent mushroom compost require no further modification. Urban waste including food scraps and grass clippings are suitable for earthworms but are best fed after mulching and mixing to produce a uniform feed stock (Edwards and Bohlen 1996).

Optimising worm products as marketable outputs of a production system encompasses most of the principles of livestock production systems, as shown below:

Inputs	Process	Outputs	Products
Feedstock (Kg/unit time)	Growing time (Wk)	Vermicast (Kg/unit time)	Vermicast ($ / Kg)
Initial worm weight (Kg)		Worms (Kg)	Meal ($ / Kg)
			Oil ($ / Kg)

Such an earthworm production system includes the concept of feed conversion efficiency (Kg Worm Out /Kg Worm In per unit of feedstock per unit of time). In this case the excreta (vermicast) has significant value but equally important is reproduction rate and its management to maximise worm dry matter production to produce the best economic returns from the system.

Nutrition of Earthworms

The gut of the earthworm is a tube extending from the mouth to the anus; it is differentiated into a buccal cavity, pharynx, oesophagus, crop, gizzard and intestine. An anterior intestine secretes enzymes, while increasing absorption of nutrients appears to occur along the length of the intestine of the worm, with the posterior intestine being the major zone of absorption (Edwards and Fletcher 1988). Some types of micro-organisms greatly increase in numbers during passage of materials through the gut, not all multiply during passage and not all appear to be used as food by the worm.

Most evidence points to that earthworms depending on a range of micro-organisms for their nutrition and fungi are probably the most important source of microbes as food. Most species of micro-organisms that occur in the alimentary canal of earthworms are the same as those in the soils in which the worms live (Edwards and Bohlen, 1996). Earthworms have demonstrated strong preferences for specific fungi. Protozoa are also important in the diet but bacteria appear much less important (Edwards and Fletcher, 1988). Although earthworms may exhibit strong preferences, *E. foetida* grew best on various mixtures of micro-organisms (Edwards and Fletcher, 1988) under sterile conditions but could live on individual cultures of certain bacteria, fungi and protozoa. There is still controversy over whether earthworm gut contains a truly indigenous microflora. Earthworm's casts tend to have higher numbers of micro-organisms than the surrounding soil. Casts are usually rich in ammonia and partially digested organic matter, likely to be a good substrate for growth of micro-organisms.

Variation in product composition

Earthworm meal produced by is very high in protein with a variable oil content (Table 1). This suggests the potential to modify at least the composition and possibly oil content through dietary manipulation. Similarly, variation in the amino acid composition of the protein indicates a need to determine to what extent this may be managed to produce specific outcomes. However no experimental data exists and research work is needed in these areas.

The dry matter content of earthworms ranges between about 15 and 20%. The fatty acid composition of the lipid extracted from worms is quite similar to the lipid composition of some fish oils, being high in φ3 polyunsaturated lipids. Animal feeding trials reported in the literature (see below) have tended to have variable outcomes and this may well reflect the variation in the composition of worm meals (Table 1). The often-noted palatability issues with higher levels of inclusion of earthworm meal may well reflect a nutritive imbalance or high level of lipids in the worm meal. There is no literature available on the effects of the composition of worm meal on animal performance. Research is required in this area to ensure worm meal can be produced, which is consistent quality with predictable performance characteristics.

Table 1. Gross composition of earthworm meals from 3 species.

	Composition range from Fisher 1988	*L. terrestis*	*E. foetida*	*D. veneta*
		from Stafford and Tacon 1988		
Dry matter g/kg meal	899-966			
Ash g/kg meal	55-245	28.7	17.2	4.3
Crude Protein g/kg organic matter (OM)	674-768	787	710	595
Ether Extract g/kg OM	51-130	29	109	200
NFE. g/kg OM		200	181	205
Gross energy kJ/gOM	16.3-20.7			

Stafford and Tacon (1988) reported a linear inverse relationship between protein and lipid concentrations. This is as expected because if one component increases then another must decrease, however there is a clear indication from the range in composition reported by Fisher (1988) that the body composition of the worms may respond to nutritional manipulation. D. veneta appears to be a very slow growing worm (see below) and has a high lipid content.

Reproduction

Earthworms are hermaphroditic and have both male and female genital openings to the exterior consisting of paired pores on the ventral or ventro-lateral side of the body. Most species reproduce by cross-fertilisation, although some species can also produce cocoons parthenogenetically. Methods of copulation are not identical for all species and fertilisation which occurs in the cocoon is external. They are semicontinuous or continuous breeders, producing ova at most times of the year. However most species produce cocoons when food supply and environmental conditions are suitable for success. The ova are deposited in cocoons, which differ in shape with species and most species will deposit the cocoon near the surface of the soil.

The time cocoons take to hatch varies considerably, ranging from 3 weeks to 5 months (see Edwards and Bohlen, 1996, for review). The time taken to reach sexual maturity from hatching differs greatly between species and has been investigated in detail for only a few species, mostly in culture (see Table 2). The potential lifespan of mature earthworms is 4-8 years although in the field they are unlikely to attain such ages.

The nature of the reproductive system provides unique challenges for genetic selection of worms for improved productivity, growth rate or body composition. The concept of breeder units is already used by commercial farmers for multiplying up populations but little information is available on methods, including genetic selection to maximise the productivity of breeder/grower units within a farming system.

Current industry systems

In Australia, the earthworm species most commonly used for intensive farming are *E. foetida* (Tiger worm), *E. andrei* (Red Tiger worm) and *Perinnyx excavatus* (Indian Blue). *E.foetida and E.andrei* are very closely related and are considered to have the same requirements and productivity potential. The Indian Blue is a tropical species and not readily found in the Mediterranean climate of Western Australia (K.Smith pers com). The reproduction rates and productivity of 4 commonly used species during culture in a range of organic matter sources are reported in Table 2. These data are a summary of an extensive series of trials undertaken at Rothamstead Research Station in the United Kingdom.

Table 2. Productivity of earthworms in animal and vegetable wastes-biomass production (from Edwards, 1988)

Species	Maximum net reproductive rate/worm/week	Mean mature weight (g) Wet	Minimum time to maturity (weeks) (from hatching)	Biomass production per worm per week (g)	Production efficiency (Biomass per Worm Wt)
Eisenia foetida	10.4	0.55	8.4	0.68	1.2
Eudrilus eugeniae	6.7	4.3	5.0	5.76	1.4
Perionyx excavatus	19.4	1.3	4.0	6.3	4.9
Dendrobaena veneta	1.4	0.92	8.1	0.16	0.2

No published data are available on optimising the composition of earthworm diets to improve production or reproduction rates.

The data in the last column of Table 2 (Productivity of earthworms) have been calculated from data in the preceding columns. Production efficiency as calculated has a 25-fold range and *P. excavatus* has a significantly higher production potential than the other species considered in the evaluation. The high reproductive rate and biomass production of the tropical earthworm *P. excavatus* make it ideally suited to worm meal production. Research on the commercial potential for cultivation of this species in Australia is required, where climatic conditions are optimal for its survival. In some climatic zones of Australia productivity benefits from careful and precise management may be considerable. Edwards (1988) defined the optimal conditions for breeding *E. foetida* (Table 3), and the major difference between *E. foetida/E. andrei* and *P. excavatus* is survival at extremes of temperature. *P. excavatus* is a tropical species which will not survive outside its optimal temperature range (9^{o}C to 30^{o}C). *E. foetida* and *E. andrei* remain the most commonly cultivated species around the world, although other species are more productive, they have a wide temperature range, are easily handled and in mixed cultures tend to become dominant. More careful management of P. *excavatus* is likely to be needed to be the dominant species in earthworm farms for most areas of Australia.

Table 3. Optimal conditions for breeding E. foetida in animal and vegetable wastes (from Edwards, 1988)

Condition	Requirements
Temperature	15-20 ^{o}C (range 4-30^{o}C)
Moisture content	89-90% (range 60-90%)
Oxygen requirement	Aerobicity
Ammonia content of waste	low: <0.5 mg/g
Salt content of waste	low: <0.5%
pH	>5 and < 9

Many vermiculture operators in Australia are relatively small operations who use a variety of growing beds, 28-day reproductive cycle with a 4 month hatching/growing cycle. After a 4-month growing cycle mature worms are manually harvested for sale. Such an operation has a high labour cost and low potential production of worms per week with operators usually not meeting optimal reproductive and growing rates. Commercial-scale production of worms suitable for meal and oil production will require a consistency of supply of worms to meet market demand. There is sufficient waste organic matter available to supply such operations, but large vermiculture operations are required to ensure a continuous supply of earthworm product.

Vermiculture products: value and uses

Worm meal

Meal produced from earthworms is high in protein with a very favourable amino acid composition compared to fishmeal and a variable oil content naturally high in omega-3 fatty acids. Extraction of very high value fatty acids may be possible as a substitute for fish oils in a range of products. Worm meal may also be a suitable meat meal replacement for ruminants if disease transfer issues can be clarified.

Worm meal is able to substitute for fishmeal in diets for monogastric animals and for fish. With current methods of extraction and preparation of the meal, 25-50% of the dietary protein could be supplied from worm meal (Edwards and Niederer, 1988).

Worm meal has successfully replaced meat meal in chicken rations (Sabine, 1978). However less encouraging results were obtained by Fisher (1988) who fed chickens 0, 72, 144 and 215g/kg worm meal from 2-4 week of age. The dietary ME, N-retention, growth rate and voluntary feed intake (VFI) were not affected when worms were included in the diet at a rate of 72g/kg, providing about 25% of dietary protein. However at higher levels of inclusion there was a small but significant depression in growth and feed conversion efficiency and a tendency for feed intake to decrease. The author suggested some worm meals may have physical or chemical characters which make them unpalatable. Better definition of worm meals and different methods of treatment appear necessary before worm meals can be included in chicken diets at higher levels. For example, Stafford (1984, cited by Stafford and Tacon, 1988) improved the acceptability of *E. foetida* by blanching the live worms in boiling water; this resulted in *E. foetida* being a suitable complete feed for fish. The extent to which the improvement was due to the heat treatment applied to the worms or the elimination of a large proportion of the noxious yellow coelomic fluid was not established in this experiment.

In two trials (Harwood and Sabine, 1978, Sabine, 1978, both cited by Edwards, 1988) earthworm protein supplements were fed to starter and grower pigs. Growth rate and feed conversion efficiency were similar to those of pigs fed on commercial rations.

Oil
The lipid content of earthworms can vary between 1 and 20% of dry matter with oil composition similar to that of some fish oils. The oil contains a wide range of fatty acids and includes relatively high levels of omega-3 polyunsaturated fatty acids. Further research is required to determine the potential to modify the lipid content and composition in worm meal, through diet and environment manipulations.

Vermicasts
At present "vermicast" is one of the main products marketed from earthworm "farming". A number of commercial operators in Australia are developing commercial products from vermicasts, suitable for gardening/horticultural use as well as marketing products for broad acre farming.

As outlined above there are well defined environmental conditions for successful production, at least *of E. foetida*, but systems suitable for on-farm operations need to be adapted to be cost effective for the type of organic matter being fed (Phillips, 1988).

Constraints to commercial production systems include:

- the need for a mechanical separator for the separating of the worms from vermicompost waste. Various trommel-type separators based on an inclined rotating cylinder made of woven mesh (Phillips, 1988) have been developed.
- Optimised systems for reproduction and for growth need to be developed particularly in terms of harvesting techniques appropriate for each. No literature appears on the sorting of the cocoons (eggs) from the worm-worked material. This appears to be an extremely difficult and requires consideration of maximised reproduction in one area of the operation while feeding worms to maximise growth in other areas of the system.

2. Objectives

1. Define and optimise the biological parameters of production systems, which include efficiency of feed conversion growth rate, composition of the biomass and reproductive efficiency.
2. Identify opportunities to manipulate the production system for specified products including markets for high added-value products.
3. With a commercial collaborator, identify costs associated with production and processing in earthworm production systems.

3. Methodology

3.1 Effect of protein supplementation on worm meal and vermicast

Earthworms and housing

Earthworms were housed in commercially available worm farms ("Can'O'Worms" Reln Industries NSW). Each experimental unit consisted of the collection tray, a single working tray and the lid. The working tray was filled with 10 litre of wet coco peat (75% moisture) prior to earthworm introduction. The experiment was carried out in a darkened controlled temperature ($21^oC \pm 2^oC$) room, with feeding and sampling carried out under red light.

Immature earthworms were sourced from a local earthworm producer, approximately 3kg of a mixture of worms, of Red (*E.andrei*) and Tiger worms (*E. foetida*) and vermicompost was added to each farm. This provided each farm with approximately 1.5kg of worm mass. The mixture of vermicast and worms was gently mixed with the coco peat and the lid placed over the top so the worms could stabilise in the farm. The worms were gradually introduced to the experimental diets commencing 1 week after worm introduction to the experimental farms.

Experimental units were randomly allocated to one of the following treatments and numbered accordingly:

Beds	**Treatment**	**Feed**
1-5	1	Control
6-10	2	Control + Antibiotic
11-15	3	Control + Inoculum
16-20	4	Control + Inoculum + Antibiotic
21-25	5	Control + Protein
26-30	6	Control + Protein + Antibiotic
31-35	7	Control + Protein + Inoculum
36-40	8	Control + Protein + Inoculum + Antibiotic

The layout of the experimental farms in the room was rerandomised every 2 weeks to minimise the effects of proximity to the door, air-conditioning vents and lights on earthworm production.

The control diet was a commercial pelleted ration (Emu pellets; Milne Feeds), the composition is reported in Appendix B. The pellets were purchased in bulk prior to the commencement of the experiment and mixed to produce a uniform diet for the experimental period.

The broad-spectrum antibiotic (Oxymav 100) was applied in solution at a ratio of 1 part antibiotic to 50 parts deionised water (8g/400ml), a spray bottle was use to apply 5ml to each bed. The amount of antibiotic applied was proportional to the volume of organic matter and did not change with changing amounts of feed offered.

The inoculum used was a powdered nutrient broth supplied by a commercial worm producer (Advanced Waste Management NSW). The inoculum was prepared using a ratio of 1 part inoculum to 200 parts deionised water. Air was bubbled through the solution for 24 hours then it was added to the control pellets and stored at room temperature for another 24 hours.

The protein source was meat and bone meal, the specifications are reported in Appendix B. Protein was added to the control diet to double the protein content being offered eg: if 100g of emu pellets (16g of protein) was provided to the treatment with no protein added, then the protein added treatments would be provided with 50g of emu pellets (8g protein) plus 50g of meat and bone meal (25.5g protein) to provide a total of 33.5g protein.

Feed was added to the worm farms *ad libitum* twice a week to maintain residue feed offered between 5 and 15%. The feed was generally spread as a strip across the centre of the bed; however for treatments with a significant volume of feed, the feed was spread across the surface of the bed. The antibiotic was applied after feeding and then all beds were watered down until the surface was wet (approximately 20 ml of water).

Analyses

All units were sub sampled weekly to determine moisture content and pH. Approximately 3g of vermicompost was sampled from each farm and dried at 65^{o}C for 24 hours to determine the moisture content.

The pH was determined using a 1:5 vermicompost/water suspension method. Approximately 5ml of vermicompost was placed into a sterile 50ml plastic centrifuge vial with 25ml of deionised water The samples were then mixed on a shaking tray for 1hour, then allowed to settle for approximately 20 mins and the pH recorded.

Feed residues were determined by visual assessment, based on calibration photographs.

The nitrogen content of worms and vermicompost was determined monthly throughout the experiment. Vermicompost nitrogen content was determined from a sub sample of vermicompost dried in the oven at 65^{o}C. Dried vermicast analysed for total nitrogen content using a LECO NS-2000 analyser. Adult worms were randomly sub sampled from a core of bedding taken from each unit and euthanased in ethanol. Dried worms were also analysed for nitrogen content using the LECO NS-2000 analyser.

Changes in the total earthworm mass and the number within each maturity category were determined monthly. Experimental units were sampled by taking a 7cm core from each farm. All worms, cocoons and vermicast were taken from the core and placed onto a tray. Under red light (to minimise stress) the worms and cocoons were separated from the vermicast. Only the numbers of unhatched cocoons were recorded. Worms separated from the vermicast were washed thoroughly under slow running water. Most of the vermicast separated from the worms freely and was easily removed; however some vermicast clung to the worms and required further washing. The worms were classified by maturity category into adults, pre-adults, immature and hatchlings. Adults were classified by the presence of a large and clearly visible clitellum. Pre-adults had no clitellum and tended to be smaller than the adults. Immature worms were no longer transparent, while hatchlings were very small and often transparent. Each category was counted, weighted and immediately returned to the experimental farm.

The most probable number (MPN) method (Woomer, 1994) was used to estimate the number of microorganisms per gram of vermicast. Briefly, using a sterile inoculating loop, approximately 0.5g of vermicast was sampled from each bed and placed into sterile 15ml centrifuge tubes. Sterile phosphate buffered saline (2ml) solution was added to each tube and vortexed thoroughly for one minute. Using sterile micro-titre plates, 180 µl of nutrient broth was placed into each well, excluding the first row. 200µl of supernatant was added to each well in the first row. The contents of the well were mixed using a clean pipette tip to withdraw and dispense several times. A subsample (20 µl) was then withdrawn and dispensed into the next well. The mixing and dispensing (with sterile

pipette tips) was repeated until the second last row. The final row was left blank to indicate the presence of contamination on the plate. The plates were incubated at 28^{o}C for 7-days. The plates were removed from the incubator and cells that were no longer transparent were visually scored. MPN tables were used to calculate the number of microorganisms per gram of vermicompost.

At the end of the experiment, a randomly selected ¼ of the unit surface area was harvested for total worm numbers, mass and cocoon numbers. A subsample of 100g of worms were collected for analysis. The worms were sealed in a plastic bag and liquid nitrogen was carefully poured over the bag ensuring no liquid nitrogen came in direct contact with the worms. The worms were stored at –80^{o}C before freeze drying (Dynavac Freeze Drier FD12). The samples were ground using a water-cooled Knifetec 1095 Sample Mill (Foss 1095). Samples were analysed by the Chemistry Centre of Western Australia.

3.2 Changing fatty acid composition of meal through feeding

Earthworms were housed in 24 worm farms ("Can'O'Worms" Reln Industries NSW). Each experimental unit consisted of the collection tray, a single working tray and the lid. The working tray was filled with 10 litres of wet coco peat (75% moisture) prior to earthworm introduction. The experiment was carried out in a controlled temperature room (21^{o}C ± 2^{o}C) and all units were placed on racks (1m high 6m long) to facilitate feeding, sampling etc.

Each experimental farm was prepared with a mixture of coco peat (1 block of soaked coco peat; 10 litres) and well-worked vermicast (approximately 1.25kg). To each bed 1kg of Tiger worms (*Eisenia foetida*) were added. The mixture of vermicast and worms was gently mixed with the coco peat and the lid placed over the top so the worms could stabilise in the farm. The worms were gradually introduced to the experimental diets 1 week after worm introduction to the experimental farms.

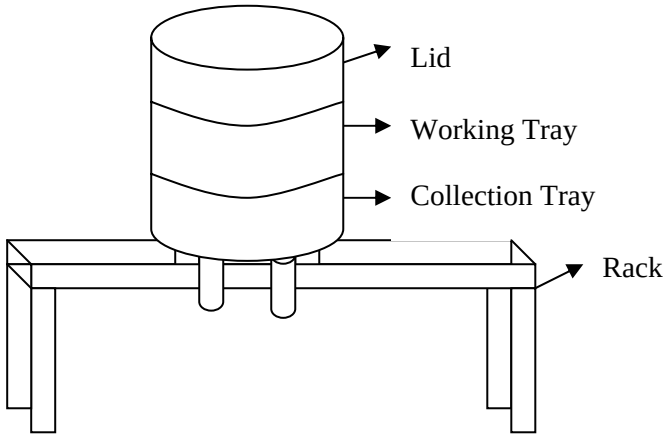


Figure 1. Diagram of an experimental farm on the rack.

Experimental beds were allocated to one of three treatment groups;

Bed		**Treatment**	**Feed**
1-8	T1	Control	
9-16	T2	control + tuna oil (10%)	
17-24	T3	control + tuna oil (10%)+ inoculum (20mls per bed)	

The layout of the experimental farms in the room was rerandomised every 2 weeks to minimise the effects of proximity to the door, air-conditioning vents and lights on earthworm production.

The control diet was a commercial pelleted ration (Emu pellets; Milne Feeds), the composition is reported in Appendix B. The pellets were purchased in bulk prior to the commencement of the experiment and mixed to produce a uniform diet for the experimental period. Feed grade tuna oil (Appendix B.) (Clover Corporation NSW) was used to provide the fatty acid supplement. The inoculum was a powdered nutrient supplement (AET Perth W.A.). The inoculum was dissolved by adding 3g of the powdered supplement to 20ml of deionised water per bed. The solution was mixed thoroughly then placed in a water bath at 30-37^{o}C for at least 24 hours, with occasional mixing.

The experimental farms were checked twice a week and fed when residues were 20% or less. Tuna oil was added to the control pellets before feeding (T2, T3) while the inoculum (T3) was added directly to the bed after feeding.

Harvest

At the completion of the experiment, all beds were harvested. The collection tray was weighed and the contents of the collection tray subsampled for dry matter determination. A subsample from the vermicompost in the working tray (200g) was taken to determine dry matter content and the electrical conductivity of the vermicompost.

All worms were harvested from each experimental bed. Clean worms were weighed to determine total mass and a subsample sorted into the maturity categories described earlier. The worms in each category was weighed and counted.

A minimum of 150g of cleaned worms from each bed were sealed in plastic bags and immersed into liquid nitrogen. Samples were stored at –80^{o}C until freeze-drying. Dried samples were ground in a Knifetec grinder before analysis for fatty acid composition.

3.3 Disposal of paunch waste through vermicomposting.

Rumen contents were used to simulate the composition of abattoir paunch material. Composting earthworms (*Eisenia foetida)* of similar weight and maturity were housed individually in 250ml plastic containers. The lids were modified with nylon gauze for airflow. Pure clean sand was used as inert bedding in each container. Worms were allocated to one of the following 8 treatments (n=25 worms per treatment):

Treatment	
	Rumen solids
1.	unwashed
2.	washed
	Rumen fluid
3.	autoclaved
4.	control
	Microbial mass
5.	control
6.	washed
7.	washed & sonication
8	Commercial inoculum

A rumen fistulated sheep was fed lucerne chaff at maintenance to provide rumen contents at weekly intervals. The rumen contents were strained to separate liquid (rumen fluid) and solid components (rumen solids). Solids were either washed thoroughly in distilled water (washed) or unwashed. Fluid was centrifuged (30min at 20000 x g) to separate the microbial population (microbial mass) from the liquid. The microbial mass was either washed with distilled water and centrifuged (washed) then placed in an ultrasonic bath (washed and sonication) or unwashed (control). A commercial inoculum of unspecified aerobic microorgansims (Worm Inoculum; Advanced Waste Management NSW) was added to water and aerated for 24h then added to cellulose in the same ratio as the microbial mass was fed. Treatment groups were fed with cellulose in the ratio of 0.08:1:2.4 microbial mass to cellulose to liquid, or 1:1 rumen solids to cellulose. Individual worms were fed *ad libitum*, approximately 1.25 g of feed each week. Liveweights of all 200 worms were recorded every 2 weeks.

3.4 Worm meal as a replacement for fishmeal in Marron diets.

See Appendix C.

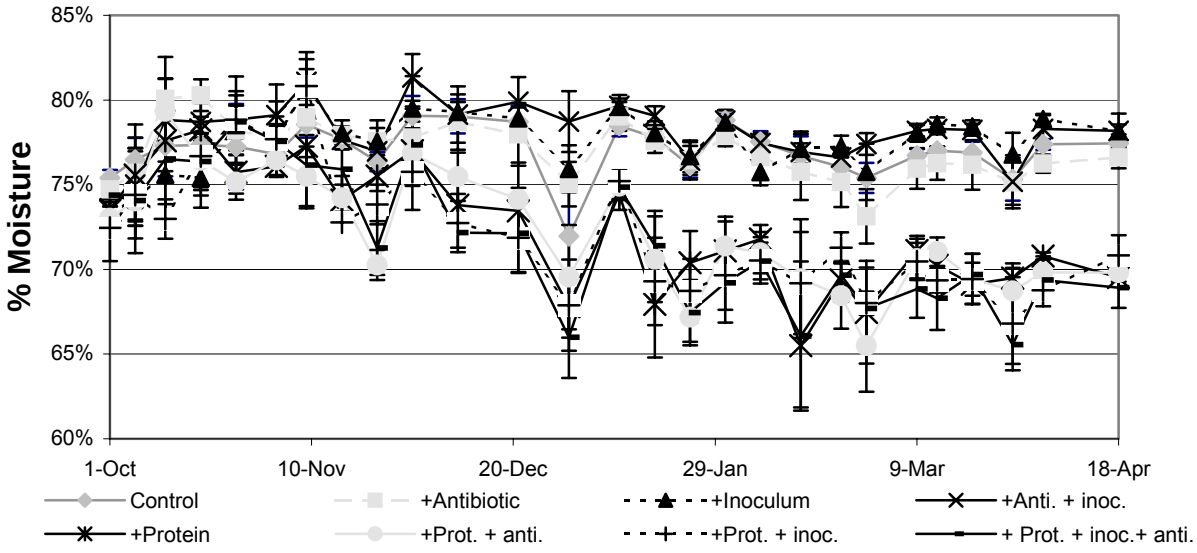
4. Results

4.1 Effect of protein supplementation on worm meal and vermicast

4.1.1 Vermicompost

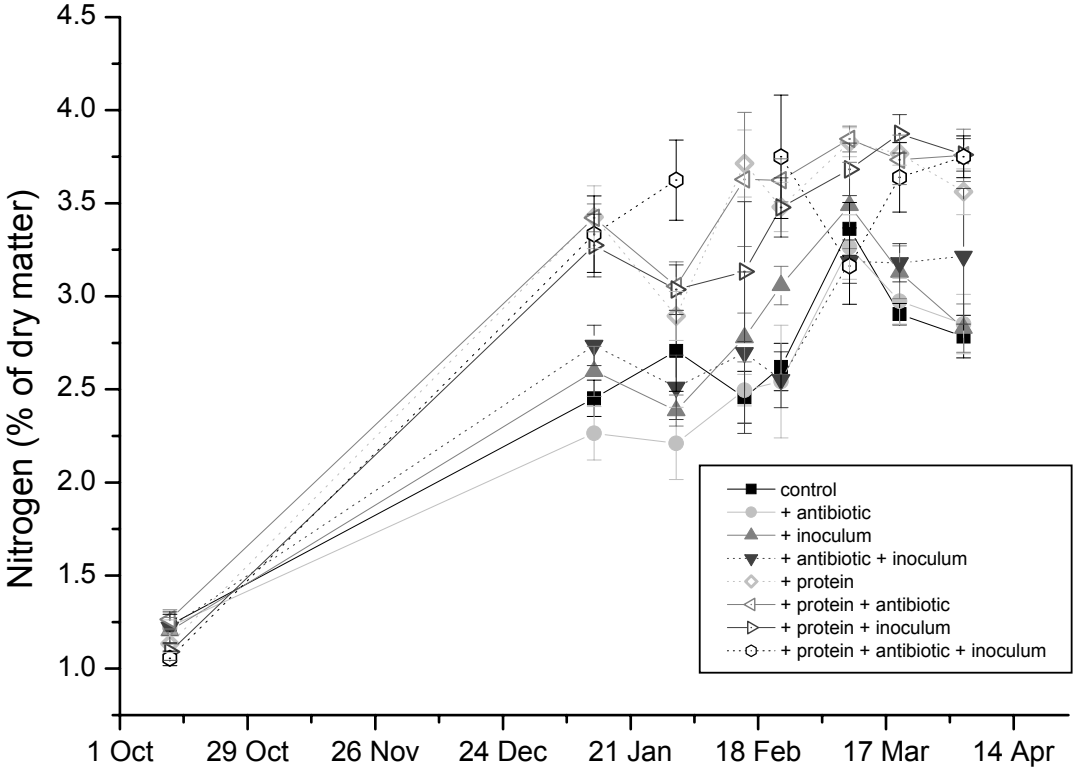
Changing the composition of the diet fed to earthworms had a significant effect on the composition of the vermicompost (Figures 1-5). The moisture content of the vermicompost was initially in the range of 75-80%, however by week 12 the moisture content of the treatments receiving additional protein (treatments 5-8) was averaged 70%, significantly lower than the 75% mean for the basal diet treatments (1-4). During the last 8 weeks of the experiment the moisture contents had stabilised at 69% for the protein group and 77% for the basal group (Figure1).

Figure 1. Mean (± sem) moisture content (%) of vermicompost in a continuous feeding experiment (n=5).



The nitrogen (N) content of the vermicompost was significantly affected by the nitrogen content of the diet fed to the earthworms (Figure 2.). The nitrogen content of the vermicompost averaged 1.2% in all beds initially, additional protein in the diet (treatments 5-8) increased nitrogen content to 3.3%, significantly higher than 2.4% N for the basal diet treatments (treatments 1-4). Although there was some variation in the treatment N content, the significant difference between basal and additional N groups remained until the completion of the experiment.

Figure 2. Mean (± sem) nitrogen content of vermicompost during continuous feeding with diets of different nitrogen content. (n=5)



The mineral content of the vermicompost is reported in Figure 3. Mineral content varied only with the addition of protein, antibiotic and inoculum treatments had no effect. Additional protein resulted in significantly less manganese and zinc, ($P<0.05$), while Ca and P levels were double those of the basal diet group at the completion of the experiment. (Figures 3a&b).

Figure 3a Mean (± sem) mineral concentration (mg/kg) in vermicasts from beds receiving different dietary treatments (n=5).

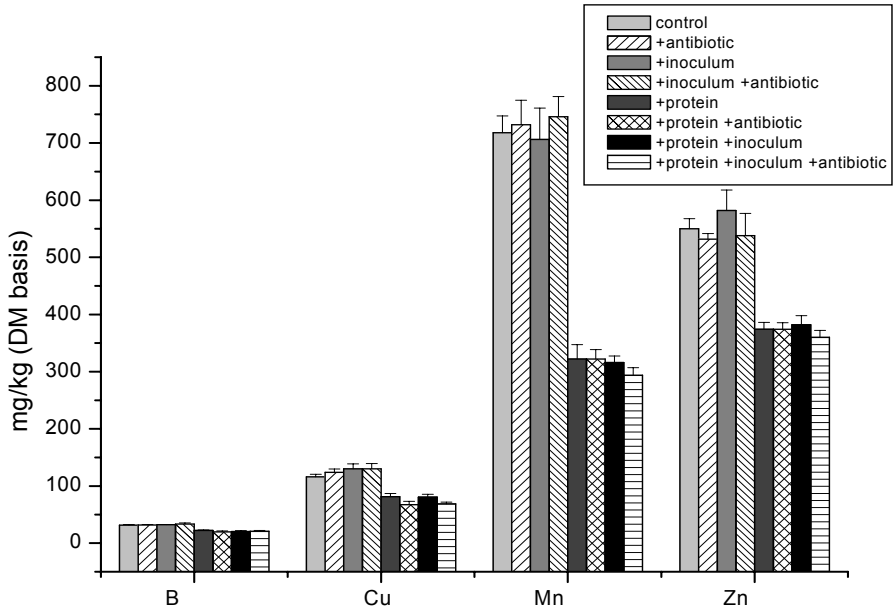
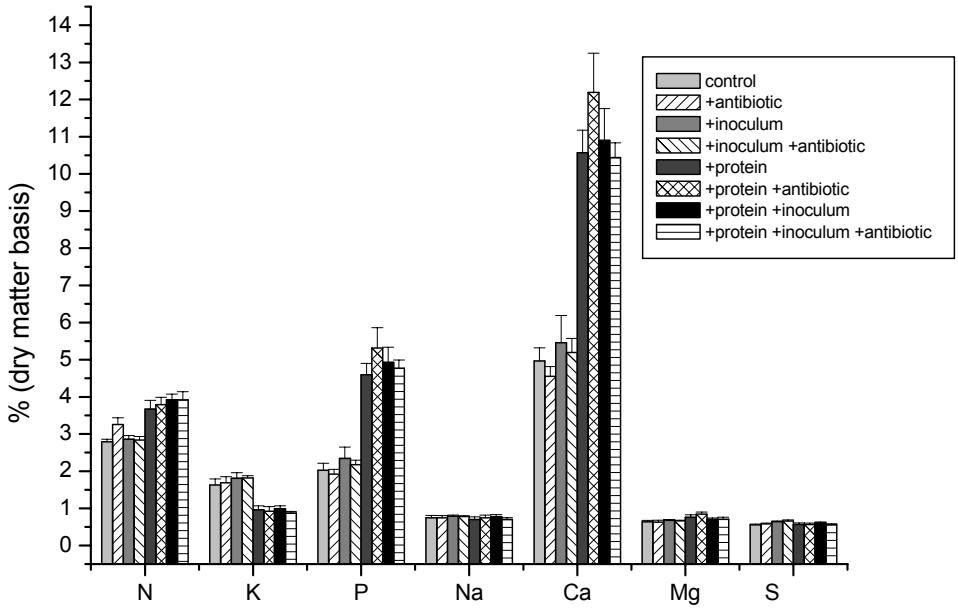
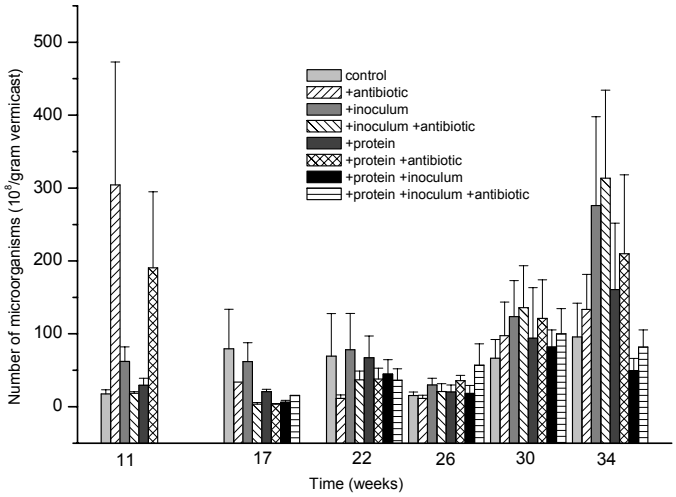


Figure 3b Mean (± sem) mineral concentration (% of dry matter) in vermicasts from beds receiving different dietary treatments (n=5).



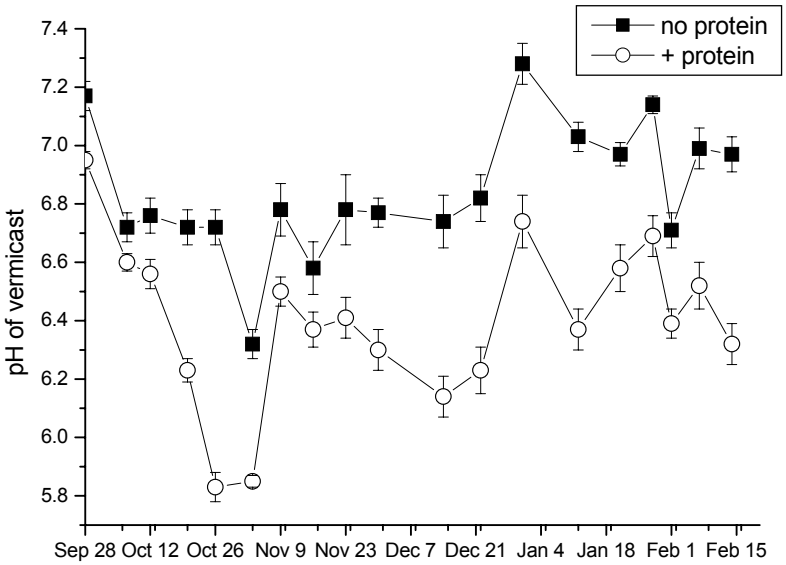
The most probable number (MPN) technique was used to estimate microbial population sizes when quantitative assessment of individual cells is not possible and is reported in Figure 4. Microbial activity tended to increase with addition of protein ($P=0.097$), however there was a significant effect of time of sampling on MPN ($P<0.05$). There was no effect of the addition of either antibiotic or inoculum on the estimate of microbial activity.

Figure 4. Mean (± sem) most probable number of micro-organisms (*n* x10^8 per g of vermicompost) in vermicompost from beds receiving different dietary treatments (n=5).



The pH of the vermicompost was measured weekly; the mean values for all beds receiving no additional protein or additional protein are shown in Figure 5. The pH of the vermicompost was between pH 6.0 and 7.0, however the addition of protein to the diet resulted in pH in the vermicompost being 0.4 units lower than the beds which did not receive protein. The addition of antibiotic or inoculum did not change the pH of the vermicompost.

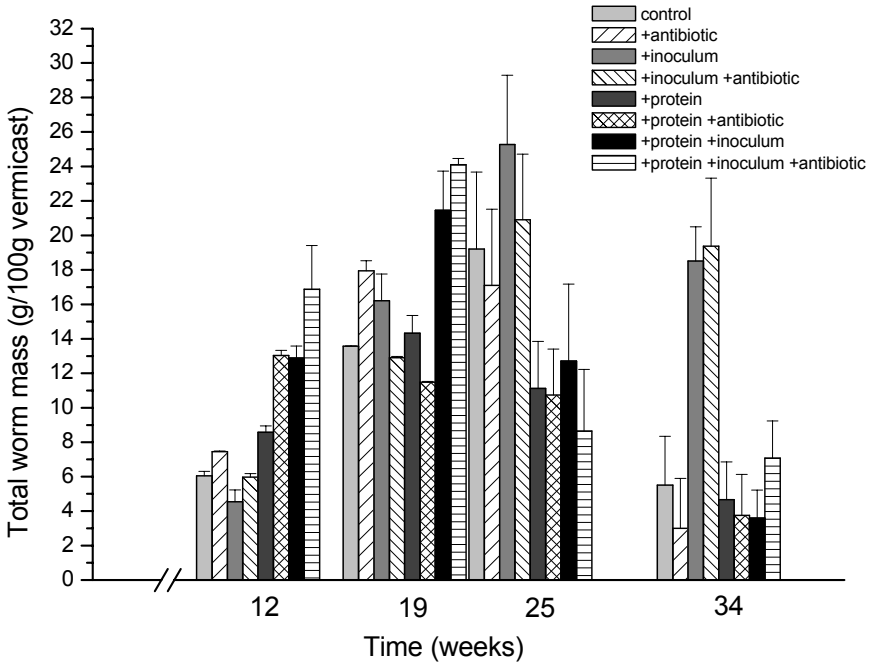
Figure 5. Mean weekly pH of vermicast in beds receiving a basal diet (no protein) or additional protein (+ protein) (n=20).



4.1.2 Earthworm production and reproduction

Four of the 10 sampling dates are reported here and for each class of worms there was a significant treatment by time interaction ($P<0.05$). However, the population dynamics within beds tended to be cyclical; for example, beds would have high numbers of preadults at one sampling and low adult numbers, then at the next sampling, the reverse would be recorded. To account for this cyclical shift in populations, total worm mass per unit area (g/100g core of vermicast) is reported in Figure 6.

Figure 6. Mean total worm mass (g/100g vermicast) in experimental units receiving different dietary treatments (n=5).



By week 12 of treatment significant differences in the total worm mass were recorded. Treatments receiving basal diets +/- inoculum and antibiotic had similar total mass of worms after 12 weeks of treatment diets. Treatments with additional protein plus antibiotic and/or inoculum were similar and had significantly more worm mass than the treatments receiving basal feed and the plus protein treatments.

By week 19 of treatment, worm mass significantly increased in the basal diet groups. Consequently, the total worm mass was similar in all treatment groups, except treatment 6 (+ protein +antibiotic) and treatment 8 (+ protein + antibiotic + inoculum) which had worm masses some 3 times higher than the other treatments..

By week 25 of treatment, the mean worm mass was similar to the mass recorded at week 19, except for treatment 6 (+ protein + antibiotic) and treatment 8 (+ protein + antibiotic + inoculum). Both treatments had suffered a highly significant (50%) decline in worm mass. Even where the mean worm mass was similar, the increasing variability in the standard errors reflects the degeneration in individual replicates.

At the final harvest only treatment 3 (+ inoculum) and treatment 4 (+ inoculum + antibiotic) remained with significantly higher worm mass. All other groups had significant and substantial declines in the total mass of worms present compared to week 25.

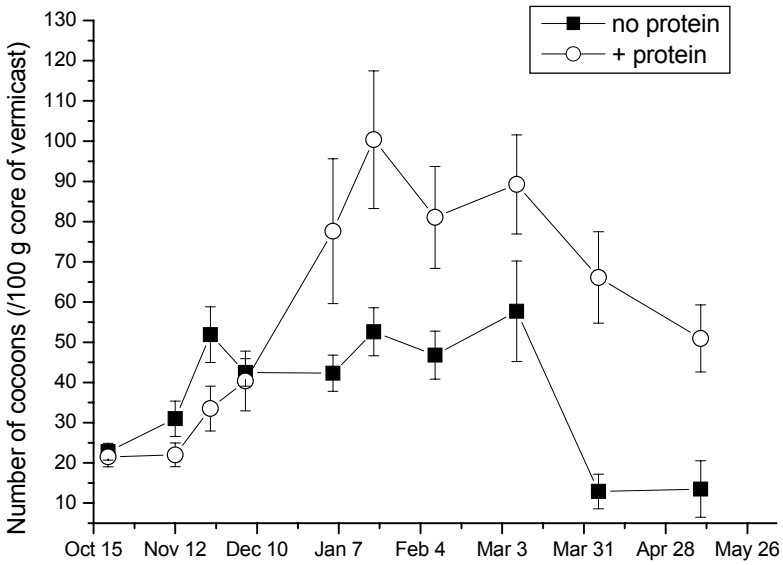
Treatments with additional protein plus antibiotic and the protein plus antibiotic plus inoculum were the most successful over the long term. These two treatments differed from the others in having a greater mass of adult worms at any stage during the trial and unlike other treatments there was not a decline in mass of adults over the final two samplings. There were no other significant differences in population dynamics linked to the success of these treatments.

Additional protein (treatments 5-8) significantly increased total worm mass ($P < 0.001$) for the first 20 weeks of the experiment. However, toward the end of the experiment the total worm mass declined significantly both compared to worm mass at week 20 and to treatments receiving control diets.

Treatments receiving basal diets did not differ when inoculum or antibiotic or a combination of antibiotic plus inoculum were added to the basal diet ($P > 0.05$).

Addition of protein to the diet significantly affected the number of cocoons, neither antibiotic nor addition of inoculum affected apparent reproduction rate (Figure 7). Combining treatment groups for analysis into no protein (treatments 1-4) or additional protein (treatments 5-8), there was an increase in the total number of cocoons in both groups although the increase was significantly greater in the treatments receiving additional protein after week 8. Over the final 12 weeks of the experiment the apparent rate of reproduction declined in both groups but significantly in the basal diet group, which had apparent reproductive failure (zero cocoons).

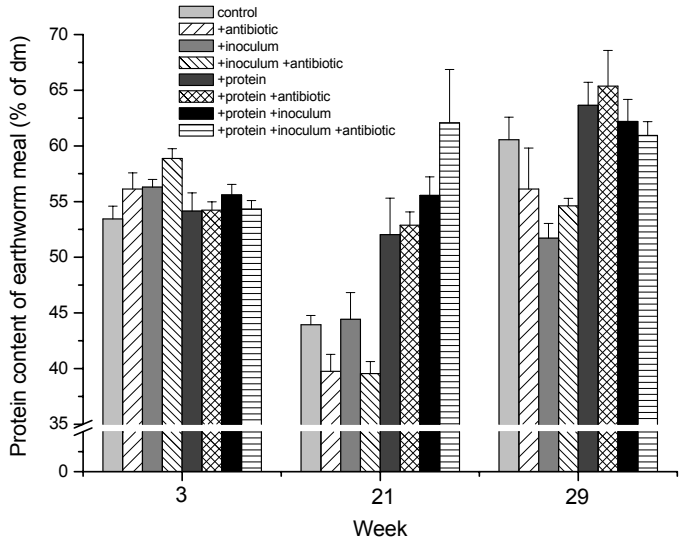
Figure 7. Mean (±sem) number of unhatched cocoons per 100g of vermicast from beds receiving a basal diet (no protein) or additional protein (+protein) (n=20).



4.1.3 Composition of earthworm meal

The average protein content of worm meal is reported in Figure 8. The addition of protein to the diet resulted in a 15-20% difference in the protein content of meal from the groups fed additional protein compared to those fed the basal diet at week 21. Initially (week 3) all treatments produced a worm meal with a protein content of 52-55%, this was maintained in the protein supplemented group while the basal groups had significantly declined in protein content ($P<0.05$). By week 29 the basal treatments had worm meal protein contents similar to initial concentrations. However the protein content of the basal treatments were on average 10% lower than the treatments receiving additional protein. The exception was the control treatment with protein content of 60%, not significantly different from the additional protein groups.

Figure 8. Mean (± sem) protein content (%) of earthworm meal from experimental units receiving different dietary treatments (n=5).



The fatty acid profile from the single bulked sample from each treatment group is reported in Table 4.

Table 4. Fatty acid composition of a sample bulked across all beds (n=5) within each treatment. Identification based on known standards and equivalent chain length comparisons.

Fatty Acid	Chain Length	Area %							
		Tr 1	Tr 2	Tr 3	Tr 4	Tr 5	Tr 6	Tr 7	Tr 8
	12 iso		1.32	1.07	0.99	1.05	1.15	0.86	1.10
Lauric	**12:00**	8.51	8.41	8.49	9.00	11.88	12.95	10.08	12.15
		1.68	1.59	2.01	1.79	1.45	1.94	1.57	1.82
	13 iso?	3.10	3.28	2.79	2.74	3.04	2.96	2.65	2.98
	13 anteiso?	1.61	1.81	1.30	1.30	1.84	1.90	1.52	1.75
	13:00	0.77	0.75	0.68	0.70	0.77	0.81		0.82
	14 iso	0.87	0.83	0.73	0.66	0.81	0.82		0.81
Myristic	**14:00**	4.23	4.08	4.72	4.84	4.48	4.69	4.20	4.57
	14:1 isomer?	8.01	7.00	9.10	10.12	9.91	10.93	9.31	10.49
	14:1	1.86	1.96	1.93	1.71	1.74	1.75	1.63	1.72
	15 antiso	1.43	1.59	1.30	1.23	1.50	1.49	1.28	1.41
	15:0?	4.82	4.27	6.02	6.84	3.07	3.32	2.63	3.01
		0.86	0.81	0.77	0.79	0.87	0.81	1.14	0.83
	15 iso	0.65	0.66						
	16 antiso	1.53	1.49		1.39	1.22	1.08	1.33	1.06
	16:00	3.30	3.22	3.45	3.37	3.35	3.22	3.29	3.18
		1.00	0.87	0.93	0.89	0.86	0.81	0.94	0.79
	16:1 isomer?	0.56				0.73	0.73	1.62	0.70
Palmitoleic	**16:1**	1.08	1.12	1.00	0.94	1.58	1.55	1.25	1.56
	17 antiso	1.22	1.34			1.41	1.21		1.27
Margaric	**17:00**	1.50	1.58	1.37	1.34	1.35	1.21	1.44	1.30
		2.94	2.61	3.07	2.94	3.01	2.91	3.38	2.66
Stearic	**18:00**	6.05	6.54	5.90	5.70	5.83	5.33	6.56	5.80
	18:1 t9	1.11	1.08	1.14	1.08	0.96	0.90	0.94	1.00
	18:1 c9	4.16	4.19	4.88	4.52	4.98	5.15	5.21	4.94
	18:1 c11	3.68	4.51	3.56	3.36	4.07	3.20	4.14	3.60
		0.53	0.68						
Linoleic	**18:2**	8.00	8.05	8.03	7.91	7.32	7.05	7.87	6.74
	18:3	0.79	0.76	0.79	0.81				0.55
CLA	**t10,c12?**	4.18	4.67	4.11	3.83	4.02	3.60	4.98	4.09
	20:2 w6	3.71	3.62	4.16	4.12	3.06	2.97	3.04	2.82
		0.68		0.77	0.76				
	20:3	1.44	1.52	1.53	1.51	1.22	1.25	1.39	1.13
	20:4	7.25	7.75	7.60	7.09	6.89	6.63	8.83	6.60
EPA	**20:5**	5.66	6.04	5.07	4.81	5.74	5.69	7.40	6.13
	Recovery	98.77	98.68	97.20	98.09	98.96	98.86	99.62	98.28

C=cis unsaturated fatty acid, t=trans unsaturated fatty acid., w=omega, Tr=treatment

The absence of replicated samples for each treatment group limits the statistical interpretation of the fatty acid composition. There was a tendency for the addition of protein in the diet to increase 12:00 and 14:1 isomer, and decrease 15:0, 18:2 and 20:2w6 fatty acids.

4.2 Changing fatty acid composition of meal through feeding

4.2.1 Electrical conductivity of vermicast

The electrical conductivity of the vermicast at harvest is reported in Table 5. There was no effect of treatments on the electrical conductivity of the beds ($P>0.05$). However there was a strong correlation ($r=-0.70$, $P<0.001$) between the subjective assessment of the motility of the earthworms within beds (Table 5) at the completion of the experiment and electrical conductivity.

Table 5. Mean (± sem) electrical conductivity (dS/m) of vermicast in beds at harvest (n=8)

	Electrical conductivity dS/m	Motility index
Control	10.9 ± 0.67	3.25±1.7
Control + tuna oil	9.9 ± 0.70	4.5±0.85
Control + tuna oil + inoculum	9.6 ± 0.72	5.0±1.25

4.2.2 Composition of earthworm meal

The total fat content of the earthworm meals was not affected by diet (Table 6). Fat content was positively correlated with the electrical conductivity of the beds ($r=0.732$, $P<0.001$). However worm meal with highest fat content came from farms with low subjective ranking of the motility of the earthworms and low feed consumption ($r=-0.776$, $P<0.001$).

Table 6. Mean (± sem) fat content (%)of earthworm meal at harvest (n=8)

Experimental diet	Fat (%)
Control	15.5 ± 1.0
Control + tuna oil	14.4 ± 0.50
Control + tuna oil + inoculum	15.2 ± 1.2

More than 70 fatty acids were present in the oil extracted from the earthworm meal (full profile in Appendix A). The major fatty acids present in the worms and those which were significantly affected by the experimental diets are reported Table 7.

Table 7: Fatty acid composition (Mean ± sem) (% of total fatty acids) of earthworm meal where worms received a basal diet (n=8) or were supplemented with tuna oil (n=16).

Common name	Fatty acid	Tuna Oil	Control diet	with tuna	P
Lauric	**C12.0**				
	Not identified		**1.5 ± 0.17**	**1.03±.11**	*
	Not identified		**3.3±0.31**	**2.45±0.20**	*
	Not identified		**0.84±0.038**	**0.98**	**
Myristic	**C14.0**	**2.81**	**2.6±0.26**	**3.3±0.178**	*
	Not identified		**0.25±0.04**	**0.38±0.026**	*
	Not identified		**1.7±0.084**	**1.36±0.056**	**
	Not identified)		**0.03±0.30**	**1.23±0.0197**	**
Palmitoleic	**C16.1 cis-9**	**0.3**	**0.74±0.11**	**1.79±0.074**	**
	Not identified		**0.47±0.056**	**0.32±0.037**	*
Margaric	**C17.0**	**1.2**	**0.74±0.087**	**2.21±0.057**	**
Stearic	**C18.0**		**7.61±0.415**	**6.85±0.27**	ns
	Not identified)		**1.24±0.104**	**0.71±0.069**	**
Linoleic	**C18.2 cis-9,12**	**1.08**	**6.11±0.39**	**5.075±0.25**	*
cis-11-Eicosenoic	**C20.1 cis-11**	**1.29**	**4.8±0.18**	**3.77±0.115**	**
Heneicosanoic	**C21.0**		**0.51±0.028**	**0.37±0.018**	**
	Not identified		**1.11±0.086**	**0.84±0.057**	*
Arachidonic	**C20.4 cis5,8,11,14**	**1.8**	**4.56±0.43**	**3.9±0.29**	ns
Eicosatrienoic	**C20.3 cis-11,14,17**	**0.17**	**0.11±0.050**	**0.1063±0.033**	ns
Brassidic	**C22.1 trans-13**	**0.84**	**4.02±0.47**	**4.08±0.31**	ns
Lignoceric	**C24.0**	**0.23**	**0.50±0.031**	**0.39±0.02**	ns
Nervonic	**C24.1 cis-15**	**0.64**	**0.22±0.079**	**0.46±0.05**	*

* P<0.05 **P<0.001 ns=not significant

The addition of tuna oil to the diet of earthworms increased the content of the saturated fats myristic and margaric acids. Tuna oil supplementation decreased the contents of stearic and heneicosanoic acids while there was no change in the lignoceric acid content.

Tuna oil addition to the diet also had a variable effect on the concentrations of monounsaturated fatty acids. Palmitoleic acid and nervonic acid contents were 200% higher in the tuna supplemented earthworm meal. There was a significant reduction in the content of eicosenoic acid content of the worm meal and no effect on the brassidic acid content. The polyunsaturated acids were unaffected by the addition of tuna oil with the exception of linoleic acid where there was a 1% unit decrease with the addition of tuna oil to the diet.

Tuna oil addition also resulted in changes to the amounts of a number unidentified fatty acids present in the worm meal.

4.3 Disposal of paunch waste through vermicomposting.

The average daily weight gain of earthworms fed components of paunch waste is reported in Table 8.

Table 8. Average daily liveweight gain (LWG) (mean ± sem[1]) for earthworms fed components of rumen paunch material for 16 weeks (n=25)

	Treatment	LWG ($mg.d^{-1}$)[2]
	Rumen solids	
1	unwashed	6.5±0.84[a]
2	washed	4.4±0.69[b]
	Rumen fluid	
3	autoclaved	7.0±0.47[a]
4	control	5.9±0.50[ab]
	Microbial mass	
5	control	2.4±0.20[c]
6	washed	2.3±0.13[c]
7	washed & sonication	2.3±0.15[c]
8	Commercial inoculum	-0.2±0.04[d]

[1] standard error of the mean

[2]means with same letter are not significantly different (p>0.05)

The earthworms gained most weight when consuming unwashed rumen solids or rumen fluid. The microbial mass alone regardless of presentation resulted in similar levels of growth but 50% the rate achieved with solids or complete rumen fluid. The commercial inoculum added to cellulose did not support growth, worms lost weight and mortalities increased in the last 4 weeks of the experiment.

4.4 Wormmeal can replace fishmeal as a protein source for Marron.

See Appendix C

5. Discussion

Paunch material appeared to be a very promising feed source for earthworms. The earthworms gained most weight when consuming unwashed rumen solids or rumen fluid. The rapid growth rates achieved here were similar to the highest recorded by feeding aerobic bacteria to *E. foetida* (Flack and Hartenstein,1984). Similar rates of growth occurred with worms fed the microbial mass alone regardless of presentation as washed or unwashed feed stock. Growth rates were half the rate achieved with solids or complete rumen fluid.

The commercial inoculum added to cellulose did not support growth, worms lost weight and mortalities increased in the last 4 weeks of the experiment. The inoculum is usually added to organic matter to stimulate composting, so the result, in the absence of organic matter was not unexpected. *E. foetida* needs micro-organisms in contrast to acellular soluble nutrients (Flack and Hartenstein,1984), and here they grew very well in the presence of rumen fluid and/or solids. Paunch material may provide a source of nutrients for aerobic composting, also creating an indirect nutrient source for the worms. Complete paunch material appears suitable for rapid growth in earthworms and washing is not necessary.

Further large-scale studies are required with earthworms for disposal of abattoir waste. Disposal of paunch material is difficult and costly problem for meat processors. Paunch waste is the gut contents from slaughtered ruminants. It tends to have a high odour, high water content and a high proportion of coarse poor quality forage. The actual composition of the paunch waste will depend on the animal type and origin for example grass vs lot fed beef.

Current methods of disposal vary from abattoir to abattoir. In many instances, the paunch waste is simply dumped on adjacent land, which is owned by the abattoir. Tightening of environmental requirements for waste management and specifically for odours and contamination of soil and water through seepage and runoff will limit the life of this disposal method. Encroaching of urban development into close vicinity of many plants will hasten the need for alternative methods of waste disposal.

Existing off site composting and earthworm farms or landfill are available, although all are costly. To transport paunch waste, water extraction is necessary and EPA licensed trucks are required, both adding significantly to the costs involved. Trucking costs alone are estimated to be around $20/tonne in Queensland, while we understand extracting a large quantity of water requires a $300 000 plant and approximately $100 000 per year in running costs. Transport and landfill costs would add another $100 000 per year to paunch disposal costs. An ideal solution would be a system operating on site, which is environmentally sustainable and profitable. Composting earthworms could provide this, through a composting system, with vermicast material being sold or spread onto adjacent paddocks and excess worms processed in the rendering plant to produce a high protein meal. It therefore appears to be reasonable to expect that a commercial disposal system could be established. It will require research to identify the major variables impacting on disposal of paunch material from Australian abattoirs using earthworms. Support exists within the waste management industry for research and development of systems for paunch waste disposal using earthworms, however despite preliminary approval we were not successful in developing a collaboration and financial support from the meat processing industry to bring this project to fruition.

Earthworms, both productivity and worm meal composition are responsive to changes in the protein and fatty acid composition in the diet. These findings support our belief that the potential exists to modify the nature of the organic matter supplied to earthworms, to produce a worm meal to meet particular market specifications. Production, reproduction and worm meal composition were all improved by increasing the nitrogen content of the diet. The amino acid analyses are given in Appendix D.

The addition of a broad-spectrum antibiotic to worm beds over an extended period did not alter the productivity of the earthworm farm. Antibiotic treatment was applied to reduce the microbial density in the organic matter of the experimental farms and permit the investigation of the contribution of micro-organisms to worm nutrition. Earthworms are believed to utilise both micro-organisms and simple nutrients for growth (Morgan, 1988; Flack and Hartenstein (1984). However detailed experiments had not previously been performed. Our pilot studies suggested a reduction the microbial population of the bed could be achieved with the broad spectrum antibiotic. However it was unlikely there was any significant depression in microbial activity in the results presented here since results from the most probably number technique showed no differences in microbial populations. The MPN values which were 20-200 $x10^8$ were of comparable magnitude to the $8x10^9$ in composting biosolids reported by Eastman (1996). Consequently we cannot draw any conclusions from our experiment on the role of microbial populations as food sources for earthworms.

Studies on nutrition will potentially lead to increased worm production, leading to increased rates of composting of organic matter. There is some evidence that the gut of worms contains enzymes, particularly cellulase (Morgan, 1988). However the relatively contribution of gut enzymes and resident micro-organisms in the gut to the nutrition of the earthworm are not fully understood. This understanding is essential to the development of any inoculum to modify the microbial populations of compost either as a direct feed source of earthworms or to increase the rate of composting of the material.

Three commercial inoculum products were investigated as part of this study. The inoculum used in the paunch study was claimed to provide a pool of micro-organisms to promote rapid growth in earthworms. Even in the presence of cellulose, the rate of weight loss in earthworms and death suggested the inoculum in the presence of carbon but not organic matter did not facilitate growth in worms. The inoculum used in the protein and tuna oil studies did provide a nonsignificant increase in productivity. Inoculation effects across all experiments were variable and not consistently significantly higher than the treatments with no added inoculum. In addition there was no effect on the composition of the worm meal or the characteristics of the vermicast.

The experimental units (farms) used in these studies may not be ideal for long-term experimentation using concentrate feeding. Significant decline in the worm mass and subjective assessment of the motility of the earthworms occurred in both long-term experiments. The decline was particularly evident in the protein supplementation experiment. The likely cause of the decline was the small size of the experimental unit, the limited volume of organic matter added to the unit and the addition of organic waste containing ammonia, organic or inorganic salts. Drainage from the beds was not a problem. The protein meal used here was 51% protein, 16% crude fat, sodium of 7% and high ash from the bone resulting in calcium of 10%, phosphorous of 4.5% and magnesium of 1.7%, similar to standard composition figures (Ostrowski-Meissner, 1987). Buildup of salts probably contributed to the decline in the productivity of the experimental units, indicated by the increasing electrical conductivity values in the tuna experiment. The build-up of salts and/or toxic metabolites from microbial activity would not be expected to cause a problem in a commercial scale operation where the earthworms can move away from contaminated areas, to fresh feeding areas or shorter production cycles are used. However larger scale testing will still be required.

The series of experiments undertaken in this project highlighted a narrower range of conditions for optimal production than in Table 3. In our environment, a pH of 6-7 and moisture content of 75-80% were required, outside this range motility and productivity dropped. This range is consistent with observations of local commercial producers.

The fatty acid composition of earthworm meal was altered by the addition of tuna oil to the diet of the earthworms. This was achieved without a change in the total fat content of the worm meal, in all treatments it remained at around 16% of total dry matter. The fat content of worm meal has been

reported to range from 5-20% of dry matter (Edwards 1988), thus the 16% reported here is in the higher end of the range. This may reflect the relatively high feeding value of the control feed used in these experiments. It is difficult to make comparisons with published literature where the composition of the organic matter fed to the worms is not reported.

Tuna oil was selected for its relatively high concentrations of mono and polyunsaturated fatty acid content and their potential value to the intensive animal industries. Many of the fatty acids present in the worm meal were less than tuna oil. A number of mono and polyunsaturated fatty acids are in fairly high concentrations in the worm meal. These fats are of special interest in terms of their nutritional benefits to humans and other animals. Tuna oil supplementation to the diet also had a variable effect on the concentrations of monounsaturated fatty acids, palmitoleic acid and nervonic acid contents. These were 200% higher in the tuna supplemented earthworm meal, yet there was a significant reduction in the content of eicosenoic acid content of the worm meal with no effect on the brassidic acid content. The polyunsaturated acids were unaffected by the addition of tuna oil with the exception of linoleic acid with a 1% unit decrease following the addition of tuna oil to the diet. Although we significantly changed the amount of a number of fatty acids, with the inclusion of tuna oil in the diet, the magnitude of the increase in %units was not large. An alternate approach, which we did not investigate, was using specific microbial populations in an inoculum to increase fatty acid availability to earthworms.

Marron (Fresh water crayfish) were successfully grown on worm meal (Appendix C). Where worm meal replaced fishmeal as the dietary protein source there was no reduction in either growth rate or intake by the Marron. The potential to use worm meal to optimise fatty acid content of the diet was not investigated. As research further defines the fatty acid and amino acid requirements of farmed crustaceans, further research on the potential of earthworm meals to supply limiting fatty acids and amino acids will be required.

The value of these fatty acids to intensive farming in particular the aquaculture industry may be significant. The outcomes of this experiment suggest that worm meal may have a role in Marron diets but will need to be evaluated in large-scale studies. The findings support those where worm meal was included in poultry diets, although higher rates of inclusion were possible with Marron diets. The nutritional value of worm meal for poultry has been evaluated, since details of the nutritional requirements of poultry are well understood (Fisher, 1988). The author reviewed a number of feeding studies and concluded as components of poultry feeds earthworms and earthworm meal have an excellent chemical composition for inclusion in poultry feeds. However above 5% worm meal in the diet, some researchers have reported reductions in feed consumption in poultry. Our findings suggest worm meals may differ significantly depending on the organic matter fed to the earthworms and worm meals should be incorporated into diets based on analysis of the meal. The potential value of worms as a protein source was first published in 1948 (Lawrence and Miller, 1948: cited by Edwards and Niederer 1988) but only since the 1970's has analysis of the tissues of worms been published (see review Edwards and Niederer 1988). Worms are an excellent source of protein and the amino acid content is comparable to other meals (Edwards and Niederer 1988). However earthworm meal contains a range of long chain fatty acids (Edwards and Niederer 1988), many of which cannot be synthesised by non-ruminants.

Earthworms have real potential to both increase the rate of composting of organic matter and to stabilise organic residues. Composting by worms decreases the proportion of anaerobic to aerobic decomposition, resulting in a decrease in methane and volatile sulphur compounds (Mitchell et al. 1980), therefore any composting utilising earthworms may be part of a strategy in greenhouse gas mitigation for the intensive agricultural sector. This aspect of earthworm culture will need further studies to quantify the beneficial greenhouse gas effects.

6. Conclusions and Recommendations

The growth and reproduction rates of earthworms are significantly affected by the nutrient content of their diet. Considerable scope exists to manipulate the composition of worm meal through the management of organic waste fed to earthworms. The composition of the vermicast was determined by the nutrient and mineral density of the diet fed to earthworms. Earthworm composting of organic waste has the potential to be environmentally attractive and cost-effective for the meat processing industry. Meat processors are likely to be well placed to process earthworms into worm meal, utilising existing processing facilities to handle intractable water and reduce their greenhouse gas outputs.

7. References

Baker, G.H. and Barrett, V.J. (1994) Earthworm Identifier. CSIRO Melbourne.

Eastman, B.R., (1996) Achieving pathogen stabilization using vermicomposting. Biocycle November 1999 62-64.

Edwards, C.A. (1988) Breakdown of animal, vegetable and industrial organic wastes by earthworms. *In*: 'Earthworms in waste and environmental management.' (ed C.A. Edwards and E.F. Neuhauser) pp 21-32. Academic Publishing, The Hague, The Netherlands.

Edwards, C.A. and Bohlen, P.J. (1996) Biology and Ecology of Earthworms 3rd edition. Chapman & Hall, 2-6 Boundary Row, London SE1 8HN, UK

Edwards, C.A. and Fletcher, K.E. (1988) Interactions between earthworms and micro-organisms in organic-matter breakdown. *Agriculture, Ecosystems and Environment* 24 235-247.

Edwards, C.A. and Niederer, A. (1988) The production and processing of earthworm protein. *In*: 'Earthworms in waste and environmental management.' (ed C.A. Edwards and E.F. Neuhauser) pp 169-180. Academic Publishing, The Hague, The Netherlands.

Fisher, C. (1988) The nutritional value of earthworms meal for poultry. *In*: 'Earthworms in waste and environmental management.' (ed C.A. Edwards and E.F. Neuhauser) pp 181-192. Academic Publishing, The Hague, The Netherlands.

Flack, F.M. and R. Hartenstein (1984). Growth of the earthworm *Eisenia foetida* on microorganisms and cellulose. Soil Biol. Biochem. 16 (5): 491-95.

Harwood, M. And Sabine, J. (1978) The nutritive value of worm meal. *Cited by:* Edwards, C.A. and Niederer, A. (1988) The production and processing of earthworm protein. *In*: 'Earthworms in waste and environmental management.' (ed C.A. Edwards and E.F. Neuhauser) pp 169-180. Academic Publishing, The Hague, The Netherlands.

Lawrence, R.D. and Miller, R.H. (1948). Protein content of earthworms. *Cited by:* Edwards, C.A. and Niederer, A. (1988) The production and processing of earthworm protein. *In*: 'Earthworms in waste and environmental management.' (ed C.A. Edwards and E.F. Neuhauser) pp 169-180. Academic Publishing, The Hague, The Netherlands.

Mitchell, M.J., Horner, S.G. and Abrams, B.L. 1980. Decomposition of sewerage sludge in drying beds and the potential role of the earthworm, Eisenia foetida. J.Environ.Qual. 9: 373-378.

Morgan, M.H. (1988) The role of microorganisms in the nutrition of *Eisenia foetida*. *In*: 'Earthworms in waste and environmental management.' (ed C.A. Edwards and E.F. Neuhauser) pp 169-180. Academic Publishing, The Hague, The Netherlands.

Neuhauser, E.F. Loehr, R.C. and Malecki, M.R. (1988) The potential of earthworms for managing sewage sludge. *In*: Earthworms in waste and environmental management. C.A. Edwards and E.F. Neuhauser SPB Academic Publishing, The Hague, The Netherlands.

Ostrowski-Meissner, H.T. (1987) Australian Feed Composition Tables National Collection (1970-1987) AFIC-CSIRO Sydney.

Phillips, V.R. (1988) Engineering problems in the breakdown of animal wastes by earthworms. *In*: 'Earthworms in waste and environmental management.' (ed C.A. Edwards and E.F. Neuhauser) pp 111-118. Academic Publishing, The Hague, The Netherlands.

Sabine, J. (1978) The nutritive value of earthworm meal. *Cited by:* Edwards, C.A. and Niederer, A. (1988) The production and processing of earthworm protein. *In*: 'Earthworms in waste and environmental management.' (ed C.A. Edwards and E.F. Neuhauser) pp 169-180. Academic Publishing, The Hague, The Netherlands.

Stafford, E.A. (1984) *cited by:* Stafford, E.A. and Tacon, A.G.J (1988) The use of earthworms as a food for rainbow trout *Salmo gairdneri. In*: 'Earthworms in waste and environmental management.' (ed C.A. Edwards and E.F. Neuhauser) pp 181-192. Academic Publishing, The Hague, The Netherlands

Stafford, E.A. and Tacon, A.G.J (1988) The use of earthworms as a food for rainbow trout *Salmo gairdneri. In*: 'Earthworms in waste and environmental management.' (ed C.A. Edwards and E.F. Neuhauser) pp 181-192. Academic Publishing, The Hague, The Netherlands.

Woomer, P.L. (1994). Most probable number counts. In: Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties SSSA Book Series, no 5.

Appendices

Appendix A: Complete fatty acid profile for worm meal from earthworms fed a control diet or supplemented with tuna oil.

			Earthworm meal supplemented	
		Tuna Oil	no tuna oil	+ tuna oil
Lauric	C12.0			
			1.5 ± 0.17	1.03±.11
			3.3±0.31	2.45±0.20
			0.84±0.038	0.98
Myristic	C14.0	2.81	2.6±0.26	3.3±0.178
			0.25±0.04	0.38±0.026
			1.7±0.084	1.36±0.056
			0.03±0.30	1.23±0.0197
Palmitoleic	C16.1 cis-9	0.3	0.74±0.11	1.79±0.074
			0.47±0.056	0.32±0.037
Margaric	C17.0	1.2	0.74±0.087	2.21±0.057
Stearic	C18.0		7.61±0.415	6.85±0.27
			1.24±0.104	0.71±0.069
Linoleic	C18.2 cis-9,12	1.08	6.11±0.39	5.075±0.25
cis-11-Eicosenoic	C20.1 cis-11	1.29	4.8±0.18	3.77±0.115
Heneicosanoic	C21.0		0.51±0.028	0.37±0.018
			1.11±0.086	0.84±0.057
Arachidonic	C20.4 cis5,8,11,14	1.8	4.56±0.43	3.9±0.29
Eicosatrienoic	C20.3 cis-11,14,17	0.17	0.11±0.050	0.1063±0.033
Brassidic	C22.1 trans-13	0.84	4.02±0.47	4078±0.31
Lignoceric	C24.0	0.23	0.50±0.031	0.39±0.02
Nervonic	C24.1 cis-15	0.64	0.22±0.079	0.46±0.05

Appendix B: Composition and proximate analysis of control diet, meat and bone meal and tuna oil used in experiments.

Emu Pellets

Protein	16.0%
ADF (Fibre)	10%
Metabolisable Energy	2300kJ
Min. Protein	16.0%
Min. Fat	3.0%
Max. Fat	5.0%
Max. Crude fibre	12.0%
Min. Calcium	1.0%
Max. Calcium	1.3%
Min. Phosphorus	0.5%
Max. Phosphorus	0.8%
Min. Sodium Chloride	0.5%
Max. Sodium Chloride	1.5%

Contents:
Oats, wheat, millmix, lupins, meatmeal, barley, lime, sand, salt, vitamin/mineral premix, kaolin, methionine, lysine.

Meat & Bone Meal

Crude protein	51%
Fat	16%
Moisture	3%
Energy content	1459kJ/g (348 cal)\

Tuna Oil (Clover Corporation)

Acid value	0.3mg KOH/g
Peroxide value	1.0 meq O2/kg
Docosahexaenoic Acid (DHA)	25.6%
Eicosapentaenoic Acid (EPA)	5.4%
Total ω-3 Fatty Acids content	34%

Antioxidants:

- Tertiary butylhydroquinone (TBHQ) 400mg/kg
- D Alpha tocopherol 800mg/kg

Appendix C: Abstract: Worm meal can replace fishmeal as a protein source for Marron.

Hewson, T. (2001). Honours Project
Animal Science Group
The University of Western Australia

To determine the value of worm meal for marron and to investigate how feeding behaviour of marron can affect feed intake and growth, two main hypotheses were tested. The first was that worm meal can replace fishmeal as a source of protein without adverse effects on growth, and that feed intake can contribute to variations in growth rates. The second hypothesis was that the manufacture feed pellets used in the experiment would become less stable in water over time, in terms of dry weight loss and nitrogen leaching, and that variations in pellet water stability may contribute to variations in the growth of marron.

Three hundred juvenile marron were housed in 30 individual tanks. A three month growth trial was conducted where marron were fed 8 isonitriogenous and isoengergetic test diets, each containing 25% protein. Diets were formulated to contain 0:70%, 10:60% 20:50%, 30:40%, 40:30%, 50:20%, 60:10% and 70:10% (treatments 1 to 8) inclusions of worm meal and fishmeal respectively, as the total content of dietary protein. Additional treatments included a positive control (0:80% worm meal fishmeal) and negative control (0:40% worm meal : fishmeal). Marron were weighed at the end of each month to determine growth per month.

No significant difference was found between the growth rates marron fed each test diet or between the growth rates in the test diets compared with the positive control, after 1,2, or 3 months of growth ($P>0.067$). Marron fed negative control grew slower ($P<0.05$) during the third month. Feed intake per animal was measured by placing 0.6g of each diet into respective tanks, and collecting, drying and weighing the refused feed after 6 hours. Marron ate less ($P>0.05$) of the negative control than in any other treatment. They ate more ($P<0.05$) of the positive control (80% fishmeal: 0% worm meal) than of treatment 8 (0% fishmeal: 70% worm meal).

Dry weight loss and nitrogen leaching over 24 hours from treatments 1 and 8 and the positive and negative controls were used as measures of pellet stability in water. The greatest rate of dry weight loss of pellets in all treatments occurred in the first hour of pellets being immersed in water ($P<0.05$) and decreased in subsequent hours. Pellets in the negative control lost less ($P<0.05$) dry weight than all the other treatments over the entire 24 hours, and treatment 8 had lost a significantly greater proportion ($P<0.05$) of dry weight than all other treatments after 24 hours. Pellets from treatment 8 lost a significant amount of nitrogen ($P<0.05$) in the first hour of pellet immersion, and there were no other significant losses of nitrogen in subsequent hours of immersion. There was no significant loss of nitrogen from other treatments ($P>0.0610$).

The separate experiment was conducted to observe marron feeding behaviour and to determine if marron fed as soon as feed was offered at different times of the day. Under conditions of low light intensity, there was no significant difference in feeding activity of marron in the morning, at, or at dusk ($P>0.241$).

Worm meal was found to be a valuable source of protein for marron. The results of the experiment showed that worm meal may replace fishmeal in diets fed to marron without adverse affects on growth.

Appendix D: Amino acid composition of earthworm meal.

Background

The importance of earthworms in processing organic matter in compost is well documented. As early as the 1940s, research suggested earthworms contained sufficient protein to be considered as animal food (Lawrence and Miller, 1948). More recently, this has been supported by a number of analyses of the body tissues of earthworms. However the composition of earthworm meals are commonly from experimentation where the primary focus was on use of earthworms for waste management. The impact of the composition of the organic waste on earthworm meal composition is not clear. In addition, manipulating the composition of the compost may increase the value of worm meal as a protein source if the quantity and/or consistency of essential amino acids are changed. Controlled experiments with consistent nutrient availability are required. Both the protein and tuna oil supplementation trials meet this requirement. Worm meals from both the protein and tuna oil supplementation experiments were analysed to determine amino acid profiles.

Results

The amino acid composition (g/100g protein) of worm meals from protein and tuna supplementation trials are shown in Table 1 for essential amino acids and Table 2 for non-essential amino acids. Comparisons with other international and Australian published analyses are given (Edwards, Sabine, 1978).

Addition of protein, antibiotic and or inoculum did not significantly change the amino acid composition of the protein. The protein content and level of individual amino acids tended to be higher in the worm meal from the control diet than from any of the other diets but this was not significant.

Supplementation of the control diet with tuna oil alone increased the arginine, threonine, aspartic acid and serine concentrations. With each of these amino acids, the increases were small, being 0.2-0.4 % units.

Discussion

Supplementation of a good quality control diet with protein or energy had minimal impact on amino acid composition of worm meals. Surprisingly the changes observed were following energy supplementation.

The results suggest care should be taken with feeding protein-rich diets since there was a tendency (not significant) for amino acid concentrations and total protein to be depressed. This supports findings of Neuhauser et al. (1980) (cited by Bouwman and Reinecke 1991) that worm growth rate and survival were sensitive to protein source and concentration.

The amino acid content of the worm meals appeared higher than other Australian work (Sabine, 1978) but more similar to the mean of published analyses summarised in late 1980s (Edwards and Neuhauser, 1988).

Worm meals produced from beds fed the same diet were very consistent in amino acid composition. Where composition was variable, the exception being where mortality rates were high and growth rates low, all analytical results were variable between replicates.

Summary

Managing the feed resource for composting earthworms can produce high quality worm meals of consistent quality. Increasing the concentration of essential amino acids in worm meals is more challenging and requires further research.

Table 9. Mean concentration (g/100g crude protein) of essential amino acids in worm meals

	Arginine	Histidine	Isoleucine	Leucine	Lysine	Phenylalanine	Threonine	Valine	Tyrosine
Protein supplementation									
Control	6.4	2.3	3.8	7.1	6.3	3.6	4.4	4.3	3.0
+ antibiotic	5.5	2.0	3.2	5.8	5.2	3.0	3.6	3.7	2.6
+ inoculum	5.9	2.2	3.7	6.6	5.9	3.4	3.9	4.1	2.9
+ antibiotic + inoculum	5.5	2.1	3.4	6.1	5.5	3.2	3.6	3.9	2.7
+ protein	4.6	1.7	2.7	4.9	4.4	2.5	2.9	3.0	2.2
+ protein + antibiotic	5.4	2.1	3.4	6.1	5.5	3.1	3.7	3.8	2.7
+ protein + inoculum	5.8	2.3	3.6	6.5	5.9	3.4	3.8	4.1	2.9
+ protein + antibiotic + inoculum	5.8	2.2	3.5	6.3	5.7	3.2	3.7	4.0	2.8
Max s.e.m	0.26	0.11	0.20	0.36	0.34	0.14	0.19	0.23	0.15
Tuna supplementation									
control	6.5	3.0	4.3	7.0	6.2	3.7	4.1	4.5	3.2
+ tuna oil	6.8	3.1	4.4	7.2	6.5	3.9	4.3	4.6	3.3
+ tuna + inoculum	6.7	3.1	4.3	7.1	6.4	3.8	4.3	4.5	3.3
Max s.e.m	0.08	0.03	0.07	0.1	0.09	0.02	0.05	0.06	0.04
Mean published analyses	6.0	2.6	4.3	7.2	6.8	3.8	5.2	4.7	
Sabine (1978) (Australia)	4.2	1.6	2.6	4.8	4.3	2.3	3.0	3.0	

Table 10. Mean concentration (g/100g crude protein) of non-essential amino acids in worm meals

	Glutamic Acid	Aspartic Acid	Alanine	Serine	Glycine	Proline
Control	12.4	8.9	5.1	4.4	5.1	3.3
+ antibiotic	10.4	7.3	4.3	3.6	4.4	2.6
+ inoculum	11.8	8.2	4.8	4.0	4.6	2.9
+ antibiotic + inoculum	11.1	7.8	4.4	3.8	4.3	2.7
+ protein	8.7	6.2	3.5	3.1	3.6	2.2
+ protein + antibiotic	11.0	7.7	4.4	3.9	4.3	3.2
+ protein + inoculum	11.6	8.3	4.7	4.0	4.6	3.1
+ protein + antibiotic + inoculum	11.5	8.0	4.6	3.9	4.5	2.8
Max s.e.m	0.66	0.41	0.24	0.22	0.22	0.20
control	16.1	8.7	6.0	4.6	6.0	4.0
+ tuna oil	16.6	9.0	6.0	4.8	5.6	4.0
+ tuna + inoculum	16.4	8.9	5.9	4.8	5.8	3.9
Max s.e.m	0.17	0.08	0.14	0.06	0.25	0.04