Establishment of Lesser Mouse Deer (*Tragulus javanicus*) Colony for Use as a New Laboratory Animal and/or Companion Animal:

1.Behavior; 2.Hematological Characteristics; 3.Reproductive Physiology; 4.Rumen Microbiology in Relation to Feed Digestibility; and 5.Metabolic Activities.

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Abstract

Five females and 5 males of the lesser mouse deer (*Tragulus javanicus*) captured from the jungles of Selangor and Pahang, Malaysia were introduced into the Rumen Microbiology Laboratory, Universiti Pertanian Malaysia. The behavior of the lesser mouse deer was different from that of domestic ruminants and was more similar to that of dog and cat. Hematological studies showed that the blood composition of the lesser mouse deer was different from that of other domestic ruminants. In many erythrocytes, 1 or occasionally 2 unique pits were observed. The wild lesser mouse deer and

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offsprings reproduced well in the laboratory. The mouse deer reached sexual maturity at about 4 to 5 months of age and adult size at 5 months. The earliest sexual maturity was observed at 166 days for the male and 125 days for the female. Estimated gestation period was 134 +/- 2 days, which was much shorter than previously reported. The rumen flora and fauna of the lesser mouse deer were unique and differed from those of other ruminants. For example, the presence of fairly large bacteria, similar to *Oval* and *Oscillospira* as well as natural occurrence of mono-fauna of protozoa and protozoa-free conditions in this animal was observed. A new species of protozoa, *Isotricha jalaludinii*, was detected. Our observations under laboratory conditions suggest that the lesser mouse deer may be suitable as a small laboratory animal for studies in domestic ruminants and/or as companion animal.

Additional key words: Ruminants, rumen, living fossil

Introduction

Studies on nutritional physiology of herbivorous domestic animals such as ruminants are very important in the field of veterinary and zootechnical sciences[2, 10, 12-15]. However, small herbivorous laboratory animals are still not established for comparative studies. It has been suggested that the lesser mouse deer could be used as an experimental animal model for ruminant studies[7, 8, 20]. From the view point of conservation and protection of wild life, it is also very important to establish a breeding colony.

The family Tragulidae comprises the three Asian species of mouse deer or chevrotain, genus *Tragulus*, and the water chevrotain of Africa, *Hyaemoschus aquaticus*[1]. Virtually unchanged during 25 million years of evolution, the mouse deer is considered to be the most primitive ruminant, known as a "living fossil", which first differentiated in the Oligocene, Cenozoic era from all other pecoran ruminants[5]. The taxonomic relationship of the Tragulidae to the other families of living artiodactyls is shown in Table 1. The lesser mouse deer, despite its name, is not closely related to the true deer. The larger mouse deer, *Tragulus napu*, weighs about 4 to 6 kg. The lesser mouse deer, *Tragulus javanicus*, is among the smallest (40-48 cm long, weighing 1.3-1.8 kg) of all hoofed animals. The body color of the lesser mouse deer is reddish-brown with an unbroken white stripe running from jaw to shoulder. Males do not have antlers but instead they have well-developed canines which extend beyond the upper lip. They occur in South-east Asia and all the Sunda Islands. The lesser mouse deer probably represents a stage in the evolution of the true deer and cattle which had fossilized in the tropical rain forest. The mouse deer lives in both primary and secondary forests. The Rumen Microbiology Laboratory of Universiti Pertanian Malaysia (UPM) has established a small lesser mouse deer colony and has initiated some studies on the reproduction, nutrition, microbial population and activities in the rumen of the lesser mouse deer.

To our knowledge, this is the first report on the successful establishment of a lesser mouse deer colony in the laboratory.

Materials and Methods

Ten lesser mouse deer (5 males and 5 females) captured from the jungles of Selangor and Pahang, Malaysia were introduced into the Rumen Microbiology Laboratory, UPM, between November 1990 and November 1991 with the permission from the Department of Protected Wild Life and National Park, Malaysia. They were kept in pairs in half of stainless steel cages measuring 46 cm (H), 116 cm (W) and 45 cm (D) with

Table 1. Taxonomic relationships of Tragulus javanicus

Order	Artiodactyla
Suborder	Suiformes
Family	Suidae. Pigs
Family	Tayassuidae. Peccaries
Family	Hippopotamidae. Hippopotamuses
Suborder	Tylopoda
Family	Camelidae. Camels and llamas
Suborder	Ruminantia
Infraorder	Tragulina
Family	Tragulidae
	Hyemoschus aquaticus. African water chevrotain
	Tragulus meminna. Indian chevrotain
	Tragulus javanicus. Lesser Malayan mouse deer
	Tragulus napu. Larger Malayan mouse deer
Infraorder	Pecora
Family	Cervidae. Deer
Family	Giraffidae. Giraffe, okapi
Family	Antilocapridae. Pronghorn antelope
Family	Bovidae. Cattle, sheep, goats, antelopes.

(Ralls *et al.*, Z. Tierpsychol., 1975)

movable divider. This divider was useful for the cleaning of the cage and pairing. Wood shavings were used as bedding material and were changed twice a week. Commercial rabbit pellet (GC-4, Oriental Yeast Co. Ltd. Japan) and water-spinach (*Ipomoae aquatica*) were given freely *ad libitum*. Sweet potato, carrot and long beans were hand-fed in the morning once a day (Fig. 1). Essentially, light was not used and the animals were kept under semi-dark conditions. The eating, drinking and other habits were observed regularly at feeding (morning) and resting (afternoon) times, while the reproductive performance and survival rates of the animals were monitored daily over a period of 40 months.

For the collection of blood samples, a 26 gauge needle was inserted into the saphenous vein. Blood was collected in Pasteur pipettes and haemotocrit capillaries. The method for hematological and scanning electron microscopic studies was described previously[9].

The rumen samples were collected with a rumen catheter and investigated. Samples for protozoa study were fixed and stained with a methylgreen-formalin-saline (MFS) solution for light microscopy. A part of the MFS-fixed sample was also used for scanning electron microscopy[11].

Fig. 1. Lesser mouse deer taking feed from hand

Results and Discussion

1. Behavior

It is generally recognized that the lesser mouse deer is extremely nervous and it is necessary to cover the cage and keep it dark when the animal is captured. However, based on our experience, the animals tended to be more nervous in complete darkness. Thus, proper handling right after capture was found to be crucial to the

subsequent survival of the animals in the laboratory. Lesser mouse deer could be hand-fed on the day they were brought into the laboratory. Hand-feeding using sweet potato, carrot and/or long beans in the morning was then practiced to help the animals adapt to their new environment (Fig. 1). Mortality of captured lesser mouse deer is usually very high, 60 - 70% (National Zoo and Institute of Medical Research in Kuala Lumpur, personal communications). However, we recorded a mortality of about 10% for captured lesser mouse deer in our laboratory. Both male and female can be good pets but probably the female is superior because she can be more tamed and emits less odor although the lesser mouse deer emits less odor as compared with other pet animals. Foster-nursed lesser mouse deer revealed that it can be trained toilet like dog and cat (Fig. 2). These characteristics together with the small size, lovely face and quietness make this animal attractive as a companion animal. The canine of the male sometimes injures the handlers and the male mouse deer emit more odor, especially the urine. Mouse deer is active during the daytime although it has been reported to be nocturnal and copulation which lasts 5 minutes is often observed during the daytime (observed under infrared rays and animals seem to recognize it)[8]. The behavior of the lesser mouse deer was different in many aspects from that of domestic ruminants[8, 19] and was more similar to that of dog, cat or rabbit (Table 2).

2. Hematological characteristics

Hematological studies showed that the blood composition of the lesser mouse deer was different from that of other domestic ruminants[9]. There is an evolutionary trend to reduce the size and to increase the number of erythrocytes for better transport of gasses[23]. The erythrocytes of the lesser mouse deer therefore appear to present more evolved mechanisms for the transport of gasses. The extremely low mean corpuscular volume (3.5 μm^3) was the smallest recorded in all known ungulates. The packed cell volume and hemoglobin concentration values were higher than in the other ungulates[22]. The more active species exhibit high hemoglobin values and elevated hemoglobin values may be common in wild animals[23]. Scanning electron microscopy revealed that the shape of the erythrocytes varied, including the presence of discoid, biconcave discoid, flat oval, spheroidal, ellipsoid rod, triangular and irregular forms. In many erythrocytes have 1 or occasionally 2 unique pits (135 +/- 65 nm; mean +/- S.D. in diameter) were observed. The pits occurred in all the examined animals which were

Fig. 2. Lesser mouse deer trained toiletting on newspaper

Table 2. Behavior of lesser mouse deer

Activity	Behavior
Rumination	In a standing position.
Eating	Does not use tongue to draw feed into the mouse.
Drinking	Uses tongue like a dog or cat.
Sitting	First drops hip and then bends the forelegs (like a dog). The forelegs are tucked in under the belly.
Stamping	Moves legs rapidly like a rabbit.
Head scratching	Uses hindlegs in a standing position.

derived from different colonies and were clinically healthy[9].

3. Reproductive physiology

Mouse deer reached sexual maturity at about 4 to 5 months of age and adult size at five months. The earliest age of the first parturition in our laboratory was 258 days. The canines of the young male do not extend beyond the upper lip until the animals are about 9 to 10 months old. Sexual cycle of the female lesser mouse deer was estimated to be 16 days from mounting action, the duration of the mating period was about 2 days where copulation occurs several times and they resume estrus unless they are pregnant. In the lesser mouse deer, pregnancy was not noticeable. The pregnant female tended to sit all the time a few days before parturition. The female lesser mouse deer have been reported to have a post-partum estrus[3, 4] and our observation showed that copulation could occur 30 minutes to a few hours after parturition. Most parturitions were observed to occur between 6:00 AM to 10:00 AM. The infant was on its feet within 30 minutes after birth. The mother ate and removed the neonate membranes. Most of the ungulate species which consume the fetal membranes and placenta generally maintain their young close to the parturition site for some days at least, while those that do not, usually lead their neonate away from the parturition site very early in the post-partum period[6]. There was no breeding season in the laboratory and parturitions were observed monthly.

All the 5 pairs of animals from the jungle and the offsprings reproduced well without major problems and exhibits relatively regular continuous parturitions (Table 3) which may compensate for their relatively long gestation

Table 3. Parturition interval of captured lesser mouse deer at Universiti Pertanian Malaysia

Animal No.	Origin	Entry	Parturition Record	Interval (days)
W- 1	Puchong	11/90	11/90[a)]	
			22/03/92	
			12/08/92	143
			26/12/92	136
			20/06/93	176
W- 3	Selangor	11/90	10/06/91	
			18/11/91	161
			17/08/92	274
			04/01/93	140
W-13	Puchong	11/90	03/08/92	
			19/12/92	136
			12/05/93	145
			22/09/93	133
W-15	Pahang	15/07/91	26/12/91	
			10/05/92	136
			20/09/92	132
			03/02/93	136
			26/07/93	173
W-23	Pahang	18/11/91	13/12/91	
			19/07/92	219
			04/12/92	138
			25/04/93	142
			20/09/93	148

a): Accurate parturition date was not available.
Data from November 1990 to February 1994.

period. Although it was reported that the gestation period of the lesser mouse deer ranged from 140 to 177 days[1, 16, 21], we observed a shorter gestation period in our colony. Apparent gestation period ranged from 136 to 164 days but actual gestation period was estimated to be 134 +/- 2 days, judging from the intervals of continuous parturitions shown in Table 3. This table also indicated that the lesser mouse deer are highly productive and that this high reproductivity supports the survival in the jungle and enables them to avoid extinction. Delay in implantation due to lactation probably occurs. Although it is considered that reproduction in the laboratory is usually difficult, we did not encounter major problems unlike in other wild animals introduced into the laboratory. The litter size in our laboratory was always one. The weight of the offspring was fairly large (120 to 190 g) in proportion to that of the mother animal (about 10%). Overall mortality of the young (newborn to weaning) in this study was about 12.8% (6/47, Table 4), although it had been reported to be as high as 75% (3/4) in the zoo[19]. Serious fighting between captive male and female lesser mouse deer which leads to the mortality of adults and youngs was also observed. The reproductive data obtained at UPM for 40 months are summarized in Table 4.

4. Rumen microbiology in relation to feed digestibility

From the view point of rumen microbiology, the rumen flora and fauna of the lesser mouse deer were unique and different from those other ruminants. Rumen contents of the lesser mouse deer was much more solid than in other large domestic ruminants. A preliminary study indicated that very active cellulolytic bacteria were present in the rumen of the lesser mouse deer, which may be ascribed to the fact that highly cellulolytic rumen microorganisms are essential for the breakdown of

Table 4. Reproductive data at Universiti Pertanian Malaysia

Breeding season	No, all year round
Estrous cycle	14 to 16 cyclic days
Mating period	2 days
Earliest age of copulation (Male)	166 days
Earliest age of conception (Estimated)	125 days
Earliest age of the first parturition	258 days
Shortest gestation period	132 days
Estimated actual gestation period	134 +/- 2 days
Earliest copulation after parturition (Post-partum estrus)	30 min. (Yes)
Delay of implantation of fertilized egg	Yes
Maximum continuous parturition within 136-day intervals	3 times (136, 133 and 136 days)
Sexual ratio	Male 25: Female 18
Litter size	1
Infant mortality before weaning	6/47

fibrous jungle forages composing the natural diet of the lesser mouse deer. Six species of ciliate protozoa belonging to two genera were detected in our laboratory (Table 5). Except for *Isotricha*, all the other five species of protozoa were similar to those found in large ruminants. The number of species detected in the lesser mouse deer was much lower than that detected in domestic ruminants. It has been reported that the composition of ciliate protozoa in wild ruminants is not varied[11]. The average number of ciliate protozoa in the lesser mouse deer was about 4.6 x 10^5/g of rumen content which was almost the same value as that recorded in healthy domestic ruminants. The isotrichids were distinctly different morphologically from the other two known species, thus requiring them to be assigned to a new species, *Isotricha jalaludinii*[11]. We often observed the natural occurrence of mono-fauna of protozoa, *Entodinium* alone or *Isotricha* alone, in the rumen of lesser mouse deer for unknown reasons. Sometimes, no protozoa was detected. Even when the animals were kept in pairs, one harboured several species of protozoa which the other only one species or no protozoa. It is difficult to develop and maintain a mono-fauna of protozoa or protozoa-free conditions. However these conditions naturally occur in the lesser mouse deer. Although no fungi had been isolated or identified from the rumen of the lesser mouse deer, we have isolated *Fibrobacter succinogenes*, *Ruminococcus albus* and *R. flavefaciens* from the rumen of the lesser mouse deer. Fairy large motile bacteria, similar to *Oval* (7-10 x 3-4 μm) and *Oscillospira* (4-6 x 20-40 μm), were present in large numbers (10^7 to 10^9 /g of rumen content) in the rumen of almost all the lesser mouse deer (Fig. 3). Considering the size of these bacteria, the biomass amount was fairly large. Attempts to isolate these large bacteria were unsuccessful. Prevot *et al.*[18] reported the effects of *Oscillospira guillermondii* on the rumen fermentation pattern in new- faunated cattle. Although few studies have been carried out on the flora and fauna, it is noteworthy to describe the presence of *Oval* and *Oscillospira* as well as the natural occurrence of mono-fauna of protozoa and protozoa-free conditions in this animal.

Table 5. Ciliate protozoa in the rumen of the lesser mouse deer

Total number	45.5 x 10^4/ml
Species identified	*Isotricha jalaludinii* *Entodinium simplex* *E. dubardi* *E. anteronucleatum* *E. nanellum* *E. convexum*

5. Metabolic activities

Studies on the voluntary feed intake, digestibility characteristics and water kinetics of the lesser mouse deer fed only lundai leaves (*Sapium baccatum*, a tropical shrub) showed that the animals consumed 3.7% M (body mass) per day. This value was rather high and it suggested that the lesser mouse deer can increase feed intake on less energy-dense diets. The apparent digestibility of dry matter (DM) of lundai leaves was high (83.8%) and comparable to the value obtained by the nylon bag technique in sheep. Urinary allantoin secretion (indicative of the outflow of microbial nucleic acids from the rumen) was high (4.7 mg allantoin/g digestible DM intake) when compared to other ruminants. This finding indicated a high net outflow of microbial

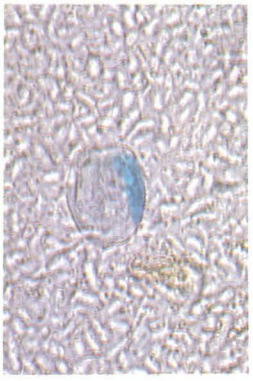

Fig. 3. Protozoa and abundance of large bacteria (*Oscillospira and Oval*) in the rumenof the lesser mouse deer. Protozoa was stained with a methylgreen-formalin-saline solution

cells from the rumen per unit of digestible energy, which is beneficial in terms of microbial protein supply to the animal. The body content of the lesser mouse deer based on a tritiated water dilution study, was 77% M, a value consistent with the very lean carcass with low lipid content[17].

Conclusion

The establishment of lesser mouse deer colony at UPM enabled researchers to study the reproductive performance, nutritional physiology and metabolic activities of the lesser mouse deer. The results indicated that it may be possible to introduce this animal as a pilot and/or bioassay test animal for studies in domestic ruminants. The information obtained could contribute to the management and conservation of the animal which is listed as a "totally protected wild animal" under the Malaysian Protection of Wildlife Act, 1972. Due to its attractive size, quietness and characteristics, the lesser mouse deer could also become a good house pet/companion animal (Fig. 4).

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(A) (B)

Fig. 4. Lovely and attractive lesser mouse deer as a house pet/companion animal
(A) Baby lesser mouse deer (22 days).
(B) Adult lesser mouse deer, the size is similar to that of a rabbit (1.6 kg).

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新しい実験動物／ペットとしてのマメジカ（*Tragulus javanicus*）コロニーの確立

1．行動；2．血液性状；3．繁殖生理；4．飼料消化に関わるルーメン微生物；5．代謝活性

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摘　要

マレイシアセランゴール州とパハング州のジャングルで捕獲された雄５頭，雌５頭のマメジカ（トラギュラスジャヴァニクス）が，マレイシア農業大学ルーメン微生物研究室に導入された。マメジカの行動は反すう家畜のそれと異なり，むしろ犬・猫のそれに類似していた。血液学的に，マメジカは他の反すう家畜と比較して異なった組成を示していた。多くの赤血球で，１個まれに２個の穴があるのが観察された。野生マメジカとその子孫は室内で繁殖良好であった。マメジカは生後４－５ヵ月で性成熟に達し，５ヵ月で成体サイズとなった。最短性成熟は雄で166日，雌で125日であった。推定妊娠期間は134＋／－２日であり，これまでの報告よりかなり短かった。マメジカのルーメン（第一胃）内微生物群は他の反すう動物と異なり，独特で興味深いものであった。例えば，オヴァールおよびオスシロスピラに類似した巨大な細菌，自然発生のプロトゾア単一相および無プロトゾア相が観察された。新種プロトゾア（イソトリカ　ジャラルデニイイ）が見つけられた。我々の室内での観察で，マメジカは反すう家畜のための実験動物としてもペットとしても有望であることが示唆された。

キーワード：反すう動物，ルーメン（第一胃），生きた化石

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