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Performance of Selected Casings as Feed Carrier for Feeding of Asian Buffalo Leech *Hirudinaria manillensis*

Jun Chin Teh, Mohd Salleh Kamarudin, Abdullah Abd-Rahim, Che Roos Saad and Ehsan Ramezani-Fard

Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

Corresponding Author: Mohd Salleh Kamarudin, Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ABSTRACT

Providing a suitable feeding delivery method is very important in the studies of the feeding behavior of medicinal leeches. In this study, several materials and membranes were tested for their suitability as blood carrier for the feeding of Asian buffalo leech *Hirudinaria manillensis*. In the first experiment, four different materials (sheep intestine skin, cotton wool, cellulose sausage casing and rubber sacs) were prepared and filled or soaked with pre-warmed cattle blood. The food was offered to leeches stocked in 10 L aquaria at 10 leeches per aquarium. All treatments were triplicated. The results showed that cotton wool and sheep intestine skin had severe blood leakage while cellulose sausage casing failed to allow leech feeding. Leeches successfully imbibed the blood with no visible leakage through the rubber sacs. In the second experiment, different solutions (150 mM sodium chloride, distilled water and 6 mg L^{-1} heparinized cattle blood) were prepared into rubber sacs. Similar feeding protocols as in the first experiment were followed. Percentages of leech that approached the sacs and the average volume consumed per individual were estimated. A little or no feeding response was observed for the sacs filled with saline and distilled water. This indicated *H. manillensis* were able to detect the blood contained in the rubber sacs and successfully imbibed the blood. Therefore, the rubber sac was found to be suitable as the blood carrier for the feeding of buffalo leeches.

Key words: Asian buffalo leech, *Hirudinaria manillensis*, feed carrier, blood feeding

INTRODUCTION

Enguang (2008) reported that blood from cattle, sheep, wild boar, frog, snake and bird are the common food source for leeches in nature. Although blood may not be the only food for leech, it is deemed to be the most efficient feed for leech growth and reproduction (Michalsen *et al*., 2007). Buffalo leech (*Hirudinaria manillensis*) has a preference of mammal blood (Sawyer, 1986; Scacheri *et al*., 1993).

The process of blood sucking involves the coordination of leech sucker, buccal cavity, jaw, teeth and salivary gland. The skin of prey is worn out by teeth on the three jaws making a 'Y' shape incision (Michalsen *et al*., 2007; Enguang, 2008).

Hirudin is secreted and the radial muscle of the pharynx wall would contracts and relaxes in a regular pace to create a vacuum. Blood is drawn out into buccal cavity and then pumped into the gut (Enguang, 2008).

Blood of slaughtered animals such as cattle and swine are used to feed leeches in the commercial breeding facilities (Kutschera and Roth, 2005; Spencer and Jones, 2007). The blood obtained is usually heparinized and presented to the leeches in animal intestine or other membrane casing (Zhang *et al*., 2008; Teh *et al*., 2011). The carrier casing must allow blood consumption without any leaking to the environment. Different animal skins such as chicken skin, mouse skin, sheep gut skin and swine intestine, are typically employed as carrier for liquid feed. Physical properties of the animal skins such as pores size would influence its performance as feed carrier and hence feeding response of the respective animals (Pothikasikorn *et al*., 2007).

Besides animal skins, cloth, parafilm and commercial membrane also have been used for leech feeding in several earlier works (Galun and Kindler, 1966; Dickinson and Lent, 1984; Kutschera and Roth, 2005). Sawyer *et al*. (1981) invented a blood feeding device using a plexiglass box with two compartments separated by a piece of cow's head skin. Heparinized cattle blood is placed in one compartment while leeches are kept in the other compartment. The leeches (*Hirudinaria manillensis*) are able to feed through the skin but it could be too thick for some smaller leeches. Despite, this study was conducted to determine the efficiency of selected materials as blood casing for leech (*Hirudinaria manillensis*) feeding.

MATERIALS AND METHODS

Experiment I: Sheep intestine skin (Eastman Outdoors, Flushing, Michigan, USA), cotton wool, cellulose sausage casing (obtained from local food supplier) and rubber sacs (SSL Healthcare Malaysia SdnBhd) were evaluated as blood carrier for the feeding of buffalo leech, *Hirudinaria manillensis*. The sheep gut was soaked in water overnight and then washed with distilled water to remove the salt. Rubber sacs was also soaked overnight in a beaker, washed and rinsed with distilled water for three times before use.

All materials were prepared and filled or soaked with 50 mL of pre-warmed heparinized cattle blood (6 mg L^{-1} heparin salt) to 40°C. Each of them was then offered to 5 leeches (mean length 50±10 mm) placed in a 10 L aquarium which was filled with 4.5 L de-chlorinated water except for the treatment of cotton wool. All leeches were starved for 30 days prior the feeding trial. All treatments were triplicated. The 1 h of feeding time was allowed. The carrier condition and leech feeding behaviour was observed. Ingestion of blood was indicated by peristaltic movements of the leech's body wall muscles (Davies *et al*., 1996). The volume of blood for successful feeding was estimated. Thirty minutes after feeding, two leeches were chosen randomly from each aquarium to be dissected by using forceps and scalpel. The fluid or content in the body of leech was observed.

Experiment II: Three different solutions (6 mg L^{-1} heparinized cattle blood, 150 mM sodium chloride and distilled water) were offered to leeches by using the rubber sacs. A similar rearing and feeding protocols were conducted. All treatments were performed in triplicate. The volume of blood for successful feeding was also recorded.

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.

RESULTS

Experiment I: There was no significant different ($p>0.05$) among the number of leech approached to the food source contained in different materials. Leakage of blood from the tested material was observed by changing the aquaria water colour to red. The sequence from the most to the least redness in aquaria was cotton wool, sheep intestine skin, rubber sac and cellulose sausage casing. The feeding through cotton wool was done in dry aquaria as the blood was seeping out of the cotton. Leeches were successfully fed through all the materials except cellulose sausage casing (Table 1).

Experiment II: Leeches approached and attached to the rubber sac filled with cattle blood and saline solution. However, there were significantly higher ($p<0.05$) approach rate and consumption volume in rubber sac filled with blood compared to that filled with saline solution. No feeding response was observed in rubber sac filled with distilled water (Table 2). The feeding took not more than 45 min. Red fluid was found in the body of leeches that fed cattle blood (Fig. 1) and the volume of blood in the rubber sac was decreased. Non feeding leeches had no red fluid in their body (Fig. 2). Water in aquaria remained visibly clear after feeding except for one replicate in which regurgitation arose from one of the leeches (Fig. 3).

Table 1: Performance of selected materials as feed carrier

Materials	No. of leech approach (%)	Volume consumed (mL individual^{-1})
Cotton wool	93.3±6.7^{a}	Unable to define*
Sheep intestine	93.3±6.7^{a}	Unable to define*
Cellulose casing	60.0±11.6^{a}	No feeding
Rubber sacs	86.7±6.7^{a}	1.22±0.09

Means with the same letter are not significantly different at $p>0.05$, *The final volume of blood was not measurable due to severe leaking

Table 2: Volume of solution consumed through the rubber sacs

Solutions	No. of leech approach (%)	Volume consumed (mL individual^{-1})
Cattle blood	86.7±6.7^{a}	1.22±0.09^{a}
Saline water	13.3±6.7^{b}	0.33±0.17^{b}
Distilled water	0.0 ±0.0^{b}	0.00±0.00^{b}

Means with the same letter are not significantly different at $p>0.05$

Fig. 1: Red fluid was found in the body of a satiated leech

Fig. 2: No red fluid was observed in non-feeding leech

Fig. 3: Regurgitation arisen from leeches after feeding

DISCUSSION

Leeches showed different feeding response towards the blood provided in different carrying materials. Leeches detected the blood contained in all carriers by similar approaching rate but not all were successfully fed. Although, the amount of blood consumed through the cotton wool and sheep intestine skin was not possible to be defined, blood ingestion was generally observed by the peristaltic movements of the leech body. Whilst, the response of leeches towards blood in cellulose casing was poor (60% approach rate) and all failed to feed. Cellulose casing may be too thick to be pierced through by leech. Different feeding responses towards the feed (blood) contained in different carriers have also reported in other blood-sucking animals especially mosquitoes (Pheng *et al*., 2005; Pothikasikorn *et al*., 2007). Animal skin is a more favorable membrane for feeding of mosquitoes (*Aedes albopictus*) compared to artificial salted sausage skin (Pheng *et al*., 2005). Meanwhile, Pothikasikorn *et al*. (2007) also reported that blood feeding response of mosquitoes *Aedes aegypti* is best with chicken skin as blood carrier, followed by mouse skin and swine intestine layer.

Leeches successfully imbibed the blood through the rubber sacs. However, a little or no feeding response was observed for the sacs filled with saline (150 mM sodium chloride) and distilled water. This indicated *H. manillensis* could readily feed through the rubber sacs if the solution offered was palatable to them. Rubber sacs obtained from local market was made of natural rubber with only 0.02 mm thickness. The sac membrane was thin enough to enable the leeches to detect the blood and pierce through the membrane and thus feed on the blood inside the sac. The water in the

aquaria remained clear, indicating a minimal leakage of blood from the rubber sacs. The good elasticity of rubber might have allowed the immediate "auto sealing" of the pierced hole after the leech detached from the sac and thus minimized the leakage which could cause wastage and affect water quality.

Hirudinaria medicinalis, another commercially cultured medicinal leech, has been reported to be able to feed through thin layer of parafilm or membrane made of ox caecum (Dickinson and Lent, 1984; Galun and Kindler, 1966). Dickinson and Lent (1984) devised test tubes fitted with a single layer of parafilm whereas Galun and Kindler (1966) directly used silverlight membrane to hold the blood. No severe leaking has been reported by the latter authors. In contrast, the sheep gut membrane used in this study severely leaked. Kutschera and Roth (2005) placed individual leeches on blood-soaked cloth instead of offering the food in the culture tank to observe their feeding behaviour without measuring the blood consumption. Their feeding method is not practical for the commercial culture of medicinal leeches as it is laborious to capture the leeches for feeding and then releasing them back to the culture tanks.

Zhang *et al*. (2008) has successfully used fresh and disinfected cattle intestine skin to hold the blood and artificial food for the feeding of *H. manillensis*. In this case, the food intake of leech was calculated from the sum of wet body weight gain immediately after feeding, rather than measuring the decreased amount of solution offered. This was probably due to the technical difficulty to measure the remaining blood. Generally, leeches can ingest 7-9 folds of its own body weight in a single meal (Tschoerner and Zebe, 1989; Elliott and Kutschera, 2011). It is easy to identify those leeches that have fed by their distinct body shape soon after the feeding (Zhang *et al*., 2008). *Hirudinaria manillensis* gains 1 g of body weight after consumption of 3 g blood (Enguang, 2008). Therefore, the sum of wet body weight gain after feeding may not accurately reflect the amount of food intake as the excess fluid would be rapidly removed from the ingested blood upon entering the gut of leech (Sawyer, 1986).

CONCLUSION

Hirudinaria manillensis were able to detect the blood contained in the rubber sacs and successfully imbibed the blood. No blood leakage was observed when the leeches detached from the sacs after the completion of the feeding. Therefore, the rubber sac was found to be suitable as the blood carrier for the feeding of buffalo leeches. However, the performance shall be further compare to live host feeding.

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