

Influence of the diet on the composition of the earthworm *Eudrilus eugeniae*

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Summary

The earthworm *Eudrilus eugeniae* is a well-known source of protein in animal feeding but there is lack of information on its fatty acid profile. This study aimed to determine its nutrient components and fatty acid profile. Earthworms were bred in substrate containing organic matter; peanut powder was added in the experimental group diet. The nutrient components (protein, ash, and minerals) of the adult worms were determined according to the Kjeldahl method and by atomic absorption spectrophotometer. The fatty acid profile was determined by gas chromatography / mass spectrometry. The dry-matter protein content significantly increased in worms fed peanut powder ($p < 0.05$) as did the minerals measured. The main fatty acid families had similar proportions in the groups. In the saturated fatty acids, C12:0 and C18:0 were present in higher proportions in both groups, whereas in monounsaturated fatty acids, C18:1 ω 9 prevailed. Significant differences ($p < 0.05$) in polyunsaturated fatty acids were observed between the two groups. An increase in the proportion of ω 6 and ω 3 was observed in the experimental group compared to the control. This study showed that peanut powder significantly influenced nutrient components of *E. eugeniae*. The fatty acid profile of this species was similar to that of most animals. Earthworms' diet can be altered to improve the nutritional value of *E. eugeniae*.

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INTRODUCTION

Earthworms are a source of nutrients for animal feeding (Heuzé et al., 2020). They are a natural feed for poultry and there is growing interest in including them in their diet. Fat represents almost 9% of the dry matter (DM) of earthworms and proteins are about 57.9% (Heuzé et al., 2020). Fatty acids (FAs) determine, in part, the nutritional value of feeds for livestock (Chilliard et al., 2008). Their transfer into food

from animal origin thus contributes to improving human nutrition. Several studies have shown that the fat content of animal diets influences the lipid content of products such as meat. Polyunsaturated fatty acids from feed could be transferred into muscles with effects on chicken meat quality (Chilliard et al., 2008). The dynamics of transformation and translocation of FAs have been studied in *Lumbricus terrestris* L. and revealed that significant changes in FA concentration and content occur at very small spatial scales inside the gut of that earthworm (Sampedro and Whalen, 2007). In some aquatic invertebrates (Sushchik et al., 2003), the impact of dietary FAs on the FA profile of tissues has been reported.

However, no study has examined the feed contribution to the FA profile of terrestrial worms, in particular *Eudrilus eugeniae*. As feed, peanuts have never been used to study the effect of fatty feeds on the FA profile of earthworms. Peanut powder as a source of fat could influence the FA profile of *E. eugeniae*. Peanut is an interesting source of fat for feeding and contains about 490 g.kg⁻¹ DM (Settaluri et al., 2012). This work aimed to study the influence of peanut powder on the fatty acid content of *E. eugeniae*, whose breeding would provide feed supplementation to poultry.

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■ MATERIALS AND METHODS

Study site

The study was conducted at the Forestry and Agricultural Research Institute (IRAF) in Libreville in Gabon. It is located between 4° S and 2°3' N, and 8° and 15° E. The average rainfall is 2000 mm/year with an average temperature of 27°C and relative humidity of 80% (Emane Mba and Edou-Minko, 2003). The experimental breeding was located in a half-open room with room temperature close to outside temperature.

Experimental design

The breeding technique (Tahir and Hamid, 2012) was described and used in India. The reference density was 1.6 kg of worms/m² or 6000 worms/m³ of substrates (Francis et al., 2003). In this report, 10 plastic containers of about three liters (23 x 19 x 8 cm) were used. The substrate was composed of a mixture of free and locally available organic materials (9% cow dung, 29% potting soil, 24% coconut fibers, 24% sawdust, and 9% meadow compost). All blends were made to obtain a carbon by nitrogen ratio of 30 (Nayak, et al., 2013). The plastic containers were randomly divided into two groups: control and treatment. The composition of substrates in each container was repeated five times in each group (n = 5). Each substrate was precomposted for 14 days. This process eliminates toxic gases and avoids mortality of earthworms in early breeding (Nayak, et al., 2013). Then ten adult worms were added to each container.

The diet was chosen for its fat content (Settaluri et al., 2012), the worms were thus fed with peanut seeds. Mature peanut seeds were obtained from local farmers. They were sun-dried and powdered into smaller than one-millimeter particles before being introduced into the breeding containers of the treated group; the control group did not receive additional feed. Peanut powder was given once or twice a week. Earthworms can consume 50–100% of their body weight in a day (Heuzé et al., 2020). The quantity of feed was equal to the biomass of earthworms in each container. At the end of the three-month rearing period, all clitellated worms were taken out from each container, gently rinsed and kept for one month at -18°C before analysis to determine the chemical composition and the fatty acid profile.

Nutrient components

The nutrient components of *E. eugeniae* was analyzed to determine crude protein, ash, calcium, phosphorous, potassium, sodium and magnesium. Crude protein was determined by the micro Kjeldahl method (ISO 937) using PYREX 500 ml Kjeldahl Nitrogen Distillation Apparatus. Minerals were determined by an atomic absorption spectrophotometer method (Mosier, 1985).

Chemical reagents and materials

Free fatty acids standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hexane and toluene were of Picograde quality and provided by Promochem (Wesel, Germany). Chromanorm-quality methanol and chloroform were provided by VWR International (West Chester, PA, USA). Sulfuric acid (95–97%) was from Acros Organics (Belgium, WI, USA). Potassium chloride, sodium chloride and potassium carbonate were from Merck (Darmstadt, Germany). Individual stock solutions of each fatty acid standard in hexane were used to prepare a pool of 23 FA standards for calibration. Nonadecanoic acid (C19:0) was used as internal standard.

Fat extraction

The fat content was determined by the Folch method (Folch et al., 1957). Two grams of the sample was weighed into a 50-ml centrifuge

tube (Greiner BioOne, Frickenhausen, Germany). The extraction was performed while shaking the tube upside-down overnight with 40-ml chloroform/methanol (2:1 v/v). The sample was then filtered through a paper filter in a new 50-ml centrifuge tube and 8-ml KCl 0.88% w/v was added. The tube was vortexed on a REAX Top vortex from Heidolph (Germany) and centrifuged at 3700 g for 10 min and a Mini-fuge T centrifuge from Heraeus (Germany). The upper aqueous phase was discarded and the lower phase (10 ml) was poured in a test tube previously dried and weighed. The solvent was evaporated in an oven at 60°C and the tube weighed to determine the fat content.

Fatty acid profile

The fatty acid profile was determined by the analysis of the fatty acid methyl esters (FAMES) by gas chromatography-mass spectrometry (GC-MS) according to Douny et al. (2015). FAMES were separated on a Focus GC gas chromatograph (Thermo Fisher Scientific) using a CP-Sil88 column (Varian, 100 m x 0.25 mm, 0.2 mm) and analyzed with an iron trap PolarisQ mass spectrometer (Thermo Fisher Scientific). The peaks were identified by comparing their mass spectrum and retention times with those of the corresponding standards. "FAMES were detected using selected ion monitoring mode in five segment windows. In each chromatographic run, different ions were monitored for each fatty acid analyzed, which allowed to perform detection and quantitative analysis: m/z 74+143 for saturated fatty acids (SFAs), and 79+91 for monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). The sum of SFAs, MUFAs, and PUFAs were individually expressed as the percentage of total fatty acids."

For quantification, an "8-point-calibration curve containing standard solutions and the internal standard was performed for each of the 23 fatty acids methyl esters determined. The response (ratio between FAMES and the internal standard peak areas) was plotted against standard concentrations. A linear regression was used and no fit weighing was applied."

Statistical analysis

In each sample, the effect of the diet on nutrient components and fatty acid profile of *E. eugeniae* was analyzed using Student's t test. Differences were considered as significant at the alpha level p < 0.05.

■ RESULTS AND DISCUSSION

Effects of peanut powder on some nutrient components of *Eudrilus eugeniae*

The effects of the diet on the nutrient components of *E. eugeniae* are presented in Table I. Peanut powder had an impact on nutrient components as most of the nutrients increased significantly (p < 0.05). Among major nutrients in animal feeding, proteins increased by about 7%. Lipids amounted to about 4% of DM. Total lipids decreased in the experimental group, compared to the control group (3.8% vs 4.3% DM). The phosphorus and potassium increased by 1 g/kg each. No significant difference was observed in sodium content between the control and experimental groups (p > 0.05).

The nutrient components of *E. eugeniae*, especially as a source of protein for animal feed, have been reported in many studies (Moreki and Tiroesele, 2012). Our experiment showed that the nutrient components of *E. eugeniae* were influenced by the diet. These results are similar to studies that state that feeding can significantly influence the nutrient components of an animal (Bourre, 2005). In our study, the external source of feed was peanut powder, which is rich in fat for animal feeding (Settaluri et al., 2012).

Table I: Effect of peanut powder on some nutrient components of *Eudrilus eugeniae* /// Effet de la poudre d'arachide sur les composants en nutriments d'*Eudrilus eugeniae*

Nutrients (g/kg DM)	CG	PPG
Crude protein	657.2 ^a ± 0.9	706.2 ^b ± 1.4
Fat	43.1 ^a ± 0.1	38.2 ^a ± 0.3
Ash	105.0 ^a ± 0.7	165.0 ^b ± 1.0
Calcium	7.9 ^a ± 0.1	6.8 ^b ± 0.1
Phosphorus	9.9 ^a ± 0.1	11.1 ^b ± 0.1
Potassium	8.9 ^a ± 0.3	11.5 ^b ± 0.2
Sodium	1.4 ^a ± 0.1	1.6 ^a ± 0.1
Magnesium	1.5 ^a ± 0.1	2.2 ^b ± 0.2

Means ± standard error; n = 5 containers; CG: control group; PPG: peanut powder group; DM: dry matter; ^{a,b} Values with different superscripts in the same row are significantly different (p < 0.05) /// Moyennes ± erreur standard ; n = 5 bacs ; CG : groupe témoin ; PPG : groupe poudre d'arachide ; MS : matière sèche ; ^{a,b} Les valeurs avec des exposants différents sur une même ligne sont significativement différentes (p < 0,05)

The protein content of *E. eugeniae* was influenced positively by the peanut powder. The presence of peanuts in the gut tract could explain the increase in protein content because peanuts are a source of protein. In our study, the gut tract of earthworms was not emptied of feed before analysis. This suggests the ability of earthworms to convert peanut protein into earthworm tissue.

The amount of lipids (about 4% of DM) was slightly less than that reported in other studies (Moreki and Tiroesele, 2012) with the earthworm *Hyperioidrilus euryaulos*. The difference could be due to the species because *H. euryaulos* is larger than *E. eugeniae*. This study revealed that total lipids in earthworms were greater than total lipids in the muscles of some cattle species (Luciano et al., 2011). *E. eugeniae* as a source of nutrients in poultry feed contains fat that can contribute to the composition of egg yolks which contain 31–33% lipids (Cherian, 2015).

Minerals were also significantly influenced by the diet, except for sodium as no significant difference was observed (p > 0.05). Earthworms are able to concentrate most of the macro minerals in their tissue and the variations observed are probably due to the diet. The nutrient components of *E. eugeniae* and all those of annelids are likely to vary depending on the composition of the substrate on which they are bred or maintained (Moreki and Tiroesele, 2012).

Fatty acid profile of *Eudrilus eugeniae* fed peanut powder

Table II shows the fatty acid profile of *E. eugeniae* fed peanut powder. Slightly more than half of the fatty acids were from the SFA family, followed by PUFA (about 30%), and MUFA. The proportion of SFAs did not differ significantly between groups, but their amount was higher in the control group (583 g.kg⁻¹ of FAs) than in the experimental group (519 g.kg⁻¹ of FAs). Among the SFAs which occurred in higher proportion in both groups were C14:0, C16:0 and C18:0. The amount of C18:0 increased significantly (p = 0.008) in the experimental group (173 g.kg⁻¹ of FAs) compared to the control group (110 g.kg⁻¹ of FAs). A decrease in the proportions of MUFAs was observed in the experimental group (130 g.kg⁻¹ FAs vs 139 in the control). Among MUFAs, C18:1ω9 had a higher proportion in both groups. Significant differences in the proportion of this FA were observed (p < 0.05) between the groups.

PUFAs increased significantly (p < 0.05) in the experimental group compared to the control group (278 vs 351 g.kg⁻¹ FAs). The amount of ω6 reached the highest proportion, followed by ω3. With regard

to ω6, no significant differences were observed between the control and experimental group. The diet induced a significant decrease in C18:2ω6 proportion in the experimental group. For C20:4ω6 the increase was significant. A significant ω-3-proportion difference was observed between the groups. Both C20:3ω3 and C20:5ω3 increased significantly in the experimental group (p < 0.05). Noteworthy, C18:3ω3, C18:4ω3, C22:5ω3 and C22:6ω3 were under the detectable limit (0.1% of total FAs); C20:5ω3 presented the highest proportion in this group (p < 0.05).

SFAs were found in greater amounts followed by PUFAs. The presence of SFAs in sufficient amounts in *E. eugeniae* can be interesting for poultry feeding as a source of energy because they will influence their content in poultry tissues (Milićević et al., 2014). In the SFA family, FAs with more than 10 carbon atoms (C12:0, C14:0, and C18:0) were largely present in *E. eugeniae*. The same observation was reported by Rouabah-Sadaoui and Marcel (1995) with *Eisenia fetida*. We also observed that in this family C16:0 and C18:0 were abundant, and their concentration increased in the experimental group, probably because of their transfer from peanuts to *E. eugeniae* tissues. These FAs are also the most abundant in peanuts (palmitic acid, 63.4 g.kg⁻¹ fat; stearic acid, 57.4 g.kg⁻¹ fat) considering the SFA family (Settaluri et al., 2012).

In the PUFA family, C20:4ω6, C20:5ω3 and C18:2ω6 were the most abundant. In the ω3 and ω6 groups they are called essential FAs because vertebrates cannot synthesize them (Settaluri et al., 2012) and many experiments in animal feeding aim to increase their proportion in animal tissues for healthier human foods. The ω3 and ω6 have a unique role in the growth, immune health, and development of the central nervous system of vertebrates (Kabeya et al., 2018). The presence of these FAs in earthworms is interesting for poultry feeding to enrich chicken products with FAs e.g. of the ω3 group, in particular

Table II: Fatty acid profile of *Eudrilus eugeniae* /// Profil des acides gras d'*Eudrilus eugeniae*

Fatty acids (g/kg FAs)	CG	PPG
MUFAs	139.4 ^a ± 3	130 ^a ± 11.1
PUFAs	277.9 ^a ± 3.5	351.4 ^b ± 20.5
SFAs	582.6 ^a ± 4	518.5 ^a ± 30.7
ω3	65.5 ^a ± 2.7	110.8 ^b ± 10
ω6	212.4 ^a ± 2.3	240.6 ^a ± 15.4
C12:0	181.1 ^b ± 10.7	96.2 ^a ± 26.8
C13:0	16.8 ^a ± 1.3	15 ^a ± 3.3
C14:0	141.6 ^b ± 4.7	89.4 ^a ± 19.6
C16:0	113.3 ^a ± 8.9	116.5 ^a ± 19.3
C17:0	13.8 ^a ± 0.8	24.7 ^a ± 4.5
C18:0	110.1 ^a ± 4.3	172.7 ^b ± 15.2
C22:0	6.3 ^a ± 0.7	4.6 ^a ± 0.1
C16:1ω7	18.7 ^a ± 1	31 ^a ± 4
C18:1ω9	120.6 ^a ± 2.9	99 ^a ± 10.6
C18:2ω6	103 ^a ± 2.3	92.9 ^a ± 8.1
C20:2ω6	27.6 ^a ± 0.6	27.2 ^a ± 2.3
C20:4ω6	81.7 ^a ± 2.4	120.4 ^b ± 6.6
C20:3ω3	19 ^a ± 1.6	33.3 ^b ± 2.3
C20:5ω3	46.4 ^a ± 1.7	77.4 ^b ± 8.5

MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated FAs; SFAs: saturated FAs; Means ± standard error; n = 5 containers; CG: control group; PPG: peanut powder group; DM: dry matter; ^{a,b} Values with different superscripts in the same row are significantly different (p < 0.05) /// MUFAs : acides gras mono-insaturés ; PUFAs : acides gras polyinsaturés ; SFAs : acides gras saturés ; Moyennes ± erreur standard ; n = 5 bacs ; CG : groupe témoin ; PPG : groupe poudre d'arachide ; MS : matière sèche ; ^{a,b} Les valeurs avec des exposants différents sur une même ligne sont significativement différentes (p < 0,05)

C18:3 ω 3 (alpha-linolenic acid, ALA), C20:5 ω 3 (eicosapentaenoic acid, EPA), and 22:6 ω 3 (docosahexaenoic acid, DHA). The amount of ALA and DHA in earthworms is much lower than in fish (< 0.1 vs 3 g.kg⁻¹ FAs and < 0.1 vs 13 g.kg⁻¹ FAs for ALA and DHA, respectively) (Bourre, 2005). However, earthworms contained high amounts of EPA (7–17 g.kg⁻¹ FAs vs 46–77 g.kg⁻¹ FAs). The unsaturated ω -3-fatty acids increased significantly in earthworms fed peanut powder ($p < 0.05$). Even though C18:3 ω 3, C18:4 ω 3, C22:5 ω 3 and C22:6 ω 3 were below the detection limit, an increase near 50% was observed with C20:3 ω 3 and C20:5 ω 3. The amounts of ω 3 are low in peanuts (Settaluri et al. 2012), therefore the increase should result from the synthesis of FAs by earthworms. The presence of C20:5 ω 3 in relatively abundant quantity in *E. eugeniae* has also been reported by some authors (Limsuwath et al., 2012), and *L. terrestris* can probably biosynthesize C20:5 ω 3. This indicates that earthworms could be a very interesting source of very long chain ω 3 FAs for animal feeding.

The peanut powder affected the amount of ω 6 in *E. eugeniae*. The increase could be attributed to peanuts which contain high amounts (320 g.kg⁻¹ FAs) of ω 6 (Settaluri et al., 2012). However, the fact that the earthworms of our experiment were rich in ω 6 could be due to the fact that many invertebrates, like earthworms, synthesize PUFAs (Kabeya et al., 2018). This result is similar to that reported by Limsuwath et al. (2012), who found that C20:4 ω 6 is among the major FAs in *L. terrestris*. C20:4 ω 6 has been influenced by the diet in addition to the fact that earthworms like sandworms are able to synthesize it (Limsuwath et al., 2012).

■ CONCLUSION

The current study showed that peanut powder had significant effects on the nutrient components and fatty acid profile of *E. eugeniae*. However, it must be acknowledged that peanuts have good nutritional components. To our knowledge, this study is the first to report the effects of peanut powder on the FA profile of *E. eugeniae*. It shows specific characteristics that could be influenced by the feeding environment but that could also result from acid biosynthesis by the earthworms. However, the study clearly showed that earthworms improved the FA profile by increasing very long chain omega-3 FAs. The FA content of *E. eugeniae* revealed that it could substitute fish meal in animal feeding as a source of protein and essential FAs. More experiments should be carried out to enrich data on the effect of diets on the nutrient value and FA profile of earthworms.

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Author contributions statement

PB and JLH designed, planned and carried out the study; PB collected data in the field and carried out the experimental breeding to feed earthworms; NM, CD and PB performed data analysis and interpretation for nutrient components; CD and PB performed data analysis and interpretation for fatty acid assay and determined the fatty acid profile of *E. eugeniae*; PB, JLH and CD wrote the first version of the manuscript; CD contributed to Materials and Methods for the determination of fatty acids; JLH, MLS and CD revised the manuscript.

Conflicts of interest

This study was carried out without any conflict of interest.

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Résumé

Byambas P., Douny C., Moula N., Scippo M.-L., Hornick J.-L.
Influence du régime alimentaire sur la composition du ver de terre *Eudrilus eugeniae*

Le ver de terre *Eudrilus eugeniae* est une source de protéines bien connue en alimentation animale mais il y a un manque d'informations sur son profil d'acides gras. Cette étude visait à déterminer ses composants en nutriments et son profil d'acides gras. Des vers de terre ont été élevés dans un substrat contenant des matières organiques ; de la poudre d'arachide a été ajoutée au régime du groupe expérimental. Les vers adultes ont été analysés pour les composants en nutriments (protéines, cendres et minéraux) selon la méthode micro Kjeldahl et le spectrophotomètre d'absorption atomique. Le profil des acides gras a été déterminé par chromatographie en phase gazeuse / spectrométrie de masse. La teneur en protéines de la matière sèche a augmenté de manière significative chez les vers nourris à la poudre d'arachide ($p < 0,05$), ainsi que les minéraux mesurés. Les principales familles d'acides gras avaient des proportions similaires dans les groupes. Dans les acides gras saturés, C12:0 et C18:0 étaient présents dans des proportions plus élevées dans les deux groupes, alors que dans les acides gras mono-insaturés, C18:1 ω 9 prédominait. Des différences significatives ($p < 0,05$) dans les acides gras polyinsaturés ont été observées entre les deux groupes. Une augmentation de la proportion de ω 6 et ω 3 a été observée dans le groupe expérimental par rapport au groupe témoin. Cette étude a montré que la poudre d'arachide influençait significativement les composants en nutriments d'*E. eugeniae*. Le profil des acides gras de cette espèce était similaire à celui de la plupart des animaux. Le régime alimentaire des vers de terre peut être modifié pour améliorer la valeur nutritionnelle de *E. eugeniae*.

Mots-clés : *Eudrilus eugeniae*, ver de terre, alimentation des animaux, protéine animale, acide gras

Resumen

Byambas P., Douny C., Moula N., Scippo M.-L., Hornick J.-L.
Influencia de la dieta en la composición de la lombriz de tierra *Eudrilus eugeniae*

La lombriz de tierra *Eudrilus eugeniae* es una fuente de proteína animal bien conocida en la alimentación animal, pero existe falta de información sobre su perfil en ácidos grasos. El objetivo del presente estudio fue el de determinar sus componentes nutricionales y su perfil de ácidos grasos. Las lombrices de tierra fueron criadas en un sustrato que contenía materia orgánica; polvo de cacahuete se agregó a la dieta del grupo experimental. Los componentes nutricionales (proteína, ceniza y minerales) de las lombrices adultas fueron determinados mediante el método de Kjeldahl y mediante espectrofotómetro de absorción atómica. El perfil de ácidos grasos fue determinado mediante cromatografía de gases/ espectrometría en masas. El contenido de proteínas en la materia seca aumentó significativamente en los gusanos alimentados con polvo de cacahuete ($p < 0,05$), al igual que los minerales medidos. Las principales familias de ácidos grasos tenían proporciones similares en los grupos. Entre los ácidos grasos saturados, C12:0 y C18:0 estuvieron presentes en mayores proporciones en ambos grupos, mientras que entre los ácidos grasos monoinsaturados predominó C18:1 ω 9. Se observaron diferencias significativas ($p < 0,05$) en los ácidos grasos poliinsaturados entre los dos grupos. Un aumento en la proporción de ω 6 and ω 3 se observó en el grupo experimental en comparación con el grupo control. El presente estudio demostró que el polvo de cacahuete influyó significativamente en los componentes nutricionales de *E. eugeniae*. El perfil de ácidos grasos de esta especie fue similar al de la mayoría de los animales. La dieta de las lombrices de tierra podría ser alterada para mejorar el valor nutritivo de *E. eugeniae*.

Palabras clave: *Eudrilus eugeniae*, lombriz de tierra, alimentación de los animales, proteínas de origen animal, ácidos grasos

