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Full Length Article

Effect of Cow Dung and Manure of Laying Hens on Growth and Reproduction of *Eudrilus eugeniae* in Gabon

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Abstract

Even though *Eudrilus eugeniae* was identified in Gabon, its vermiculture has never been experimented. The objective of the current study was to compare the effect of cow dung and manure of laying hens, associated to one of three carbon substrate, on weight and population of this worm. Each substrate was sown with twenty (20) adult worms, 0.7 to 1 g weight. The weight of worms was collected at the beginning and at the end of breeding. The number was collected weekly until 60 days of breeding. The average number of worms was multiplied by 2.38 (60 ± 6.78 in cow dung, 40 ± 6.81 in manure of laying hens, 43 ± 8.23 in control). The average weight of worms was 27.19 ± 1.21 g; 24.41 ± 1.20 g; 22.41 ± 1.44 g, respectively. These results suggest that the cow dung is better than manure of laying hens for vermiculture of *E. eugeniae* in Gabon. © 2017 Friends Science Publishers

Keywords: *Eudrilus eugeniae;* Manure; Laying hens; Cow dung; Gabon

Introduction

Eudrilus eugeniae is a diggerworm of Tropical areas (Ansari and Saywack, 2011) and native from Africa. It was indexed in Ivory Coast (Coulibaly and Zoro Bi, 2010), in Ghana, in Nigeria, in Cameroon (Oboh *et al*., 2007), and in Gabon (James and Divina, 2012), this is why it's called the African Night Worm (Edwards and Bolhen, 1996). This invertebrate is commonly used in the process of vermicompost in tropical and subtropical countries (Vijaya *et al.*, 2012). The demographic growth in tropical countries has an environmental impact, with an important production of organic waste (Temgoua *et al.*, 2014). In order to find solutions to waste management, severalways are explored. Among them, organic treatment by vermicomposting (Yadav and Garg, 2011). This process allows earthworms, suchas *E. eugeniae*, to accelerate mineralisation of organic waste in simple element as Nitrogen, Phosphorus, Potassium (Morin, 1999; Karmakar *et al.*, 2012). The vermicompost is thus an alternative to classical methods of lands fertilization in the tropical countries (Karmakar *et al.*, 2012). These earthworms also represent a source of protein for animal feed (Munroe, 2006).

Use of the earthworms in animal feedstuff implies to produce worms in area where they should be used. To better know *E. eugeniae* and to develop its lombriculture, several studies were carried out and aimed at describing the internal anatomy (DaDong *et al.*, 2007; Butt and Grigoropoulou, 2010), the cycle of reproduction (Parthasaeathi, 2007; Sivasankari *et al.*, 2013), growth and reproduction in various substrates (Coulibaly and Zoro Bi, 2010; Tahir and Hamid, 2012; Nayak and Sahu, 2013), and reproductive organs (Vijaya *et al.*, 2012; Moraes *et al.*, 2012). Lombriculture of *E. eugeniae* was carried out in several countries of the tropical area (Coulibaly and Zoro Bi, 2010; Tahir and Hamid, 2012; Francis *et al.*, 2003). But its breeding was never carried out in Equatorial zone, and particularly in Gabon where coconut scrap abounds on beaches. The potential of *E. eugeniae* in vermicomposting of different vegetable and animal wastes has been reported (Coulibaly and Zoro Bi, 2010; Tahir and Hamid, 2012), but to now, no study was conducted with manure of laying hens breeded on the ground or *Leucaena leucocephala* leaflets. So it was hypothesized that cow dung or manure of laying hens associeted or not with coconut fiber or *Leucaena leucocephala* leaflets would affect differently the growth and the reproproduction of *E. eugeniae* in equatorial zone.

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Materials and Methods

Study Site

The study was conducted at the Forestry and Agricultural Research Institute (IRAF), at Libreville in Gabon. It's situated between latitudes 4°S -2°3N and longitudes 8°E and 15°E (Meka M'allogho, 2013). The average rainfall is 2000 mm/year, with average temperature of 27°C and relative humidity of 80% (Emane Mba and Edou, 2003). The experimental breeding was located in a half-open room whith a room temperature close to outside.

Biological Materials

Earthworms (EW) all were picked, by digging and manual removing on the same plot of land, to 10 cm of depth, in order to reduce the variation of the biotype (Vijaya *et al.*, 2012). The species was identified using the keys of identification of adult worms and descriptions of *E.eugeniae* made by Sims and Gerard (1985), Dhiman and Battish (2005), Dominguez and Edwards (2010), Ansari and Saywack (2011). Earthworms were packaged and transported in plastic trays with a little soil sampling of their natural environment.The hens manure (HM) was a dry mixt of 55% hens drops, 25% wood chips and 20% water. It was collected in a poultry farm in Libreville. A nitrogen content of 2.8% dry matter (DM) was determined by the Kjeldahl method (ISO 937). The cow dung (CD) was harvested on Libreville stockyard, then dried and ground. Nitrogen content was 3.6% DM. Leaflets of *L. leucocephala* were harvested from shrubs and sun-dried over 2 days. Then they were crushed by hand to obtein green waste. Coconut fibers were collected on the beach at Libreville. Coarse fibers were cut and then passed to oven for 48 h. The dried fibers were passed to the mill and reduced into particules of about 0.5 cm. The Okoume sawdust was collected in a wood factory. Manure compost of 4 months old was used as pre-compost to stimulate the compositing process in the bins. All blends were made to obtain a C/N ratio of 30 (Bruyeer and Simus, 2012). The C/N ratio of different organic matter used in substrates is listed in Table 1.

Experimental Design

The breeding technique was described by Francis *et al*. (2003), Coulibaly and Zoro Bi (2010) and Tahir and Hamid (2012). Eighteen (18) plastic containers of 9.8 liter (L: 33 cm, W: 23 cm, H: 13 cm) were divided in six lots of substrate as shown in Table 2. The basic substrate was composed of mold, sawdust and 0.5% of Precompost. Half of them were randomly mixed with cow dung and the other half with hens manure. Among these sub-groups, one third was mixed with coconut fiber, another third with *L. leucocephala* leaflets and the other third not. The susbrates compositions are indicated in Table 2.

Table 1: The C/N ratio of ingredients of the substrates

Sample	C/N
Mold	10
Pre compost	12
Sawdust	200
Cow dung	20.95
Laying hens manure	10.89
Coconut fiber	24.71
Leucaena leucocephala leaflet	9.08

Table 2: Expérimentatal design

Source of Nitrogen	Bins	Substrates	Abberviations
	Control=	M+S+P+CD	CDC
Cow dung	Treatment=	M+S+P+CD+CF	CDCF
	Treatment=	M+S+P+CD+LL	CDLL
	Control=	M+S+P+HM	HMC
Laying hens manure	Treatment=	M+S+P+HM+CF	HMCF
	Treatment=	M+S+P+HM+LL	HMLL

Legend: M: Mold, S: Sawdust, P: precompost, CD: cow dung, CDC: CD control, CF: coconut fiber, LL: Leucaena leaflets, HM: hens manure, HMC: HM control

The bins were distributed randomly in the breeding room. For ventilation, funds and lids trays were perforated (holes of 3 mm) with a drill. The walls of the bins were painted in black to prevent the passage of light (Fig. 1).

Each substrate was pre-composted for 14 days (Nayak and Sahu, 2013). This process eliminates toxic gases and avoid mortality of earthworms in early breeding (Coulibaly and Zoro Bi (2010). Then twenty (20) adult worms (0.85±0.15 g) were added to each bin. The reference density was 1.6 kg of worms/m² or 6000 worms/m³ of substrate (Francis *et al.*, 2003).

The total weight of worm population was noted at the beginning and at the end of breeding. The number of worms was registered every 15 days, by manual sorting. Worm population was weighed with a digital scale with a precision of ± 0.01 g. The manual mixing of the substrate was carried out in parallel, in order to evacuate volatile gases, toxic to earthworms (Coulibaly *et al.*, 2011).

Statistical Tests

Data were analyzed with the SAS statistical software following a mixed model, including the effects of nitrogen and carbon sources and their interactions over time in type 1 autoregressive covariance structure.

The overall weight changes were analysed according to a general linear model including the effect of the source of carbon, that of nitrogen and their interaction.

Least square means were compared according to Student's t tests. Differences were considered as significant at the alpha level $P<0.05$.

Results

Population of Adults and Youngs *E. eugeniae* According to Carbone Sources

Changes in number of adult worms were observed in different substrates (Fig. 2A). These data revealed that in the control group the reduction of number of adult worms was 50%. In the other two groups, the decline of population was reduced by about 40% at the end of the experiement, the difference between group being not significant (P=0.75). However, a significant difference was observed between the number of adult worms in the control substrate and those of the two carbon sources in the 3rd and 5th week of culture ($P<0.05$).

Juvenile earthworms in the different substrates appeared from the 5th week of breeding (Fig. 2B) and their numbers increased linearly and significantly, but more in the FC group, 40 units/week vs about 25 in the two other groups ($P<0.05$) that were not differentiated.

Population of Adults and Youngs *E. eugeniae* According to Nitrogen Sources

The evolution of the population of adult earthworms as influenced by the nitrogen source is depicted in Fig. 3A. The adult population declined in both nitrogen sources. However, the highest decrease was observed with eartworms breeded in the HM (50%) while in the CD a decrease of 35% was observed. The differences were significant ($P<0.05$) from the 5th to the 9th week of breeding.

The worms appeared from the 5th week (Fig. 3B) and their number increased linearly in both groups but more strongly with cow dung (35 units/wk. Therefore in the 9th week, 143 young specimens were observed in substrate of CD but only 91 specimens were observed in HM. This difference between the CD and HM curve was significant ($P<0.05$).

Weigth of *E. eugeniae* in Different Susbtrates

Table 3 shows population worm weight changes observed in the different groups. The initial weight did not vary between batches. It increased on average by 2.4. No difference was observed between nitrogen sources, although numerically greater increase was observed in the system with cow dung where the weight was multiplied by 2.5. Within the three sources of carbon, differences tended to appear. The starting weights were multiplied by the highest value (2.7), in the group with coconut fiber.

The average gain of biomass (AGB) observed in the overall experimental environment was about 26.2 g. In the CD system, it was numerically higher of 4 g, compared to that observed in the HM system.

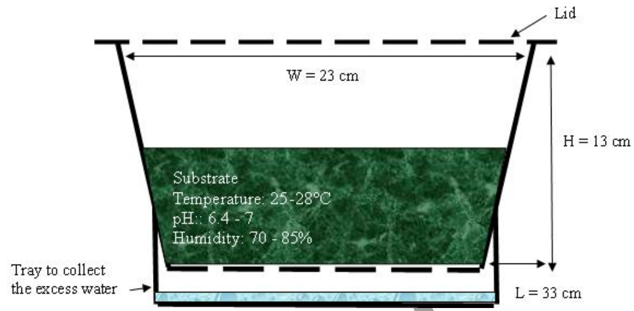

Fig. 1: Experimental vermicomposting system for *E. eugeniae* (adaped from Tahir and Hamid, 2012)

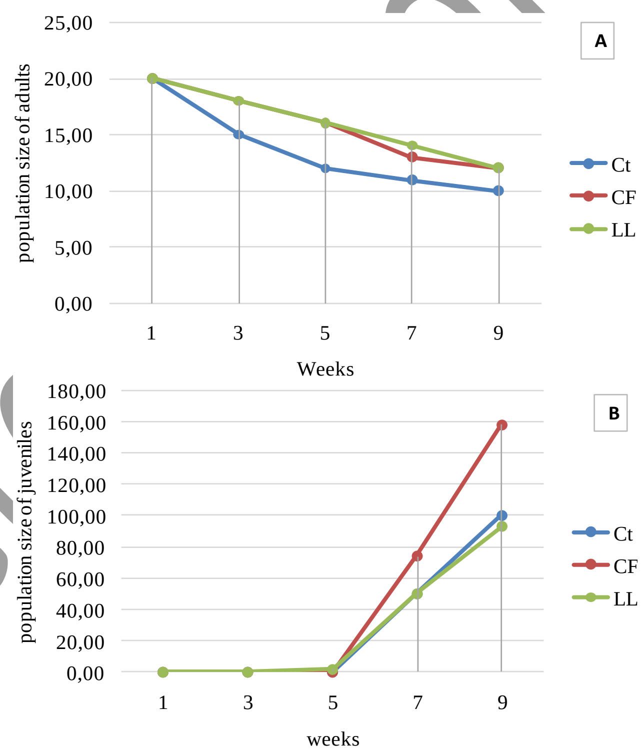

Fig. 2: Evolution of the population size of adults and juveniles *E. eugeniae*, breeded in substrates enriched in different carbon sources (control, coconut fiber and *L. leucocephala* leaflets)

Legend: Ct: control; CF: coconut fibers; LL: *L. leucocephala* leaflet

Among the various sources of carbon, coconut fiber had the highest biomass gain, at about 10 g more than in the other two groups ($P<0.05$). The biomass gain observed in substrate containing *L. leucocephala* leaflets, was superior to the control but not significantly.

The interaction between the two main factors tended to be significant. Biomass gains in the 3 groups of CD system did not differ significantly ($P>0.05$). Among the three groups of the HM system, the one with coconut fibers had the best AGB, followed by the one with *L. leucocephala* and the control (39.7 ± 5.17 g, 20.2 ± 6.33 g and 13, 4 ±5.17 g, respectively).

Table 3: Growth performance of *E. eugeniae* breeded in substrates containing cow dung or manure of laying hens, associated or not with either coconut fiber or *Leucaena leucocephala* leaflets

	N		C			P>F		
	CD	HM	Ct	CF	LL	N	C	N*C
Initial weight (g)	18.9±1.89^{a}	19.78±1.88^{a}	19±2.32^{a}	19.33±2.32^{a}	19.67±2.32^{a}	NS	NS	NS
Δ weight	28.1±2.99^{a}	24.4±3.23^{a}	21.0±3.66^{a}	33.5±3.66^{b}	24.4±4.09^{a}	NS	+	+
Weight gain per week (g)	3.28±0.21^{a}	2.85±0.21^{b}	2.45±0.62^{a}	3.91±0.61^{b}	2.82±0.61	NS	+	+

Within a N and C source, means with same letter are not significantly different at p>0.05

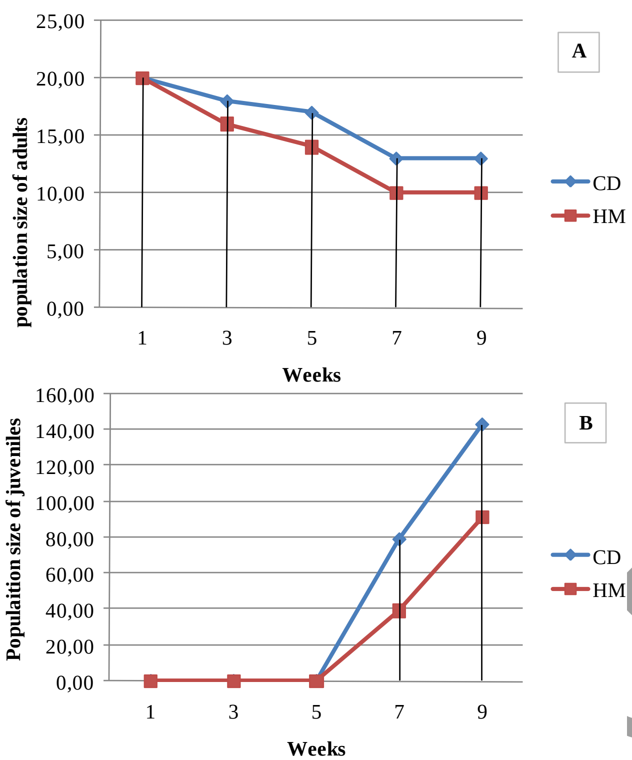

Fig. 3: Evolution of the population of adults and juveniles earthworms belonging to *E. eugeniae* species breeded in two N sources (cow dung and manure of laying hens)
Legend: CD: Cow dung; HM: hens manure; CD: cow dung; HM: hens manure; Ct: control; CF: coconut fiber; LL: *L. leucocephala* leaflet; NS: non-significant; « + »: bias

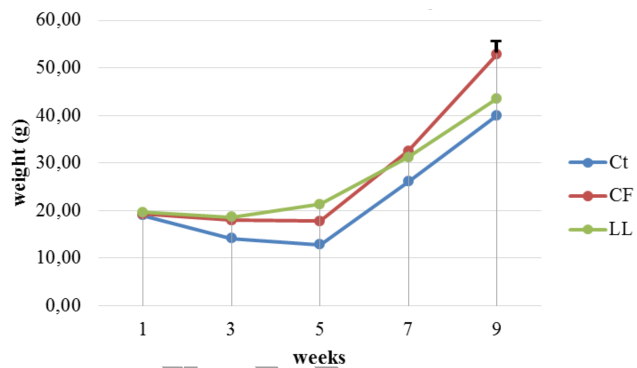

Fig. 4: The growth of *E. eugeniae* in differents breeding substrates (control, Coconut fiber and *L. leucocephala* leaflet)
Legend: Ct: control; FC: coconut fibers; FL: *L. leucocephala* leaflet; T: upper standard deviation

The average weekly gain (AWG) of 3.28 ± 0.21 g, observed with worms raised on CD system was significantly higher than that observed in the HM system (P<0.05). When considering the carbon sources, the difference in AWG were significant also. The worms raised on coconut fibers had significantly higher value than the control group (P<0.05) but neither difference was observed between Ct and LL nor between CF and LL (P>0.05).

Weigth Growth of *E. eugeniae* in Different Susbtrates

The evolution of weight Biomass of *E. eugeniae* on the breeding period is reported in Fig. 4. Carbon source did not affect the average weight of worms up to the third week (P>0.05). From the 5th week, a significant difference was observed between weight of worms raissed in the control substrate and that raised on *L. leucocephala* leaflets (P=0.012). At week 7, the worms raised in the substrate receiving coconut fibers showed the highest weight, followed by that of *L. leucocephala* substrate and control substrate. The difference between the control group and the substrate containing coconut fibers observed in weight reflected a trend (P=0.06). The weight of the worm measured at the 9th week breeding, in the substrate with coconut fiber was significantly higher than the other two groups, (P>0.05) and (P>0.001) in the groups with *L. leucocephala* and control respectively. No significant differences were observed between the control and the substrate with *L. leucocephala* (P>0.05).

Discussion

The increase of the population and weight of earthworms, are essential indicators during vermiculture. The initial population of 20 EW was multiplied on average by 2.4 after 60 days of breeding but some adult worm's mortality were observed. The curves of mortality of adult worms presented the same slope in the various substrates. This global reduction of number of worms could be due to the presence of poison gases such as the CO_2 or H_2S stemming from the microbial activity during the decomposition of the organic matter (Coulibaly and Zoro Bi, 2010; Tahir and Hamid, 2012). The mortality of adult earthworms in control group was higher at 10% when compared with that observed in groups raised with additional carbon source. The introduction of coconut fibers or *L. leucocephala* leaflet was able to improve physic chemical quality of the substrate so as to favor the viability of worms raised in these substrates. The quality of the substrate indeed influences positively worms (Suthar, 2009a). The young earthworms were observed from the 5^{th} week and their number increased very quickly from the week 7 in all substrates, although it remained significantly higher in the group with coconut fibers (P<0.05), with regard to the control group.The observation of the juveniles earthworms from the 5^{th} week is similar to that made by Coulibaly and Zoro Bi (2010) with *E. eugeniae*. They noticed that the production of cocoons started 5 weeks after the beginning of the breeding. This time would allow the earthworms to adapt themselves to their new breeding environment, to couple, to produce cocoons and to give birth to small worms becoming easily countable in few days. The relatively fast multiplication of earthworms would be also result from the temperature of the breeding environment of *E. eugeniae* (Dominguez *et al.*, 2001; Francis *et al.*, 2003). This experience was realized in the equatorial zone under an average temperature of 27°C. Coconut fibers had a significant effect (P<0.05) on earthworms by increasing by 40 units their population, against 20 units for those raised in the control substrates and that with *L. leucocephala* who did not differ significantly. This positive effect of coconut fiber on *E. eugeniae* had been reported by Tahir and Hamid (2012). Coconut fibers would favor adequate humidity and the aeration of the bredding midia which are, among others, essential conditions to development of earthworms (Sierra *et al.*, 2013). The weack growth of worms raised in the substrate with *L. leucocephala*could has been impacted by possible toxic effect of mimosine or toxic compounds such as saponins (Graham *et al.*, 2014).

The results showed a global mortality of 42% of adults worms raised on cow dung and manure of laying hens and their respective curve of survival decrease in same way during the period of breeding. However, earthworms raised in manure of laying hens recorded a mortality rate significantly higher than in the presence of cow dung (P<0.05). This loss could be associated to the quality of the manure of laying hens because of medicinal treatments they receive in farm, mainly vermifuges whom residues can be found in poultry droppings. This report differs from that made by Coulibaly *et al.* (2011). Their source of nitrogen was droppings while in the current study it is manure of poultry reased on the ground, which is composed of droppings additioned of the shaving which forms the litter. These results reveal nevertheless that in the conditions prevailing in the region, the cow dung allowed better viability of adults earthworms. The population of juveniles earthworms observed from the 5^{th} week increased very quickly and more significantly in the system with cow dung (P<0.05). It suggests that the cow dung would favor also better the reproduction of earthworms comparing with the manure of laying hens. Coulibaly and Zoro Bi (2010), nevertheless classified cow dung at the third place behind poultry droppings and sheep dung for the production of cocoons. For rate of hatching it was ranked at second place behind poultry droppings. This difference would stem on one hand, on use of manure instead of poultry droppings, and on other hand, on presence of residues of medicine in the manure of laying hens.

The average population weight observed in the various substrates was multiplied by 2.4 and did not differ significantly between the two sources of nitrogen. However, the higher numerical value, obtained with worms raised on cow dung is an additional element suggesting that this one would favor better body growth of earthworms compared to manure of laying hens. Between the various sources of carbon, coconut fibers gave the highest weights. These results differ from those reported by Coulibaly *et al.* (2011) for the biomass but are similar to those reported by Tahir and Hamib (2012) who observed best weight growth with worms raised on coconut fibers. The average gain of biomass for CD system was superior of 4 points, compared with that of HM system and suggests that cow dung would be a better substrate for growth of *E. eugeniae* in equatorial region. Coulibaly *et al.* (2011) ranks it at the second palce after poultry droppings for biomass gain with *E. eugeniae*.The average weekly gain reported in Table 3 is globally close to AWG reported by Tahir and Hamib (2012) with *E. eugeniae*. However, cow dung distinguishes itself significantly from manure of laying hens (P<0.05). And coconut fibers provided a significantly better value compare to that of control (P<0.05) by contratste to *L. leucocepala*. This implies that the cow dung would be better for the growth of earthworms. Within the association of CD and CF, coconut fibers would allow earthworms to express better their growth potential. The tendency of coconut fibers to improve weekly weight gain was reported by Tahir and Hamib (2012).

The weight growth curves of earthworms, although incomplete, follow a regular evolution. As reported by Coulibaly and Zoro Bi (2010), no difference was observed between groups for average weight before the 5^{th} week of breeding (P>0.05). In their study, they show that curve of growth of *E. eugeniae* accelerates after 5^{th} week of breeding in all the substrates. By 7^{th} week, differences of weight observed between control group and that with coconut fibers reveal a tendency (P=0.06). At the 9^{th} week the weight measured in the group with FC showed significantly higher than that of the group with *L. leucocephala* (P<0.05) and the control (P=0.001). It reveals that there would be a combined effect of time and substrate on the weight of *E. eugeniae*. The process of decomposition being faster in hot climate (Francis *et al*., 2003), then the mineralization of the organic matter would have favored the production of nutrients available for *E. eugeniae* but also microorganisms which are food sources for earthworms (Gomez-Bradon *et al.*, 2012).

The difference observed between the avarage weigth of earthworms raised in coconut fiber compared to that of *L. leucocephala* at the 9th week, suggest that CF help *E. eugenaie* to have better weigth gain.The same obeservation have been made by Tahir and Hamid (2012) with *E. eugeniae*. The presence of coconut fibers combined with the effect of the time would have improved quality of substrate which influences development of earthworms (Suthar, 2009a). While coconut fibers, richer lignine, should degrade more slowly and influence negatively the earthworms. This situation could be attributed to the fact that coconut fibers were very finely crushed (Tahir and Hamid, 2012) and allowed a better aeration of breeding environment contrary to *L. leucocephala* leaflet which facilitates compaction of the substrate and bad aeration.

Overall, these results suggest that cow dung is a good source of nitrogen for vermiculture, including promoting weight gain and reproduction. In this study, the best position of CD before HM would be due to manure used. After analysis, it content low nitrogen (3%), because of loss of element resulting from its outside storage and to abundance of wood chip. In these conditions, the content of nitrogen would become then lower than the reference value of 3.4% for dry manure of laying hens, whereas droppings, for the same type of hens, presents a rate of 6.01% nitrogen (Chevalier *et al*., 2005; Seydoux *et al*., 2006). These factors would explain, partially, why the obtained results differ from those reported by Coulibaly and Zoro Bi (2010) with poultry droppings. Indeed the richness of poultry droppings in nitrogen when compared with the cow dung would influence the growth, the production and the reproduction of *E. eugeniae* (Deka *et al*., 2011). But in the condition of this experiement the richest source of nitrogen was cow dung with 3.6%. Munroe (2006) reported also that nitrogen would be nutritionally benefic to earthworms. There were probably also possible residues in veterinary product in laying drop that could adversely affect the growth and reproduction of *E. eugeniae*. Coconut fibers, as for it, would be a good source of carbon for this type of farming in the equatorial zone by promoting aeration and maintaining humidity of substrate.

The period of breeding for this experiment was 60 days, because of material constraints. Other experiments with *E. eugeniae* were conducted on 50 days and led to similar results for the weight gain (Tahir and Hamib, 2012). To reach a more complete curve of growth, a period of 3 or 4 months would be necessary (Parthasarathi, 2007; Coulibaly and Zoro Bi, 2010; Deka *et al.*, 2011). It would allow to determine weight curve inflection point in order to provide the optimum time to stop breeding and get the best biomass of earthworms for poultry feed.

Conclusion

The culture of *E. eugeniae* is possible in Gabon, with two sources of nitrogen. The present study intended to show that the lombriculture of *E. eugeniae* could be carried out in equatorial conditions with manure of laying hens or cow dung associeted or not with coconut fibers or *L. leucocephala* leaflets. The results obtained in the various treatments with the population, the weight and the average weight gain of earthworms are similar to those of previous studies with the same species. Moreover, the resulting values reveal that the use of cow dung is more appropriate than manure of laying hens and that association cow dung-coconut fibers is well adapted to this type of breeding in equatorial zone. They also confirm that *Eudrilus sp.* can reproduce very quickly in equatorial zone under ambient average temperature of 27°C which is similar to data brought back in other experiments.

This experience should be repeated over a longer period to align itself with the durations of breedings realized with *Eudrilus sp*, and substitute the manure by droppings of laying hens as source of nitrogen.

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