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Effects of Feeding Frequency on the Survival, Growth and Body Composition of Buffalo Leech, *Hirudinaria manillensis*

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ABSTRACT

The growth and survival of leeches are highly affected by environmental changes particularly the availability of food. In this study, Asian buffalo leeches *Hirudinaria manillensis* were stocked in 10 L aquaria at 10 leeches per aquarium and randomly assigned to five different feeding frequencies (once every 3, 6, 12 and 24 days) and no feeding. The leeches were fed with prewarmed cattle blood in rubber sacs. The results showed that leech fed every 3 days had the highest average growth both by day 30 (82.8%) and day 60 (115.8%). This followed by those fed every 6, 12 and 24 days. By day 60, no significant differences in growth were found among the feeding leeches except those fed at every 24 days which have a significantly much lower growth. Starved leeches (FS) had a negative growth throughout the experiment. The weight lost in starved leeches was due to the depletion of reserved energy in the body. The protein content of all leeches was extremely high (about 80%) and no significant differences in the body composition of among treatments were found. The study also showed that leech survival was not significantly affected by feeding frequency. A feeding interval of 6-12 days was recommended for the commercial culture of leech *H. manillensis*.

Key words: Asian buffalo leech, *Hirudinaria manillensis*, feeding frequency, starvation

INTRODUCTION

Availability of preferential host for leech can be restricted due to climate, mobility and other factors in nature. The harsh condition of environment such as drought and winter may threaten or put stress on leech life (Kalarani *et al.*, 1993). Leech has to detect, attach and feed the target host and then drop off rapidly to the water pond before the host moves away (Michalsen *et al.*, 2007). Besides precision and agility, leeches must have incredible energy storage ability to sustain over a long period between meals. Leech sucks blood directly through the skin of the host, usually until fully satiated or gorged and then drops off (Orevi *et al.*, 2000; Kutschera and Roth, 2005; Michalsen *et al.*, 2007). The amount of food intake and digestion period is highly dependent on the complexity of the given diets and the physiological condition of leech (Orevi *et al.*, 2000; Zhang *et al.*, 2008). For instance, juvenile leech has difficulty to pierce through the mammal's skin while in some cases there might be constraints on morphology and sensory capacities of leech to encounter with their prey (Smith and Davies, 1995; Spencer and Jones, 2007).

The amount of blood ingested by a starved leech in one meal can be 7-9 times of their body weight (Dickinson and Lent, 1984; Tschoerner and Zebe, 1989). In certain cases, leeches that are fed to satiation at monthly interval may have a significantly lower weight gain compared to starved leeches fed in one single meal (Orevi *et al.*, 2000). Leech can be remained satiated for the next 3 to 18 months for digestion process while full depletion of the stomach may takes 4 to 21 months (Dickinson and Lent, 1984; Michalsen *et al.*, 2007). However, the gorged leech is known to have a higher risk of mortality probably due to digestive complication and the cannibalistic behavior itself (Kutschera and Roth, 2005; Teh *et al.*, 2011).

Spencer and Jones (2007) found that temperature has a crucial effect on the frequency of feeding of leech *Hirudo medicinalis*. A feeding interval of 8-12 months is found suitable for leeches kept at 10-20°C. At higher temperatures of 20-35°C, a feeding interval of 3-6 months is recommended. Zhang *et al.* (2008) fed *Hirudinaria manillensis* every 10 days in another study but they did not explain why that feeding frequency was chosen. *Hirudinaria manillensis* is being commercially cultured by small scaled farmers in Peninsular Malaysia for alternative medicinal purposes (Teh *et al.*, 2011). To date, no information is available on the optimal feeding frequency for *H. manillensis*. In this study, the effects of feeding frequency on the survival, growth and body composition of leech *H. manillensis* was determined.

MATERIALS AND METHODS

Culture condition: Leech *H. manillensis* was starved for at least four weeks before the experiment. Ten leeches of similar size (mean length 50±10 mm) were chosen randomly and placed into 15 units of aquaria which were filled with 4.5 L dechlorinated water. Temperature, DO, pH and ammonia content were measured and retained at 25-32°C, 7.6 mg L⁻¹, 7.8 and 0.05 mg L⁻¹, respectively. The leeches were acclimatized to the culture conditions for three days.

Experiment design: Five different feeding frequencies (once every 3, 6, 12, 24 days and no feeding) randomly assigned to the aquaria in triplicates. Preheated heparinized cattle blood (6 mg L⁻¹) was filled into rubber sac and fed to the leeches (Table 1) for a period of 60 days. The leeches were allowed to feed for 60 min and uneaten food was then removed. The weight of leeches was measured at the beginning, 30th day and the end of the experiment. Dead leeches were removed and recorded daily. A 100% water change was performed once every week.

At the end of the experiment, the leeches were sampled for body composition analysis. The crude protein, crude lipid, fiber and ash contents were determined following AOAC (1997) methods.

Statistical analysis: All data were analyzed by one-way ANOVA while differences between means were tested with Tukey test at p = 0.05 using SAS 9.1 (SAS Inc.). Percentage data were arcsine transformed prior to the analyses.

Table 1: Schedule of feeding for leech *H. manillensis*

Treatment	Feeding interval (days)	No. of feeding in 60 days period (times)
F03	3	20
F06	6	10
F12	12	5
F24	24	2
FS	No feeding	0

RESULTS

Leech fed every 3 days (treatment F03) had the highest average growth (Table 2) both by day 30 (82.8%) and day 60 (115.8%). This was followed by F06, F12 and F24 also had positive average growth during the first 30 days. Leeches under treatment F03, F06 and F12 continued to grow with increased average growth, except treatment F24 which showed a decreased growth on day 60. No significant differences in growth were found among F03, F06 and F12. Starved leeches (FS) had a negative growth throughout the experiment. Leech mortality was higher during the first 30 days than the second 30 days of experiment. Nevertheless, the leech survival was not significantly affected by feeding frequency.

The results of body proximate analysis are shown in Table 3. The protein content of all leeches was high (about 80%). However, there was no significant difference among the body composition of leeches.

DISCUSSION

Leech which lives in a highly variable environment needs a sufficient accumulation of energy reserves. Depending on availability of food, leeches change in their energy deposition and utilization to sustain the body needs (Kalarani *et al.*, 1993). On first 30 days, feeding leeches at every 3 days gave significantly better growth than other treatments. However, in a longer period of 60 days, the growth of leeches was similar for feeding frequency at every 3, 6 and 12 days. This is consistent with the growth pattern shown by *Nepheleopsis obscura* where asymptotic biomass is reached after 38 weeks regardless of their feeding frequency at three, two, or one feedings per week (Smith and Davies, 1995).

Starvation period between meals has an important influence on the size of the next meal and thus accounts for the growth of leech (Wrona and Calow, 1988). The growth of leeches fed every 24 days was significantly lower than a more frequent feeding. In fact, the growth decreased from 15% by day 30 to 13% by day 60. The decrease in the feeding efficiency is probably due to the

Table 2: Effect of different feeding frequency on leech at day 30 and day 60

Treatment	F03	F06	F12	F24	FS
Initial body weight (g)	2.14±0.03 ^a	2.20±0.06 ^a	2.12±0.06 ^a	2.18±0.06 ^a	2.18±0.05 ^a
Day 30					
Body weight (g)	3.91±0.12 ^a	3.35±0.14 ^b	3.42±0.11 ^{ab}	2.50±0.11 ^c	1.84±0.04 ^d
Weight gain (%)	82.80±2.90 ^a	52.40±2.40 ^b	61.50±3.70 ^b	14.80±7.60 ^c	-15.50±2.60 ^d
Survival (%)	70.00±0.00 ^a	53.30±3.30 ^a	60.00±5.80 ^a	63.30±8.80 ^a	56.70±3.30 ^a
Day 60					
Final body weight (g)	4.62±0.14 ^a	4.12±0.18 ^a	4.06±0.32 ^a	2.44±0.21 ^b	1.39±0.09 ^c
Weight gain (%)	115.80±2.90 ^a	87.20±3.60 ^a	91.70±13.8 ^a	12.60±12.9 ^b	-36.40±2.90 ^c
Survival (%)	70.00±0.00 ^a	50.00±5.80 ^a	56.70±6.70 ^a	60.00±10.0 ^a	56.70±3.30 ^a

Means within a row and followed by a same letter are not significantly different at $p > 0.05$

Table 3: Proximate composition (% dry matter) of leeches following different feeding frequencies

Treatment	F03	F06	F12	F24	FS
Crude protein	80.12±0.82 ^a	81.58±2.84 ^a	82.00±1.86 ^a	81.26±3.36 ^a	79.25±2.01 ^a
Crude lipid	0.96±0.10 ^a	1.13±0.10 ^a	1.27±0.27 ^a	0.99±0.09 ^a	0.88±0.32 ^a
Crude fiber	0.34±0.17 ^a	0.23±0.09 ^a	0.20±0.07 ^a	0.25±0.06 ^a	0.24±0.02 ^a
Ash	4.12±0.66 ^a	2.74±0.76 ^a	2.69±0.46 ^a	2.02±0.81 ^a	1.95±0.50 ^a

Means within a row and followed by a same letter are not significantly different at $p > 0.05$

prolonged period of starvation between meals. Similar observations were made on other fresh water leeches such as *N. obscura* and *Alboglossiphonia heteroclita* where lower food ingestion rate is accounted for a reduced growth rate at a low feeding frequency (Wrona and Calow, 1988; Smith and Davies, 1995).

Starved leeches and those fed at every 24 days were significantly smaller than other leeches. The weight lost in starved leeches was due to the depletion of reserved energy in the body. This was consistent with the finding of Barnes and Bick (1983) where the weight of leech *Haemopsis marmorata* decreases from an average 1.5 g/animal to less than 1.0 g in 80 days. Histochemistry study of *H. marmorata* showed that body shrunken during starvation is due to depletion of triglyceride in the coelomocyte cells (Barnes and Bick, 1983). In the natural environment, leeches usually de-grow in order to survive over winter (Dratnal *et al.*, 1992). Leeches have been reported to survive up to two years of starvation (Michalsen *et al.*, 2007).

Although, the body composition was similar in all treatments, starved leeches had the lowest protein content and crude lipid content. This suggested that protein and lipid were metabolized and used as a source of energy during the long 60 days starvation. In conjunction with the energy allocation strategy, smaller body size has a lower cost of resting metabolism and hence enhances the potential of a leech to survive for a prolonged starvation (Smith and Davies, 1995; McCue, 2010). It was also reported that the longer the starvation period, the lower the antithrombotic activity in the saliva of leech as to preserve energy and thus it is recommended that the starvation period shall not more than 4 months for effective utilization of leech *H. manillensis* (Ghawi *et al.*, 2012).

CONCLUSION

Feeding leeches at 3, 6 and 12 days feeding intervals provided a good growth and survival. A feeding frequency of every 6-12 days interval was recommended for the commercial culture of Asian buffalo leeches, *H. manillensis*.

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