Studies regarding the effects of the redworm meal utilization as a protein substitute in Japanese quail diets

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Abstract. The aim of this research was to highlight the effect of utilizing redworm meal, *Eisenia foetida* (red manure worm) as a protein substitute for soybean meal protein in the diet of Japanese quail chicks (*Coturnix coturnix japonica*). The research was carried out on three experimental lots, in which the control group (CD) was fed with a compound fourage specific for the age category of the species, and in the experimental groups the soybean meal protein from the same compound fourage was substituted in a proportion of 10% (D_1) and 20% (D_2) respectively with protein from redworm meal. The experiment has demonstrated that the use of earthworm protein ensures an accumulation of body weight superior to the control group. Thus, group D_1 recorded the highest weight increase compared to the control group, the difference being statistically very significant ($p < 0.001$), followed closely by the D_2 group. There were no significant differences between the experimental groups ($p > 0.05$). The D_1 group also recorded the highest body weight gain and the D_2 group had the superior feed conversion ratio. During the experiment no mortality occurred within the three groups. In conclusion, the use of redworm meal in diets of the quail chicks is justified in a proportion of 10%, because it resulted in superior growth performances.
Key Words: *Eisenia foetida*, redworm meal, quail, nutrition.

Introduction. In the last decades, many studies about organic waste management had focused on the potential role of vermiculture in the improvement of problems occurred in this field (Gupta & Garg 2008; Abbasi et al 2009; Manyuchi et al 2013; Van Schaik et al 2016; Bhat et al 2018).

The species *Eisenia foetida* Sav. belongs to the phylum Annelida, the Olligochaeta class and Lumbricidae family (Schultz & Graff 1977; Venter & Reinecke 1988). It is encountered in areas and heaps with organic matter, like waste of various origins and animal manure (Garg et al 2006; Aira & Dominguez 2009; Bernal et al 2009; Malafaia et al 2015; Cestonaro et al 2017). The main activity of the redworm consists in processing the substrate or organic matter through the digestive tract and eliminating the content in the form of excrement, as a durable, fine, porous, granulate and insipid matter called humus, through a vermicomposting process (Frederickson et al 2007; Hait & Tare 2011; Amouei et al 2017; Mupambwa & Mnkeni 2018). In addition to composting organic matter in valuable organic fertilizer, a conversion into biomass is made, a process in which an important part of the protein from the feed of farm animals eliminated by the digestion process is recovered (Arancon et al 2005; Gomez-Brandon et al 2013; Ravindran & Mnkeni 2016; Vodounnou et al 2016).

The intensive breeding of the *Eisenia foetida* species can be managed due to the biological adaptation capacity and through maintaining optimum environmental conditions (Sivasankari 2016). Currently, vermiculture is recognized as a biotechnological application of ecological and nutritional interest (Gupta & Garg 2009) with two main objectives: an alternative for recycling residual organic matter (Khwairakpam & Bhargava 2009; Gomez-Brandon & Dominguez 2014) and an economical unconventional protein source (Bou-Maroun & Cayot 2015; Das et al 2016; Bhat et al 2017).

Literature studies about the chemical-nutritive composition of redworm meal reveals a high protein content (60-68% CP) with a high biological value (lysine 4.44%, methionine 1.2%, ME 3258 Kcal kg^{-1}) (Istiqomah et al 2009; Bahadori et al 2015). The fat content is also noteworthy (7-10% CF), as is the ratio of fatty acids, and also the vitamin and mineral balance (Schultz & Graff 1977; Sabine 1983; Gunya et al 2016). The nutritional profile is comparable to fish meal, but more valuable than vegetable protein sources frequently used in farm animal feed, like soybean meal, sunflower meal or fodder yeasts (Zhenjun et al 1997; Sogbesan & Ugwumba 2008; Rezaeipour et al 2012).

In poultry nutrition, the presence of a quality protein is essential to support growth and production processes. Because of its nutritional value, most research in this field propose the redworm meal as an alternative source of protein for replacing soybeans meal in farm animal diets, respectively in poultry diets (Sogbesan & Ugwumba 2008; Tiroesele & Moreki 2012; Gholami et al 2016; Zang et al 2018). For broiler pullets, the partial replacement of soybean and fish meals with redworm meal has resulted in a superior growth rate, a lower feed conversion index and a higher final weight of chicks (Loh et al 2009). The results obtained from a different research on poultry show that the utilization of redworm meal in diets increases feed conversion ratio, protein digestibility, weight gain and improves meat quality (Jang Ho 2009; Bahadori et al 2015, 2017).

Due to its nutritional characteristics, redworm meal can successfully replace the protein sources in fodders for poultry nutrition. The purpose of this research was to establish the effects of partial soybean protein replacement with redworm (*Eisenia foetida*) biomass protein in diets of Japanese quail chicks (*Coturnix coturnix japonica*).

Material and Method

The location and research objectives. The experiment was conducted over a period of 21 days, starting in January 11, 2016, in the Ecology and Environmental Protection Laboratory of the Faculty of Animal Science and Biotechnologies from University of Agricultural Science and Veterinary Medicine, Cluj-Napoca. By replacing the protein of soybean meal (a component of the specific compound fodder for quail chicks) with protein obtained from redworm biomass (*Eisenia foetida*), the following were observed: the percentage of body mass accumulation, feed consumption, daily weight gain and feed conversion index.

The biological material and ambiance parameters. A total of 63, three days-old Japanese quails (*Coturnix coturnix japonica*) were acquired from a private farm specialized in breeding the species. Three experimental lots were made (CD, D_1 and D_2) consisting in 21 quails per group. The distribution in lots was randomly performed, in cages similar to those from industrial breeding, provided with equipment used in growing technology: grill flooring, manure collecting trays, feeder (3 cm per head front), nipple drinker systems and brooder lamp. The optimal microclimate for this age category was ensured: temperature 35-37°C, humidity 70-75% and light 18 hours/day. Growing cages were prepared one day before populating with biological material.

Preparation of experimental treatments. For quail chicks feeding, nutritional and energy-protein balanced compound feed was made, consisting in: corn, soybean meal, sunflower meal, vegetal oil, mineral-vitamin premix (MVP). The experiment consisted in three applied treatments: a control diet (CD), composed of specific combined fodder and two experimental diets (D_1 and D_2), in which the soybean protein was substituted by 10% (group D_1) and 20% (lot D_2) respectively, with redworm meal protein.

For obtaining the redworm proteic meal, the method proposed by Ibanez et al (1993) was followed. The worms were harvested from half-fermented manure obtained from dairy cows reared in a free-range system. The biomass obtained was subjected to a water manual washing process. Next, redworm drying and dehydration process was carried out by placing them in the Memmert oven, on a glass support, at a temperature of 42°C, for 48 hours.

In the case of the two experimental diets (D_1 and D_2), the proportion of the ingredient inclusion in the combined fodder structure was realized by respecting an energy-protein balance and a nutritional value similar to the control group (CD): 25% CP and 2900 kcal kg^{-1} ME (Table 1).

Table 1

The structure of the combined feed, expressed as a percentage of ingredient participation in the mixture and the nutritional value

Specification	*CD*	*D_1*	*D_2*
Corn (%)	45.00	47.00	49.00
Soybean meal (%)	43.50	38.80	34.00
Sunflower meal (%)	5.00	5.00	5.00
Vegetal oil (%)	4.00	3.70	3.50
Mineral-vitamin premix (%)	2.50	2.50	2.50
Redworm meal (%)	-	3.00	6.00
Total (%)	100.00	100.00	100.00
Nutritional value			
Metabolizable energy (kcal kg^{-1})	2900	2902	2907
Crude protein (% DM)	25.08	25.08	25.03
Lysine (%)	1.30	1.31	1.32
Methionine (%)	0.38	0.40	0.40

After dehydration, the dry biomass was milled, resulting the redworm meal, which was included in the combined feed structure in proportion of 10% and respectively 20%. In fact, redworm meal was included in the feed mixture in the proportion of 3% (D_1) and 6% (D_2), respectively (Figure 1).

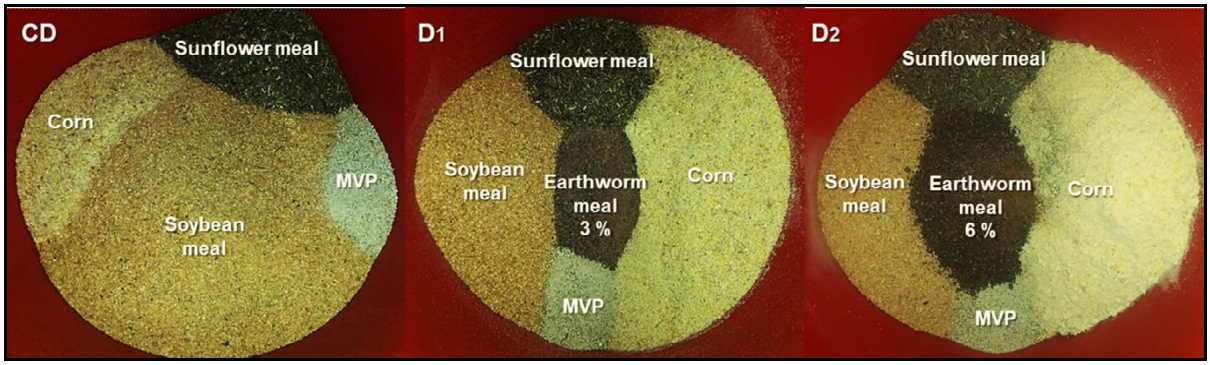

Figure 1. Inclusion of the proteic redworm meal in the structure of feed compound (MVP = mineral-vitamin premix).

Feeding of quail chicks was realized *ad libitum*, in feeders with protective grills, specific to each age category. By daily weighing of the feed dose administered to each group, the average daily feed consumption and the feed conversion ratio specific for the growing period from 3 to 21 days were evaluated.

The growth dynamics and body mass evolution were established based on individual weighing from each group, for 3 days-old of quails (at start of the experiment) and over the growth period, respectively at the age of 7, 10, 14 and 21 days. For estimating the average weight gain, the final body mass mean from each lot was reported at the number of growing days. The last evaluation corresponds, according to the technology (Soares et al 2003), to crossing from diets based on starter feed, characterized by a high protein level (25% CP), specific for quail pullets, to the grower feed, specific for the next breeding phase (22-23% CP).

The data obtained were statistically processed with the Graph Pad Inst 3 program and the test of differences between two averages ("t-test" $p < 0.05$) was utilized.

Results and Discussion. The results on body mass evolution indicate that the substitution of 10% and 20% of soybean meal protein with redworm meal protein, determine a superior growth of quail chicks from those groups, when compared to the control group (Table 2). These differences were obvious after just 4 days of administrating redworm meal in the diet, so for quail chicks from group D_2 there was a doubling of body weight mass. Similar results were also obtained for group D_1. After 11 days of feeding, quail chicks from these two groups realized a 402% and 404% growth rate compared to the initial weight, respectively. The growth rate has been maintained above the control group throughout the experiment.

Table 2

Average body weight registered at quail chicks (g) and relative growth rate from the first day of experiment and up to 21 days age of quails

Evaluation	*Age of chicks (days)*	*Average individual body weight (g)*			*Percentage of growth (%)*		
		CD	*D_1*	*D_2*	*CD*	*D_1*	*D_2*
I	3	16.32	16.27	16.22	100	100	100
II	7	28.92	31.35	32.42	177	193	200
III	10	38.78	44.97	44.25	238	276	273
IV	14	53.81	65.47	65.45	330	402	404
V	21	103.10	121.19	119.47	632	745	737

These results highlight the nutritional quality of redworm, which supports a high rate of growth, especially in growing youth animal organism. The protein of redworm has the quality to support the intensive growth process of quail chicks, due to the appreciable amino acid content (Khan et al 2016), such as methionine and lysine, limitative at monogastric animals.

At the quail age of 3 days, there were no significant differences ($p > 0.05$) between the mean of the body weight for the three groups. The first differences ($p < 0.01$) were register at the second evaluation, between the control group and group D_2, respectively at the chick age of 7 days (Table 3).

Table 3

Average body weight mass and statistical significance of differences registered at quail chicks over the 3 to 21 days growth period

Comparation	*$X \pm s_X$*	*$X \pm s_X$*	*d*	*t*
		Evaluation I		
CD vs D_1	16.32 ± 0.57	16.27 ± 0.43	0.05	0.05
CD vs D_2	16.32 ± 0.57	16.22 ± 0.46	0.10	0.13
D_1 vs D_2	16.27 ± 0.43	16.22 ± 0.46	0.10	0.07
		Evaluation II		
CD vs D_1	28.92 ± 0.96	31.35± 0.93	2.43	1.81
CD vs D_2	28.92 ± 0.96	32.42 ± 0.79	3.50	2.82**
D_1 vs D_2	31.35 ± 0.93	32.42 ± 0.79	1.07	0.87
		Evaluation III		
CD vs D_1	38.78 ± 1.07	44.97 ± 1.36	6.19	3.55***
CD vs D_2	38.78 ± 1.07	44.25 ± 1.48	5.47	2.98**
D_1 vs D_2	44.97 ± 1.36	44.25 ± 1.48	0.72	0.35
		Evaluation IV		
CD vs D_1	53.81 ± 1.16	65.47 ± 1.84	11.66	5.37***
CD vs D_2	53.81 ± 1.16	65.45 ± 2.30	11.64	4.52***
D_1 vs D_2	65.47 ± 1.84	65.45 ± 2.30	0.02	0.01
		Evaluation V		
CD vs D_1	103.1 ± 2.65	121.19 ± 2.71	18.09	4.79***
CD vs D_2	103.1 ± 2.65	119.47 ± 3.24	16.37	3.90***
D_1 vs D_2	121.19 ± 2.71	119.47 ± 3.24	1.72	0.40

$p < 0.05$ (* significant); $p < 0.01$ (** distinctly significant); $p < 0.001$(*** very significant); d = difference; t = calculated value.

Furthermore, the evolution of the body weight for chicks from the three lots reveals significant differences ($p < 0.001$) between the control diet and diets with 10% and 20% proteic redworm meal inclusion. These differences expressed by growth performances are similar to those obtained by Prayogi (2011) in quail chick diets. The superiority of D_2 group to D_1 during the first feeding days may be owed to the more intense growth of quails in the first days after hatching and therefore to the more efficient usage of worm protein included in highest proportion in fodder mixture.

In general, the quail pullets of group D_1 and D_2 have shown a similar evolution of body weight, without significant differences in growth ($p > 0.05$). However, absolute mean values indicate the superiority of chicks from group D_1 (121.19 g) and it can be stated that utilizing more than 10% redworm meal protein in diets at this category is unjustified. The results obtained are similar to those obtained by Prayogi (2011), which also show superior growth performance in the case of 10% fish meal substitution with earthworm meal.

Feed consumption, based on daily average consumption for each lot, shows that the group who did not benefit from redworm meal had the lowest daily feed consumption (11.17 g day^{-1}) (Figure 2). More consumption of fodder at quail groups fed with redworm meal, 12.35 g day^{-1} at D_1 and 12.14 g day^{-1} at D_2 also explains their superior growth. Fodder with high digestibility is easily assimilated by the organism, accelerates metabolism and energy use, which implies a higher feed consumption (Hill 1979). These affirmations are demonstrated by the feed conversion ratio, superior to lots D_2 (2.11) and D_1 (2.12). The results are better than those obtained by Prayogi (2011) where the conversion ratio was 2.68. The highest daily body weight gain was recorded in group D_1 (5.82 g day^{-1}) followed by D_2 (5.73 g day^{-1}). Similarly, Rezaeipour et al (2014), and recently Zang et al (2018) obtained the highest BWG for inclusions of 10% and 5% earthworm meal protein in broiler chick diet.

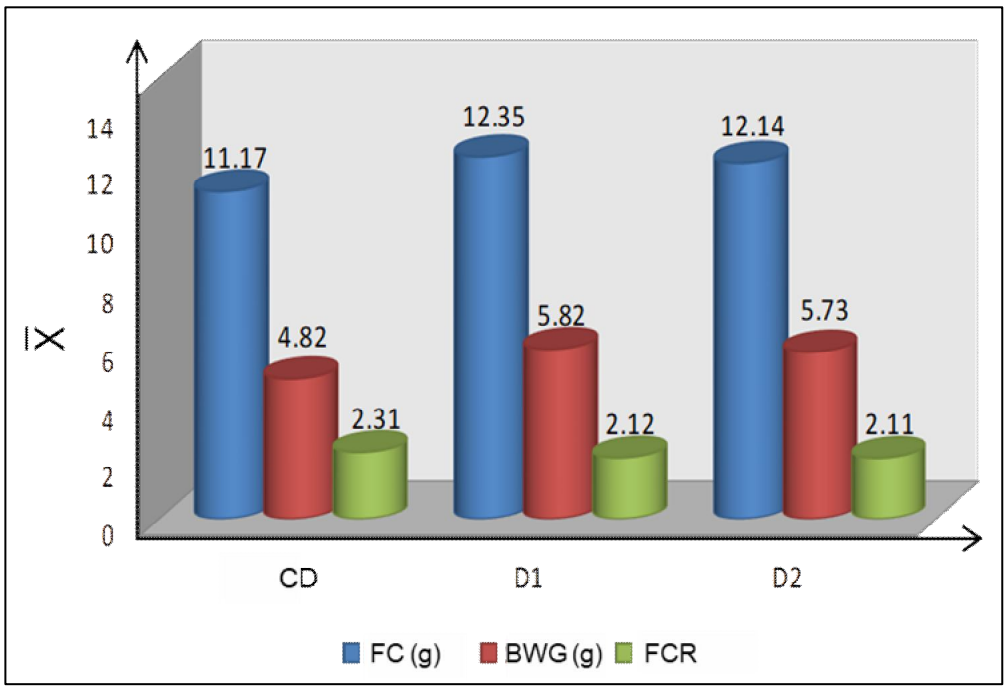

Figure 2. Production and consumption parameters for quail chick groups (CD, D_1, D_2): FC = feed consumption; BWG = body weight gain; FCR = feed conversion ration).

According to Ignacio et al (1993), redworm meal *Eisenia foetida* is relatively deficient in amino acids with sulfur and tryptophan, but the appearance of juvenile plumage and the degree of feather development were superior to chick lots supplemented with redworm meal.

During the experiment there were no mortalities in any of the groups, which shows that the proteic redworm meal does not contain antinutritive factors for poultry

growth (Reinecke et al 1991). Other studies have reported that earthworm meal contains certain antibacterial bioactive compounds (Julendra et al 2012), hepatoprotectors (Balamurugan et al 2008), antimicrobials and anticancerigens (Cooper et al 2004). Zang et al (2018) demonstrated that for broiler chicks the inclusion of earthworm meal in diets increases the activity of some antioxidant enzymes in the liver ($p < 0.05$), and Bahadori et al (2017) showed that earthworm meal determines the increased meat quality and reduction of intestinal pathogenic microflora.

Conclusions. The chemical composition of redworm meal has important and proven qualities that justify its use in poultry breeding and farm animals, both as a protein supplement and for the replacement of fish or soybean meal.

In the present study, substitution in percent of 10% of soybean meal protein with redworm meal protein for the Japanese quail chicks (*Coturnix coturnix japonica*) had the best growth performance ($p < 0.001$) and a superior body weight gain (5.82 g).

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