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Food Substrates and Population Density in the Breeding of Californian Red Worm (*Eisenia Foetida*)

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Abstract: The effect of food substrate and population density on the rearing of californian red worms (Eisenia foetida) was evaluated. A completely randomized design (CRD) was used, with an AxB factorial arrangement, with twelve treatments and two replications, making a total of 24 experimental units (EU). Factor A was the food substrate, with four levels (a1=bovine compost, a2=sheep compost, a3=guinea pig compost and a4=alpaca compost) and factor B was the population density, with three levels (b1=200 worms/EU, b2=400 worms/EU and b3=600 worms/EU) of clitellate worms. The EU were wooden beds of 0,4 m length, 0,4 m width and 0,2 m height, with a capacity of 32 L. In each bed, 25 kg of compost from each food substrate was incorporated, to subsequently inoculate the worms. After 90 days, the biological and productive development capacity was evaluated. It was determined that the alpaca and sheep compost demonstrated a greater biological and productive development capacity. In terms of population density, level b3 stood out (600 worms/EU); however, in terms of humus production, although there were significant differences ($p>0.01<0.05$) between the levels, this was not higher than 3%.

Key words: Manure, compost, bedding, inoculation, reproduction, humus

Introduction

Climate change is becoming an increasingly visible and palpable threat, as it directly influences local climate variability and indirectly influences the availability of natural resources, with an emphasis on poverty (Intergovernmental Panel on Climate Change IPCC), 2001 and 2007, cited by Cuellar & Salazar, 2016; Maza-Villalobos et al., 2023). The Andes have shown greater sensitivity, favoring soil erosion processes, loss of vegetation cover, variability in rainfall, alteration in crop dynamics, etc. (Lozano et al., 2021). Therefore, a new conception of agriculture has arisen that is oriented not only by productive aspects, but also by socio-environmental aspects, which has led to the need for a change towards a more sustainable agricultural model (Bedoya & Julca, 2021) that is in line with environmental quality, whose main problem is not to achieve high yields. but a long-term stabilization (Altieri, 1999), where the soil constitutes the basis of its productivity, in this sense, conserving the soil is an essential task (Sáenz, 2020). Likewise, soil organic matter (OM) is an essential constituent of the soil system (Bedoya-Justo & Julca-Otiniano, 2021), since due to its constitution and properties it is directly responsible for most of the physicochemical and biological processes of the soil (Raison & Rab, 2001, cited by Medina *et al.*, 2017; Trinidad & Velasco, 2016); it is a crucial element for the regulation of many processes related to agricultural productivity (Martínez, 2019). The application of OM to the soil provides an integrative effect, which promotes soil health and quality, with a preventive approach to agricultural management, improving soil properties so that the agroecosystem is self-regulating, self-sufficient, resistant to degradation and resilient (Céspedes & Millas, 2015; Martínez, 2017; Collantes et al, 2019; Jiménez et al., 2019). An alternative for adding OM to the soil is the incorporation of worm humus or vermicompost; high-quality organic fertilizer, due to the benefits of its application to the soil (Damian et al., 2018; Sarmiento *et al*., 2019; Ojeda-Morales et al., 2023).

Vermiculture is a clean and easy-to-apply process for recycling biodegradable waste, producing a high-quality fertilizer (Schuldt, 2006; Romero et al., 2015; Gutiérrez et al., 2020). It is a zootechnical activity, easy to integrate into agricultural systems (Rodríguez, 2005; Romero et al., 2015). *Eisenia foetida*, known as the Californian red worm, is the most widespread species in the practice of vermiculture (Reines et al., 1998), given its hardiness, tolerance to environmental factors, reproductive potential and crowding capacity, living in captivity and without escaping (Rodríguez, 2005). Earthworms are microphages, feeding on bacteria, protozoa, microalgae and fungi, which they ingest with the substrate. This conglomerate of organisms abounds in the manure of properly matured animals (Limachi, 2018), which is why they constitute the essential basic means to start a worm culture (Schuldt, 2006). The digestion of OM by earthworms results in an organic fertilizer called humus (Gutiérrez et al., 2020). The amount of humus that can be produced on a given surface and at a given time is related, among other factors, to the population density of earthworms (Schuldt, 2006). Likewise, the yield of vermicompost can be variable, because the characteristics of the substrate directly affect the state and multiplication of this organism since they depend to a large extent on the type and quality of the food (Rodríguez-Flores et al., 2021), so the effect of food sources on the population dynamics and productive capacity of earthworms must be known (Durán & Henríquez, 2009; Romero et al., 2018). Therefore, the research aimed to evaluate the effect of food substrate and population density on Californian red worm breeding.

Materials and Methods

The research was carried out at the National University of San Antonio Abad del Cusco (UNSAAC), located in the district of San Jerónimo, province and department of Cusco, at an altitude of 3225 meters above sea level, geographical coordinates 13°33´11.10" S and 71°52´29.33" W, and UTM coordinates 8'499 994.02 N and 188 844.83 E. The area has two well-marked climatic periods: rainy (November-March) and dry (April-October), with January being the month in which it rains most intensely (156.32 mm/month). The highest temperature (21.7 °C) occurs in October, while the lowest temperature (-1.6 °C) occurs in July (Paucar et al. 2018; National Meteorology and Hydrology Service of Peru [SENAMHI], 2018).

A completely randomized experimental design (DCA) was used, with a factorial arrangement of AxB, making a total of 12 treatments and two replications (Table 1).

Board 1

Factors, levels and treatments studied

Factor A Food substrate Levels	Factor B Population density Levels	Treatments
a1: Beef compost	b1: 200 pcs/EU	T1
	b2: 400 units/EU	S2
	b3: 600 units/EU	S3
a2: Sheep compost	b1: 200 pcs/EU	S4
	b2: 400 units/EU	S5
	b3: 600 units/EU	S6
a3: Guinea pig compost	b1: 200 pcs/EU	S7
	b2: 400 units/EU	S8
	b3: 600 units/EU	S9
a4: Alpaca compost	b1: 200 pcs/EU	S10
	b2: 400 units/EU	S11
	b3: 600 units/EU	S12

EU: Experimental unit.

In the preparation of the compost, cow manure (Bos Taurus), sheep (Ovis aries), alpaca (Vicugna pacos) and guinea pig (Cavia porcellus) were used; in addition to quinoa harvest stubble, agricultural land, ash and water; the proportion of quinoa stubble in relation to manure being 50%. The procedure consisted of incorporating a layer (30 cm) of quinoa stubble, on which a layer of manure (30 cm) was added, on top of which farm soil and ash were spread as a sprinkle; and then moisten it and cover it with plastic. After 15 days, the first turning was carried out. From that moment on, this work was repeated weekly, under room temperature, for a period of 60 days.

The breeding beds were made of wood. Each bed constituted an experimental unit (EU), whose dimensions were 0.4 m long, 0.4 m wide and 0.2 m high, with a capacity of 0.032 m3 (32 L). For the inoculation of the worms, first, 25 kg of the compost was incorporated in each EU. Subsequently, the worms were inoculated at the density corresponding to each treatment, where the worms that were inoculated had developed and visible the clitellar ring, a sign of their sexual maturity (Durán & Henríquez, 2009). Likewise, the EUs were covered with raschel mesh, and to guarantee the appropriate biological conditions, humidity and temperature control were carried out.

At 90 days, samples of 500 g were taken from each EU and the biological development capacity (number of eggs, juvenile and adult worms) and productive capacity (humus weight and humus conversion) were evaluated. Analyses of variance (ANOVA) were performed with the corresponding data, and when the sources of variation in the ANOVA were significant, comparisons of the mean treatments were made using Tukey's significance test (0.05).

Results

Number of cokes. - According to ANOVA, no differences ($p>0.05$) were determined in the source of variation of the interaction of the AxB factors. On the other hand, within factor A (food substrate) differences ($p<0.01$) and differences ($p>0.01<0.05$) were found in factor B (population density); therefore, it was necessary to apply the Tukey test (0.05) to determine the difference between the mean levels of the main factors of A and B respectively, due to the statistical non-significance of the interaction of the AxB factors (Gabriel et al., 2021); where alpaca and sheep compost reached the highest averages with 119 and 104 cocones respectively, surpassing guinea pig and beef compost, which achieved averages of 58 and 56 cocones respectively; likewise, the density of 600 and 400 earthworms/EU reached averages of 94 and 83 cocons, respectively, surpassing the density of 200 earthworms/EU, which achieved an average of 75 cochons (Table 2).

Table 2
Tukey's test (0.05) for the number of cocones in factors A and B

Food substrate	Average (g)	Population density	Average (g)
a4	119a	b3	94a
a2	104a	b2	83from
a1	58b	b1	75b
a3	56b		

A: Food substrate, B: population density
a1: Vaccine compost, a2: Sheep compost, a3: Compost de cuy, a4: Alpaca compost
b1: 200 lombrices/UE, b2: 400 lombrices/UE, b3: 600 lombrices/UE
Equal letters within the column are statistically similar ($p>0.05$)

Number of juvenile worms. - With ANOVA, for the sources of variation of factors A, B and AxB, they were significant ($p<0.01$). When performing the single-effect ANOVA of AxB (Gabriel, 2021), differences ($p<0.01$) were found in factor A with respect to factor B levels (b1, b2, and b3). Also in the case of factor B for each level of factor A, differences ($p<0.01$) were obtained in levels a2, a3 and a4, however, for level a1 no differences were determined ($p>0.05$). When applying the Tukey test (0.05), for the number of juveniles, significant differences were observed for the food substrates (Factor A) when combined with the different inoculation densities, with the highest average being found in the combination a4b2 (alpaca compost and 400 worms/UE) with 175 juveniles. Similarly, for Factor B (inoculation densities) differences were determined when combined with the different levels of food sources, achieving the highest average b3a4 (600 earthworms/EU and alpaca compost) with 175 juvenile worms (Table 3).

Table 3
Tukey's test (0.05) for the simple effects of factor A at each level of factor B; and the simple effects of factor B at each level of factor A, on the number of juveniles

Factor		Average	Factor		Average
A	B	g	B	A	g

a4	b1	83d	b3	a2	122c
a2	b1	64d	b2	a2	116c
a3	b1	20e	b1	a2	64d
a1	b1	5f	b3	a3	110c
a4	b2	175a	b2	a3	61d
a2	b2	116c	b1	a3	20e
a3	b2	61d	b3	a4	175a
a1	b2	20e	b2	a4	164b
a4	b3	164b	b1	a4	83d
a2	b3	122c			
a3	b3	110c			
a1	b3	22e			

A: Food substrate, B: population density
a1: Vaccine compost, a2: Sheep compost, a3: Compost de cuy, a4: Alpaca compost
b1: 200 lombrices/UE, b2: 400 lombrices/UE, b3: 600 lombrices/UE
Equal letters within the column are statistically similar (p>0.05)

Number of adult worms.- There were no statistical differences (p>0.05) in the interaction of AxB factors; but the main effects of A and B differences were found (p<0.01). By applying Tukey's test (0.05) for A and B, it was possible to determine that the best food substrates were alpaca and sheep composts, reaching 27 and 25 adult worms respectively. On the other hand, with the population density of 600 earthworms/EU, 30 adults were achieved (Table 4).

Table 4
Tukey's test (0.05) for the number of adult worms in factors A and B

Food substrate	Average (g)	Population density	Average (g)
a4	27a	b3	30a
a2	25a	b2	21b
a1	18b	b1	15c
a3	18b		

A: Food substrate, B: population density
a1: Vaccine compost, a2: Sheep compost, a3: Compost de cuy, a4: Alpaca compost
b1: 200 lombrices/UE, b2: 400 lombrices/UE, b3: 600 lombrices/UE
Equal letters within the column are statistically similar (p>0.05)

Worm yield.- When processing ANOVA, no differences (p>0.05) were determined in the interaction of AxB factors . However, differences were found for the effect of the main factors A and B (p<0.01). By applying Tukey's test (0.05) for the effects of A and B, it was determined that alpaca and sheep compost achieved yields of 24 and 21 g, respectively. For inoculation density, it was found that 600 worms/EU reached a yield of 25 g (Table 5).

Table 5
Tukey's test (0.05) of worm performance in factors A and B

Food substrate	Average (g)	Population density	Average (g)
a4	24a	b3	25a
a2	21from	b2	19b
a1	18b	b1	13c

a3	13c

A: Food source, B: inoculation density
a1: Vaccine compost, a2: Sheep compost, a3: Compost de cuy, a4: Alpaca compost
b1: 200 lombrices/UE, b2: 400 lombrices/UE, b3: 600 lombrices/UE
Equal letters within the column are statistically similar (p>0.05)

Conversion of substrate into vermicompost.- Statistical differences were determined, according to ANOVA, for A (p<0.01) and B (p>0.01<0.05). When applying Tukey (0.05) for A and B; Alpaca and sheep compost achieved the highest humus yields with 14.94 (59.76%) and 14.22 kg (56.88%), respectively. Likewise, for population density, it was found that 600 earthworms/EU achieved a higher yield with 12.58 kg (50.32%). The differences observed express the benefits of composted alpaca manure with the highest population density (600 earthworms/EU), for humus production, with sheep manure composting showing the same tendency (Table 6).

Table 6
Tukey's test (0.05) of humus yield in factors A and B

Food substrate	Average (kg)	Conversion (%)	Population density	Average (kg)	Conversion (%)
a4	14,94a	59,76a	b3	12,58a	50,32a
a2	14,22a	56,88a	b2	12.18b	48.72b
A1	10.20b	10.20b	b1	11.88b	47.52b
A3	9.50b	38.00b			

A: Food source, B: inoculation density
a1: Vaccine compost, a2: Sheep compost, a3: Compost de cuy, a4: Alpaca compost
b1: 200 lombrices/UE, b2: 400 lombrices/UE, b3: 600 lombrices/UE
Equal letters within columns are statistically similar (p>0.05).

Discussion

Raising worms for humus production offers agriculture an alternative to improve the physical, chemical and biological properties of the soil (Díaz et al., 2020), in addition to protecting crops from diseases and pests, as well as from sudden changes in temperature and humidity in the environment; likewise, it increases the assimilation of nutrients and the capacity to retain water (Bedoya-Justo & Julca-Otiniano, 2021; Contreras & Cocoletzi, 2023).

Biological development.- The food substrates from alpaca and sheep breeding were the ones that had the greatest influence on reproduction, with a population density of 600 earthworms/EU, this is corroborated by Bollo (1999) and Ferruzi (1986), cited by Durán & Henríquez (2009); and Alcívar (2023), who mention that despite the adaptability of the different species of earthworm, The characteristics of the substrate or growth material directly affect the state and multiplication of this organism. However, the salinity levels of the substrates used should be observed, which, in this case, the one from cattle had an EC of 6.46 dS/m and the guinea pig 5.51 dS/m; higher than those of alpaca (1.89 dS/m) and sheep (2.21 dS/m); and this could have affected reproduction; as Díaz (2002) states, that the high content of salts does not favor the necessary firing, in addition those containing sodium are phytotoxic, devaluing vermicomposting.

In the juvenile population, it was found that the highest population occurred with the density of 600 earthworms/EU, in all food substrates; obtaining 164 individuals for alpaca compost, 122 for sheep compost, 110 for guinea pig compost and 22 with cow compost, respectively. This is related to what was reported by Durán & Henríquez (2009), and Alcívar (2023), where they mention that both the size of the individuals and their reproduction rate are influenced by the type of substrate, and in our case the alpaca and sheep substrates offered better conditions for the reproduction of earthworms. However, this contrasts with what was mentioned by Baltierra (2003), regarding that population density did not show a statistically significant effect (p>0.05) with respect to the number of cocoons and number of young worms for Eisenia sp.

In the case of adult worms, it was found that alpaca compost offered better conditions for the development of worms, followed by sheep compost, which would be related to the lower salt content of alpaca compost (1.89 dS/m) and sheep compost (2.21 dS/m), in relation to the other substrates. Durán & Henríquez (2009) and Canales et al. (2020) mention that high pH and EC values produce stress conditions and have a negative impact on fertility, recording decreases of up to 25% in the reproduction rate. Also in terms of inoculation density, it was observed that the greater the number of individuals inoculated, the higher the reproduction rate.

For the weight of worms, no differences were found ($p>0.05$), neither at the level of factors nor between levels of both factors; which shows that food substrates and inoculation population density did not influence the weight of the worms; however, average ranges of 0.79 to 0.84 g were achieved, which is within what was reported by Schuldt (2004). 0.3 to 1.4 g above the average of 0.34 to 0.66 g achieved by Durán & Henríquez (2009) and León et al. (1992) of 0.13 to 0.21 g.

Productive capacity.- In the yield of earthworms, there are no significant differences ($p>0.05$) at the level of interaction between factors, but differences between the levels of the factors are observed, thus in the food substrate factor an average of 24 g of worms per EU was achieved with alpaca compost followed by sheep compost with 21 g. 18 g in beef and 13 g in guinea pig. This shows that alpaca manure compost favored the yield of worms up to 45% higher than that of guinea pig, and 25% compared to that of cattle. For the inoculation density factor, we found the same trend with 25 g for 600 individuals (level 3), 19 g in the case of level 2, and 13 g in level 1. This shows that the greater the number of individuals inoculated, the greater the yield, although it should be observed that over time the food decreases and the population increases, which brings with it productive consequences, as stated by Schuldt et al. (2004); Muraira-Soto et al. (2023) and Rincones (2023), that whatever the management strategy of vermicultures, the degree of crowding of earthworms in the substrate has a greater impact on production than other factors.

In the conversion of the substrate into vermicompost, a yield of 14.94 kg of humus (59.76% conversion) was achieved with alpaca compost; 14.22 kg (56.88% yield) with sheep compost; 10.20 kg (40.8% conversion) in cattle compost and 9.5 kg in the case of guinea pig compost (conversion of 38%), these results are similar to what was mentioned by Rodríguez (2005). who reports conversions of 48.52% (using banana pseudo stems); likewise, Chura (1999), cited by Enríquez & Soto (2017), which used manure from cattle, horses, camelids, sheep and goats and reported a yield of 49 to 57%; although they are higher than what was obtained by Sánchez (2003) with 26% and 23%, using mixed semi-composted banana and cachaça harvest by-products. Likewise, the population density of inoculation 600 worms/EU reached a yield of 12.58 kg (50.32%), surpassing the density of 400 and 200 worms/EU that achieved 12.18 (48.72%) and 11.88 kg (47.52%) of humus, respectively.

Conclusions

Alpaca and sheep composts showed a greater capacity for biological development in number of pigs, juvenile and adult worms; likewise, in the productive capacity they achieved a higher yield in the weight of worms and a 58.27% conversion of humus. In the biological dynamics and humus production, the inoculation density of 600 earthworms/EU stood out. The results obtained demonstrate that it is possible to use inputs from the area, such as alpaca and sheep manure, quinoa harvest stubble, among other biodegradable waste, as food sources for the development of vermiculture and produce humus, high-quality organic fertilizer, which can be used to incorporate into the soils in the Andes and develop sustainable agriculture.

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