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Proximate evaluation of redworms (*Eisenia foetida*) as an alternative protein ingredient to fish meal

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Abstract
Current study evaluated the proximate composition of redworm meal, an alternative protein source to fish meal in fish feeds formulation. Proximate analysis results of crude protein, crude lipids, moisture, ash and crude fibre content in redworms varied insignificantly from fish meal ($P \geq 0.05$). Redworms had highest crude protein content of $62.29 \pm 1.13\%$ against $57.71 \pm 0.77\%$ of fish meal. Crude lipids had mean values of $6.34 \pm 0.48\%$ and $5.51 \pm 0.19\%$ for redworms and fish meal respectively. Redworms' moisture content was $7.97 \pm 0.06\%$ compared to $6.07 \pm 0.06\%$ of fish meal. Redworms recorded mean ash content of $12.23 \pm 0.12\%$ compared to $9.25 \pm 0.23\%$ of fish meal. Crude Fibre content of $3.02 \pm 0.80\%$ and $5.15 \pm 0.08\%$ were recorded in redworms and fish meal respectively. Findings of this study indicates that Redworms have almost similar nutritional values with fish meal hence a potential animal protein to supplement fish meal. When adopted for year-round mass production, redworms could result into increased aquaculture production while enabling sustainable fisheries.

Keywords: Aquaculture, protein ingredients, redworm meal, fish meal

Introduction

In aquaculture, fish feeds account for upto 50-60% of the total production costs in the semi-intensive and intensive farming systems [1]. High cost of fish feeds usually results from inclusion of animal-based proteins which are very key in promoting fish growth. This is due to their high-quality protein content, high palatability, digestibility, and balanced amino acid (AA) profiles [2]. However, most of the animal protein ingredients used in fish feeds are relatively expensive and geographically unavailable to most farmers [3, 4].

In Kenya, Fishmeal (FM), has been heavily relied upon as a source of animal protein in formulation of fish feeds [4], However, challenges coined to its seasonal availability increases the overall cost of fish feeds. This coupled to competition for its use from the livestock sector as feed has contributed to its increased demand while threatening the sustainability of fisheries ecosystems [3]. Increasing fishmeal demand has resulted to increased costs and adulteration of fish feeds by inclusion of low-level proteins by merchants. This has become a common problem which negatively affect the growth of fish, consequently reducing profits to farmers [4].

To promote aquaculture in the country, a paradigm shift towards the use of a readily available, cheap and sustainable source of protein feed for fish is necessary [5]. There is a need for an alternative protein source to reduce over-reliance on FM protein in fish feeds given that its supply is declining rendering it unreliable and expensive in fish diets [6].

Current study aimed to evaluate and compare the proximate composition of redworms, *Eisenia foetida* against fish meal. Findings from this study will inform on the use of redworms as an alternative protein ingredient to fishmeal in on-farm formulated fish feeds. Previous studies have documented redworms (*E. foetida*) as a good source of animal proteins in feeds. This is due to their high amount of protein, essential amino acids, fats and minerals. According to Guerrero [7] and Kasye [8], redworms has been used as a viable ingredient in the formulation of feeds for livestock e.g. pigs and poultry. Studies by Satchell [9] on proximate analysis of dried *E. foetida* shows that have a protein content of 50-60%, 7-10% fats, 8-20% carbohydrates and mineral composition of 2-3%, These has made *E. foetida* be explored in diets of livestock feeds across Asia With this success, however, little information has been documented on its use in aquaculture feeds [10].

Although there is scarce information on the use of *E. foetida* in fish feeds production, there is

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documented evidence showing that, redworms have been used in capture fisheries as baits for sport fishing and as food for ornamental fish. According to Sharma *et al.* [11] *E. foetida* have been explored though at small scale as live feeds in fish farming and as an intermediate feed for juveniles especially for carnivorous species such as catfish.

Sharma *et al.* [11] indicates that *E. foetida* have a low feeding costs, breed easily in artificial culture environments and have high reproduction rate. They can easily be cultured by farmers to form a reliable source of protein which can be fed to fish directly or can be processed to be used as an ingredient in on farm formulated fish feeds. The key environmental factors that affect redworms growth and reproduction include sufficient oxygen, temperature, light, moisture, aeration, pH, food and bedding materials. According to Sherman [12], redworms survive in a pH range of 6.8 to 7.2. Adequate moisture through bed aeration should be maintained to help redworms breathe through their skin. Beds need to sustain a moisture range of 60 to 85 percent and temperature range of 12 ˚C to 26 ˚C for successful reproduction. Feeding redworms is relatively cheap as they consume animal manure, compost, food scraps, shredded or chopped cardboard or paper, or almost any decaying organic matter or waste product. Horse, rabbit, swine, dairy, or steer manures are excellent food items [13].

Materials and Methods
Description of the Study Area
The study was conducted at the Agro-Science Park's Fish Farm within Egerton University (figure 1). The university is situated at an altitude of 1,800m above sea level and has a geographical reference of S 0˚22'11.0", E 35˚55'58.0" within the Kenyan Rift valley at Njoro, Nakuru County. Temperatures in Njoro region range between 17˚C and 22˚C on average and drop to 11˚C during cold season. Average annual rainfall received in the area is upto 1,200±100 mm.
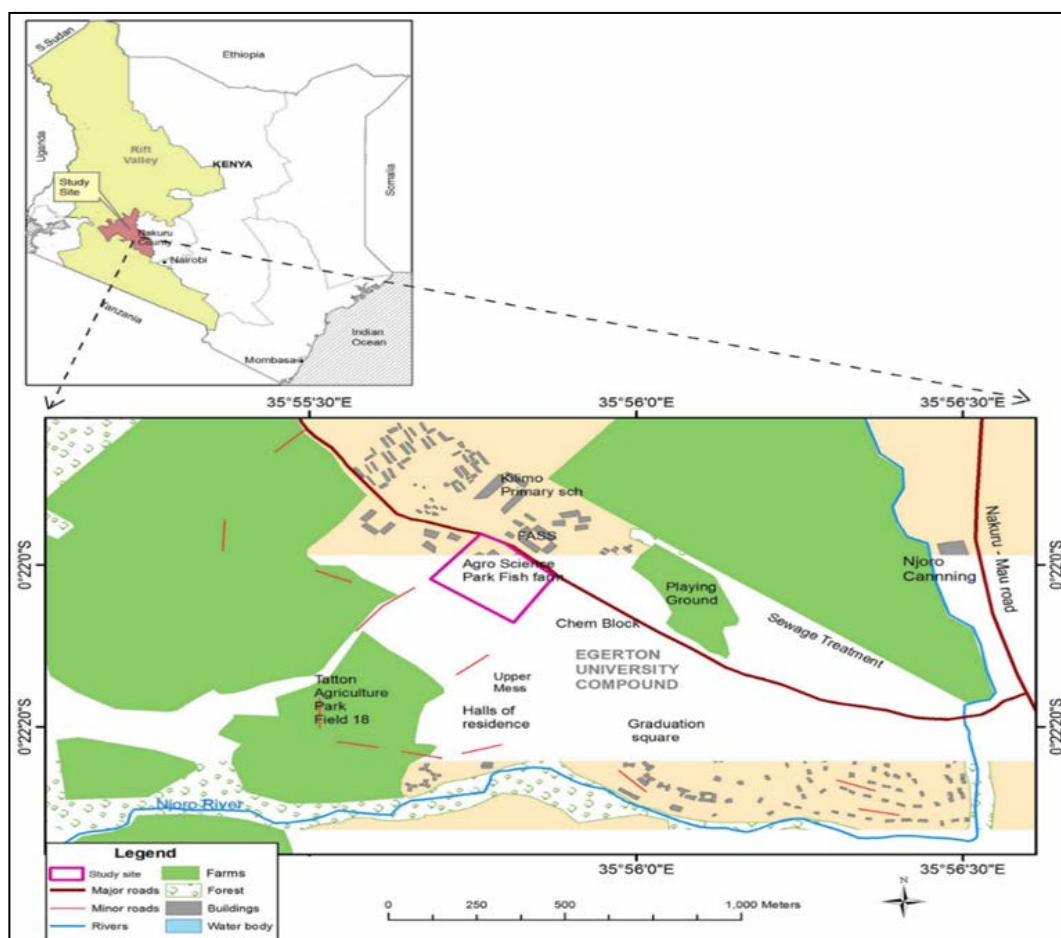

Fig 1: A map of Egerton University showing the site where experimental was set up

Culturing the redworms
The redworms were cultured in black plastic containers within a sheltered house structure to ensure easy management and control of their movement. Dried grass was mixed with goat and chicken dung to prepare indoor compost beds. The beds were watered daily to facilitate decomposition and maintain moisture conditions. A black polythene paper was used to cover the top of the bed to maintain temperature and prevent direct light to the worms since they are photophobic [14]. After every three weeks, dried grass was added to the bed and mixed with previously decomposed organic matter for feeding the worms.

Harvesting and processing of redworms
Harvesting of the worms was done manually by hand picking as described by Jameson & Venkataramanujam [14]. The bedding materials containing the earthworms were dug using a garden rake to expose the worms which were then handpicked and placed into plastic containers. Processing of worms to be used as fish feed ingredient was done by thoroughly rinsing the worms in clean water followed by a 30-minute waiting period for the worms to evacuate undigested matter from their guts. Final rinsing of worms was done in clean water followed by oven-drying at 80˚C for 3 hours and grinded using a mortar and pestle for proximate analysis.

Proximate analysis of redworms and fish meal
Proximate analysis was carried out to determine the chemical composition of redworms and fish meal. The analysis comprised of chemical tests to determine the moisture, crude protein, lipid, crude fiber and ash content.

Determination of Dry Matter (DM) and moisture content in the feed ingredients
Dry Matter (DM) and moisture content were determined using the drying method described by the Association of Official Analytical Chemists [15]. A cleaned crucible was dried for one hour in an oven previously heated to 105˚C and later allowed to cool in a desiccator. The crucible was weighed (W1) and approximately 2g of the finely ground feed sample added. The crucible containing the feed sample was oven dried for 2 hours at 105 ˚C followed by cooling in a desiccator. The sample was reweighed to determine the final weight (W2). The DM and moisture content in the sample were calculated according to formulae described by AOAC [15].

$$\% \, DM = \frac{(W2-W1)}{Wf} \times 100 \qquad (1)$$

Where, W_1 is the weight of empty dish (g), W_2 is the weight of dish and feed sample after drying (g) and W_f is the weight of the feed used in grams.

$$\% \, Moisture = 100 - \% \, DM \qquad (2)$$

Determination of Crude Protein (CP)
Determination of crude protein was carried out using Semi-micro Kjeldahl method [16]. Approximately 0.5g of grounded feed ingredient sample (W), was weighed in digestion tubes. The samples were digested using Kjeldahl digester for 3 hours at an average temperature of 420 ± 20˚C and allowed to cool for 10–20 minutes. Cooled samples were distilled using 50ml of 40% NaOH. The distillate containing ammonia was trapped in 4% boric acid. A mixed indicator was then added and titrated against 0.02N HCL to the endpoint (indicated by colour change from colorless to pink). The percentage of CP in the sample was calculated using equation 3 as described by Balthrop *et al.* [16].

$$\% \, CP = \frac{\frac{a \times b}{1000} \times 14 \times 100 \times 6.25}{c} \qquad (3)$$

Where: a is the amount ml of HCL used, b is the Normality of the HCL used for titration, c is the weight of analyzed feed sample, 14 is a constant indicating the molecular weight of nitrogen and 6.25 is the Conversion factor from nitrogen content into crude protein content.

Determination of Ash Content in dried feed ingredients
Ash content in the dried feed samples was determined through incineration method as described by Balthrop *et al.* [16]. The incineration crucibles were pre-dried, cooled and weighed (W1). Approximately 2g of the grinded feed powder was added into the crucibles. The contents were incinerated for 3 hours in a muffle furnace at a temperature of 550 ˚C. The crucibles containing the residue were cooled in a desiccator and re-weighed (W2). The percentage ash content was calculated using the equation given by by Balthrop *et al.* [16].

Where: W_f is the weight of the feed used (g), W_1 is the weight of empty dish (g) and W_2 is the weight of dish and residue after incineration (g).

Determination of Crude Fats in feed ingredients
The Soxhlet method was used to determine crude fats according to procedures described by AOAC [15]. Approximately 2g of feed sample was weighed into an extraction thimble and covered with a fat-free cotton wool. The extraction thimble was transferred into an extractor. Dried conical flask was weighed (W1) and 95ml of petroleum ether added. The extractor was then connected to the conical flask before heating started. Crude fats in the sample were extracted for 6 hours. The solvent was distilled until the flask was nearly free from the solvent. The flask was left in a fume hood overnight to evaporate all the solvent. The flask with residue was dried for 1.5 hours, cooled and re-weighed (W2). The percentage Crude Fat was calculated using equation 5 described by Balthrop *et al.* [16].

$$\% \, crude \, fat = \frac{W2-W1}{Wf} \times 100 \qquad (5)$$

Where: Wf is the weight of the feed used (g), W1 is the weight of flask (g), and W2 is the weight of flask and fat residue (g).

Determination of Crude Fibre content in the feed ingredients
Crude fibre was determined using filtration method as described by Balthrop *et al.* [16]. Approximately 2g of finely ground feed sample was weighed in a filtration unit for pre-treatment using petroleum ether. The content was digested in 150ml of 0.15N sulphuric acid by boiling for 30 minutes, cooled then filtered and washed with 10ml acetone. The residue was boiled in 150 ml of 0.23M potassium hydroxide for 30 minutes, cooled, filtered and washed with 10ml acetone. The washed residue was then transferred onto a crucible, oven dried for 4 hours at 105 ˚C and re-weighed after cooling in a desiccator (W1). The residue was then incinerated at 550˚C for two hours in a muffle furnace, cooled and crucible weighed again (W2). The percentage crude fibre was calculated using equation 6 described by Balthrop *et al.* [16].

$$\% \, crude \, fibre = \frac{(W2-W1)}{Wf} \times 100 \qquad (6)$$

Where: Wf is the weight of the feed used (g), W1 is the weight crucible and residue after drying (g), and W2 is the weight crucible and residue after incineration (g).

Data management and analysis
Microsoft excel 2016 was used to store and analyse data. Descriptive statistics: means and standard deviation (SD) were used in reporting the findings. One way ANOVA was used to compare means of proximate results for the two feed ingredients. In all statistical analyses, 5% level ($P< 0.05$) of significance was used.

Results
Proximate Analysis of Feed ingredients
The proximate results of crude protein, lipids, moisture, ash and crude fiber expressed in terms of percentages are shown

in Table 1. Proximate results of crude protein obtained varied insignificantly. (One Way ANOVA, df = 6, p = 0.084). Redworm meal had the highest percentage mean crude protein level of 62.29% compared to 57.71% of fishmeal.

Proximate results of crude lipids in the two feed ingredients also varied insignificantly (One Way ANOVA, df = 6, p = 0.066). Redworm meal had a mean lipid content of 6.34% while fishmeal had mean lipid content of 5.51%.

The moisture content between the two feed ingredients was statistically insignificant (One-way ANOVA, df = 6, p = 0.059). Redworm meal recorded a mean moisture content of 7.97% followed by fishmeal with mean moisture content of 6.07%.

There was no significant difference in mean ash content among the analyzed feed ingredients (One Way ANOVA, df = 6, p = 0. 051). Redworm meal had a mean ash content of 12.23% followed by fishmeal with a mean of 9.25%.

Proximate analysis of crude fibre in the two feed ingredients varied insignificantly (One Way ANOVA, df = 6, p = 0. 059). Fish meal and redworms recorded mean values of 5.15% and 3.02% respectively. Crude fibre results indicated that fish meal had highest crude fibre content when compared to redworms.

Table 1: Results of proximate analysis for experimental feed ingredients. Values indicate % means ± standard deviation

Feed ingredient	Proximate composition (%)				
	Protein	Lipids	Moisture	Ash	Fibre
Fishmeal	57.71±0.77[a]	5.51±0.19[a]	6.07±0.06[a]	9.25±0.23[a]	5.15±0.08[a]
Redworms	62.29±1.13[a]*	6.34±0.48[a]*	7.97±0.06[a]*	12.23±0.12[a]*	3.02±0.80[a]

Values with different superscripts in the same column are significantly different $P< 0.05$), the highest value in column is indicated by *

Discussion
Proximate Analysis of Feed ingredients
Redworms *E. foetida* had the highest protein content followed by fishmeal. Fish meal recorded a mean protein content of 57.71% which is not significantly different from a mean of 54.34%, value obtained by Al-Mahmud *et al.* [17] and Munguti *et al.* [4]. Redworms' proximate analysis yielded a mean protein content of 62.29%. This result is similar to those of Vodounnou *et al.* [18], who recorded a mean protein of 59.00%,

In the current study, redworms had the highest lipid content when compared to fish meal. These values are in line with ones reported by Tom [19] who recorded mean lipid content of 5.6% when evaluating fish meal. Studies by Dynes [20] working on *E. foetida* recorded a mean lipid content of 10%, a value that was higher than one obtained in the current research. On the other hand, Bhuiyan *et al.* [21] analyzing *E. foetida* recorded a mean lipid content of 6.04% which is similar to the results obtained in this study. This study has shown that redworms, *E. foetida* have higher percentage protein and lipids extract than fishmeal. The observed variations in proximate compositions from other studies might be associated with the differences in specific-ecology, food, seasons, life stages and reproductive states as reported by Ntukuyoh *et al.* [22].

Redworms, *E. foetida* had the highest ash content of 12.23% followed by fishmeal with a mean of 9.25%. Other studies on the same ingredients have documented values similar to the ones obtained in this study. For instance, studies by Kedar *et al.* [23], documented ash values of 8.8% for *E. foetida*, 9.02% for fishmeal. Furthermore, his study reported ash content of 12.7% in *E. foetida* and 15.3% in fishmeal. Disparities observed in the results might have resulted from the harvesting and processing procedures exposing the ingredients to mix with soil Preston *et al.* [24].

Highest crude fibre content of 5.15±0.08% was recorded for fish meal while redworms recorded a mean crude fibre content of 3.02±0.80%. it is worthy to note that findings of this study were lower than values reported by Kedar *et al.* [23] who obtained a fibre content of 7.8% and 5.8% in *E. foetida* and fish meal respectively. The observed differences in the two findings might have resulted from differences in specific-ecology, food, seasons, life stages and reproductive states Ntukuyoh *et al.* [22].

Proximate analysis for moisture content revealed that redworm meal had the highest moisture content of 7.97% followed by fishmeal with a value of 6.07% It is worthy to note that moisture levels obtained in all the feed ingredients used in this study did not exceed the recommended maximum level of 10% an indication that the ingredients were not under dried.

All the proximate analysis values obtained in the current study were within the acceptable range to qualify the feed ingredients as suitable in formulation of fish feeds. According to Kinyuru *et al.* [25] fish feed should maintain certain standards to qualify as aquaculture feed. The feed should have moisture content of less than 10%, a crude fat/lipids range of 5 to 15%, crude fibre content ranging from 5 to 20%, ash content of less than 10% and protein composition ranging from 28 to 50%. Similar standards have been documented by Craig & Helfrich [26] who recommends that fish feed should contain a protein range of 18 to 50%, lipids from 10 to 25%, carbohydrate 15 to 20%, ash < 8.5% and moisture content of <10% alongside minerals and vitamins. In this study, both redworm meal and fishmeal had proximate results within the recommended values.

Conclusions
Findings of this study indicates that Redworms have almost similar nutritional values with fish meal hence competes favorably to be a potential animal protein to supplement fish meal. Culturing redworms can therefore be adopted for year-round mass production of protein ingredient on fish farms. Embracing culturing of redworms could result into increased aquaculture production while enabling sustainable fisheries.

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