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AGE ESTIMATION OF *RATTUS ARGENTIVENTER* FROM EYE LENS WEIGHT

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Abstract

Attempts were made to correlate eye lens weight and other body measurements of laboratory-bred Rattus argentiventer *(rice field rats) with their ages. Eye lenses were fixed in 10% formalin, dried at 120°C and weighed. The rats were sacrificed at varying ages up to 112 days. Transformed age and lens weights were highly correlated (r = 0.9486). Incorporation of the other measurements did not cause marked statistical improvement over the equation derived from using lens weight alone. In the case of live animals, particularly domestic rats, the equation derived from transformed age on total length was considered to be fairly reliable. However, in the case of the field rat, caution should be exercised because the error increases with age as the growth rate decreases.*

Introduction

Aging criteria for small mammals are of interest to ecologists and mammalogists because these are necessary for determining the age structure of populations. There are many methods for determining age in mammals, which involve measurements of the genital system, skeletal development, tooth wear and eye lens size. The lens weight method of determining age has been developed in recent years and tested on a number of animals with varying degrees of success; however, variable results have been obtained with small mammals, particularly rodents[1,2]. In both sexes of *Rattus norvegicus* lens weight increases during the period from birth to 550 days[3]. The dry weight of the lens of the cottontail rabbit increases continuously throughout life, so that the weight of the lens could be used as an indicator of age[4]. The precision of estimates of age of jack rabbits based on lens weight decreases as age increases[5]. For *R. villosissimus* and *R. fuscipes* the errors of prediction increase absolutely as the age increases. Predicted ages of less than 150 days were reasonably accurate whereas predictions above 300 days were less accurate. The variation in lens weight has been attributed to differences in nutritional status and body condition[6]. In contrast, lens weight was not correlated with body condition in *Myocaster coypus*[7]. Differences in lens growth of *Micotus pinetorum* were not attributable to sex, season of birth or laboratory generation[8].

Little has been published in regard to the problem in the genus *Rattus* in Southeast Asia. The objective of the present work was an attempt to correlate eye lens weight and other body measurements of laboratory-bred *R. argentiventer* with age.

Materials and Methods

Animals

Wild rats were dug out of burrows in the rice fields. Male and female rats with body weights over 80 g were selected for breeding. F_1 known-age litters were divided into 9 groups. Seven groups of rats ranging from 14 to 54 days old, equally spaced 7 days apart, were used in the study. At 56 days after birth to 112 days, when rats are supposedly mature, they were examined once a month.

Caging

A total of 40 male-female pairs of rats were kept in wire cages, 28 x 46 x 44.5 cm in size. Rice straw and bamboo tubes were provided as nesting materials. The animals were given water *ad libitum* and fed rice grain with food additive. Rice plants at the booting stage were fed to them once a week. After they had been paired for 20 days, each female was observed daily for signs of pregnancy, such as enlargement of the abdomen and mammary development. Young rats were separated from the mother on the day of sacrifice or when 30 days old (weaning age). They were then divided into sex groups and kept in captivity until the day of sacrifice.

Eye Lens Measurement

A total of 65 female and 74 male rats were examined at various ages. Eyeballs were removed immediately after the rat had been killed with chloroform, and preserved in a vial containing 10 percent formalin. The left and right eye lenses were treated separately. They were stored at room temperature (26–28°C) for 10 - 14 days[9]. Following fixation, each eyeball was rinsed in distilled water and the lens was dissected out. Each lens was gently rolled on filter paper to remove surface moisture. Lenses were dried in individual open-topped vials in an oven at 120°C for 16 hours. On removal from the oven, lenses were allowed to cool to room temperature over silica gel in a desiccator. Each lens was weighed on a Mettler H51 AR analytical balance to the nearest 0.01 mg, with silica gel present in the weighing compartment.

Procedures for Body Measurement

The dead specimen was extended on its back over a ruler and total length (TL) was measured from the tip of the nose to the tip of the tail.

Tail length (T) was measured from the base of the anal region to the tip of the tail.

Hind foot length (HF) was measured from the heel to the tip of the longest toe and did not include the claw.

Ear length (E) was measured from the lowest margin of the orifice to the farthest point on the margin of the ear flap in as natural a position as possible.

Statistical Analysis

The statistical methods used in the analyses of these data were single and multiple regression analyses to investigate the relationships between transformed age and six variables measured for each rat. Data for each sex were analysed separately. The analysis is divided into three parts :

(i) Transformed age in relation to lens weight, using simple linear regression.

(ii) Transformed age and six measured variables (body weight, total length, tail length, hind foot length, ear length and lens weight), using stepwise regression.

(iii) Transformed age and five external measurements (not including lens weight), using stepwise regression.

The relationship between age and lens weight is not linear. A logarithmic transformation of age produced an approximately linear relationship. Transformed age was derived by taking the logarithm of age after adding the gestation period as a constant : $\log_{10}$ (age + gestation period) where the mean gestation period of *R. argentiventer* is 22.9 days[10]. This transformation has been found not only to improve greatly the goodness-of-fit of the age-prediction equation but also to reduce the heterogeneity of variance and non-normality of errors in this type of data[5,7]. Mathematical models describing these relationships were derived by computer, BMDP 2R[11].

Results

There were no significant differences between right and left lens weight. Thus, measurements of both eye lenses were pooled together and the mean weight of each pair was used for analysis.

Transformed Age and Lens Weight

The results of the simple linear regression analysis of transformed age on lens weight are presented in Table 1. Transformed age and lens weight had been shown to be highly correlated ; the correlation coefficients(r) are 0.9586 and 0.9351 for male and female rats, respectively. Simple regression equations that fitted for males and females were not significantly different. Thus for practical purposes, ages of both males and females can be estimated from the same equation.

The equation is $\log_{10}$ (age + 22.9 days) = 1.4267 + 0.0449 (mean lens weight).

The standard error of the intercept and slope are 0.0113 and 0.0013, respectively, and the corelation coefficient(r) is 0.9486. The slope and intercept of the regression are highly significant. The equation was used to estimate the mean value of age based on the observed average value of mean lens weight, and the results are presented in Table 2, Figure 1.

Transformed Age and Six Measured Variables

The results of the stepwise regression procedure for selecting variables in the regression equation are presented in Table 3(a). Because the admitted variables in the equations for male and female rats show differences, the pooled equation for both sexes cannot be produced.

Transformed Age and Five External Measurements

For some age predictions, it is desirable that animals should not be killed[12]. Hence, five external measured variables were analysed by using stepwise regression for selecting variables in the regression equation the results of which are presented in Table 3(b). Because of the admitted varables in the equations for male and female rats show some differences, a pooled equation for both sexes cannot be produced. For practical purposes a single predictive equation for both sexes which includes as few variables as possible is desirable. Thus, the repeated analyses by using the simple linear regression of transformed age on total length were carried out. The results are presented in Table 4. A single equation for both sexes using total length alone as a predictor is : $\log_{10}$ (age + 22.9 days) = 1.2459 + 0.0029 (total length). The standard error of the intercept and slope are 0.0178 and 0.0001, respectively, and the correlation coefficient (r) is 0.9392. The slope and intercept of the regression are highly significant. The results of using this equation to estimate the mean value of age based on observed mean value of total length are shown in Table 5 and Figure 2.

Discussion

The eye lens technique for determining age has been applied to several other species of rodents. Some workers used the mean weight of each pair of lenses for the equation[7,8]. It was reported that there was no difference in the eye lens weight of the right and left lens[3,6]. In the present study, similar results were also obtained ; thus the mean eye lens weight of each pair was used for statistical analyses.

The results of this study indicated that there was a very close correlation between age and lens weight. No significant difference between the regression equations for males and females using transformed age on lens weight, was observed. A single predictive equation for both sexes is recommended for practical use. The present results were in accordance with those for *Microtus pintorum* and *M. montanus.* The pooled equation relating transformed age to lens weight for both sexes was found to be slightly better than in the case of *Myocastor coypus*[7]. On the other hand, in *Microtus arvalis*[13] the body size reflected the sex difference in lens weight. For *Mus musculus* the slight differences in calculated regressions for the and the females appeared to be genetically controlled[2].

It was reported that in the black-tailed jack rabbit, inclusion of ear and foot measurements into the equation of transformed age on lens weight gave a somewhat better estimation of age ; however, this was not applicable to old animals[14]. In this present study, incorporation of sex and body measurements caused no marked statistical improvement over the equation derived from transformed age on lens weight. Hence, the simple regression equation of transformed age on dry lens weight was a satisfactory basis for this study. However, removal of the animals from a study population necessarily limits the application of this technique. Simple regression equations derived from transformed age on total length were considered to be good for live animals, but they should be applied to field rats with caution because the error increases with age as the growth rate decreases. Comparison between observed age with estimated age derived from lens weight (Table 2) and total length (Table 5) indicated that the error of estimated age of the last age group (112 days) showed great differences in both equations. The ages are 12.1 days for lens weight and 24.1 days for total length. Thus, the difference of estimated age derived from these two regression equations are 12 days.

This preliminary study has shown that dry lens weight could be a reliable age indicator for *R. argentiventer*. Further studies on this field rat are in progress.

TABLE 1. RESULTS OF SIMPLE LINEAR REGRESSION ANALYSES RELATING TRANSFORMED AGE TO LENS WEIGHT OF *R. ARGENTIVENTER*.

Sex	Intercept	Regression coefficient	F value in regression	Correlation coefficient (r)
Male	1.4221	0.0454	815.61[a]	0.9586
Female	1.4327	0.0442	438.60[a]	0.9351
Male & Female	1.4267	0.0449	1230.28[a]	0.9486

A single predictive equation can be used for combining sexes ($F_{cal\,(2,135)} = 1.10$).

[a] highly significant at 0.01 level of probability.

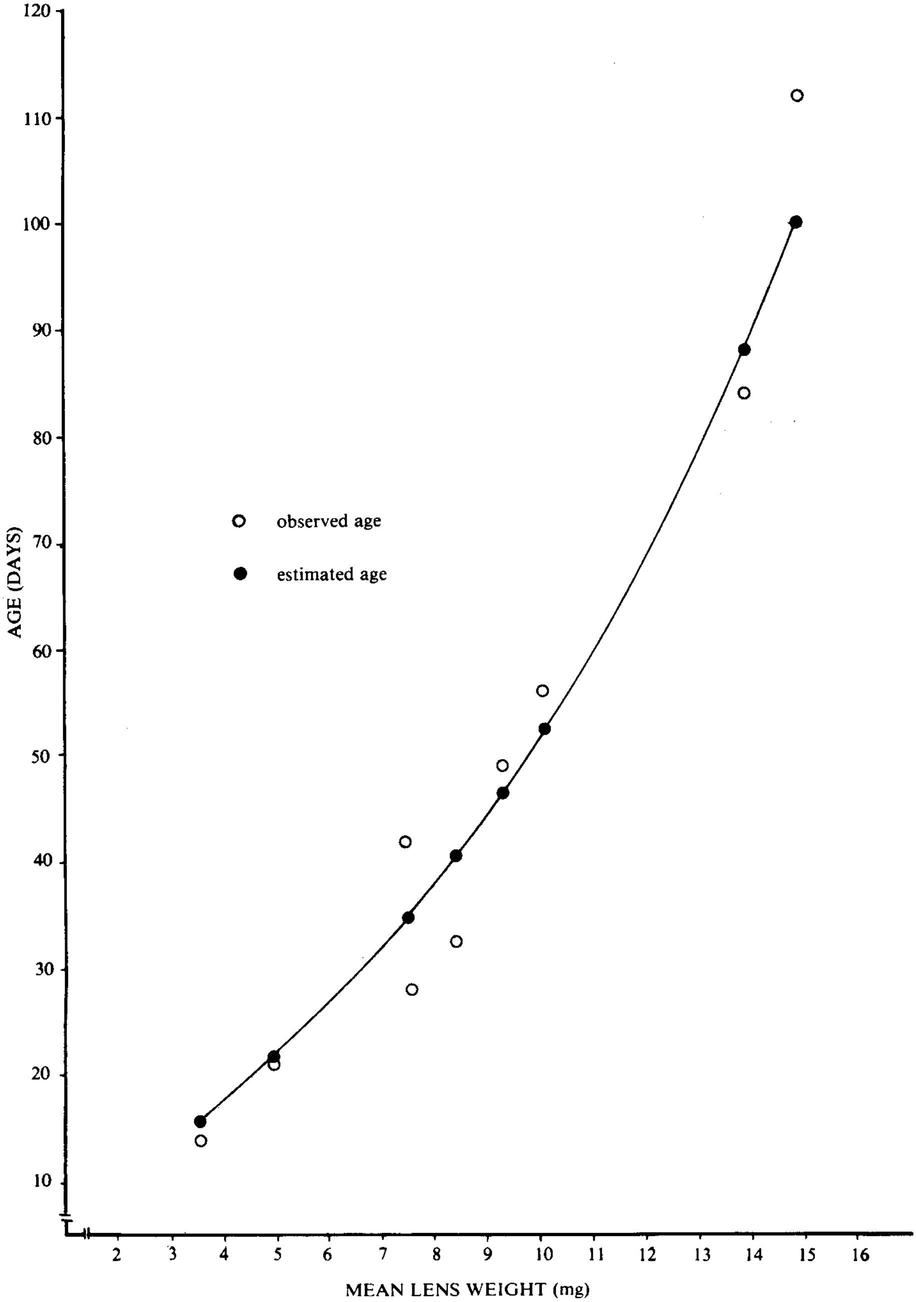

Figure 1. Regression curve in original scale derived from equation $\log_{10}$ (age + 22.9) = 1.4267 + 0.0449 (mean lens weight) for *R. argentiventer*.

TABLE 2. ESTIMATED MEAN VALUE OF AGE BASED ON OBSERVED AVERAGE VALUE OF MEAN LENS WEIGHT FOR *R. ARGENTIVENTER* USING SIMPLE REGRESSION EQUATION FOR BOTH SEXES :

$\log_{10}(\text{age} + 22.9) = 1.4267 + 0.0449 \text{ (mean lens weight)}$

Number of animals		Average mean lens weight (mg)	Observed age (days)	Estimated age (days)	95 % Confidence interval	
Male	Female				Lower age limit	Upper age limit
9	8	3.5579	14	15.7	14.4	17.0
9	5	4.9569	21	21.7	20.5	22.9
9	9	7.5980	28	35.7	34.6	36.8
9	9	8.3789	35	40.6	39.4	41.8
9	9	7.3833	42	34.4	33.3	35.5
8	9	9.2547	49	46.6	45.3	48.0
9	9	10.0658	56	52.7	51.1	54.4
7	3	13.8005	84	88.3	84.3	92.6
5	4	14.7533	112	99.9	94.8	105.2

TABLE 3. RESULTS OF STEPWISE REGRESSION PROCEDURE FOR ANALYSES RELATING (1) TRANSFORMED AGE TO SIX MEASURED VARIABLES[a], (2) TRANSFORMED AGE TO FIVE EXTERNAL VARIABLES[b] FOR *R. ARGENTIVENTER.*

Sex	Intercept	Regression	coefficient	Partial F value in regression	Coefficient of determination (r^2)
(1) Male	1.3893	Lens weight	0.0354	70.29**	
		Tail length	0.0013	6.54**	0.9257
Female	1.3030	Total length	0.0018	41.74**	
		Lens weight	0.0167	13.29**	0.9249
(2) Male	1.2406	Total length	0.0029	455.29**	0.8635
Female	1.3559	Total length	0.0037	89.76**	
		Hind foot length	0.0114	5.86**	0.9167

a) = body weight, total length, tail length, hind foot length, ear length and lens weight.

b) = body weight, total length, tail length, hind foot length and ear length.

** = highly significant at 0.01 level of probability.

TABLE 4. RESULTS OF SIMPLE LINEAR REGRESSION ANALYSES RELATING TRANSFORMED AGE TO TOTAL LENGTH OF *RATTUS ARGENTIVENTER*

Sex	Intercept	Regression coefficient	F value in regression	Correlation coefficient (r)
Male	1.2406	0.0029	455.29**	0.9292
Female	1.2523	0.0028	628.17**	0.9533
Male & Female	1.2459	0.0029	1024.32**	0.9392

A single equation can be used for combining sexes ($F_{cal_{(2,\ 135)}} = 0.45$)

$$\log_{10}(\text{age} + 22.9 \text{ days}) = 1.2456 + 0.0029 \text{ (total length)}$$

** highly significant at 0.01 level of probability.

TABLE 5. ESTIMATED MEAN VALUE OF AGE BASED ON OBSERVED MEAN VALUE OF TOTAL LENGTH FOR *R. ARGENTIVENTER* USING SINGLE REGRESSION EQUATION FOR BOTH SEXES :

$$\log_{10}(\text{age} + 22.9) = 1.2495 + 0.0029 \text{ (total length)}$$

Number of animals		Average total length (mm)	Observed age (days)	Estimated age (days)	95 % confidence interval	
9	8	115.089	14	14.8	13.4	16.2
9	5	129.466	21	18.6	17.2	19.9
9	9	175.165	28	33.2	32.0	34.4
9	9	195.220	35	41.1	39.8	42.4
9	9	198.945	42	42.7	41.4	44.1
8	9	208.054	49	46.8	45.3	48.3
9	9	211.110	56	53.0	51.3	54.9
7	3	272.851	84	84.0	79.9	88.2
5	4	278.222	112	87.9	83.5	92.4

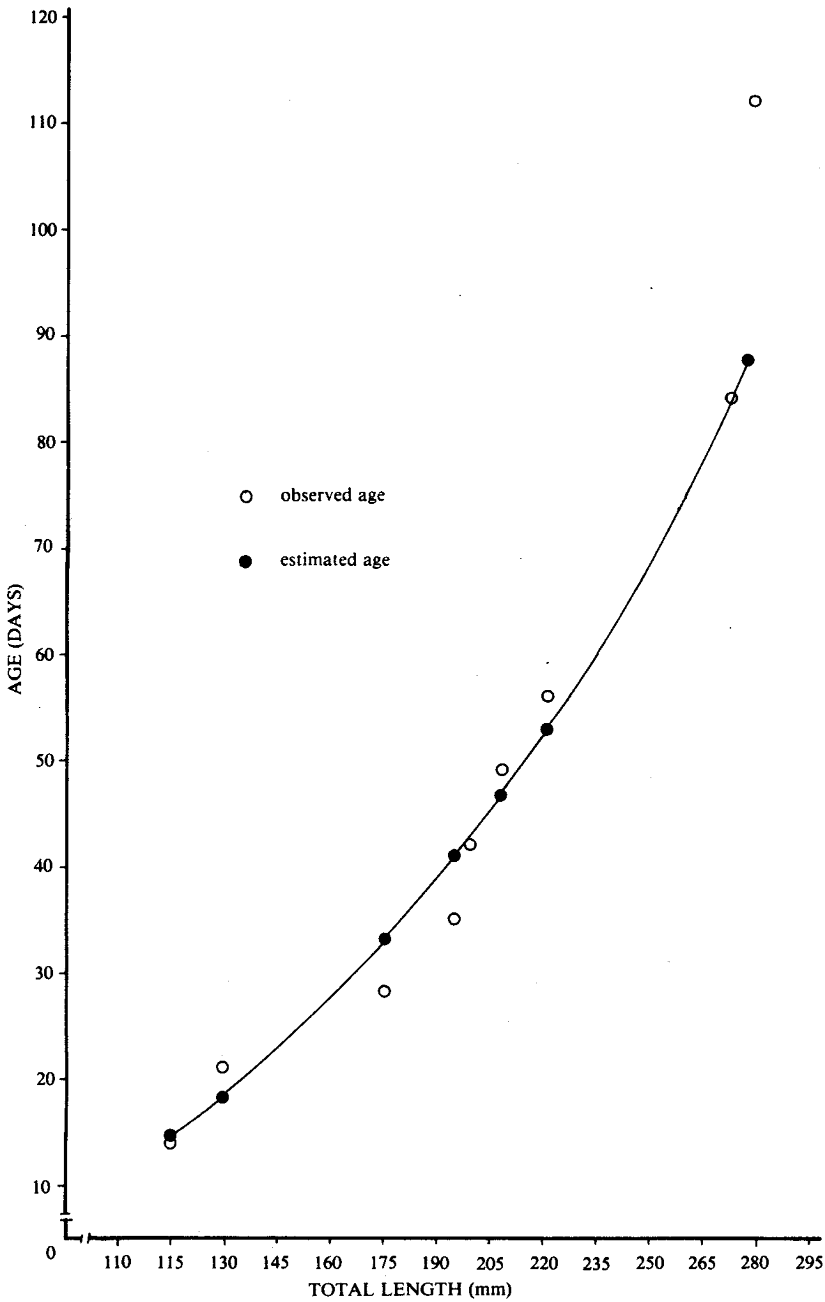

Figure 2. Regression curve in original scale derived from equation $\log_{10}$ (age + 22.9) = 1.2459 + 0.0029 (total length) for *R. argentiventer*.

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References

1. Hagen, A., Stenseth, N.C., Ostbye, E. and Skar, H.J. (1980) The eyelens as an age indicator in the root vole. *Acta Theriol.* **25**, 39 - 50.
2. Berry, R.J. and Truslove G.M. (1968). Age and eye lens weight in the house mouse. *J. Zool.* **155**, 247-252.
3. Donaldson, H.H. and King H.D. (1937) On the growth of the eye in three strains of the Norway rat. *Amer. Anat.* **60**, 203 - 229.
4. Lord, R.D. (1959) The lens as an indicator of age in cottontail rabbits. *J. Wildl. Manage.* **23**, 358 - 360.
5. Connolly, G.E., Dudzinski, M.L, and Longhurst W.M. (1969) The eye lens as an indicator of age in the black - tailed jack rabbit. *J. Wildl. Manage.* **33**, 159 - 164.
6. Myers, K., Carstairs, J. and Gilbert, N. (1977) Determination of age of indigenous rats in Australia. *J. Wildl. Manage.* **41**, 322 - 326.
7. Gosling, L.M., Huson, L.W. and Addison, G.C. (1980) Age estimation of coypus *(Myocaster coypus)* from eye lens weight. *J. Appl. Ecol.* **17**, 641 - 647.
8. Gourley, R.S. and Jannett, F.J. (1975) Pine and montane vole age estimates from eye lens weights. *J. Wildl. Manage.* **39**, 550 - 556.
9. Friend, M. (1967) Some observations regarding eyelens weight as a criterion of age in animals, *NY Fish Game J.* **14**, 91 - 121.
10. Yenbutra, S. and Boonsrong, P. (1973) Biology of *Rattus argentiventer*. *J. Agric. Sci.* **6**, 429 - 436. (In Thai).
11. Dixon, W.J. and Brown, M.B. (Eds.) (1979) Biomedical Computer Programs P-series (BMDP-79). University of California Press, London. 880 pp.
12. Collier, B.D., Cox, G.W. miller, P.C. (1973) Dynamic Ecology, Prentic-Hall International, Inc., London. 562 pp.
13. Martinet, L.(1966) Determination de l' age chez le campagnol de champs *(Microtus arvalis* Pallas) par le pesse due cristallin. *Mammalia,* **30**, 425 - 430.
14. Tiemeier, O.W., (1965) The black - tailed jack rabbit in Kansas. I. Bionomics. Technical Bulletin, *Kansas agricultural Experiment Station.* **140**, 5 - 37.

บทคัดย่อ

การประเมินอายุของหนูนาโดยใช้น้ำหนักของเลนส์ตา และลักษณะอื่น ๆ เช่น น้ำหนักตัว ความยาวลำตัวถึงปลายเท้า ความยาวหาง ความยาวตีนหลัง และใบหู จากหนูนาที่ได้รับการผสมพันธุ์และเลี้ยงดูในห้องทดลอง (อายุ 14-112 วัน) จำนวน 139 ตัว แช่ลูกตาหนูในฟอร์มาลินเข้มข้น 10 % นาน 10 - 14 วัน แล้วแกะเอาเลนส์ตาไปอบแห้งที่อุณหภูมิ 120 องศาเซลเซียส จึงนำไปชั่งน้ำหนัก พบว่าการแปลงข้อมูลจากอายุ (วัน) เป็น $\log_{10}$ (อายุ + ค่าเฉลี่ยของระยะเวลาอุ้มท้อง) มีความสัมพันธ์อย่างสูงกับน้ำหนักของเลนส์ตาในหนูทั้งสองเพศ สมการเส้นตรงรีเกรสชั่น มีค่าสัมประสิทธิ์ของสหสัมพันธ์ (r) = 0.9486 เมื่อนำข้อมูลจากการวัดขนาดของหนูดังกล่าวเข้ามาคำนวณในสมการด้วย พบว่า ค่า r^2 ไม่แตกต่างจากสมการเส้นตรงรีเกรสชั่น ที่ใช้น้ำหนักของเลนส์ตาเพียงอย่างเดียว ในกรณีที่ต้องการประเมินอายุของสัตว์มีชีวิตโดยไม่ต้องฆ่าสัตว์ สมการของเส้นตรงรีเกรสชั่นที่ใช้ความยาวลำตัวถึงปลายหางพอที่จะใช้ประเมินอายุของหนูได้ แต่การใช้จะต้องระลึกอยู่เสมอว่าความคลาดเคลื่อนของการประเมินอายุเพิ่มขึ้นเมื่ออัตราการเจริญเติบโตของสัตว์ลดลง