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The Use of Wastes from Various Agricultural Processes as Foods in Earthworm Cultures

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Abstract

Earthworms are dentritivorous and geophagous animals. The quality of food provided in their cultures is a crucial parameter for a successful rearing. Many wastes of agro-industrial operations contain plenty of nutrients and consist abundant and cheap stuff for earthworm diets. We tested the suitability of nine types of easily accessible materials as foods for the anecic earthworm species Octodrilus complanatus. The food quality was assessed based on the body weight changes at 28, 56 and 84 days. Most of these materials can be utilized as organic fertilizers, and for this reason some important soil parameters were estimated too. The diets were: olive leaves, olive leaves with olive mill wastewater (OMW) applied at doses equivalent to 40m^3 ha^{-1} and 80m^3 ha^{-1}, plain OMW, cow manure, cow manure with whey applied at a dose 80m^3 ha^{-1}, cotton gin residues, lawn clippings and vine leaves. The rearing substrate was natural soil.

All the diets, except plain OMW, were equivalent and promoted earthworms' growth. The best diets were the cotton gin residues and the combination of olive leaves + 40 m^3 ha^{-1} OMW. Plain OMW induced lethal effects on earthworms. The diets influenced soil pH, organic matter, total N, respiratory activity and C/N ratio in a similar manner but some significant differences were detected. Among them whey induced an increase in electrical conductivity and total salts which however, did not exceed the normal limits in the natural soil.

Keywords: *earthworm cultures; earthworm diet; earthworm growth; Octodrilus complanatus; agricultural wastes; soil amendments*

1. Introduction

Earthworm rearing is undertaken in cases that it is desirable to obtain large populations in order to benefit from the use of the animals or from their activities. It is the rule for vermicompost production [1,2,3,4]. There are many commercial vermicomposting units in Europe and all over the world [2,5,6] as well as in Greece. Similar to this endeavor is the practice of vermicomposting all agricultural byproducts and crop residues by the owner of a farm and to convert them into organic fertilizer for the next cropping season, in a circular production model [5]. This is a recommended procedure for farmers that produce according to the sustainable system both in developed and developing countries in order to lower the inputs of the chemical fertilizers and to reduce the cost of production [5,7].

Due to the desirable effects of earthworms in land, their culturing may aim to reinforce the natural populations of the soils through earthworm inoculations, mainly in fields that return low yields or produce under the organic management [8,9,10,11]. Moreover, a good and promising strategy to increase earthworm populations in the fields is to create favorable conditions so that the earthworms will thrive and proliferate in situ and return the beneficial functions that characterize their presence [12]. One measure that can be taken to achieve this goal is to amend the soil with organic materials that qualify as diets for earthworms [13,14,15].

Culturing of the earthworms is a technique through which we can study their behaviour and which can assist in uncovering unknown aspects of the biology and physiology of this precious group of animals and their interference in the balance of the natural soil processes [11,16,17]. It is also indispensable in order to obtain synchronous and genetically identical individuals to be used in ecotoxicological trails [1, 18,19].

The most widely used species that are reared in cultures belong to the guilt of epigeics because this can process organic matter irrespectively of the presence of inorganic soil which is a prerequisite for vermicomposting and, in general, it simplifies the procedure of culturing significantly [20,21,22,23]. Nevertheless, representatives of anecics and endogeics have been elaborated for rearing and succeeded to produce sustainable cultures [14,24].

The most crucial parameter for the success of earthworm rearing is the type of organic matter that is provided for nourishment [10,13,17,25]. A good food is the one that accelerates growth and builds big and prolific individuals [1,10,13,26]. The diet of the earthworms should include all the necessary elements and active compounds for their life at desirable concentrations, provide them with adequate energy for their functioning and be devoid of toxic substances. Among the latter, the most common ones are heavy metals, polyphenols at high concentrations, pesticides and antibiotics or excess of ammonia. These may result in bioaccumulation [27,28] and negative reproduction performance [29,30], induction of oxidative stress and neurotoxisity [31,32], loss of weight, oxidative stress [33] and cease of reproduction [2] and direct lethal effects [2], respectively. Particularly polyphenols, when included in high concentrations, can cause lowering in palatability and rejection of food [22,34]. It is known [35,36] that, when a diet has a balanced composition regarding energy and protein, it is more desirable for earthworms and this ends in a successful rearing. The above can be expressed by the ratio C/N which is considered best in the range 25-30 [25,36].

Ideally the potential diet of earthworms should be a cheap and readily available material similar to those that constitute the diet of the earthworms in nature. From the other hand, there are many other very nutritive materials that are produced through certain agro-industrial processes which have not been tested as earthworm diets. Some of these materials are traditionally used as efficient food sources of various animal groups other than earthworms e.g. cotton gin residues and whey [37,38]. Residues that come from natural procedures and originate from very common raw materials in the ordinary environment of earthworms, are expected to be promising diets for them, like the leaves of olive and vine or the lawn clippings. From the other hand, agro-industrial by-products that have been authorized to be recycled into orchards in an attempt to save water and reduce chemical fertilizer inputs [31], like Olive Mill Wastewaters (OMW), have not been tested for their effects on earthworms' nourishment adequately. The above are expected to contribute to earthworm nutrition substantially, as they do for plant nutrition.

The present study was designed to fulfill a double aim: a) to test a set of solid and liquid wastes for their performance as foods of the very common anecic earthworm species in Greece, *Octodrilus complanatus* (Dugès, 1828), in order to obtain information for its culturing and b) to have indications on the suitability of the above wastes to be used as soil amendments in agricultural land, because soil organic fertilization is multiply beneficial when it is also capable to augment the earthworm populations as food supplements. The materials used were residues from some very important and common branches of the rural business activities and some of these have not been tested as potential earthworm diets or soil amendments before.

2. Methodology

2.1. Soil and animals

The experiment was performed in plastic rectangular boxes with a size 13.5 x 19.3 x 10.0cm (width, length, height) and a free cross area 260.55cm^2. At the bottom of each box small holes were drilled to facilitate drainage and at their lid a wide opening, sealed with mess cloth was made to permit air renewal. Each box was filled with 1,300g of air dried and sieved up to 2mm natural soil, originating from the olive orchard of Agricultural University of Athens (A.U.A.). Some important soil properties are presented in table 1.

Table 1. *Some important physicochemical parameters of the soil used as experimental substrate to test the effects of various diets on the growth of the earthworm species O. complanatus.*

Parameter	Value	Parameter	Value
granul. Class*	L	$CaCO_3$ (%)	33.20
sand (%)	40.30	E.C.* (mS cm^{-1})	0.94
silt (%)	40.00	total salts (%)	0.04
clay (%)	19.70	total N (mg g^{-1} d.w.)	0.72
pH	7.54	W.H.C.* (% d.w.)	45.07

**granul. class: granulometric class, E.C.: electrical conductivity, W.H.C.: water holding capacity*

For the determination of the soil properties, at the beginning and the end of the experiment, the following methodologies were used: 1) granulometric analysis on the basis of Bouyoucos method [39] 2) soil pH in aqueous solution (1:1) by means of pH – 2005 pH meter, SELECTA, Spain [40] 3) total soil $CaCO_3$ with the Bernard apparatus [39] 4) total soil organic matter using Walkley – Black chromic acid wet oxidation method [40] 5) specific electrical conductivity (E.C.) of the aqueous solution (1:2) and T=25^oC, by the organ 5-EaSy, Mettler Toledo, Switzerland [40] 6) total salts were assessed from EC according to the empirical equation (0.128 x EC x SP)/200, where SP=water content in saturation (% d.w.) 7) total N using the Keldhal method [40] 8) water holding capacity (W.H.C.) as a weight difference after free drainage for 3 hours under a glass cover [41] and 9) soil microbial respiration by means of a modified laboratory Birch & Friend apparatus [42].

The choice of the species *O. complanatus* was based on its broad distribution in the Mediterranean area and its high dominance and frequency in Greek cultivated soils, that have acknowledged it as good bioindicator of human interventions. Moreover, its big body facilitated in experimentation and biological studies. The individuals used in this study were obtained from the rearing maintained in the laboratory of Agricultural Zoology and Entomology of A.U.A. In each experimental box, 4 individuals were placed. Three of them were at the reproductive stage with well-developed clitella. When preparing the clusters of earthworms, an effort was made to have equal total body weight in all boxes. The measurement of the initial body weight were taken after 24 hour fasting on a wet filter paper in order to expel the majority of the content of their alimentary track. The animals were gently dried by placing them on a soft absorbing paper and weighted in accuracy 0.01g with a laboratory scale (Portable, Sartorius AG, Göttigen, Germany).

2.2. Food Types

2.2.1 Solid wastes

The solid wastes used were the following: olive leaves, residues of cotton gin, lawn clippings, cow manure and vine leaves.

Fresh olive leaves were the byproducts of the olive harvest and were obtained from a conventional olive mill close to Athens. Manure was obtained from the A.U.A. cattle stable and originated from animals under a semi-intensive conventional rearing system. The residues of cotton ginning originated from a plant that was processing conventional cotton harvests. The lawn clippings were obtained from the yard of A.U.A and the vine leaves came from an organic vineyard and were collected from the soil during their autumn shed.

It was decided that a small period of natural preconditioning was necessary for all solid materials before they can be used as diets safely [14]. For this purpose, the foods were piled on the floor of an unheated greenhouse for 20 days without adding moisture. The temperature during this period fluctuated from 10 – 15^oC. Subsequently the materials were stored in plastic bags in a place with low temperature (about 15^oC) until their use. The solid foods were offered to the earthworms after being grinded with a kitchen blender, because it is known that small particles can be more easily ingested by earthworms [13,24]. The size of the particles offered as foods were less than 3mm.

In table 2 some important parameters of the solid food types are presented.

Table 2. *Some important chemical parameters of the solid materials used as foods of the earthworm species O. complanatus*

Parameters	olive leaves	cotton residues	lawn clippings	vine leaves	manure
C (%d.w.)	50.75	46.64	45.07	51.22	46.81

ash (%d.w.)	12.50	19.60	22.30	11.70	19.30
total N (%d.w.)	1.29	2.32	2.47	0.84	2.55
C/N	39.30	20.10	18.25	60.98	18.36

2.2.2. Liquid Wastes

Two liquid wastes of the agricultural industry were tested as foods: OMW and whey.

OMW were used in the present experiment as amendment of the olive leaves at two doses. The first dose was being equal to the maximum permitted dose for liquid fertilization according to the Hellenic official law ($80m^3$ ha^{-1}) while the half of the above ($40m^3$ ha^{-1}) was the second one dose. Motivated from the positive effects they had on earthworm growth when used together with vine leaves [43], plain OMW were also tested for their suitability as exclusive and compulsory diet for earthworms. The dose of plain OMW was decided to be the double of the higher dose ($160m^3$ ha^{-1}) in order to cover the nutritional requirements of the earthworms. After their collection on November, OMW were preserved in the refrigerator at 3^oC until their use in February of the next year.

The whey used in the present study consisted of a mixture of sheep and goat milk and was obtained from a creamery in the area of Marathon, approximately 60 km from Athens. This was a heated residue of a double (feta cheese and anthotyros) production. It was stored in the freezing, till its use. The whey was provided in the diet at a rate equivalent to the maximum dose of OMW ($80m^3$ ha^{-1}) as an amendment of manure, due to their similar origin. The choice of this dose was in accordance with the maximum dose selected for OMW to have comparable treatments.

Some properties of the OMW and the whey are presented in table 3.

Table 3. *Some physicochemical characteristics of the liquid wastes used as foods of the earthworm species O. complanatus*

Physicochemical characteristics	OMW	Whey
specific weight (g. ml^{-1})	0.9896	1.018
moisture (%) w/v	90.29	94.75
C (%) w/v	7.607	6.117
dry weight (% v)	8.67	7.05
total N (g l^{-1})	0.50	0.34
E.C. (mS cm^{-1})	9.11	13.68
C/N	88.10	104.20
pH	4.97	6.38
fat (%)	not estimated	0.467
proteins (%)	not estimated	0.490
lactose (%)	not estimated	5.267

The determination of the parameters of the solid (table 2) and liquid (table 3) ingredients of the diets was done at the laboratories of Dairy and Agricultural Chemistry & Soil Science of the A.U.A. using the following methodologies and analytical organs: 1) Ash and organic matter determination according to the loss-on-ignition method [40]. Incineration took place in a furnace at 500^oC for 24 hours and dehydration in other furnace at 60^oC for 48 hours for the solid materials and at 105 ^oC for 24 hours for the liquids [41]. 2) Organic C was estimated by using the empirical equation: (organic C) = (organic matter) X 1.724 [40]. 3) Total N was measured by the Keldhal method [40]. 4) electrical conductivity (E.C.) was measured at $T=25^oC$, by the organ: 5-EaSy, Mettler Toledo, Switzerland. 5) pH of the OMW was measured with a pH-meter, type: pH – 2005 pHMETER / J.P.SELECTA, s.a. SPAIN. pH of the whey was assessed with the aim of the organ: MilkoScan 133/Telso. 6) fat, protein and lactose in the whey were determined by the organ: MilkoScan 133/Telso.

In total, nine different foods and combinations of foods, were tested: olive leaves, olive leaves + OMW in dose $80m^3$ ha^{-1}, olive leaves + OMW in dose $40m^3$ ha^{-1}, plain OMW at dose $160m^3$ ha^{-1}, cow manure, cow manure + whey in dose $80m^3$ ha^{-1}, residues of cotton ginning, lawn clippings and vine leaves. Due to the long duration of the experiment, which lasted for 3 months, no real control (no food provision) was included, in order to avoid the inevitable sacrifice of the animals which would definitely die from starvation or loose so much weight that they would not be able to recover again.

2.3. Experimental Set up and Procedure

The soil into the plastic boxes was wetted with deionized water equal to 40% of the dry soil weight (w/w), except in the boxes that received liquid wastes where lower volume of water was applied. The boxes remained in a non-heated room for 2 days, and on the third day the groups of the 4 earthworm individuals were placed on the soil surface of each box together with 4g of food. The liquid amendments were provided in 2 equal portions, by dispersing on the soil surface, one day after the introduction of the earthworms and 1.5 months from the initiation of the experiment. The dose of $160m^3$ ha^{-1} equals to $416.8cm^3$ of liquid per box. These were applied in three equal sub-doses at times as follows: the first at the initiation of the experiment, the second on the 22^{th} day and the third on the 45^{th} day (1.5 months from initiation), by dispersing on the soil surface. The earthworms were fed with solid foods in weekly intervals by placing enough quantity of each grinded food on the soil surface so as the earthworms could feed whenever they needed to, as they would do in nature. Every 3 – 4 days the water losses due to evaporation were assessed by weighing the boxes by means of a cooking scale (Gold – PYREX®, France) having 1g accuracy and replaced by spraying deionized water.

The boxes were kept in an unheated room where the temperature was measured to fluctuate from 14 to 23^oC during the entire experimental period. The light regime was 24 hours darkness in order to stimulate food consumption because it is known that earthworms are active only in darkness.

During the first days of the experiment, dead individuals appeared on the soil surface. These were removed from the box on a daily basis and replaced by new, healthy individuals till the 7^{th} day. Their counting was necessary to calculate mortality, though the influence of the diet on body weight was estimated on the basis of the number of living specimens per box at the end of the experiment.

The growth of the earthworms was assessed on the 28^{th}, 56^{th} and 84^{th} day, by emptying the boxes and weighting the earthworms. After the weight changes were measured, the soil and the earthworms were returned in the boxes.

On the 84^{th} day the experiment finished and the earthworms were subjected to the final measurement of their body weight. In order to record the influence of the foods on reproduction, the soil was searched carefully for cocoons and newborns. Finally, a sufficient quantity of the soil of each unit was preserved in the refrigerator, at 3^oC, until it was analyzed to determine the influences of food on the soil parameters.

2.4. Statistical Analysis

The experimental design was a factorial and was analyzed according to the complete randomized model. The factors were two: a) time with 3 levels (28, 56 and 84 days) and b) food with 9 levels. Four replicates were used per treatment. The growth of the earthworms under the influence of the different foods was estimated by the mean body weight change of the group of the 4 individuals per replication from the initial weight. Due to the weight differences of the groups at the initiation of the experiment, even though insignificant, the comparisons were accomplished using the percentages of the initial weight changes. The analysis was performed using a two-way ANOVA after the data of the percentages of the weight increase of each group were arcsine transformed. The influences of the food on the soil parameters were tested using one-way ANOVA. The analysis was done with the statistical computer software JMP [44] and the means were compared by the Tukey HSD criterion at a level of significance α=0.05 but Dunnett was used too. All data are presented as means and standard errors (mean ± S.E.).

3. Results

3.1. Influence of the Diet on Earthworms

The percentages of earthworm deaths in each food which were counted at the beginning of the experiment and during the monitoring of the body weights are presented in table 4. To assess the survival under the various diets the treatment "vine leaves" was selected to become the control, due to its high preference by the earthworms, high digestibility and lack of toxic substances (e.g. polyphenols).

Table 4. *Cumulative mortality rates of the earthworm species O. complanatus fed by various organic materials in three consecutive monthly periods (% ± S.E.).*

Food type	Total individuals	28 days	56 days	84 days
olive leaves	16	0.0 ± 0.0^a	0.0 ± 0.0^a	6.3 ± 6.3^a
olive leaves + 40	17	10.0 ± 10.0^a	16.3 ± 9.8^a	16.3 ± 9.8^a
olive leaves + 80	17	5.0 ± 5.0^a	5.0 ± 5.0^a	11.3 ± 6.6^a

plain OMW	18	75.0 ± 10.4[b]	100.0 ± 0.0[b]	100.0 ± 0.0[b]
manure	16	18.8 ± 11.9[a]	18.8 ± 11.9[a]	25.0 ± 17.7[a]
manure + whey	17	16.3 ± 9.8[a]	16.3 ± 9.8[a]	21.3 ± 14.2[a]
cotton gin residues	16	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]
lawn clippings	16	12.5 ± 12.5[a]	18.8 ±18.8[a]	25.0 ± 17.7[a]
vine leaves (control)	16	0.0 ± 0.0[a]	0.0 ± 0.0[a]	18.8 ± 11.9[a]

Values of a certain column followed by the same letter do not differ statistically from the control according to Dunnett (a=0.05).

Mortality rates showed a tendency to rise with time in all foods except in cotton residues. The ANOVA testing showed significant difference in all time periods, including the final cumulative percentage of death ($F_{8,27}$=6.80, P<0.0001). The comparisons of the Dunnett test resulted in an only significant difference between the plain OMW and the control sample. Plain OMW when used as exclusive and compulsory diet at the dose of $160m^3ha^{-1}$ caused severe death to the earthworms from the first sub-dose and when all the quantity was provided, no earthworms could survive. In order to justify the reasons of the deaths in the treatment of plain OMW, soil samples were analyzed to determine pH and electrical conductivity. The estimated means confined into the natural limits for plants and the majority of soil organisms' growth and took values 7.50 ± 0.01 and 3.29 ± 0.10 mS cm^{-1} respectively. Both liquid wastes when used as additives in the earthworm diet did not affect survival negatively, since no increase percentages were recorded during the second measurement (in 56 days), which followed the application of the 2nd sub-dose (in 45th day). Olive leaves, cotton residues and vine leaves had low percentage death at least during the two first time periods.

In figure 1 the mean individual body weight changes as a percentage of the initial value in each period of monitoring are presented. The analysis was performed excluding the treatment plain OMW due to the death of the individuals. All the foods induced similar effects in the body weight from the initial, as it is depicted in the figure. Nevertheless the influence of plain OMW was negative. Also, the ANOVA of the mean body weight changes on the basis of the whole experiment was not significant ($F_{23,72}$=0.94, P=0.54) at 0.05 level.

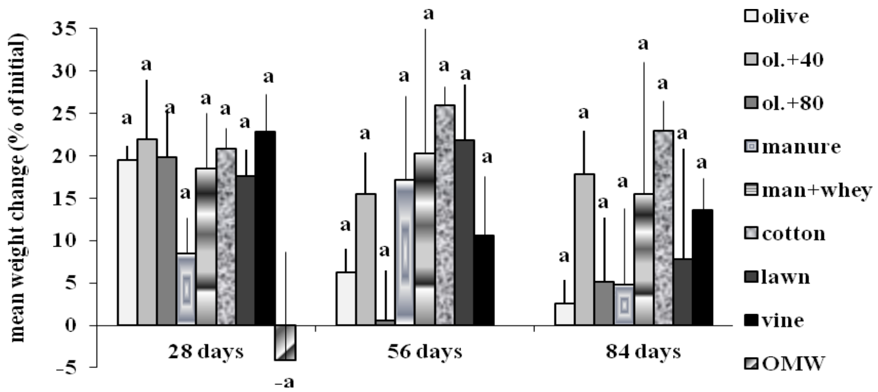


Figure 1. *Mean individual body weight changes of the earthworm Octodrilus complanatus with diet as a percentage of the initial body weight in three consecutive months (% means ± S.E.). Columns connected with the same letter do not differ significantly (Tukey HSD, a=0.05)*

The mean individual body weights and their statistical comparisons, according to ANOVA and Tukey HSD in each time and totally, are presented in table 5. It is obvious that the performance of either doses of OMW as an additive did not differ significantly from whey according to Tukey HSD in all periods and totally. Moreover, the body weight increased with time in all diets except OMW. These increases were significant with olive leaves + 40 ($F_{3,12}$=3.80, P=0.0399), cotton residues ($F_{3,12}$=9.78, P=0.0015) and vine leaves ($F_{3,12}$=5.24, P=0.0153) (the comparisons are not shown in the table).

Table 5. *Mean individual body weight of the species O. complanatus fed by various organic materials at the beginning, after 28, 56 and 84 days and the mean total effect of the diet (g ± S.E.).*

Food type	0 days	28 days	56 days	84 days	total
olive leaves	4.46 ± 0,07[a]	5.34 ± 0.11[a]	4.74 ± 0.06[a]	4.58 ± 0.18[a]	4.89 ± 0.22[a]
olive leaves + 40	4.39 ± 0,05[a]	5.36 ± 0.29[a]	5.08 ± 0.24[a]	5.18 ± 0.20[a]	5.21 ± 0.22[a]

olive leaves + 80	4.37 ± 0.04[a]	5.24 ± 0.23[ab]	4.38 ± 0.23[a]	4.60 ± 0.32[a]	4.74 ± 0.22[a]
plain OMW	4.41 ± 0.02[a]	3.18 ± 1.16[b]	0.00 ± 0.00[b]	0.00 ± 0.00[b]	1.06 ±0.22[b]
manure	4.35 ± 0.04[a]	4.73 ± 0.20[ab]	5.10 ± 0.40[a]	4.56 ± 0.36[a]	4.79 ± 0.22[a]
manure + whey	4.38 ± 0.03[a]	5.07 ± 0.41[ab]	5.28 ± 0.79[a]	5.13 ± 0.64[a]	5.16 ± 0.22[a]
cotton gin residues	4.38 ± 0.02[a]	5.29 ± 0.11[a]	5.52 ± 0.10[a]	5.38 ± 0.13[a]	5.39 ± 0.22[a]
lawn clippings	4.37 ± 0.04[a]	5.15 ± 0.17[ab]	5.33 ± 0.31[a]	4.73 ± 0.60[a]	5.07 ± 0.22[a]
vine leaves	4.36 ± 0.05[a]	5.35 ± 0.13[a]	4.80 ± 0.27[a]	4.95 ± 0.19[a]	5.03 ±0.22[a]

Values of a certain column connected with the same letter do not differ statistically according to Tukey HSD (a=0.05).

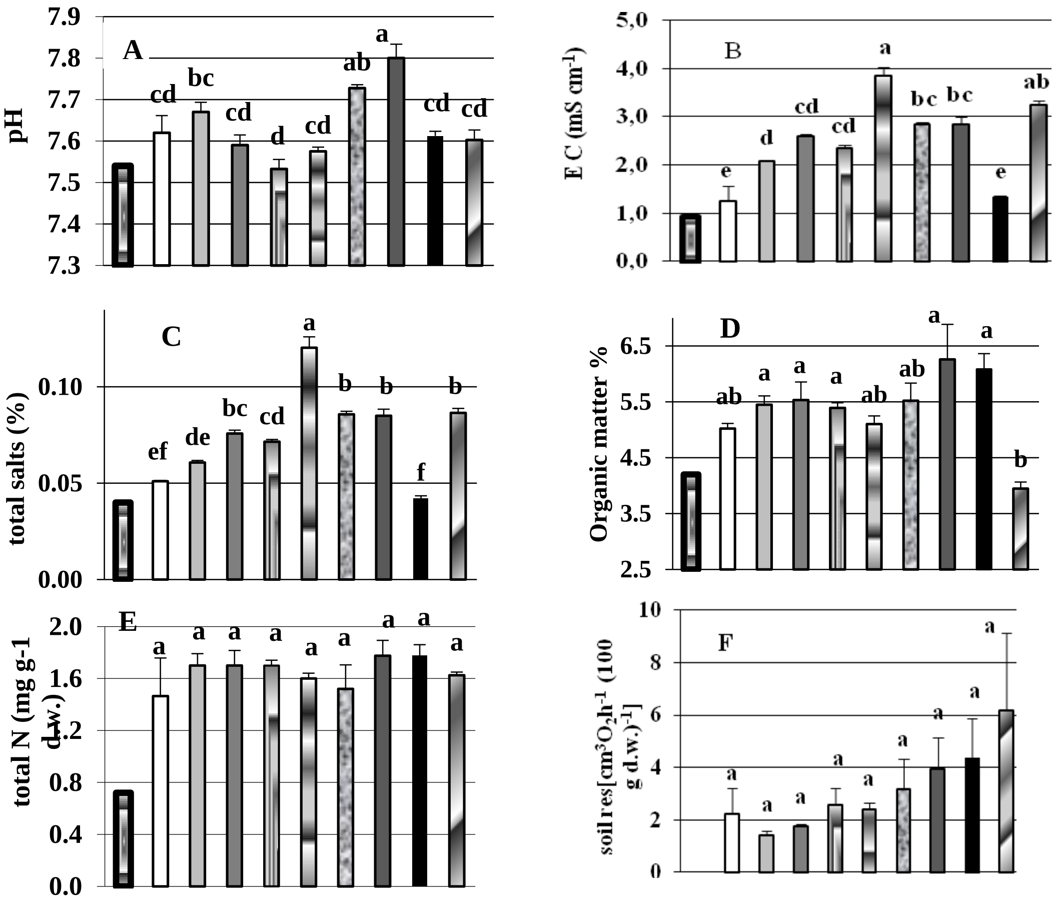
The number of cocoons and young juveniles found in the soil of the cultures are given in table 6. All treatments had cocoons or juveniles, except of olive leaves + 80, manure + whey and plain OMW.

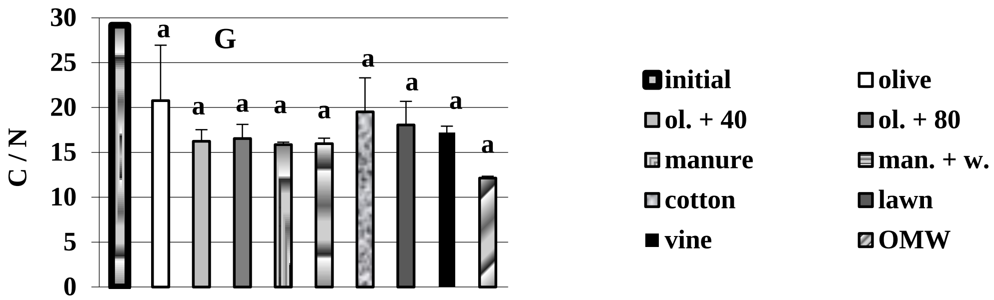
Table 6. *Reproduction of the earthworm species O. complanatus after 84 days nurishment with various diets.*

diet	offsprings	diet	offsprings
olive leaves	2 cocoons	**manure + whey**	–
olive leaves + 40	2 cocoons	**cotton residues**	2 cocoons
olive leaves + 80	–	**lawn clippings**	1 cocoon
OMW	–	**vine leaves**	1 cocoon
manure	1 cocoon + 1 juvenile		

3.2. Influence of the Foods on Soil

In figures 2A-G the infuences of the various diets on some important soil parameters at the end of the experimental time (84 days) have been plotted along with the initial value. The statistical comparisons of the means, after Tukey HSD, are depicted in the same figure (α=0.05, Q=3.3647).





Figures 2. *Changes of some important soil parameters as a result of nourishment of the earthworm species O. complanatus with various diets for a period of 3 months (means ± S.E.). A. pH, B. Electrical conductivity, C. Total Salts, D. Organic matter, E. Total Nitrogen, F. Soil respiration rate G. C/N ratio. Columns connected with the same letters do not differ significantly (Tukey HSD, a=0.05)*

As far as pH is concern (fig. 2A), all treatments, even the plain OMW, resulted in slight increases from the initial value after 84 days of application. The ANOVA ($F_{8,27}$=11.40, P<0.0001) was significant for pH in which lawn clippings caused the highest rise, differing from all other foods except from cotton residues (Tukey HSD, a=0.05).

All foods raised soil electrical conductivity in comparison to the initial (fig 2B), but none of them surpassed the upper limit for agricultural cropping, namely 4mS cm^{-1}. The ANOVA for testing the means of the foods was significant ($F_{8,27}$=41.76, P<0.0001). The combination "manure + whey" caused the highest rise, followed by plain OMW. Olive and vine leaves caused significantly lower rises in E.C. compared to the other foods (Tukey HSD a=5%). The same was observed for soil total salts ($F_{8,27}$=69.83, P<0.0001) (fig. 2C).

All soils had higher organic matter than the initial value (fig. 2D). The ANOVA ($F_{8,27}$=5.33, P=0.0005) separated the foods concerning soil organic matter enrichment, but the test of Tukey HSD revealed that only plain OMW differed significantly from most of the other foods. The soil concentration in total N in all treatments including plain OMW increased from the initial value (fig. 2E), but the soils did not differ regarding this parameter ($F_{8,27}$=0.63, P=0.75). All foods caused a reduction in C/N ratio compared to the soil initial value (fig. 2G). No statistical differences were detected according to ANOVA ($F_{8,27}$=0.84, P=0.57).

No statistical differences were found between foods considering soil microbial activity ($F_{8,27}$=1.29, P=0.29) (fig. 2F). Plain OMW had apparently, but not statistically, much higher O_2 consumption compared to the rest foods.

4. DISCUSSION

4.1 Influence of the Foods on Growth of *O. complanatuss*

Taking into account the small groups of experimental animals in the boxes, the mortality rates recorded in the various diets, except in plain OMW, were not high. In manure, during the first period, slightly higher deaths were recorded 18.8 ± 11.9% than these of the other diets. Although this material had undergone preconditioning in order to be cleared of deleterious volatile compounds and undesirable organisms [14], it may retain some hazardous ingredients that had a lethal effect on earthworms. Since the changes of the soil characteristics that were induced by the foods, as measured at the end of the trials (figures 2A-G), did not surpassed the "normal limits" for the growth of the plants, the deaths probably were not caused by such environmental shifts. One potential cause of mortality is the discomfort or the injuries that were made in earthworms' bodies while handling them for counting, as it is suspected from the increase deaths in the consecutive periods.

Having excluded the hazardous influence of soil parameters on earthworms in the diet with plain OMW, we can presume that deaths in this case were due to some biotoxic substances that OMW contain in high concentrations, such as polyphenols, fats and high total phosphorus [45,46,47]. Earthworms cannot tolerate high doses of this waste, which was proved to be repulsive according to [48]. Indeed, it is known [31,32,49] that the mandatory feeding with OMW may have caused oxydative stress, neurotoxicity and death. Moreover, the organic ingredients of the OMW are mainly organic sugars, organic acids, phenolic compounds, polyalcohols, pectins and oil [50]. Celluloses are included in very low concentration, since they are water insoluble and cannot be separated from the solid residue during the oil extraction process. Anecic earthworms basically consume litter and, although they prefer to ingest litters rich in protein and soluble carbohydrate [2,35], they are capable of breaking down cellulose by their own enzymes [2,51] which is their main resource of energy. Another adverse property of OMW is that, due to their high concentration in suspended solids, they may reduce soil porosity [52,53] and inhibit the aerobic soil functions [54] when added in high dose. The accumulation of solid material on soil surface was observed by us during the present experiment, too.

OMW as an amendment of olive leaves had a positive influence on mean body weight, especially when used in the lower dose that induced significantly increase in mean body weight with time in addition to the positive influence on mean body weight compared to the plain olive leaves (table 5, figure 1).

All the foods, except plain OMW, were equally capable to increase the mean body weight and non significant differences were observed between them in each time period. Vegetative and animal origin foods were not differenciated on the context of body weight change. This is not in agreement with the findings of [55], who concluded that horse manure had a positive effect on the body weight and the number of cocoons produced from the species *Lumbricus terrestris* as compared to the leaves of birch. The above differenciation may be species depending or can be attributed to the specific nature of the experimental materials.

The use of whey improved, though not significantly, the positive effect of manure on mean body weight. This was evident because whey contains many nutritive ingredients, like proteins and lactose (table 3), although it has a very high ratio C/N (104.2). Since it is the first time that this residue is used as diet of the earthworms, there is dearth of information in the literature. Nevertheless, it is known [56] that earthworms accepted this liquid dairy waste and were able to process it and render an almost unpolluted liquid when used as part of the biofilter along with microorganisms, with or without plants. High salinity is the negative attitude of whey (table 3) which, though, had no negative impact on earthworm growth. However, the salty nature of whey may be the reason for not obtaining offspring in this treatment (table 6), since it is known [57] that high salinity levels affect negatively reproduction.

The best food for growth and survival, though not significantly, was the residues of cotton gin. This material is rich in energy because it includes many cytarinaceous ingredients, like fibres and small cellulotic hairs that were expelled from the seed of cotton [58]. To our knowledge, this is the first time that this material is used as diet for earthworms, so the literature has no data on its performance. Nevetheless, it is known that cellulose as diet of the earthworms has significant influence on various biological parameters of their organism. For example, [8] concluded that the use of paper pulpe as food for the anecic *L. terrestris* accelerated growth more than manure and increased survival. Other researchers [10], who studied the influence of various foods using the epigeic species *Eisenia andrei* Bouché, 1972, found that the addition of cellulosic materials (cardboard, paper) in a protein diet enhanced reproduction, though it caused a reduction in the maximum body weight. In a study [13] that explored the influence of food type on the growth and reproduction of tropical species, it was found that the addition of cellulolitic material, namely sawdust, improved growth and reproduction of earthworms, this was species specific and was attributed to the enzymatic system of their body.

If we exclude cotton residues, the rest of the foods of vegetative origin (lawn clippings, vine leaves and olive leaves) had minor differences among each other regarding their influence on body weight.

In all diets, a clear decrease in the earthworm body weight was detected on 56 or 84 days. This decline in weight may be attributed to the reproductive effort of the earthworms, since most of the experimental boxes included offspring (table 5). It is known [29,59] that reproduction is an energy costing stage of life, especially for amphimictic species as it is *O. complanatus*, because it spends high amounts of mucus, sperm and investment on mate acquisition. Particularly for the last measuring, on 84 days, it may have come as a result of the late season (May) and the changes in environmental conditions, mainly the rise in temperature. From a study [60], it became apparent that during the dry and hot season the species *O. complanatus* looses weight and changes its physiological reactions. The maximum temperature measured in the culturing room was 23^{o}C. Other researchers [16] reported a decline in mean body weight of *L. terrestris* in high temperatures. The importance of this environmental parameter in the culturing of soil dwelling earthworm species has been marked by [19,24].

4.2 Influence of the materials used as diets on soil parameters

Only in few soil parameters, namely pH, EC and total salts, had the various foods significantly influenced the natural soil of the experimental boxes. Nevertheless, even in these cases the recorded values clearly fell into the range of "normal" levels for agricultural use. The above gives indications that the tested materials can be considered as suitable for disposal in agricultural soils. There was only in one case an ambiquity against "manure + whey" that obtained levels of EC and total soil salts marginally close to the upper limit of normal range (4mScm^{-1}, 0.2% respectively). All the parameters altered from the initial values as an effect of foods in similar way as has been reported by other researchers who tested various organic substances [4,61,62,63,64,65,66,67].

5. Conclusion

All tested materials and their combinations proved to be suitable as diets of *Octodrilus complanatus* in cultures, except plain OMW at dose 160m^3 ha^{-1} which is unsuitable. The best food was cotton residues which promoted growth better than the others and supported reproduction. Whey, when incorporated in the diet at doses up to 80m^3 ha^{-1}, is a promising additive that enhances growth but more research is needed to consolidate this finding. OMW favored growth of earthworms when added in low doses (up to 80m^3 ha^{-1}), too.

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