# EFFECTS OF FEEDING RATE ON DENSITY, BIOMASS AND PROTEIN COMPOSITIONS OF OLIGOCHAETE (*Limnodrilus hoffmeisteri* Claparede, 1862)

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## ABSTRACT

*L. hoffmeisteri is an aquatic invertebrate, belonging to the class Oligochaeta and family Tubificidae, used as an important live food for feeding larval stages of freshwater species. This study was carried out to provide scientific knowledge for L.hoffmeisteri culture as well as optimal feeding ration affected on density, biomass and protein compositions. L. hoffmeisteri was cultured under flow-through in concreted trench system (160 x 25 x 20 cm) with mud bottoms for 5 weeks. They were fed a mixture of 33.3% soybean meal, 33.3% corn meal and 33.3% rice bran at feeding rations of 5%, 10%, and 15% of body mass.*

*The results showed that different feeding rations significantly effect on the density, biomass and protein compositions of L. hoffmeisteri. Specifically feeding ration of 15% resulted in the highest density (64 ± 5 individual/cm²), biomass (133.90 ± 9.24 mg/cm²), protein (% of dry biomass) (52.34 ± 1.35 %). Conversely, the lowest density (5± 1 individual/cm²) and biomass (10.24 ± 1.18 mg/cm²) were recorded in the control treatment (not fed). The lowest protein (% of dry biomass) (45.76 ± 1.18 %) was recorded in the treatment with feeding ration of 5 %. In conclusion, feeding at 15% of body mass/day displayed as a suitable ration for L. hoffmeisteri.*

*Keyworms: L. hoffmeisteri worms, feeding rations, culture*

## I. INTRODUCTION

*L.hoffmeisteri* is one of many species of aquatic worms that is widely distributed throughout the world [5], tolerating a wide variety of environmental conditions. In Vietnam, these worms can be found in fish ponds, river and wastewater ditches [8].

*L. hoffmeisteri* is a small species with body size about 20-35 mm long and plays an important role to freshwater aquaculture [9]. Furthermore, this species in high in nutritional values (5575 cal g- on a dry weight basis [2]) and highly digestible for aquatic animals.

*L. hoffmeisteri* is mainly used as food in aquarium fishes and have been reported as an important live food in larval rearing of many commercially important fishes, particularly for catfish and another fish such as gray eel-catfish and crab [10,11].

In Vietnam, current total supply of these worms mainly comes from wild caught source which is unreliable and insufficient to meet the demand. Information related to culture of *L. hoffmeisteri* in Vietnam is not known. Therefore, the present study was undertaken to determine the effects of feeding ratio on density, biomass and protein compositions of *L. hoffmeisteri* worms.

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## II. MATERIALS AND METHODS

### 1. Experimental worms and system

*L. hoffmeisteri* worms were collected from waste water ditch of Vinh Ngoc district, Khanh Hoa province in Vietnam. The collected worms were rinsed and cultured at the Nha Trang University Laboratory. The worms were cultured in a flow-through system over 2 month period to achieve quantity (450g) for experiments.

All experiments were conducted from early November to mid-December 2015 for 5 weeks. The worms were cultured in indoor concrete culverts (160 cm x 25 cm x 10 cm) system to protect from rain and sunlight. Prior to the experiment, the culverts were with clean freshwater. Each culvert was connected to flow-through-system. Substrate was made by mud layer of 1 cm thickness.

**2. Experimental design**

Four treatments of feeding rations were used in this experiment including the 0% (negative control, not fed), 5.0%, 10.0% and 15.0% of body mass. With 6 replicates each. All worms were fed daily with the same mixture feed at 08:00 am. Ingredients of mixture feed were 33.3% soybean meal, 33.3% corn meal and 33.3% rice bran. A sample of feed was sent to the Biotechnology institute - Nha Trang University of for analysis (Table1).

**Table 1: Percentage of five ingredients in mixture feed in the culture of *L.hoffmeisteri.* (The data analysis were provided by the Biotechnology Institute – Nha Trang University)**

| Ingredient | Mixture feed |
|---|---|
| Crude protein | 17.41 |
| Crude lipid | 11.70 |
| Ash | 12.24 |
| Crude fiber | 5.58 |
| Cacbonhydrat | 21.20 |

**3. Inoculation of *L. hoffmeisteri* Oligochaete**

Water flow was adjusted one day before inoculation of worms to the culverts. The collected *L. hoffmeisteri* worms were inoculated at the density of 5 individual/cm² and spread over the media homogeneously as much as possible in each of the culvert.

**4. Periodic supply of feed**

The supply of feed was done following a date of worms' inoculation. The amount of food was changed once every 7 days. When feeding, water flow was stopped. Amount of food was spread throughout the culvert. Then, the water flow was reopened after 30 minutes.

**5. Methods of data collection and Statistical Analysis**

*5.1. Water quality*

Continuous water flow was maintained to keep the dissolved oxygen in suitable level (>3 mg $L^{-1}$) for development *L. hoffmeisteri*. Water temperature (ºC), dissolved oxygen (mg $L^{-1}$) and pH of the culture culverts were measured twice a day at 8:00 and 14:00. Using a portable dissolved oxygen meter (Model YSI Pro20, USA).

*5.2. Sampling*

Worm samples were collected after 7, 14, 21, 28 and 35 days of inoculation. Each sample involved water and media (4x2 cm²) from five randomly selected sites of each culvert. They were rinsed off with clean water . After that, unwanted particles was removed by using forceps and dropper. Finally, *L. hoffmeisteri* oligochaetes were dried with blotting tissue. They were weighted by Mettler Electric balance (KD-TBED 320) to the nearest 0.0001g. Number of individuals was recorded for each sample to calculate average biomass and density.

The biomass quality of *L. hoffmeisteri* worms was analyzed as biochemical content (% of dry weight) and fatty acid. Samples were dried at 80ºC in the incubator and kept in vacuum bag until analysis. Total protein of *L. hoffmeisteri* was determined by "Kjeldahl method"; moisture content and ash in the sample *L. hoffmeister* worms were determined by "AOAC 950.46 – 1995" and "AOAC 923.03 – 1995", respectively. Fatty acids were determined by gas chromatography (GC) and processed by software GC A.08.03 ChemStation (Agilent Technologies © Inc., Santa Clara, USA)

*5.3. Statistical analysis*

Data were presented as mean values ± standard deviation. One-way ANOVA was applied to analyze the differences of density, biomass and protein compositions of the worms. Differences were regarded as statistically significant when significance level less than 0.05.

## III. RESULTS AND DISCUSSION

**1. Results**

*1.1. Water quality*

Water temperature in the experiments were ranged from 29 to 31ºC in which the average temperature was 30.2 ± 0.8ºC. pH in the treatments was ranged from 6.8 – 7.8. Fluctuation of temperature and pH were negligible and did not affect growth and development of the *L. hoffmeisteri* populations.

The dissolved oxygen was ranged from 3.5–5.0 mg $L^{-1}$. Fluctuation of dissolved oxygen was negligible and suitable for

growth, development and reproduction of the *L. hoffmeisteri* populations. This is because normal development of the embryo of species of tubificids requires a minimum oxygen content of 2.5-7.0 mg $L^{-1}$ [7]. The culture system was provided the high dissolved oxygen content (≈ 3mg $L^{-1}$) not only maintained the highest worm density but also ensured the highest fecundity [6].

*1.2. Effect of feeding rations on biomass of L. hoffmeisteri population*

During the experiment, the biomass of *L. hoffmeisteri* population in the negative control increased very slowly. Conversely, the biomass of *L. hoffmeisteri* population when this species fed at the 5%, 10% and 15% feeding rate of this food had increased rapidly. In the first 2 weeks, the biomass of *L. hoffmeisteri* population were not affected by feeding rate and showed quite similar value between treatments (those of 10%, 15% were respectively 53.87 ± 11.47, 54.39 ± 6.86 mg/cm²). However, on the day of sampling (21, 28, 35) the feeding rate had affected the biomass of *L. hoffmeisteri* population. At the 35th day, the treatment that *L. hoffmeisteri* population were fed at ration of 15% had highest biomass (133.90 ± 9.24 mg/cm²), followed by the ration of 10% and 5% (111.41 ± 7.52, 88.37 ± 10.42 mg/cm², respectively). Conversely, lowest biomass (10.23 ± 1.18 mg/cm²) of this species were recorded in control treatment (not fed) and significantly lower than those in other treatments (P<0.05) (Figure 1).

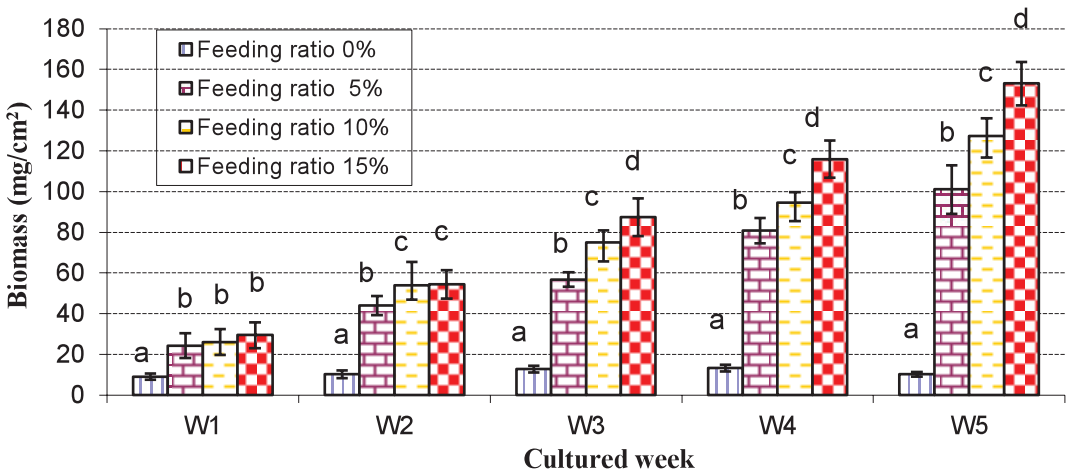


**Figure 1: Biomass of L. hoffmeisteri populations at different feeding rations**

*1.3. Effect of feeding rations on density of L. hoffmeisteri*

Density of *L. hoffmeisteri* population in the control treatment was increased slightly in the 3rd week after that it gradually reduced in the 5th week. Conversely, *L. hoffmeisteri* population density of the other treatments had increased continuously throughout the entire experimental period. From 3rd weekend to 5th weekend, the feeding rations had affected the population density of *L. hoffmeisteri* population. At 5th week, The treatment that *L. hoffmeisteri* were fed at feeding ration of 15% had highest density (64 ± 5 individual/cm²) and significantly higher than those in other treatments (P<0.05). Conversely, lowest density (10 ± 3 individual/cm²) was recorded in control treatment (not fed) (Figure 2).

*1.4. Effect of feeding rations on biochemical ingredients of L.hoffmeisteri*

The biochemical ingredients of dry *L. hoffmeisteri* were showed in Table 2

Protein ingredient was highest (52.34 ± 1.35 %) in treatment that *L. hoffmeisteri* population were fed at ration 15% of body mass/day and significantly higher than those in other treatments (P<0.05). Conversely, the lowest protein ingredient (45.76 ± 1.18 %) was recorded when feeding *L. hoffmeisteri* population with ration 5% of body mass/day.

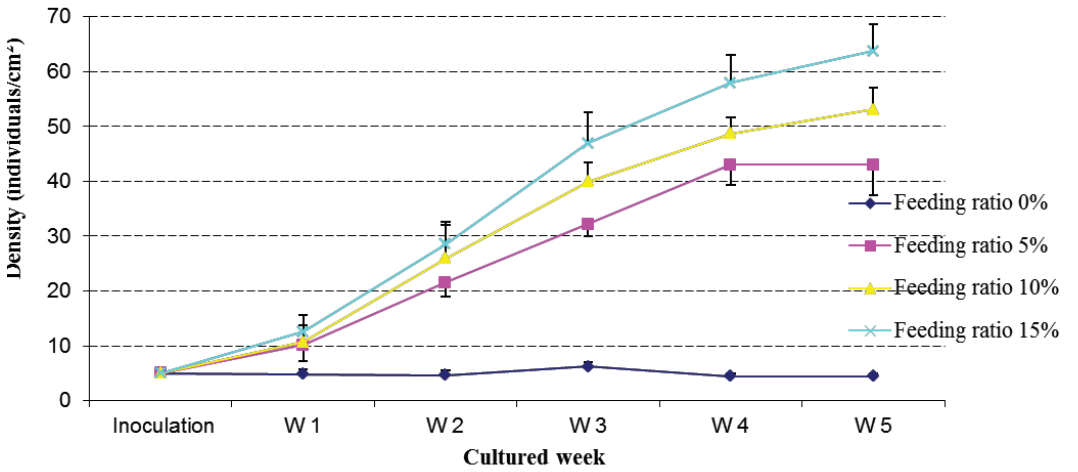


**Figure 2: Density of L. hoffmeisteri populations at different feeding rations**

**Table 2: The biochemical ingredients of dry *L. hoffmeisteri***

| Ingredient (%) | Feeding Ration (%) | | | |
|---|---|---|---|---|
| | 0 | 5 | 10 | 15 |
| Crude protein | 48.93 ± 2.79$^{ab}$ | 45.76 ± 1.18$^{a}$ | 51.97 ± 1.94$^{b}$ | 52.34 ± 1.35$^{b}$ |
| Crude lipid | 14.40 ± 2.95$^{ab}$ | 15.96 ± 0.97$^{ab}$ | 13.00 ± 2.00$^{a}$ | 17.08 ± 0.83$^{b}$ |
| Ash | 3.78 ± 0.37$^{ab}$ | 4.14 ± 0.13$^{b}$ | 4.00 ± 0.20$^{b}$ | 3.52 ± 0.13$^{a}$ |
| Crude fiber | 1.24 ± 0.03$^{a}$ | 1.26 ± 0.19$^{a}$ | 1.43 ± 0.18$^{a}$ | 1.34 ± 0.15$^{a}$ |
| Cacbohydrat | 11.23 ± 1.11$^{a}$ | 12.30 ± 1.30$^{a}$ | 12.80 ± 1.50$^{a}$ | 11.2 ± 1.11$^{a}$ |

Lipid ingredient was also highest (17.08 ± 0.83 %) in treatment that *L. hoffmeisteri* population were fed at ration 15% of body mass/day. However, there was no significant difference between the treatment 15% and the two treatment 10% and 0% (51.97 ± 1.94, 48.93 ± 2.79 % respectively) (P>0.05). The lowest lipid ingredient (13.00 ± 2.00) was recorded when feeding *L. hoffmeisteri* population with ration 10 % of body mass/day and significantly lower than those in other treatments (P<0.05) (Table 2)

The fatty acids ingredients (mg/g) of dry *L. hoffmeisteri* population were significant different between the treatments. The HUFA was highest (4.58 ± 0.18 mg/g) in the treatment that *L. hoffmeisteri* population were fed at the ration 15% of body mass/day, followed by the ration 5% of body mass/day (4.17 ± 0.17 mg/g) and significantly higher than those in other treatments (P<0.05). The lowest HUFA (3.37 ± 0.18 mg/g) was recorded when this species fed at the 10% ration (Table 3).

Saturated fatty acids (SFA) and unsaturated fatty acids with a double bond (MUFA) were highest (2.51 ± 0.11, 3.26 ± 0.16 mg/g, respectively) in the treatment 15% of body mass/day, and significantly higher than those in other treatments (P<0.05). Docosa hexaenoic acid (DHA) was quite similar between treatments. DHA was also highest (2.21 ± 0.20 mg/g) in the treatment with the ration of 15% but no significantly higher than those in other treatments (P>0.05) (Table 3).

The ingredient percentage of SFA, MUFA and HUFA were highest in treatment that *L. hoffmeisteri* population was fed at the ration 15% of body mass/day. Conversely, lowest SFA, MUFA and HUFA (11.51 ± 0.97, 16.06 ± 0.85, 24.43 ± 1.02, respectively) were recorded when feeding this species with ration 5 % of body mass/day) and significantly lower than those in other treatment (P<0.05). While, the ingredient percentage of PUFA, EPA and DHA were quite similar and no significant differences were found between treatments (Table 4).

The Table 4 showed that, the percentage of total fatty acids received little attention in

**Table 3: The fatty acids (mg/g) of dry *L. hoffmeisteri***

| Ingredients (mg) | Feeding Ration (%) | | | |
|---|---|---|---|---|
| | 0 | 5 | 10 | 15 |
| **SFA** | $2.03 \pm 0.33^{a}$ | $1.97 \pm 0.17^{a}$ | $1.83 \pm 0.10^{a}$ | $2.51 \pm 0.11^{b}$ |
| **MUFA** | $2.79 \pm 0.29^{a}$ | $2.74 \pm 0.14^{a}$ | $2.65 \pm 0.13^{a}$ | $3.26 \pm 0.16^{b}$ |
| **PUFA** | $2.69 \pm 0.62^{ab}$ | $3.23 \pm 0.13^{b}$ | $2.19 \pm 0.19^{a}$ | $3.00 \pm 0.15^{b}$ |
| **HUFA** | $4.04 \pm 0.43^{b}$ | $4.17 \pm 0.17^{bc}$ | $3.37 \pm 0.18^{a}$ | $4.58 \pm 0.18^{c}$ |
| **EPA** | $1.02 \pm 0.30^{ab}$ | $1.28 \pm 0.18^{b}$ | $0.79 \pm 0.10^{a}$ | $1.18 \pm 0.08^{b}$ |
| **DHA** | $1.92 \pm 0.31^{a}$ | $2,21 \pm 0.20^{a}$ | $1.75 \pm 0.16^{a}$ | $2.15 \pm 0.2^{a}$ |

**Table 4: The percentage of total fatty acids in dry *L. hoffmeisteri***

| Ingredient (%) | Rate (%) | | | |
|---|---|---|---|---|
| | 0 | 5 | 10 | 15 |
| **SFA** | $13.29 \pm 1.75^{ab}$ | $11.51 \pm 0.97^{a}$ | $14.08 \pm 0.77^{bc}$ | $15.74 \pm 0.72^{c}$ |
| **MUFA** | $18.28 \pm 1.94^{ab}$ | $16.06 \pm 0.85^{a}$ | $20.41 \pm 1.04^{b}$ | $20.42 \pm 1.00^{b}$ |
| **PUFA** | $17.38 \pm 1.8^{a}$ | $18.93 \pm 0.79^{a}$ | $16.87 \pm 1.50^{a}$ | $18.79 \pm 0.93^{a}$ |
| **HUFA** | $26.45 \pm 1.77^{ab}$ | $24.43 \pm 1.02^{a}$ | $25.97 \pm 1.39^{a}$ | $28.69 \pm 1.12^{b}$ |
| **EPA** | $6.56 \pm 1.13^{a}$ | $7.51 \pm 1.08^{a}$ | $6.10 \pm 0.81^{a}$ | $7.39 \pm 0.50^{a}$ |
| **DHA** | $12.49 \pm 0.39^{a}$ | $12.92 \pm 1.2^{a}$ | $13.51 \pm 1.23^{a}$ | $13.47 \pm 1.25^{a}$ |

previous studies of *L. hoffmeisteri*. Average free amino acid concentration of L.hoffmeisteri is 7.78 (nmol/mg) [3].

**2. Discussion**

Limitted information on the culture of *L. hoffmeisteri* is available in the literature. Our study, presents a culture system that is different from that of other *L. hoffmeisteri* studies in the literature, most having used glass beakers with substrates that consisted of a mixture of mud and organic matter in laboratory condition [12]. The study was to test whether large individuals of *L. hoffmeisteri* produce more eggs and/or cocoons than small individuals and to assess the influence of two granulometric fractions of sand on the reproduction and growth of *L. hoffmeisteri* under laboratory conditions [4]. Under laboratory conditions, the space was very small. Each experimental unit consisted of 600 ml glass beakers [12]. The experiment was conducted in 250-mL beakers containing 100 mL of sand, 100 mL of water [4]. Therefore, density and wet weight of the *L. hoffmeisteri* population increased slowly. These results indicate that the production system that we describe is more efficient and can produce a larger mass of *L. hoffmeisteri* more quickly than previously described systems in laboratory conditions.

This study showed that feeding rate significantly effected on *L. hoffmeisteri* density and biomass. However, feeding rate had little effect *L. hoffmeisteri* protein compositions. In general, density and biomass were highest at the 15% feeding ration and statistically with other feeding ration (0, 5, 10 %). Protein compositions was also highest at the 15% feeding ration but no statistically with 0, 10 % feeding ration. Little information on the effect of ration on *L. hoffmeisteri* density, biomass and protein compositions is available. In previous, *L. hoffmeisteri* culture studies in laboratory condition, olygochaete were provided low organic matter or high organic matter [12]. Other oligochaete culture studies, oligochaete were provided an excess of food [6,1].

## IV. CONCLUSION

5th weekend, Biomass and density of *L. hoffmeisteri* population were highest (133.90 ± 9.24 mg/cm², 64 ± 5 individual/cm², respectively) in the treatment when were fed at ration 15 % of body mass/day, followed at the 10 % ration (111.41 ± 7.52 mg/cm², 54 ± 4 individuals/cm²). The lowest density (5± 1 individual/cm²) and biomass (10.24 ± 1.18 mg/cm²) were recorded in the control treatment (not fed).

The protein compositions of *L. hoffmeisteri*

population were significanty different between the treatments. Protein ingredient was highest (52.34 ± 1.35 %) in treatment when were fed at ration 15% of body mass/day, followed at the 10% and 0% ration. The Protein ingredient was lowest (45.76 ± 1.18 %) at the 5% of feeding ration.

## V. ACKNOWLEDGEMENTS

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