UNIVERSITI PUTRA MALAYSIA

MORPHOPHYSIOLOGICAL STUDY OF DIGESTIVE SYSTEM OF BARKING DEER (Muntiacus muntjak Raf.)

I KETUT MUDITE ADNYANE

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2011

MORPHOPHYSIOLOGICAL STUDY OF DIGESTIVE SYSTEM OF BARKING DEER (*Muntiacus muntjak* Raf.)

By

I KETUT MUDITE ADNYANE

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, In Fulfillment of the Requirement for the Degree of Doctor of Philosophy

November 2011

DEDICATION

I dedicate this to my affectionate wife Eka Krisna Shinta Liber Sigai, DVM and son Edvan Vajra Adnyane who have inspired me to the higher ideals of life. I also dedicate to my father and mother, who are always support and pray for me.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

MORPHOPHYSIOLOGICAL STUDY OF DIGESTIVE SYSTEM OF BARKING DEER (*Muntiacus muntjak* Raf.)

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I KETUT MUDITE ADNYANE

November 2011

Chairman : Professor. Md. Zuki bin Abu Bakar @ Zakaria, PhD

Faculty : Veterinary Medicine

The morphophysiology of the gastrointestinal tract of barking deer (*Muntiacus muntjak* Raf.) was studied macroscopically and microscopically by scanning electron microscopic, histochemical and immunohistochemical methods. The results showed that composition of teeth was similar to other small ruminants such as goats and sheep, except for the presence of canine teeth at the upper jaw. The tongue was elongated with a rounded apex and contained four types of lingual papillae: filiform, fungiform, vallate and large conical papillae. The stomach was divided into rumen, reticulum, omasum and abomasum. The rumen was sac-like in shape. The reticulum was relatively large (12.33 ± 2.08 cm) in length, while the omasum was oval and small (5.00 ± 0.50 cm) in

length. On the mucosal surface of the rumen, ruminal papillae were densely distributed in all areas of the rumen. The length of intestine was relatively short (around 8.68 m) compared to the intestine of sheep and goat or other herbivores with ratio of the body length to the intestine was 1:9. The mucosal surface of the small intestine showed the presence of mucosal fold with intestinal villi, while the large intestine had only the mucosal folds. Unlike other ruminants, abomasum of barking deer possessed no cardiac glands. The salivary, stomach and intestinal glands contained acidic and neutral mucopolysaccharides, and glycoconjugates at various intensities. Chromogranin A, serotonin, gastrin, cholecytokinin, somatostatin and glucagon hormones-producing endocrine cells were present in the stomach and intestinal mucosa. The endocrine cells in the intestines were generally spindle shaped with long cytoplasmic processes ending into the lumen (open cell type) while in the stomach and large intestine, the cells were occasionally round or spherical in shape (close cell type). An uncommon distribution pattern of endocrine cells in the gastrointestinal tract of barking deer was noted for cholecystokinin- and glucagon-immunoreactive cells. Overall, the morphology of the digestive organs revealed that barking deer is concentrate selector eaters.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia. Sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KAJIAN MORFOFOSIOLOGI KE ATAS SISTEM PENCERNAAN KIJANG (*Muntiacus muntjak* Raf.)

Oleh

I KETUT MUDITE ADNYANE

November 2011

Pengerusi : Professor. Md. Zuki bin Abu Bakar @ Zakaria, PhD

Fakulti : Perubatan Veterinar

Morfofisiologi saluran pencernaan dari kijang (*Muntiacus muntjak* Raf.) diperiksa secara makroskopik dan mikroskopik menggunakan mikroskop pengimbas elektron, melalui kaedah histokimia dan kaedah imunohistokimia. Hasil kajian menunjukkan bahawa komposisi gigi kijang adalah serupa dengan haiwan ruminan kecil lain seperti kambing dan biri-biri, kecuali kewujudan gigi taring di rahang atas. Lidah kijang memanjang dengan hujung yang bulat dan mempunyai empat jenis papilla iaitu: papilla filiform, fungiform, valat dan papilla kon besar. Perut kijang terbahagi kepada rumen, retikulum, omasum, dan abomasum. Rumen mempunyai bentuk seperti kantung, retikulum secara relatifnya lebih besar (12.33 ± 2.08 cm), manakala omasum

berbentuk oval dan bersaiz lebih kecil (5.00 ± 0.50 cm) . Pada permukaan mukosa rumen, papila rumen diagihkan secara padat ke semua bahagian rumen. Usus kijang secara relatifnya adalah lebih pendek (sekitar 8.68 m) berbanding dengan usus biri-biri dan kambing serta haiwan herbivora lain dengan nisbah panjang tubuh ke atas usus sebanyak 1:9. Permukaan mukosa usus kecil menunjukkan kehadiran lipatan mukosa dengan vili usus, manakala usus besar hanya menunjukkan lipatan mukosa sahaja. Tidak seperti ruminan lain, abomasum kijang tidak memiliki kelenjar kardiak. Kelenjar air liur, perut dan usus mengandungi mukopolisakarida berasid dan neutral, dan glikokonjugat pada intensiti yang berbeza. Chromogranin A, serotonin, gastrin, cholecytokinin, somatostatin dan sel-sel endokrin yang menghasilkan hormon glukagon ditunjukkan di dalam perut dan mukosa usus. Sel-sel dalam usus umumnya berbentuk spindel dengan unjuran sitoplasma panjang berakhir di dalam lumen (jenis sel terbuka), manakala di dalam perut dan usus besar, sel-selnya kadangkala berbentuk bulat atau bujur (jenis sel tertutup). Pola taburan yang luar biasa direkodkan bagi kolesistokinin dan sel imunoreaktif glukagon pada sel endokrin di saluran pencernaan kijang. Secara keseluruhan, dari pemerhatian morfologi organ-organ pencernaan kijang menunjukkan jenis pemakan konsentrat.

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I certify that a Thesis Examination Committee has met on **11 November 2011** to conduct the final examination of **I Ketut Mudite Adnyane** on his thesis entitled **"Morphophysiological study of digestive system of barking deer (*Muntiacus muntjak* Raf.)"** 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 Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Mohamed Ali bin Rajion, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Mohd Zamri bin Saad, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal examiner)

Dahlan bin Ismail, PhD
Professor
Department of Animal Science
Universiti Putra Malaysia
(Internal examiner)

Yasuro Atoji, PhD
Professor
Gifu University
Japan
(External examiner)

SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 25 January 2012

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Md Zuki bin Abu Bakar @ Zakaria, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Noordin bin Mohamed Mustapha, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Srihadi Agungpriyono, PhD
Associate Professor
Faculty of Veterinary Medicine
Bogor Agricultural University (IPB)
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

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

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I KETUT MUDITE ADNYANE

Date: 11 November 2011

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LIST OF ABBREVIATIONS

AB	:	Alcian blue
ABC	:	Avidin-biotin-peroxides complex
Con A	:	Concanavalin agglutinin
DAB	:	3.3'-diamonobenzidine
DBA	:	Dolichos biflorus agglutinin
g	:	Gram
H & E	:	Hematoxylin & Eosin
kV	:	Kilo Voltage
LCA	:	Lens culinaris agglutinin
µl	:	Microlitre
µm	:	Micrometer
ml	:	Mililitre
PAS	:	Periodic acid Schiff
%	:	Percent
PNA	:	Peanut (*Arachis hypogaea*) agglutinin
SBA	:	Soybean (*Glycine max*) agglutinin
SEM	:	Scanning electron microscopy
WGA	:	Wheat germ (*Triticum vulgaris*) agglutinin
UEA	:	Ulex europaeus agglutinin
α-D Fuc	:	Fuccose
α-GalNac	:	N-acetyl-D-galactosamine
α-D Glc	:	Glucose
α-D Man	:	Mannose
β-D GlcNAc	:	N-acetyl-D-glucosamine
5-HT	:	Serotonin

CHAPTER 1

INTRODUCTION

Indonesia and Malaysia are known as countries of megabiodiversity and rich with substantial biological resources, which many of them have not been explored. Nevertheless, the populations of several animal species tend to decrease year by year. This condition has been caused by habitat damage due to exploitation of the forest for settlement and plantations, forest fires, and uncontrolled hunting activities (Corbet and Hill, 1992; Wilson and Reeder, 2005; Farida *et al.*, 2006).

The common barking deer or Indian muntjak (*Muntiacus muntjak*) also known as a kijang, kakad or muntjak is a ruminant animal belonging to the genus *Muntiacus*, family *Muntiacinae* within the order *Cervidae*. The animal inhabits the rain forests, areas of dense vegetation, hilly country, and monsoon forests in southern and southeastern Asia, from eastern part of Pakistan through India and Nepal, across Southeast Asia (including Malaysia and Indonesia) and southern China (Lekagul and McNeely, 1977; Ohtaishi and Gao, 1990; Long, 2003; Farida *et al.*, 2006).

There are ten species recognized under genus *Muntiacus,* and currently there are fifteen known sub-species of *Muntiacus muntjak*. The barking deer is the oldest of all known deer and are therefore prehistoric; since they retain characteristics, which have disappeared in our own native species of deer. The barking deer is the smallest of our resident deer and is still not fully understood, which makes the study of them immensely interesting. A mature male animal may reach a height of 43-46 cm at the shoulder and weight around 15-25 kg. The female is slightly smaller. Barking deer bucks have long pedicles from which relatively small antlers grow. Both sexes have canine teeth or tusks (Whitehead, 1972; Lekagul and McNeely, 1977; Ma *et al.*, 1986; Ohtaishi and Gao, 1990; Long, 2003).

It is stated that barking deer is more omnivorous, feeding on herbs, fruit, bird's eggs, small animals, sprouts, seeds and grasses. They use their canines to bite and their forelegs to deliver strong blows in order to catch small warm-blooded animals (Whitehead, 1972; Farida *et al.*, 2006). This is an interesting feeding behavior that is different from those of the common ruminants. To our knowledge, no report is available to support and describe the interesting and peculiar aspect of the digestive function of this species.

Morphological features of the gastrointestinal tract can reflect the digestive function. External and internal structures of the organs can suggest specific functions of the area. In ruminants such as cattle, sheep and goat, the forestomach is a very important site of fermentation and digestion that provide important volatile fatty acids and amino acids for the animal tissues. Internal structures of the stomach reflect specific function for those purposes. The density and morphology of the intestinal villi also reflect absorption process and passage of the ingesta. Enzymes, mucins and saliva are secreted by stomach and intestinal glands. These functions can be studied by observing the histochemical characteristics of the glands (Tamate *et al.*, 1971; Barry, 1977; Saber and Hofmann, 1984; Hofmann, 1988; Yamamoto *et al.*, 1991; Rowell-Schafer *et al.*, 2001; Mackie, 2002a; Soveri and Nieminen, 2007; Hofmann *et al.*, 2008; Clauss *et al.*, 2009).

Furthermore, gut hormones regulate digestive functions. Various hormones are secreted by many kinds of gut endocrine cells that are scattered among the intestinal epithelium. Distribution and frequency of occurrence of the various gut hormone-producing cells in the digestive tract reflect the characteristic regulation of the digestive function (Bordi *et al.*, 1991; Castaldo *et al.*, 1991; Castaldo and Lucini, 1991; Falkmer, 1993; Hiratsuka *et al.*, 1996; Groff and Youson, 1997; Baltazar *et al.*, 1998; Dall'Aglio *et al.*,

1999; Agungpriyono *et al.*, 2000; Ahlman and Nilsson, 2001; Eerdunchaolu *et al.*, 2001; Ku *et al.*, 2002; Cinar *et al.*, 2006).

Morphological studies of the digestive tract of domestic ruminants such as cattle, buffalo, goat and sheep have been well documented. In wild ruminants, such studies have been partly reported for certain species such as lesser mouse deer (*Tragulus javanicus*), sambar deer (*Cervus unicolor unicolor*) and the Japanese serow (Agungpriyono *et al.*, 1992; Agungpriyono *et al.*, 1995; Stafford, 1995; Yamamoto *et al.*, 1998).

Despite so many studies on the genetic and taxonomy of barking deer, there are only few biological and physiological information available on the organ system of the animal, especially the digestive system. So far, no information has been reported on the morphology of the gastrointestinal tract of barking deer, *Muntiacus muntjak*. Therefore, the current study was conducted with the following objectives:

1. to define the gross morphological characteristics of the digestive system of barking deer.
2. to define the histological structure of the digestive organs with special reference to the histochemical properties of the stomach and intestinal glands.

3. to define the distribution of glycoconjugates that reflect the glandular activities in the stomach and intestinal glands.
4. to define the distribution and frequency of gut endocrine cell that produce the digestive regulating hormones.
5. to describe a possible digestive function, feeding habit and food type of the animal based on morphological, histochemical, and immunohistochemical findings.

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