

Effect of diet on feeding and casting activities of earthworms (*Drawida nepalensis*) and response of crop growth

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Abstract: This study was undertaken to assess the feeding and casting activities of *Drawida nepalensis* fed on finger millet (*Elusine coracana*), wheat (*Triticum aestivum*) and grass (*Cynodon dactylon*) under controlled laboratory conditions. Food consumption varied from 7.8 to 12.2 mg day⁻¹ and weight-specific food consumption rates obtained were 20.2 to 25.2 mg g⁻¹ live worm day⁻¹ on the three substrates tested. Casts were produced on the surface and sides of the containers and cast production rates varied from 14.7 to 16.7 mg g⁻¹ live worm⁻¹, whereas mean weight-specific cast production rates for all food substrates was 15.5 mg live worm day⁻¹. The C:N ratio of the casts (8.39) was higher than the non - ingested soil (7.82). Plant growth parameters were higher in the earthworm treatment compared to control, although the difference was not significant. The dry weight of shoots in the earthworm treatment showed an increase of 43.1 % and 32.6 % in finger millet and wheat, respectively, whereas the dry weight of roots showed an increase of 26.6 % in finger millet and 26.9 % in wheat in the earthworm treatment. Earthworms preferred grasses and produced high cast with the same. These results demonstrate the potential of *D. nepalensis* to increase the plant biomass in the glasshouse experiments. Compared to the control treatment, plant biomass was higher in the earthworm treatment.

Resumen: Este estudio se realizó para evaluar las actividades de alimentación y producción de humus de la lombriz de tierra *Drawida nepalensis* alimentada con mijo de dedo (*Elusine coracana*), trigo (*Triticum aestivum*) y pasto (*Cynodon dactylon*) en condiciones controladas de laboratorio. El consumo de alimento varió de 7.8 a 12.2 mg día⁻¹ y las tasas de consumo de alimentos específicas del peso variaron de 20.2 a 25.2 mg g⁻¹ de lombriz viva día⁻¹ en los tres sustratos estudiados. El humus de lombriz fue producidas en la superficie y los lados de los contenedores y las tasas de su producción variaron de 14.7 a 16.7 mg g⁻¹ lombriz viva, mientras que la tasa promedio de producción de humus específicas del peso para todos los sustratos fue de 15.5 mg lombriz viva día⁻¹. La relación C:N del humus (8.39) fue mayor que en el suelo que no fue ingerido (7.82). Los parámetros de crecimiento de las plantas fueron mayores en el tratamiento de lombriz de tierra que en el control, aunque la diferencia no fue significativa. El peso seco de los brotes en el tratamiento con lombrices mostró aumentos de 43.1 % y 32.6 % en el mijo de dedo y el trigo, respectivamente, mientras que el peso seco de las raíces mostró un incremento de 26.6 % en el mijo de dedo y de 26.9 % en el trigo en el tratamiento de

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lombrices. Las lombrices de tierra prefirieron el pasto y con él produjeron mucho humus. Estos resultados demuestran el potencial de *D. nepalensis* para aumentar la biomasa de las plantas en los experimentos de invernadero. En comparación con el control, la biomasa vegetal fue mayor en el tratamiento con lombrices.

Resumo: Este estudo foi realizado para avaliar as atividades de alimentação e produção de húmus da minhoca *Drawida nepalensis* alimentada com capim (*Elusine coracana*), trigo (*Triticum aestivum*) e grama (*Cynodon dactylon*), sob condições controladas de laboratório. O consumo de alimentos variou entre 7,8-12,2 mg dia⁻¹ e as taxas de peso de consumo específico de alimentos variaram de 20,2 a 25,2 mg g⁻¹ de lombriga viva dia⁻¹ nos três substratos testados. O húmus da lombriga foi produzido à superfície e nos lados dos recipientes e as taxas da sua produção variaram de 14,7-16,7 mg g⁻¹ por lombriga viva, ao passo que as taxas médias de produção de húmus específicas do peso para todos os substratos foi de 15,5 mg lombriga viva dia⁻¹. A relação C: N do húmus (8,39) foi maior do que no solo que não foi ingerido (7,82). Os parâmetros de crescimento das plantas foram maiores no tratamento com a minhoca do que em relação ao controle, embora a diferença não tenha sido significativa. O peso seco dos rebentos no tratamento com as lombrigas mostrou um aumento de 43,1 % e 32,6 % no capim e trigo, respectivamente, enquanto que o peso seco das raízes mostrou um incremento de 26,6 % no capim e 26,9 % no trigo no tratamento com as minhocas. As minhocas preferiram a grama e com ele uma produção elevada de húmus. Estes resultados demonstram o potencial da *D. nepalensis* para aumentar a biomassa das plantas nas experiências em estufa. Em comparação com o tratamento de controle, a biomassa vegetal foi maior no tratamento com minhocas.

Key words: Cast production, *Drawida nepalensis*, earthworms, food substrates, litter consumption, plant growth.

Introduction

Invertebrate ecosystem engineers largely determine physical properties of soil by their bioturbation activities in the upper 10 to 30 cm of the soil profile (Blanchart *et al.* 1999; Cook & Linden 1996; Folgarait 1998; Jouquet *et al.* 2006). Earthworms are the most important soil invertebrates in the soil ecosystem in terms of biomass and activity (Chaudhuri *et al.* 2008; Joshi *et al.* 2010; Rombke *et al.* 2005). They dig burrows, galleries, and chambers that result in voids of diverse shapes and sizes, and produce a large diversity of solid macroaggregated structures (Bossuyt *et al.* 2005; Tapia-Coral *et al.* 2006; Velasquez *et al.* 2006). They are, therefore, key organisms in the delivery of ecosystem services by soils (Lavelle *et al.* 2006). Several studies have emphasized the importance of earthworms in the consumption and degradation of litter. For example, Edwards & Lofty (1977) reported that earthworms can consume 10 - 30 % of their own weight per day. Earthworms contribute to cycling, translocation and accumulation of nutrients by casting at the soil surface, an essential function within earthworm communities

required for maintenance of their living space. Earthworm casts consist of mixed inorganic and organic materials from the soil, along with partially digested plant residues and intestinal mucus that are voided after passing through the intestine. According to Spain *et al.* (1992), Pashanashi *et al.* (1996), Asawalam & Hauser (2001) and Bisht *et al.* (2006), earthworm casts can positively affect plant growth in the tropics, most likely due to higher concentrations of plant-available nutrients in casts than in uningested soil. Thus, the activities of earthworms have been shown to enhance plant yield (Curry & Boyle 1987) because of their beneficial effects on soil properties (Edwards & Bohlen 1996; Lee 1985).

The effects of earthworms on soil biological processes and fertility are associated with their ecological categories (Brown *et al.* 2000; Edwards & Bohlen 1996; Lee *et al.* 1985). Epigeic earthworm species live on or near the soil surface, consume plant litter and litter inhabiting organisms and ingest little or no soil. (Lavelle *et al.* 1997). Anecic earthworms live in permanent or semi-permanent vertical burrows in the mineral soil layers, feed on organic matter mixed with soil

particles. Endogeic earthworms typically live in mineral soil horizon, making horizontal burrows mainly at a depth of 15 cm. They consume more soil than other ecological categories and are often called soil organic matter feeders or geophagous. Because of low assimilation efficiency, endogeic species ingest and void large amount of inorganic soil particles in the form of casts and thus play an important role in respect of soil turnover.

The present study was carried out with soil and earthworms collected from the Narayankoti landscape in the Garhwal region of western central Himalayas, which includes both natural and human-managed ecosystem. We evaluated the effect of different diets on the endogeic earthworm *Drawida nepalensis*. This species was selected because it is abundant in the study site, with better adaptation and tolerance to various disturbances during crop rotation. *D. nepalensis* had a wider distribution and higher population densities relative to other earthworm species found in the study sites (Joshi *et al.* 2010). The objectives of the present study were to analyze: (i) the effects of three litter types on feeding and casting activities of earthworms provided with a surplus food; (ii) changes in properties of cast soil, and (iii) effects of earthworm casts on crop growth.

Materials and methods

The soil was collected from an agricultural field at Narayankoti (29° 30' 3"N and 79° 77' 02"E; altitude 1200 - 1800 m above mean sea level). The soil had a particle size distribution of 14 % sand, 51 % silt and 35 % clay. The soil was air-dried, crushed and passed through a 2-mm-mesh sieve. Soil (3 kg air-dried, sieved) was placed into a wooden box (30 cm wide × 30 cm long × 25 cm deep). The boxes were placed in a glass house at 25 - 30 °C for 60 days and the soil maintained at a gravimetric moisture content of 30 - 40 %.

The three litter diets consisted of leaves of grass (*Cynodon dactylon*), finger millet (*Elusine coracana*) and wheat (*Triticum aestivum*). These were collected from the study site and cut into 1 - 2 cm pieces. The leaves were moistened, stored at room temperature, and allowed to decompose for 10 days (Edwards & Lofty 1977) prior to use. Several studies have suggested that micro-organisms are essential for earthworm nutrition (Dechaine *et al.* 2005; Shipitalo *et al.* 1988).

Adults of *D. nepalensis* were collected from the study site by hand sorting, after digging a trench

that was 25 cm wide, 25 cm long, and 15 cm deep at each of the 10 sampling points, and cultured in the laboratory in the same soil. Five earthworms were added to each wooden box, treated with three litter diets. Five replicates per litter diet were maintained. The worms were fed the decomposing leaves (350 g wet weight), which were placed on the soil surface, and surface casts were collected weekly, dried and weighed. Decomposing leaves were added to each box and sample of each food type was kept to determine equivalent dry weight. The experiment was carried out for 60 days. After completion of the experiment, the boxes were emptied and the earthworms were separated, washed and weighed. The remaining food material was separated from the soil aggregates and weighed for each treatment, and food consumption and cast production rates were estimated. Surface cast and cast produced at the sides of the container were collected manually.

Casts and non-ingested soil without litter and earthworm were analyzed for pH using a digital pH meter and a 1:2.5 soil/water suspension. Organic matter was determined using the complete oxidation method (Nelson & Sommers 1975). Soil nitrogen was analyzed by the microKjeldahl method (Allen *et al.* 1974). Available phosphate was measured spectrophotometrically by the molybdenum blue method after extraction with sodium bicarbonate solution (Watanabe & Olsen 1965). Potassium was determined by flame photometer method after extraction with ammonium acetate buffer in 1:25 soil: extractant ratio at pH 7.

To analyze the effects of *D. nepalensis* on plant growth two treatments were applied: (i) a control treatment without earthworms, and (ii) an earthworm treatment with five clitellate individuals. Earthen pots (30 cm diameter, 25 cm height) were filled with sieved soil brought from the study site in June 2008. All pots were maintained at 25 - 30 % soil moisture in a glasshouse. Five seeds of finger millet and wheat were sown per pot. After 15 days, 3 plants were maintained per pot. After 3 months the plants were harvested and the root and shoot weight determined.

The correlation between feeding and casting rates among finger millet, wheat and grass was calculated as a simple correlation coefficient (*r*). Differences in parameters of different diets, characteristics of soil and cast was tested using one-way ANOVA. A two-way ANOVA was used to examine differences between the earthworm and control treatment across different samples.

Table 1. Food consumption, weight change, and cast production of *Drawida nepalensis* under glasshouse conditions for 60 days (n = 5; mean \pm SE).

Parameters	Finger Millet	Wheat	Grass	Significant/ Not significant
Initial weight (g live worm ⁻¹)	0.386 \pm 0.016	0.421 \pm 0.019	0.485 \pm 0.020	Not significant
Final weight (g live worm ⁻¹)	0.565 \pm 0.04	0.508 \pm 0.05	0.632 \pm 0.08	Not significant
Change in weight (g worm ⁻¹)	0.179	0.087	0.147	Not significant
Change in weight (%)	+46.37	+20.66	+30.3	Not significant
Mean growth rate (mg g ⁻¹ live worm ⁻¹)	7.2 \pm 0.7	6.3 \pm 1.7	6.6 \pm 1.3	Not significant
Weight specific growth rate (mg g ⁻¹ live worm ⁻¹)	18.65 \pm 1.1	14.96 \pm 3.2	13.60 \pm 3.3	Not significant
Food consumption (mg day ⁻¹)	7.8 \pm 0.8	9.6 \pm 1.7	12.2 \pm 1.8	Not significant
Cast production (mg g ⁻¹ live worm ⁻¹)	5.8 \pm 0.8	6.2 \pm 0.9	12.2 \pm 1.8	Not significant
Weight-specific cast production (mg g ⁻¹ live worm ⁻¹)	15.02 \pm 0.8	14.7 \pm 0.6	16.70 \pm 1.4	Not significant

Table 2. Changes in soil properties of non-ingested soil and casts produced by *Drawida nepalensis* under glasshouse conditions (n = 5; mean \pm SE).

Soil characteristics	Non-ingested soil	Earthworm cast
pH	5.24 \pm 0.8	6.05 \pm 1.2*
Organic matter (%)	3.96 \pm 0.21	4.82 \pm 0.86*
Organic carbon (%)	2.27 \pm 0.42	2.77 \pm 0.32*
Nitrogen (%)	0.29 \pm 0.01	0.33 \pm 0.02*
Phosphorus (%)	0.038 \pm 0.004	0.052 \pm 0.003*
Potassium (%)	0.018 \pm 0.001	0.027 \pm 0.002*
C:N ratio	7.82 \pm 1.1	8.39 \pm 1.6*

*Significantly different at $P < 0.05$

Results and discussion

The food consumption rate (mg dry food g⁻¹ initial live worm weight day⁻¹) was greater for earthworms fed wheat leaves and grass than those fed finger millet leaves (Table 1). No significant correlations were observed between initial earthworm biomass and weight-specific food consumption for any food substrates. Consumption rates ranged from 7.8 to 12.2 mg dry weight day⁻¹ and weight-specific food consumption was recorded as; 20.2 mg g⁻¹ live worm day⁻¹ for finger millet, 22.8

mg g⁻¹ live worm day⁻¹ for wheat and 25.2 mg g⁻¹ live worm day⁻¹ for grass. According to Satchell (1983) food palatability is the most important factor influencing food consumption of earthworms, which is also influenced by the durability of the food particles (Bostrom & Lofs Holmin 1986). Higher food consumption by *D. nepalensis* of grass than wheat and finger millet leaves suggests greater palatability and preference for grasses, although the differences were not significant. The consumption rates obtained in the present study probably overestimate actual values, since other food consumption processes, such as decomposition, were not taken into account.

Changes in the weight of earthworms were not related to diet, although earthworms ingested lower amounts of finger millet leaves, yet they showed a higher growth rate in comparison to grass and wheat diets. However, the initial weight for worms fed with finger millet was 20 % less than their counterparts in grass (Table 1). The average growth rate of *D. nepalensis* (6.3 to 7.2 mg day⁻¹) was lower than the rate reported for *L. terrestris* and *L. rubellus* (epigeic species) (10 mg day⁻¹) (Devliegher & Verstraete 1997; Shipitalo *et al.* 1988). However, they were similar to those for *Octolasion tyrtaeum* (endogeic) (5.8 to 7.4 mg day⁻¹) (Bisht *et al.* 2006).

For all diets, granular composite irregular surface casts that formed a coherent mass were produced. Mean weight-specific cast production rate for all food substrates was 15.5 mg live worm day⁻¹ (Table 1). The cast production rates of *D. nepalensis* (14.7 to 16.7 mg g⁻¹) were low compared to 0.5 to 1.5 g g⁻¹ live worm day⁻¹ reported for *Apporrectodea caliginosa* (endogeic) (Zhang *et al.* 2009) and 12.6 to 24.1 mg g⁻¹ live worm day⁻¹ reported for *O. tyrtaeum* (Bisht *et al.* 2006), but higher than the 4.2 to 6.7 mg g⁻¹ live worm day⁻¹ reported for *A. alexandri* (endogeic) (Kaushal *et al.* 1993).

Nutritional properties of cast and non-ingested soil

The mean pH of casts was higher than non-ingested soil (Table 2). The organic matter, organic carbon, nitrogen, phosphorus and potassium content of cast soils increased significantly ($F_{1,6} = 7.54$, $P < 0.05$) by 21.7 %, 22.0 %, 13.7 %, 36.8 % and 50.0 %, respectively, relative to non-ingested soil. Earthworms are known to profoundly affect microbial activities and organic matter dynamics in soil that passes through their gut, which seems to be the most important factor in the development of sustainable earthworm distribution (Edwards 2004; Joshi *et al.* 2010; Lowe & Butt 2002). The C:N ratio of casts was significantly ($F_{1,6} = 7.54$, $P < 0.05$) higher than that of non-ingested soil. Several studies have reported that earthworm casts have a higher C:N ratio than non-ingested soil (Lavelle *et al.* 1992; Lee 1985). The activity of endogeic earthworms in the humid tropics accelerates initial soil organic matter turnover through indirect effects on soil C as determinants of microbial activity. Due to selective foraging of organic particles, the gut contents are often enriched in organic matter, nutrients and water compared with uningested soil, and can foster high level of microbial activity (Haynes *et al.* 1998; Kale 2008). In addition, a higher concentration of extractable P and K was observed in earthworm casts produced by *D. nepalensis* (Table 2). Similar effects have been reported by several workers (Bisht *et al.* 2006; Kaushal *et al.* 1993; Sharpley & Syres 1977), which could be due to enhanced earthworm activity and to the physical breakdown of plant material. The increase in extractable P levels in cast soil was probably caused by modifications of the pH in earthworm casts. This might be due to the difference in Ca content and organic matter. Sabrina *et al.* (2009) also reported that Ca and

organic matter of certain residues are able to correct the acidity of cast. Thus the pH of cast becomes higher and simultaneously modifies P content in cast then in uningested soil. It indicates that the P produced by earthworms in their casts was the labile P in inorganic fraction. Earthworms in the field incorporate P from litter or other organic sources (i.e. undecomposed plant or root material, earthworm faeces) which is not normally measured in the analysis of uningested soil (Jimenez *et al.* 2003). Increases in the extractable K content of casts relative to surrounding soil may be due to the displacement of K⁺ ions from the edge sites of clay minerals by NH₄⁺ ions generated by enhanced mineralization of organic N (Basker *et al.* 1992). According to Edwards & Bohlen (1996) the elevated levels of cations often observed in earthworms cast in field can also be due to selective feeding by earthworms on materials enriched in those cations.

Effects of earthworms on crop growth

The results of the crop growth experiment showed that, compared to the control treatment, the dry weight yield of plant biomass was not significantly higher in the earthworm treatment (Table 3). Although the differences were not significant, the dry weight of shoots in earthworm treatment increased by 43.1 % for finger millet and by 32.6 % in for wheat relative to the control, whereas the dry weight of roots increased by 26.6 % in finger millet and 26.9 % in wheat (Table 3).

Table 3. Dry weight (g dry wt.) of finger millet and wheat plants after 90 days of growth in treatments with and without earthworms under glasshouse conditions (n = 5; mean ± SE).

	Crop			
	Finger millet		Wheat	
	Root	Shoot	Root	Shoot
Control	6.4 ± 1.34	10.2 ± 2.1	5.2 ± 1.1	8.6 ± 1.13
Earthworm	8.1 ± 1.82	14.6 ± 4.2	6.6 ± 1.01	11.4 ± 3.4
Significant/ Not significant	Not significant	Not significant	Not significant	Not significant

There is considerable evidence from pot trails that earthworms promote increases in plant growth. For example, the presence of *Millisonia anomala* increased the growth of maize (Spain *et al.* 1992), *Allolobophora parva* increased the growth of barley plants (Temirov & Valiakhmedov 1988),

and *Apporrectodea trapezoids* appeared to increase the shoot weight of wheat by 53 % and grain yield by 35 % (Stephens *et al.* 1995). Marked effects of earthworms on plant growth have also been demonstrated in small-scale field experiments, with dry matter increases ranging from 20 - 100 % (Bisht *et al.* 2006; Curry & Boyle 1987; Stephens & Davoren 1995).

Conclusions

The results of our feeding and casting experiments indicated that earthworm preferred grasses over wheat and finger millet leaves, and produced high amounts of casts with the same. Compared to the control treatment, plant biomass (root & shoot) was higher in the earthworm treatment. Further field and glasshouse trials are required using a range of earthworm densities and environmental conditions in order to establish the optimum conditions under which enrichment of soil with *D. nepalensis* can increase crop yields. Further understanding of the biology and ecology of earthworms will help in developing the strategies that may influence the soil biota and crop performance.

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