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Effect of cocoa on growth and reproduction of *Rattus tiomanicus*

(Kesan koko terhadap pertumbuhan dan pembiakan *Rattus tiomanicus*)

C. H. Lee*

Key words: growth, effect, cocoa, reproduction, rat

Abstrak

Pertumbuhan dan pembiakan tikus belukar *Rattus tiomanicus* telah dikaji di dalam makmal dengan makanan pelet tikus dan isi kelapa (Diet I) serta makanan pelet tikus, isi kelapa dan biji koko basah (Diet II). Dengan kedua-dua corak diet, satu perhubungan yang linear antara pertumbuhan dan berat badan tikus diperhatikan sehingga minggu ke-20. Tikus jantan membesar lebih cepat daripada tikus betina. Tikus didapati poliestrus dan membiak sembilan kali dalam setahun dengan purata 4 anak/kali. Pertumbuhan, umur pembiakan dan kadar kebuntingan tikus didapati lebih baik apabila diberi makan tambahan biji koko. Ini membayangkan potensi penambahan bilangan tikus yang cepat di ladang koko dan mengakibatkan kehilangan hasil yang teruk.

Abstract

Growth and reproduction of *Rattus tiomanicus* was studied in the laboratory on a diet of rat pellet, coconut meat, with and without fresh wet cocoa beans (Diet I and Diet II). On both diets, a linear relationship between growth and body weight until the 20th week was noted with the males growing slightly faster than the females. The rat was polyoestrus with nine litterings in a year and averaging 4 youngs/litter. Better growth, fertility age and pregnancy rates were found on a diet supplemented with cocoa beans. This reflects the potential of a rapid build-up of rat population in a cocoa field thereby causing severe losses.

Introduction

Growth and age is an essential prerequisite of animal life (Morris 1972). It is important to population studies, to relate damage and animal population structure, and the implementation of control measures. In rodents, lens weight (Thomas and Bellis 1980; Rowe et al. 1985), body weight (Southwick 1958), size (Evans 1949) and pelage condition (Smith 1968) have been used to estimate the age; separating the animals to infant, juvenile, sub-adult and adult classes. Lens weight, described as the best for mice age estimation (Rowe et al. 1985) because of its high reliability and accuracy (correlation coefficient of 0.96–0.97), was however noted to vary due to genetical differences (Berry and Truslove 1968), especially for highly artificial inbred strains. Morris (1972) cautioned the use of this parameter albeit the relevance of the data of such highly artificial inbred strains. Furthermore, sex affects the accuracy of the age estimation as male animals have slightly heavier lenses than females at a given age (Rowe et al. 1985). The need to sacrifice the animals to obtain the lens weight would lead to only one reading from each animal.

*MARDI Research Station, Hilir Perak, P.O. Box 25, 36307 Sungai Sumun, Perak, Malaysia
Author's full name: Lee Choon Hui

In contrast, age and body weight or age and tooth wear or age and pelage condition are characters that can be assessed in living animals, thereby providing continuous information on the age structure of free-living population. The age and tooth wear relationship is too tedious an assessment that may affect the behaviour of the animals in the field, whereas age and pelage condition would provide very limited information with no differentiation between adults and sub-adults.

The age and body weight relationship is preferred and frequently used because of its convenience, ease to perform and the animals can be easily differentiated as juveniles, adolescence (sub-adults) and adults. In rats and mice, growth is linearly related to body weight (Harrison 1951; Krebs et al. 1969). This has enabled the estimation of an animal's age with relative ease using a regression of age and body weight. Harrison (1956) indicated that Malaysian rat population was stable, had a constant age structure and the members had a similar growth rate. His studies were conducted on rats in forest and oil palm habitats. He considered that variation might be negligible as the Malaysian climate was quite uniform and breeding was continuous throughout the year. Kamarudin (1982) envisaged that the growth rate of *R. tiomanicus* would differ due to differences in food types, habitat and cover; and thus he undertook breeding of *R. tiomanicus* in the laboratory with laboratory chow, coconut meat, cocoa and bones as food. There was no study on breeding with laboratory chow, coconut meat and bones excluding cocoa as a food for comparison. He reported reproductive data similar to those of Harrison (1951, 1956) for wood rats in oil palm and forest habitats, with slight differences only in the mean number of youngs, fertility age and pregnancy rate. He categorized rats whose body weights were <59 g as juveniles, 60–89 g sub-adults and above 90 g adults. This study was carried out to examine the age and weight relationship of *R. tiomanicus* when raised on food with and without cocoa supplement. Reproductive data of the animals were also elucidated.

Materials and methods

Eight pregnant females of *R. tiomanicus* live-trapped from the cocoa-coconut fields of MARDI Hilir Perak were housed singly in laboratory cages measuring 45 cm x 45 cm x 30 cm. They were separated into two groups (four in each group) and fed laboratory rat pellet, coconut meat (Diet I) and water ad libitum. One of the groups was provided with fresh wet cocoa beans as supplement in addition to the above food items (Diet II).

The youngs were recorded at birth, weighed weekly until weaning, and subsequently weighed at 4-week intervals. At weaning, they were separated from their mothers, paired and provided with the same food as given to their mothers. Care was taken to prevent sib mating. The time and duration of births were noted. Youngs from these births were subjected to the same treatments. Body weight was recorded until death or 52 weeks whichever was earlier. Weights of females were excluded starting from 3 weeks prior to birth to avoid false correlation of weight to age. In each feeding regime, t-test was used to compare the body weight between 20 males and females at each age interval. Comparison of the rat body weight using t-test was also made at each age interval with 40 rats from each feeding regime. Regression analysis on the relationship of age to weight was undertaken from a sample size of 100 animals for each feeding regime.

Results

The newborns weighed between 3.8 g and 4.0 g. Steady growth was recorded for both males and females fed Diet I (*Table 1*). The females had slightly lower body weight at each age interval but it did not differ significantly from that of males ($t = 0.19$ to 1.55, D.F. = 38, $p = 0.15$ to 0.85). Similarly,

Table 1. Mean body weight of *Rattus tiomanicus* fed two diets at various ages

Age (weeks)	Body weight (g)		D.F. = 38 t value	p value
	Male	Female		
Diet I				
Birth	3.9 ± 0.1	3.9 ± 0.1		
1	9.0 ± 1.5	8.7 ± 1.5	0.63	0.50
2	16.8 ± 3.9	16.4 ± 3.8	0.33	0.75
3	23.2 ± 5.0	22.7 ± 5.5	0.30	0.75
4	30.9 ± 6.8	30.5 ± 6.3	0.19	0.85
8	65.6 ± 8.8	62.2 ± 10.0	1.14	0.25
12	74.1 ± 11.2	70.5 ± 11.9	0.99	0.30
16	83.6 ± 12.0	78.1 ± 10.4	1.55	0.15
20	93.6 ± 13.7	87.0 ± 13.4	1.54	0.15
24	98.8 ± 17.3	93.1 ± 18.2	1.02	0.30
28	107.5 ± 16.4	100.5 ± 13.2	1.49	0.15
32	112.4 ± 17.2	106.2 ± 12.6	1.30	0.20
36	110.6 ± 15.2	105.6 ± 12.9	1.12	0.25
40	108.2 ± 15.7	101.9 ± 11.6	1.44	0.15
44	112.1 ± 13.4	107.7 ± 9.5	1.20	0.27
48	114.7 ± 14.9	109.1 ± 12.3	1.30	0.20
52	114.6 ± 16.3	108.6 ± 11.1	1.36	0.18
Diet II				
Birth	3.9 ± 0.1	3.9 ± 0.1		
1	9.6 ± 1.5	9.3 ± 1.5	0.63	0.50
2	16.8 ± 3.2	16.2 ± 3.6	0.56	0.58
3	26.3 ± 4.5	25.7 ± 4.7	0.41	0.68
4	36.0 ± 7.5	35.1 ± 4.2	0.47	0.66
8	70.8 ± 10.0	66.5 ± 8.4	1.47	0.15
12	89.1 ± 13.9	83.2 ± 12.6	1.41	0.15
16	102.6 ± 17.5	94.8 ± 16.7	1.44	0.15
20	110.3 ± 19.2	102.1 ± 18.9	1.36	0.18
24	116.1 ± 19.8	106.7 ± 17.2	1.60	0.13
28	122.0 ± 22.9	112.4 ± 20.5	1.40	0.15
32	130.2 ± 28.5	118.8 ± 26.9	1.30	0.20
36	128.0 ± 17.5	120.9 ± 18.4	1.25	0.23
40	120.2 ± 21.3	117.5 ± 20.8	0.38	0.70
44	123.2 ± 19.3	115.6 ± 21.3	1.18	0.25
48	125.7 ± 21.3	116.8 ± 12.8	1.60	0.13
52	125.8 ± 21.4	117.2 ± 15.7	1.45	0.25

Diet I : Laboratory rat pellet and coconut meat
Diet II : Laboratory rat pellet, coconut meat and wet cocoa beans

steady growth was recorded for both males and females fed Diet II (*Table 1*). The females also had slightly lower body weight at each age interval and it did not differ significantly from that of males ($t = 0.38$ to 1.60, D.F. = 38, $p = 0.13$ to 0.70). Steady growth of animals occurred whether fed upon Diet I (*Figure 1*) or Diet II (*Figure 2*). Both the feeding regimes resulted in a curvilinear growth pattern with weight declining slightly beyond the 28th week. The weight gain in the early age tended to be higher than that when older (*Table 2*). At 28th week and beyond, weight gain was small and at times weight loss was noted.

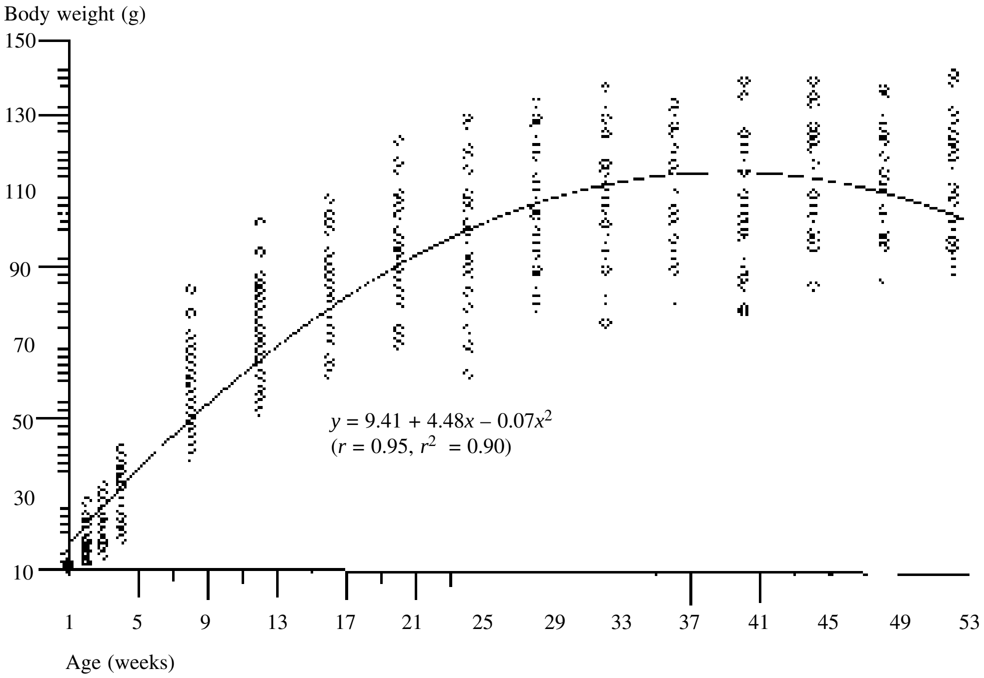

Figure 1. Relationship between age and body weight of ***Rattus tiomanicus*** *fed laboratory rat pellet and coconut meat (Diet I)*

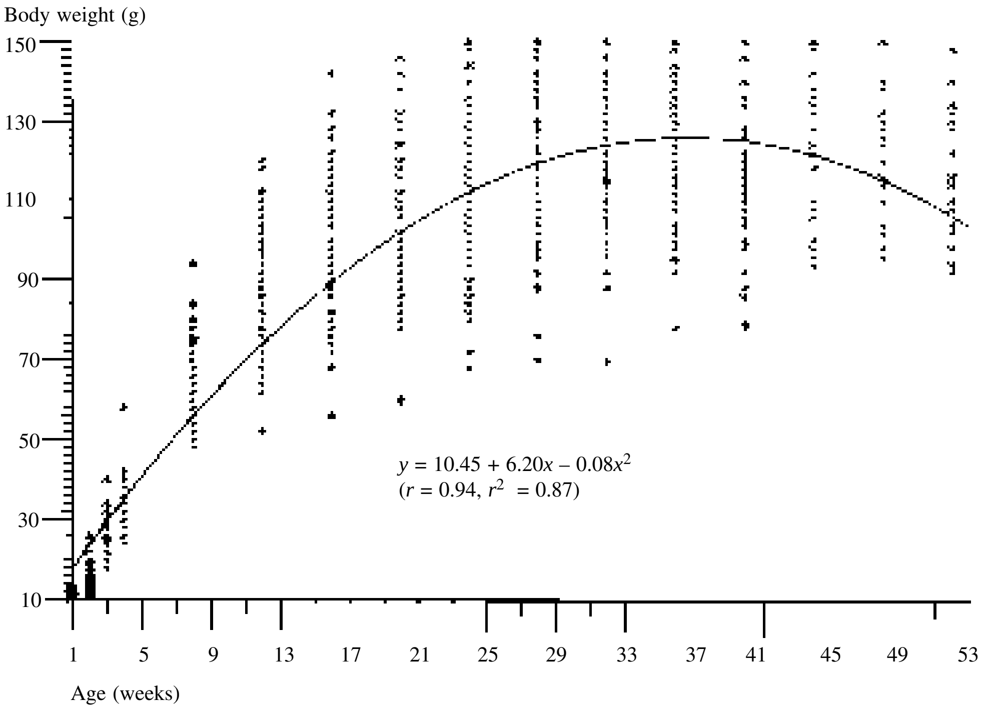

Figure 2. Relationship between age and body weight of ***Rattus tiomanicus*** *fed laboratory rat pellet, coconut meat and wet cocoa beans (Diet II)*

Growth regressional analysis

Quadratic regressional analysis of age to body weight for rats fed Diet I was (where y = body weight, x = weeks)

$y = 9.44 + 5.12x - 0.07x^2$
($r = 0.96$, $r^2 = 0.91$) for females,

$y = 9.58 + 5.76x - 0.07x^2$
($r = 0.95$, $r^2 = 0.90$) for males, and

$y = 9.41 + 5.48x - 0.07x^2$
($r = 0.95$, $r^2 = 0.90$) for both sexes pooled together.

Regressional analysis of age to body weight showed that a linear relationship was evident until the 20th week for all the animals, with

$y = 10.44 + 4.31x$ ($r = 0.94$, $r^2 = 0.88$)

For rats fed Diet II, the quadratic relationship between age and body weight was

$y = 11.46 + 5.69x - 0.07x^2$
($r = 0.94$, $r^2 = 0.88$) for females,

$y = 9.35 + 6.74x - 0.09x^2$
($r = 0.94$, $r^2 = 0.88$) for males, and

$y = 10.45 + 6.20x - 0.08x^2$
($r = 0.94$, $r^2 = 0.87$) for both sexes combined. A linear relationship was similarly evident until the 20th week and the equation was

$y = 10.78 + 5.32x$ ($r = 0.94$, $r^2 = 0.89$).

Similar weight gains were noted in the first 2 weeks of rats fed Diet I and those that fed Diet II (*Table 2* and *Table 3*). Subsequently, rats that fed on Diet II showed better growth than those fed Diet I (*Table 1* to *Table 3*). There were significant differences in body weight between both treatments from the third until the 16th week (t = 1.83 to 4.5, D.F. = 38, $p = 0.001$ to 0.075). At weaning age, i.e. 28 days (fourth week), animals fed Diet II weighed 36.0 ± 7.5 g for males and 35.1 ± 4.2 g for females, and were heavier than the males (30.9 ± 6.8 g) and females (30.5 ± 6.3 g) fed Diet I. At 32 weeks, the rats fed Diet II weighed 130.2 ± 28.5 g for males and 118.8 ± 26.9 g for females as compared with 112.4 ± 17.2 g for males and 106.2 ± 12.6 g for females fed Diet I (t = 1.68, D.F. = 38, $p = 0.10$). In both feeding regimes, 50% of the males (10/20) were scrotal and half the females (10/20) had perforated vagina by the seventh week after birth. The body weight of rats fed Diet II was 66.8 ± 6.3 g for males and 63.9 ± 9.9 g for females; for rats fed Diet I, the body weight was 62.8 ± 6.8 g for males and 58.2 ± 9.9 g for females.

On the average, 50% (10 out of 20) of the females were visibly pregnant for the first time at 20 weeks old (body weight 102.1 ± 18.9 g) for rats fed on cocoa supplement while the rats without cocoa supplement had first pregnancy at 24 weeks old (body weight 93.1 ± 18.2 g). Mortality of half of the adults occurred by mean age of 32 weeks for males (25/50 animals) and 38 weeks for females (16/32 animals) fed Diet I; for those fed Diet II, the mean age was 36 weeks for males (20/40 rats) and 43 weeks for females (16/32 rats). In both feeding regimes, the males had heavier body weight compared with the females towards old age (animals beyond 44 weeks old). However, the females lived longer than the males as three females survived until the 18th month as compared with 17 months for a single male.

Reproduction

The four wild-caught pregnant females fed Diet I had 11 young males and 10 females, while the other four wild-caught pregnant females fed Diet II also had 11 young males but 12 females. Pairing of the young males and females (no sib mating) within each feeding regime throughout the study had 10 rat couples for Diet I and 11 rat couples for Diet II.

In the Diet I feeding regime, one pair each recorded 0, 1, 4, 6 and 9 pregnancies while three pairs had three pregnancies and two pairs had two pregnancies. The mean number of youngs born to nine females for all pregnancies recorded under the Diet I feeding regime was 3.9 ± 1.2 with a total of 130 youngs comprising 60 males and 70

Table 2. Mean weight gain of *Rattus tiomanicus* fed two diets

Age (weeks)	Wt. gain (g), Diet I	Wt. gain (g), Diet II	D.F. = 78 t value	*p* value
Birth	0	0		
1	4.8 ± 1.5	4.8 ± 1.5		
2	7.7 ± 3.0	7.2 ± 2.7	0.78	0.45
3	6.4 ± 2.8	9.4 ± 3.1	4.54	0.001*
4	6.4 ± 4.7	7.5 ± 3.8	1.15	0.25
8	27.7 ± 9.4	31.4 ± 8.7	1.83	0.075
12	15.4 ± 7.8	19.9 ± 8.4	2.48	0.015*
16	8.8 ± 9.7	12.8 ± 8.0	2.00	0.03*
20	8.4 ± 7.2	8.6 ± 8.6	0.11	0.90
24	6.0 ± 14.3	5.8 ± 7.9	0.08	0.90
28	7.3 ± 9.8	4.5 ± 5.2	1.65	0.10
32	–0.5 ± 8.7	2.5 ± 7.2	1.68	0.10
36	4.2 ± 7.1	3.6 ± 5.6	0.42	0.67
40	–2.3 ± 7.3	–3.2 ± 23.4	0.23	0.80
44	3.4 ± 5.4	2.7 ± 6.1	0.54	0.59
48	2.0 ± 5.9	2.0 ± 4.9	1.60	0.13
52	–0.2 ± 4.0	0.1 ± 4.8	0.30	0.75

Diet I : Laboratory rat pellet and coconut meat
Diet II : Laboratory rat pellet, coconut meat and wet cocoa beans
40 animals (20 males and 20 females) in each diet regime
*significant differences

Table 3. Growth and development of *Rattus tiomanicus* fed two diets

	Diet I		Diet II	
	Male	Female	Male	Female
Birth weight (g)	3.9 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.9 ± 0.1
At weaning				
Weight (g)	30.9 ± 6.8	30.5 ± 6.3	36.0±7.5	35.1 ± 4.2
Age (weeks)	28 (26–35) days for all animals			
When 50% testis scrotal/ vagina perforated				
Weight (g)	62.8 ± 6.8	58.2 ± 9.9	66.8±6.3	63.9 ± 9.9
Age (weeks)	7 (5–9) weeks for all animals			
When 50% females pregnant				
Weight (g)	–	93.1 ± 18.2	–	102.1 ± 18.9
Age (weeks)	–	24 (19–28)	–	20 (16–25)
When 50% adult mortality				
Weight (g)	112.4 ± 17.2	106.2 ± 12.6	130.20 ± 28.5	118.8 ± 26.9
Age (weeks)	32(28–45)	38(30–52)	36(30–45)	43(32–56)

Diet I : Laboratory rat pellet and coconut meat
Diet II : Laboratory rat pellet, coconut meat and wet cocoa beans
Recordings from 20 animals in each set
Values in brackets indicate range

females (*Table 4*). Variation in the number of youngs per litter ranging from two to six was noted from the first litter to the sixth litter. The mean number of youngs was 2.8 at first birth to a maximum of 5.0 at litter number six. Very few females gave litters beyond the fourth birth. The earliest first litter was recorded on the 140th day while the last was on the 306th day, and the mean age of the female producing the first litter

Table 4. Litter size and interval between littering of laboratory bred *Rattus tiomanicus* fed two diets

Litter no.	Females sampled	No. of youngs	Littering interval (days)	Total no. of male youngs	Total no of female youngs
Diet I					
1	9	2.8(2–4)	204.0(140–306)	13	14
2	8	4.2(2–6)	66.5(46–76)	18	16
3	6	4.5(3–6)	57.5(55–60)	12	15
4	3	4.3(4–5)	70.0(49–110)	5	8
5	2	4.0(2–6)	74.0(63–85)	3	5
6	2	5.0(4–6)	62.0(56–68)	5	5
7	1	4.0	58.0	1	3
8	1	4.0	62.0	2	2
9	1	3.0	65.0	1	2
Total				60	70
Diet II					
1	11	4.8(4–6)	185.0(100–320)	25	28
2	8	4.0(1–5)	63.4(31–80)	16	16
3	4	4.7(4–5)	58.0(50–64)	11	13
4	4	4.3(4–5)	60.2(43–72)	8	9
5	3	2.7(1–4)	63.7(54–72)	2	3
6	3	2.7(2–4)	59.0(46–77)	1	4
7	2	3.5(3–4)	62.5(60–65)	3	4
8	1	3.0	59.0	1	2
9	1	3.0	62.0	1	2
Total				68	81

Diet I : Laboratory rat pellet and coconut meat
Diet II : Laboratory rat pellet, coconut meat and wet cocoa beans
Values in brackets indicate range
M:F = 60:70, x^2 = 0.769, D.F. = 1, p = 0.70
M:F = 68:81, x^2 = 1.134, D.F. = 1, p = 0.80
Both M:F ratio showed no significant difference

was 204 days. The interval between litters was variable with the shortest being 46 days between the first and the second litters of one female and 110 days being the longest between the fourth and the fifth litters of another female. The performance of a given female was variable. The litter size ranged from two to six as there was none with a litter size of one (*Table 5*). All youngs were raised beyond the weaning phase. The mean litter size weaned was 3.9 ± 1.2 and ranged from two to six youngs per litter throughout a female's life span.

All the 11 pairs of rats in the Diet II feeding regime gave birth to youngs. One pair each had 4, 6, 7 and 9 pregnancies while four pairs had two pregnancies and three pairs had a single pregnancy. The mean number of youngs born to 11 females fed Diet II was 4.0 ± 1.2 with a total of 149, of which 68 were males and 81 females (*Table 4*). The mean number of youngs varied from 4.8 recorded at the first birth to 2.7 at the fifth birth although the number of youngs varied from one to six. The earliest first litter was recorded on the 100th day and the latest was on the 320th day, hence the mean age of the female producing the first litter was 185 days. The shortest and longest intervals between litters were 31 and 80 days respectively, both noted between the first and the second litterings. The performance of a given female was also variable. Mortality of youngs prior to

Table 5. Survival of laboratory-bred *Rattus tiomanicus* according to litter size and diet

Litter size	No. observed	Youngs weaned (%)	Av. no. weaned/litter
Diet I			
2	3	100.0	2.0
3	10	100.0	3.0
4	10	100.0	4.0
5	6	100.0	5.0
6	4	100.0	6.0
Diet II			
1	2	100.0	1.0
2	1	50.0	1.0
3	7	80.0	2.4
4	13	96.2	3.8
5	12	100.0	5.0
6	2	100.0	6.0

Diet I : Laboratory rat pellet and coconut meat
Diet II : Laboratory rat pellet, coconut meat and wet cocoa beans

weaning was noted in litter size with 2, 3 and 4 youngs (*Table 4*). The mean litter size weaned was 3.9 ± 1.3 and ranged from one to six youngs per litter throughout a female's life span.

Discussion

There is a good linear relationship between body weight and growth, and a regression of age and body weight can be used to estimate the animal's age. Morris (1972) stated that weight is the most obvious indicator of size and for an increase in size that is directly correlated with increasing age, the body weight can be taken as an age index. Findings reflecting a good linear relationship between body weight and growth have also been noted for *R. exulans* (Wirtz 1973), *R. norvegicus, R. rattus* (Hirata and Nass 1974) and *Microtus* spp. (Krebs et al. 1969). The growth of rat appears to occur at a very regular rate until a certain size and the weight, then, would fluctuate around that size. Until this weight, the body weight of the rat can be an acceptable indicator of age. In this study, regular growth of *R. tiomanicus* until the 20th week (body weight 120 g) was similar to that reported by others (Harrison 1956; Kamarudin 1982). For the Norway and the Polynesian rats, the age-weight relationship also holds true until the 20th week (Hirata and Nass 1974).

Considering the body weight of animals feeding on both the Diet I and Diet II, the rats in this study may be categorized as juveniles when their body weight was <60 g, corresponding to age of 7 weeks (as 50% testis scrotal/vagina-perforated animals were >60 g) and animals of body weight >90 g as adults (50% females pregnant were >90 g), and animals of 60–89 g as sub-adults. Categorizing animals >90 g as adults would be within the linear relationship of body weight to the age of 20 weeks. Similar findings were reported by Kamarudin (1982) when he raised *R. tiomanicus* on a diet of laboratory chow, coconut meat, cocoa and bones.

Growth of male *R. tiomanicus* was slightly faster than the females and they reached a greater average maximum size with both types of diet. Harrison (1951) reported that the males of *R. tiomanicus* were larger and heavier than the females in both forest and oil palm areas. Similarly in Hawaii, Wirtz (1973) reported that males of *R. exulans* were larger and heavier than the females.

The evaluation of food supplemented with cocoa reflects the potential growth and development of the rat population in cocoa plantings. Enhanced rat growth noted with the above diet showed the potential of a rapid build-up of the animal population in the field thereby causing severe losses. Weaver et al. (1975) found cocoa at low concentrations to be a growth promoter. They reported that cocoa with theobromine concentrations below 100 mg per metabolic weight unit in the daily rations of mice enhanced growth. On the contrary, large proportions of cocoa in the diet of rats were noted to cause death.

The laboratory study showed that *R. tiomanicus* is polyoestrus and could breed throughout the year. On both diets, the number of litters recorded did not exceed nine and averaging about 4 youngs/litter. Similar findings on *R. tiomanicus* were also noted by others (Harrison 1951; Kamarudin 1982). In contrast, *R. argentiventer* easily produced 9 litters/year with 6 youngs/litter (Lam 1983).

Slightly better growth rates, fertility age and pregnancy rates were noted in *R. tiomanicus* fed a diet supplemented with cocoa beans. Harrison (1951) reported that the fertility and the pregnancy rates of *R. tiomanicus* in oil palm areas were lower than those in the forests. He attributed these variations and fluctuations to habitat differences. Despite the variations, the reproductive data for laboratory-bred *R. tiomanicus* were within the ranges of those obtained by Harrison (1951, 1956). Such variations and differences between rats of the same species in different habitats have been noted by Jackson and Barbehenn (1962) in the Pacific islands. They attributed these variations to nutritional and environmental factors and not genetic variations. This is substantiated in the present study as the rats were from the same population but fed different diets.

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