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Performance of Selected Chemical Compounds in Eliciting Feeding of Asian Buffalo Leech, *Hirudinaria manillensis*

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ABSTRACT

The understanding of appetitive and foraging behavior of medicinal leeches is important for successful breeding and culture of healthy leeches. This study was conducted to determine the response of Asian buffalo leech, *Hirudinaria manillensis* for combinations of selected chemical compounds. Five solutions with different combinations of sodium chloride, arginine, glycine and glucose were filled into rubber sacs and fed to the leeches in 3 replicates each. Heparinized cattle blood was used as the control. The percentages of leech that approached the sacs and the average volume consumed per individual were estimated. After feeding, the survival of leeches in each treatment was monitored daily for seven days. The results showed that a solution containing 150 mM sodium chloride, 1 mM L-arginine, 50 mM glycine and 1 mg mL^{-1} glucose gave significantly higher ($p<0.05$) approach percentage and feed consumption than other solutions. Its performance was equivalent to the cattle blood. Poor feeding consumption was observed when only L-arginine or glycine were added to the saline solution. Nevertheless, mortality up to 40% was observed among the feeding leeches while the survival of non-feeding leeches was nearing or at 100%.

Key words: Asian buffalo leech, *Hirudinaria manillensis*, feeding response, chemical stimuli

INTRODUCTION

Traditionally, leeches have been widely used for blood-letting therapy and medication especially in China, Philippines, Thailand and Malaysia (Hassan, 2008; Tan, 2008; Zhang *et al*., 2008). Several important enzymes found in leeches, such as bufrudin and hyaluronidase, have been reported to substantially contribute to the treatment of myocardial disease, thrombotic events and tumors (Electricwala *et al*., 1991; Zaidi *et al*., 2011). The findings have created increasing interest on the potential applications of leeches and their extracts in the medical field including the Asian buffalo leech *Hirudinaria manillensis*. Leeches are now being commercially cultured for medical purposes and for the production of health products (Spencer and Jones, 2007).

A lot of research has been made on the European medicinal leech, *Hirudo medicinalis* (Sawyer, 1986; Brodfuehrer *et al*., 2006; Michalsen and Roth, 2007; Spencer and Jones, 2007; Claflin *et al*., 2009). Leeches can imbibe blood up to ten fold of its own weight during a single meal. There are distinct chemical cues acting on chemosensory structures on the dorsal lip of leeches that activate the entire complement of appetitive and ingestive feeding behaviors

(Kornreich and Kleinhaus, 1999; Lai *et al*., 2010). Stringent requirement of chemical stimuli in the feeding of European medicinal leech *H. medicinalis* has been identified (Galun and Kindler, 1968; Dickinson and Lent, 1984; Elliott, 1986; Kornreich and Kleinhaus, 1999). The European leech will imbibe an artificial blood composed of glucose and sodium chloride but the intake is less than half that of blood. Another study showed that an amino acid, L-arginine, is also an important chemical cue. When used in combination with sodium chloride, the solution activates all phase of feeding behavior in *H. medicinalis* and can sufficiently substitute the natural blood (Galun and Kindler, 1968; Elliott, 1986).

Similar to *H. medicinalis*, *H. manillensis* is also a freshwater sanguivorous leech with three jaws and belongs to the family of Hirudinidae (Harding, 1927; Michalsen and Roth, 2007; Zaidi *et al*., 2011). However, *H. manillensis* is found in the warm, humid and tropical climates and shows an evolutionary change that differs from *H. medicinalis* which can only be found in temperate regions (Sawyer, 1986; Michalsen and Roth, 2007). Unlike *H. medicinalis*, *H. manillensis* specializes in feeding on mammalian blood (Electricwala *et al*., 1993; Hassan, 2008). Hence, significant differences in taste and foraging pattern might be expected between the two species.

Little information is available on the feeding requirement of Asian medicinal leech, particularly *H. manillensis*. Glycine has recently been reported to be a good chemo-attractant in artificial feed that enhances the feeding consumption of *H. manillensis* (Zhang *et al*., 2008). However, the test diet also contains other premix substances, in which no specific amino acid cue can be defined as elicitor for the feeding response. Meanwhile, Zulhisyam *et al*. (2011) reported that a compound feed containing a mixture of compost, zeolite, phosphorus, calcium, soil and other ingredients can be used as an artificial diet for the leech. Therefore, the present study was designed to identify chemical stimuli which are essential to elicit feeding response of *H. manillensis*. This information is essential in developing an artificial diet for the commercial culture of *H. manillensis*. This diet could also be an ideal alternative food for leech culture and breeding to ensure that their quality is as disease-free as possible for medical uses.

MATERIALS AND METHODS

H. manillensis were obtained from a local supplier. The leeches were starved for four weeks prior to the start of the experiment. Ten leeches (mean total length 40±10 mm) were randomly chosen and placed into 10 L aquaria filled with 5 cm depth of de-chlorinated fresh water (4.5 L). Temperature, DO and pH were monitored and retained at 32°C, 7.6 mg L^{-1} and 7.8, respectively. A preliminary experiment with random combination of four potential chemical based on literatures was conducted (Galun and Kindler, 1966; 1968; Kornreich and Kleinhaus, 1999; Zhang *et al*., 2008). Based on the preliminary results, five treatments with different combination of chemical solutions were prepared (Table 1) and fed to the leeches in

Table 1: The chemical composition of test solutions

Treatment	Solution	References
A	150 mM sodium chloride	Galun and Kindler (1966)
B	150 mM sodium chloride and 1 mM L-arginine	Galun and Kindler (1968)
C	150 mM sodium chloride and 50 mM glycine	Elliott (1986)
D	150 mM sodium chloride and 1 mM L-arginine and 50 mM glycine	Kornreich and Kleinhaus (1999)
E	150 mM sodium chloride and 1 mM L-arginine and 50 mM glycine and 1 mg mL^{-1} glucose	Zhang *et al*. (2008)

separate aquaria. All solutions (30-50 mL) were heated to 37°C and filled into rubber sacs (Mister Classic 003, SSL Healthcare Malaysia Sdn Bhd). Rubber sacs were soaked overnight in a beaker and then rinsed with distilled water for three times before use. The filled sacs were then gently placed into the aquaria for the leeches to feed.

Following the method of Elliott (1986), the number of leeches that attached to the sac for more than ten seconds was recorded. If the leeches did not readily approach the sac, they were persuaded to taste it by pushing the leeches toward the sac. A negative result was recorded only after three unsuccessful trials. The volume consumed by each leech was recorded by measuring the volume of sac before and after feeding. Heparinized cattle blood (6 mg L^{-1} heparin salt) was used as control. Each treatment was triplicated.

After feeding, the survival of leeches was observed daily for a period of seven days. Dead leeches were removed immediately and 100% water changes were performed. 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

The performances of different chemicals in stimulating the feeding of *H. manillensis* are shown in Table 2. The best feeding response (66.7%) was obtained when cattle blood was used, followed by E, D, B and A. The feeding response to E (a combination of sodium chloride, L-arginine, glycine and glucose) was not significantly lower ($p>0.05$) than the blood. The poorest response (6.7%) was observed for C.

In terms of feed consumption, only the control and E gave satisfying results (0.86-0.91 mL individual). Feeding consumption was not significantly different between those two treatments. In general, leeches with a higher feeding consumption had a higher mortality rate while those with zero or almost zero consumption had an extremely high survival percentage (96.7-100%).

DISCUSSION

Pig guts are commonly used as casings to hold blood and artificial blood for the feeding of leeches in the laboratory trials and commercial production (Kornreich and Kleinhaus, 1999; Michalsen and Roth, 2007). This study showed that *H. manillensis* was able to readily feed on feeding solution through a thin rubber membrane. With the right stimulus or a combination of stimuli, the leeches would consume the solution offered.

During the study, leeches showed a typical feeding response by moving towards a potential source of food, probing, attachment and ingestion. In fact, chemical stimuli could have played an

Table 2: The performances of selected chemicals and combinations in stimulating feeding of *H. manillensis*

	Treatment					
Parameters	Control	A	B	C	D	E
Approach (%)	66.70±8.82^{a}	20.00±5.77^{bc}	20.00±5.77^{bc}	6.70±3.33^{c}	23.30±6.67^{b}	46.70±6.67^{ab}
Volume consumed (mL individual)	0.91±0.05^{a}	0.07±0.01^{bc}	0.11±0.01^{b}	0.00±0.00^{c}	0.29±0.11^{b}	0.86±0.02^{a}
Survival (%)	60.00±11.55^{a}	96.70±3.33^{bc}	96.70±3.33^{bc}	100.00±0.00^{c}	96.70±3.33^{bc}	80.00±5.77^{ab}

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

important role to determine the palatability of food or prey for the leeches. The combination of sodium chloride, L-arginine, glycine and glucose produced a similar feeding response and consumption as the control cattle blood. In fact the survival was higher than that produced by the blood.

A combination solution of sodium chloride and L-arginine was among the least favored diets among *H. manillensis*. In contrast, a solution containing 150 mM NaCl and 1mM arginine is sufficient to activate the entire feeding response and the consumption of *H. medicinalis* and indeed comparable to the blood (Elliott, 1986; Kornreich and Kleinhaus, 1999).

Zhang *et al*. (2008) reported that glycine is a good chemo-attractant which enhances the feeding ratio among *H. manillensis*. However, they did not clearly state the amount or concentration of glycine that is required for the optimal intake. In addition, the effect of arginine was not studied although bovine blood plasma included in their designed artificial diet contained 4.2% arginine (dry matter basis). In this study, glycine in sodium chloride produced almost no feeding respond and consumption among *H. manillensis*. The addition of glycine to arginine-sodium chloride seemed to increase feeding consumption although the feeding response remained the same. This suggested that glycine may have a cumulative effect with other stimuli in enhancing the food intake of *H. manillensis*.

In this paper, not all leeches responded to the feeding solutions including the cattle blood. Those leeches were considered as non-feeders. Non-feeders (6.67%) have also been reported in several chemosensory researches of *H. medicinalis* (Elliott, 1986; Kornreich and Kleinhaus, 1999). These non-feeders may be simply not hungry as leeches are able to store food in the gut and live for over a year without feeding. Alternatively, more physiological conditions in addition of other stimuli such as tactile and thermal cues (Dickinson and Lent, 1984) might be required to stimulate those leeches to feed.

Nevertheless, the survival of leeches after a satisfactory feeding was poor. Even with the cattle blood, 40% were found death during the seven days period of post-feeding. This is probably due to the tendency of overfeeding. Overfeeding has also often led to the death of *H. medicinalis* in captivity (Michalsen and Roth, 2007). The experimental setting may have allowed for a much more peaceful and extensive feeding compared to the natural habitat. As there is not enough anticoagulant saliva to dissolve the clot, regurgitation and hardening nodules are often observed on the satiated leech that eventually cause death. Indeed, a satiated leech may also be exposed to the risk of being attacked by other hungry or aggressive leeches. Since not all leeches fed during the experiment, their peers may subsequently attack those leeches that initially fed causing injury or even fatality to both.

Besides, it was also reported that air bubbles in the feed are life-threatening to leeches (Spencer and Jones, 2007). The air bubbles trapped in the rubber casing might be accidentally taken up by leech which could lead to digestive difficulty and death. Occasionally, symptoms of partial sloughing, swelling or curling behavior are observed on the leeches that are about to die even though they are not fed. Indeed, Egyptian freshwater leeches infected by peritrich ciliates (*Epistylis* sp.) show histopathological damages on the epidermis, dermis and cuticle of the leech body wall at the area of attachment. The presence of this parasite in high numbers would cover a large area of body and impede gas exchange, leading to suffocation and death. Some possible parasites affecting local leeches had also been reported, such as parasitic protozoans and flatworms (Zulhisyam *et al*., 2011). However, further diagnostic test should be carried out to determine the actual cause of death so that prevention or recovering method could be developed.

CONCLUSION

A solution containing sodium chloride, L-arginine, glycine and glucose had successfully triggered the feeding response and consumption among the Asian buffalo leech, *H. manillensis* comparable to the cattle blood. However, the high mortality of leech associated with post-feeding may need to be investigated.

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