UNIVERSITI PUTRA MALAYSIA

EFFECTS OF SELECTED ANIMAL BLOOD AND ARTIFICIAL DIETS ON SURVIVAL, GROWTH, AND CHEMICAL COMPOSITION OF ASIAN BUFFALO LEECH, Hirudinaria manillensis

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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November 2012

Chair: Mohd Salleh Kamarudin, PhD

Faculty: Agriculture

Hirudinaria manillensis is well-known as Asian medicinal leech which have been applied in traditional medicine and blood-letting therapy. *H. manillensis* which is also known as buffalo leech is a haemophagic fresh water leech that widely distributed in paddy field of Southeast Asia including Philippines, Southern China and Malaysia. This study investigated the effects of various casings as feed carriers (sheep intestine skin, cellulose sausage casings and rubber sacs), two different blood sources (cattle and poultry), feeding frequencies (once every 3, 6, 12, 24 days and no feeding), and some chemical compounds (sodium chloride, L-arginine, glycine, glucose) on growth, survival and body composition of Asian buffalo leech *H. manillensis*. Among the casings tested, the rubber sac has been found to be more suitable as a feed or blood carrier for *H. manillensis*. The leeches were able to detect the blood contained in the rubber sacs and successfully imbibed the blood. No blood leakage was observed after the leeches detached the sacs following completion of the feeding. The leeches fed with cattle blood had a significantly higher weight gain

(88%) than poultry blood fed ones (39%). FCR was more efficient with cattle blood (2.57) compared to that of the poultry blood (4.19). However, no significant difference ($P>0.05$) was found on survival and body composition of the leeches fed on either blood. The leeches fed every 3 days had the highest growth (116%) followed by those fed every 12 days (92%), every 6 days (87%) and every 24 days (13%). However, there was no significant difference ($P>0.05$) among the growth of leeches that were fed every 3, 6 or 12 days. Therefore, a feeding regime of every 6 to 12 days interval was recommended for the culture of the leech *H. manillensis* as it was more cost effective from management view. A solution containing a combination of sodium chloride (150 mM), L-arginine (1 mM), glycine (50 mM) and glucose (1 mg/ml) invoked a feeding approach and food consumption similar to that of the cattle blood. Mortalities up to 40% were observed among all feeding leeches while survivals of the non-feeding leeches were always close to 100%. Overfeeding might be one of the reasons for this problem but a further research has to be carried out to find out the actual cause(s) of such heavy deaths among the feeding leeches.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

KESAN DARAH HAIWAN PILIHAN DAN DIET BUATAN TERHADAP KEMANDIRIAN, PERTUMBUHAN DAN KOMPOSISI KIMIA LINTAH KERBAU, *Hirudinaria manillensis*

Oleh

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Hirudinaria manillensis merupakan lintah perubatan Asia yang digunakan dalam bidang perubatan tradisi dan terapi pembekaman. *H. manillensis* yang juga dikenali sebagai lintah kerbau, adalah lintah air tawar hemofagik yang boleh dijumpai di sawah padi di sekitar Asia Tenggara termasuk Filipina, Cina Selatan dan Malaysia. Kajian ini telah menyiasat kesesuaian pelbagai bahan karung makanan cecair (kulit usus biri-biri, sarung sosej selulosa dan sarung getah), kesan dua jenis darah haiwan (lembu dan ayam), kesan kekerapan pemakanan (setiap 3, 6, 12, 24 hari dan tidak diberi makan), dan kesan perangsang pemakanan lintah dengan kombinasi sebatian kimia terpilih (natrium klorida, L-arginina, glisina dan glukosa) ke atas pertumbuhan, kemandirian dan komposisi badan lintah kerbau *H. manillensis*. Di antara bahan karung yang diuji, karung getah didapati paling sesuai digunakan sebagai karung makanan atau darah untuk lintah *H. manillensis*. Lintah didapati berjaya mengesan darah yang terkandung dalam karung getah dan menghisap darah itu. Tiada kebocoran darah dari permukaan karung getah dapat dilihat selepas lintah

selesai makan. *H. manillensis* yang memakan darah lembu mencapai penambahan berat badan (88%) bererti ($P<0.05$) lebih tinggi daripada lintah yang diberi darah ayam (39%). FCR darah lembu (2.57) adalah lebih cekap ($P<0.05$) berbanding darah ayam (4.19). Bagaimanapun tiada perbezaan bererti ($P>0.05$) dari segi kemandirian dan komposisi badan lintah yang memakan darah lembu atau darah ayam. Lintah yang diberi makanan setiap 3 hari mempunyai pertumbuhan (116%) yang paling tinggi, diikuti oleh lintah yang diberi makanan setiap 12 hari (92%), setiap 6 hari (87%) dan setiap 24 hari (13%). Namun tiada perbezaan bererti ($P>0.05$) antara pertumbuhan lintah yang diberi makanan setiap 3, 6 atau 12 hari. Justeru, pemberian makanan setiap selangan 6 hingga 12 hari disarankan untuk kultur lintah *H. manillensis* kerana ia lebih kos efektif dari segi pengurusan. Campuran cecair yang mengandungi natrium klorida (150mM), L-arginina (1mM), glisina (50mM) dan glukosa (1mg/ml) didapati berjaya membuatkan lintah menghampiri dan menghisapnya pada kadar yang setanding dengan darah lembu. Kematian lintah sehingga 40% diperhatikan di kalangan lintah yang makan berbanding lintah yang tidak makan yang kerap mencapai hampir 100% kemandirian. Pengambilan makanan yang berlebihan mungkin antara penyebab kemandirian yang rendah ini. Penyelidikan yang selanjutnya harus dijalankan untuk mengenalpasti punca sebenar kematian tinggi di kalangan lintah yang makan.

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I certify that a Thesis Examination Committee has met on 28 November 2012 to conduct the final examination of Teh Jun Chin on her thesis entitled "Effects of Selected Animal Blood and Artificial Diets on Survival, Growth, and Chemical Composition of Asian Buffalo Leech, *Hirudinaria manillensis*" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

TEH JUN CHIN

Date: 28 November 2012

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LIST OF ABBREVIATIONS

USA	United States of America
UK	United Kingdom
ANOVA	Analysis of Variance
S.E.	Standard Error
FDA	Food and Drugs Administration, USA
NFE	Nitrogen-free Extract
DO	Dissolved Oxygen
NaCl	Sodium Chloride
Arg	Arginine
Gly	Glycine
Glc	Glucose
DNA	Deoxyribonucleic Acid
FCR	Food Conversion Ratio
WG	Weight Gain
SGR	Specific Growth Rate

CHAPTER 1

INTRODUCTION

1.1. Background of Leech

Leeches are widely used as a model animal in toxicology, physiology, neurobiology, biochemist, histology and many other fields of study. The history of clinical and medical use of leech can be traced back to 5th century BC. It was then considerably used in 19th century while the usage went into a decline in the early 20th century (Whitaker *et al.*, 2004; Papavramidou & Christopoulou-Aletra, 2009).

Leech salivary gland secretion contains unique bioactive compounds such as hirudin, destabilase, bdellins, eglins and hyaluronidase. These bioactive components are found very useful for prevention and treatment of various cardiovascular and dermatological diseases (Nikonov *et al.*, 1999; Baskova & Zavalova, 2001; Baskova *et al.*, 2001). Following the recognition by US Food and Drug Administration (FDA), *Hirudo medicinalis* has been used as "medical device" in plastic surgical and hirudo therapy in United States of America (Rados, 2004). Leeches of other species such as *Hirudinaria manillensis* are also getting into the spotlight for their potential value in health care and pharmaceutical products.

H. manillensis is haemophagic fresh water leech which is widely distributed in paddy field of Southeast Asia including the Philippines, Southern China and Malaysia (Harding & Moore, 1927; Enguang, 2008). It is also commonly known as

buffalo leech and being traditionally used for leech therapy by local people. Indeed, the huge interest in *H. manillensis* is emerging as a result of discovery and development of its unique protein extracts that are shown to have important thrombolytic effect and can potentially be the best drug for treatment of cardiovascular and cerebrovascular diseases (Steiner *et al.*, 1990; Latif, 1994; Hayson, 2005).

Not as the perception of most people about leeches, blood is not the only food for them. *H. manillensis* is predacious leech which is equipped with jaws and proboscis at their mouth (Pfeiffer *et al.*, 2005). Leeches can be fed a variety of different invertebrates such as insects (water bugs, gnats and mosquito larvae,), oligochaetes (both aquatic black worms and earthworms), amphipods and different kinds of mollusks including pond snails and freshwater clams (Galun & Kindler, 1968; Govedich & Bain, 2005). The predacious leech can be either engulfs the whole prey or uses its jaws to intrude and suck up the juice of its prey.

Leeches are bred commercially with blood of animals such as eel, catfish, bullfrog, cattle, goat, poultry and swine (Michalsen *et al.*, 2007; Hassan, 2008). Leeches are fed either the live animals or the blood packed in sausage casing (Kornreich & Kleinhaus, 1999; Spencer & Jones, 2007). In Malaysia, live eel or catfish held in the rat traps are put into the leech ponds or tanks. The leeches suck the fish blood to death (Hassan, 2008). This process may takes up to a few hours and thousands of leeches are fed. This method is rather torturing and the welfare of the fishes is seriously neglected.

In fact, the quality of leeches especially for medicinal use must be of highest standard and they must be free from possible contaminants and diseases. Feeding with fresh blood can expose the leeches to potential risk of various diseases. For instance, the fresh cattle blood may carry virus of mad cow disease and has been banned from use in livestock feed in USA and Europe (Phuong, 2004; Fumiere *et al.*, 2009). Besides, some animals are prohibited for certain religions. For example, leeches that feed on swine blood are considered "najis muhalazah" and cannot be used by Muslims.

Therefore, the understanding of appetitive and foraging behavior of medicinal leeches is important for successful breeding and culture of healthy leeches. In this research, suitability of different casings as feed carrier, blood sources from selected live stocks and different feeding frequencies were studied for their effects on survival, growth and body composition of leech. The response of leech *H. manillensis* towards combinations of selected chemical compounds in blood was also evaluated.

1.2. Objectives of the Research

The general purpose of this research was to improve the feeding of leech *H. manillensis* by study on the suitability of different materials as feed carrier and then the effects of selected animal blood, feeding frequency and performance of selected chemical compounds on leech feeding responses. The specific objectives were:

1. To study suitability of different materials (sheep intestine skin, cellulose

sausage casing and rubber sac) as feed carrier for the Asian Buffalo leech.

2. To study the effects of selected animal blood (cattle and poultry) on the survival, growth and chemical composition of leech.
3. To study the effects of feeding frequency on survival, growth and chemical composition of leech.
4. To study the performance of selected chemical compounds in eliciting feeding responses of leech.

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