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The effect of food texture on eruption rate and length of incisor teeth in squirrel (*Funambulus pennantii*)

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Abstract

The purpose of this study was to evaluate the effect of food texture on incisor length and eruption rate in squirrels (*Funambulus pennantii*). The current study was carried out from July 1 to August 30, 2016. A total of 15 animals were divided into three groups, with five animals in each group. The groups were named A, B, and C. These groups were divided according to the type of food provided to animals as "hard food," "soft food," and "mixed food." This study reveals that the consistency of the diet affected the incisor length by decreasing or increasing the eruption rate. The hard food increased the eruption rate, which lowered down the incisor size (decreased lower teeth 8.56 ± 0.02 mm to 7.56 ± 0.07 mm). The other group, which was fed soft food, showed increased incisor size (increased lower incisor from 8.54 ± 0.02 mm to 10.36 ± 0.05 mm) with a decreased eruption rate. This study also showed that when animals were regularly provided with hard or soft food in the beginning, they showed a greater response, but later on, they maintained this by their homeostatic action. When hard food was provided to the individuals at the start (days 10 and 20), they showed a high eruption rate with a significant decrease in length (from 8.56 ± 0.02 mm to 7.64 ± 0.10 mm) and after that (days 40 and 60) they showed a low eruption rate with a little change in length (from 7.64 ± 0.10 mm to 7.56 ± 0.07 mm). A similar response was found with soft foods; they also showed a significant response to the food texture. At the start of the experiment, there was a low eruption rate with an increase in incisor size (from 8.54 ± 0.02 mm to 9.72 ± 0.03 mm), but after day 20, they showed a little difference in incisor length (from 9.72 ± 0.03 mm to 10.36 ± 0.05 mm)

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Introduction

A bone responds to the forces acting on it by remodeling its microstructure to combat failure. Such a response has not been documented for tooth enamel, despite the extraordinary stress associated with food breakdown. Enamel matrix is arranged in rods (prisms) of hydroxyapatite crystals grouped in different patterns to resist fracture and tooth failure (Purk, 2017). In most vertebrates, enamel formation is completed prior to dental eruption and usage, so there is no possibility of modifying the microstructure. Approximately, 2,400 mammal species today (and many other fossils) have steadily

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growing teeth that are potentially capable of structural change (Hua et al., 2015b). In these species, the epithelial cells proliferate and differentiate themselves into ameloblasts continuously, enabling the extension of enamel and dental growth throughout life. It is uncertain that the microstructure of the enamel and the strength of the teeth differ according to the diet of these species.

Rodents have continuous growth of incisors and molars to compensate for wear as growth rate. The incisors and molars of rats exhibit two different development patterns. The incisors form enamel and erupt continuously throughout their lives, while the molars will no longer erupt after having formed completed roots (Warshawsky et al., 1981; Morris et al., 2022).

Numerous studies have investigated factors that may affect the pace at which rat incisors erupt, and the impact of shortening rat incisors on that of eruption rate has been assessed (Burn-Murdoch, 1990; 1995; Risnes et al., 1995; Law et al., 2003). For example, it has been stated that the eruption rates are assessed by the total lengths of upper and lower incisors, relative to their lengths (Burn-Murdoch, 1995).

Incisor eruption in fully developed rodents occur at a rate equal to attrition, making it possible to compare rates of incisor longitudinal growth and to deduce differences in rate of attrition (Rinaldi and Cole, 2000). The incisors are suitable for studying the mechanisms of cell connections in general as well as the process of development because of how quickly they erupt (0.2-0.5 mm/day) (Wang et al., 2007). However, this is the first study to determine the effect of food texture on the eruption rate of growing teeth in squirrels. In this study, we examined the effect of food texture on the eruption rate of persistent teeth growth. For this, we considered 15 striped palm squirrels (*Funambulus pennantii*) for 60 days to provide a further evidence for better understanding of the effect of food on tooth eruption in squirrels.

Materials and Methods

Ethics considerations, study site, experimental design, and groups

The Ethical Committee of the Institutional Board granted permission for this research project. In this study, a total of 15 striped palm squirrels (*Funambulus pennantii)* were used. The animals were caught from the sports grounds of Government High School Talkot, Shujabad (South Punjab), Pakistan. These sports grounds are rich in flora. The specimens were caught in cages.

Experimental design

In this study, the effect of the texture of food on eruption rate and length of incisor teeth in squirrels was evaluated. The fifteen animals were randomly divided into 3 groups with five animals in each group. For the collection and experimental procedure, three metal cages were made having length, width and height 60 cm, 45 cm, and 30 cm, respectively. The cage floors were of metal sheet and remaining sides were of fine metal gauze for aeration purpose to maintain normal environment. The specimens in the metal cages were housed in a lab for 60 days.

Experimental procedure

Animals were fed regularly before the initiation of the trial, and they were maintained in the lab for a week to get familiar with the surroundings. After feeding them with their appropriate feed for a week, the first reading was taken. At the start, first two readings were taken with a gap of 10 days. The last two readings were taken with the gap of 20 days.

Grouping

Group A

Individuals kept in Group A were fed hard pellets. They were fed with hard dry seeds and nuts of different plants. Sometimes they were also fed with dry bread which involved high incisor use.

Group B

The specimens kept in Group B were treated as experimental specimens and they were provided with soft food which plays low or zero activity of incisor teeth. The food of this group was fruit flesh like mango, apricot, damson and soft bread.

Group C

The Group C individuals were treated as the control group. They were fed with mixed food of both experimental groups. Food material of this group consisted of hard food like seeds and nuts and also soft food like fruit flesh and bread.

Methods of measurement

Diazepam was used for anesthesia purpose to take the readings accurately and easily. A dose of 0.25

mL was applied in the hind leg muscles of each individual. After 20 minutes of application of anesthesia, the specimens became unconscious, so the reading was taken easily.

The incisor of the specimens was measured with the help of a vernier caliper. The first reading was taken on the day first and the second reading was taken after ten days. The third reading was taken after 20 days of the initiation of the experiment. The fourth and fifth readings were taken after 40 and 60 days, respectively, from the start of the experiment. After the fifth reading, the specimens were released at the place from where they were caught.

Statistical analysis of data

All the data generated by the experiment were subjected to analysis of variance to compare the three groups of animals using the SPSS 20 software. The differences among the mean values were evaluated through the Duncan's Multiple Range test at the 5% probability level.

Results

The statistical analysis shows that there was a significant difference in size of the upper teeth, between the first and the last readings. In the upper tooth size, there was no significant difference between the three groups at the first reading which was taken at day 10 after the start of the experiment; however, there was a minute change in length and in between the group soft and hard foods (**Tables 1 & 2**). The readings taken at day 20 showed the significant results of the hard feed groups as compared to the control group, but the soft feed groups showed no significant changes.

The results obtained at days 40 and 60 indicated significant differences among these three groups. The group fed with hard food showed high eruption rate, but the group fed on soft food showed a positive increase in incisor length as compared to that of the control group (**Table 1**). When the readings taken at day 40 and 60 were compared with the first and second readings taken on day 10 and 20, a gradual decrease in the incisor length and eruption rate was observed.

In the lower teeth, the growth rate and eruption rate were determined (**Table 1**). The results indicated significant differences among the three groups fed with different feeds. The group fed with hard food showed a significant positive eruption rate and a negative change in incisor length from the first day to the day of final reading (**Table 1**). The group fed with soft food showed a positive increase in incisor length and a negative response to the eruption rate.

While comparing the results of different periods within the groups, significant differences were observed from one period to the next. The group A fed on hard food showed the positive eruption rate and a significant decrease in the incisor length with a total change ranging from 8.56 ± 0.02 mm to 7.56 ± 0.07 mm (**Table 1**). The group fed with hard food showed the rate of positive eruption in each period with a reduction of the incisor length in different periods. In the start of the experiment, the eruption rates in the first and second periods at day 10 and 20 were high, however, a gradual decrease in eruption rate was observed in the third and fourth periods.

Group B fed with soft feed showed a positive increase in the lower incisor length and a decrease in the rate of eruption. The size of the incisor indicated a positive increase in length from 8.54 ± 0.02 mm to 10.36 ± 0.05 mm with a significant difference (**Table 1**). During each period, a positive increase in the incisor length and a small eruption rate were observed, but the rate of eruption increased gradually, whereas in contrast the incisor size decreased. There was a significant difference between readings 2 and 3 as compared to that in the 4^{th} and 5^{th} readings.

Table 1. Lower teeth length (mm) of three groups of *Funambulus pennantii* fed with different types of feed

	Lower teeth length (mm)				
Group	**Reading 1 (Day 0)**	**Reading 2 (Day 10)**	**Reading 3 (Day 20)**	**Reading 4 (Day 40)**	**Reading 5 (Day 60)**
Hard feed (A)	8.56 ± 0.02	8.14 ± 0.05*	7.64 ± 0.10*	7.52 ± 0.14*	7.56 ± 0.07*
Soft feed (B)	8.54 ± 0.02	8.90 ± 0.03*	9.72 ± 0.03*	10.18 ± 0.08*	10.36 ± 0.05*
Mixed feed (C) - Control	8.54 ± 0.02	8.50 ± 0.03	8.54 ± 0.02	8.54 ± 0.05	8.54 ± 0.02

*differs significantly at the 5% probability from the first reading.

Table 2. Upper teeth length (mm) of *Funambulus pennantii* fed with different types of feed

	Upper teeth length (mm)				
Group	**Reading 1 (Day 0)**	**Reading 2 (Day 10)**	**Reading 3 (Day 20)**	**Reading 4 (Day 40)**	**Reading 5 (Day 60)**
Hard feed (A)	3.06 ± 0.02	3.04 ± 0.02	2.98 ± 0.08*	2.94 ± 0.05*	2.90 ± 0.07*
Soft feed (B)	3.06 ± 0.02	3.08 ± 0.02	3.12 ± 0.08	3.16 ± 0.05*	3.14 ± 0.05*
Mixed feed (C) - Control	3.08 ± 0.03	3.08 ± 0.03	3.08 ± 0.08	3.10 ± 0.07	3.08 ± 0.04

*differs significantly at the 5% probability from the first reading.

Effect of food texture on incisor growth and eruption rate

The overall results show that the texture of the food had a positive effect on the growth of the incisor and the eruption rate. The experimental specimens demonstrated a positive response to the food texture. The continuously growing teeth increased in length, because of a low rate of eruption with soft foods and a high rate of eruption with hard foods. However, they had gradually maintained their tooth size mediated through different mechanisms.

Groups comparison for weight

The three groups compared for weight showed no significant results (**Table 3**). When the three groups were compared for weight, they showed no significant differences. The comparison of hard, soft or mixed foods among the three groups did not reveal any significant differences. A decrease in weight in each group from day first to the end of trial was observed, however, it was not statistically significant (**Table 3**). At the start of the trial, the animals gradually lost their weight in each group, but eventually they maintained their weight.

Table 3. Mean weight (g) of *Funambulus pennantii* fed with different types of feed

	Weight (g)				
Groups	**Reading 1 (Day 0)**	**Reading 2 (Day 10)**	**Reading 3 (Day 20)**	**Reading 4 (Day 40)**	**Reading 5 (Day 60)**
Hard feed (A)	102.60 ± 1.12	98.20 ± 4.35	92.20 ± 4.18	98.00 ± 1.84	97.40 ± 1.02
Soft feed (B)	113.40 ± 5.50	106.00 ± 5.92	101.40 ± 3.95	101.20 ± 3.05	102.60 ± 3.21
Mixed feed (C) - Control	111.00 ± 6.22	102.20 ± 1.56	97.60 ± 4.43	98.60 ± 2.87	100.40 ± 3.47

Discussion

Rodents have the constantly growing teeth. They have the ability to maintain their teeth length and eruption rate with the exception of texture of the diet. They have the capacity for cell division, which adds new cells and replace the old ones. The current study also found that squirrels grew new cells and continually increased the size of their incisors. They have the homeostatic ability to add new cells with more or less hardening material to maintain their incisor length with respect to consistency of food. Burn-Murdoch (1993) noted that the diet alters incisor activity from more active to less active. In our study, the diet change also altered tooth eruption rate, but in both cases the tooth length was increased. It was also revealed that the dietary texture affects the rate of tooth eruption and the incisor length. The group provided with hard food showed the high eruption rate as compared to the group fed on soft or mixed food, but in the group feeding on soft food showed low eruption rate and increased incisor length. The results of this study are similar to another study (Taylor and Butcher, 1951; Mancinelli and Capello, 2016) wherein it was reported that food texture had an effect on incisor length. However our findings contradict with those of Addison and Appleton Jr (1915) and Weinreb et al. (1967).

The findings presented in **Tables 1** and **2** demonstrate that when hard food was continually fed, the eruption rate was higher, but the tooth size reduced to some extent. However, when hard food was regularly provided, the eruption rate was initially higher, but the tooth decreased noticeably. Data recorded at days 10 and 20 demonstrate that when the same diet was continuously provided, the effect was less pronounced and the eruption rate was found to be slowed down. This happened due to the reason that the incisors are very hyperactive, and the hard material may have deposited there over time, causing this condition (Hua et al., 2015a).

According to this study, the group that consumed soft food showed reduced incisor activity and a low eruption rate with longer incisors. The incisors showed a significant increase in length due to their less activity, but they showed a significant increase in length from 8.54 $\pm$ 0.02 mm to 9.72 $\pm$ 0.03 mm with a larger difference of 1.18 mm during the first and second periods, i.e., after 10 and 20 days, and there was a less significant increase in incisor length from 9.72 $\pm$ 0.03 mm to 10.36 $\pm$ 0.05 mm with a smaller difference of 0.64 mm during the third and fourth periods (days 40 and 60). This may have been due to the homeostatic activity of the individuals.

The groups showed (**Table 3**) no significant difference in weight. The weights of all three groups were reduced at the start, with minor differences that were not statistically significant. The slight change in weight of individuals of all three groups may have been due to environmental stress. No significant change in animal weight due to different types of food as observed in the current study is in accordance with that reported elsewhere (Smith and Warshawsky, 1975, 1976; Burn-Murdoch, 1995), in which it was observed that food texture had no significant effect on animal weight. However, the slight change occurring in body weight due to different types of food could be due to a change in physical consistency of the diet model when switching from one group to another or to the specimen's eating habits (Burn-

Murdoch, 1990; Risnes et al., 1995; Helland and Nordbotten, 2021).

Conclusion

The effect of food texture on incisor length and eruption rate of squirrel (*Funambulus pennantii)* was evaluated. This study observed no significant difference in weight between the control and experimental groups due to different types of food provided. The early slight change in weight in all groups could have been due to environmental stress. Future studies focusing on other factors may help us better understand the process, but results presented here make it clear that diet does not play a role in changing the body weight of squirrels.

Author(s), Editor(s) and Publisher's declarations

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The authors declare no conflict of interest.

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Contribution of authors
Research superior(s): NS. Conceptualization and designing the study: SMA. Conduction of experiment: HRK. Data collection, visualization and interpretation: GM. Proof reading and approval of the final version: MS.

Ethical approval
This work has been approved by the ethical committee/review board of the Islamia University of Bahawalpur, Punjab, Pakistan.

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of experiment, all materials were properly discarded to minimize any types of bio-contamination(s).

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Authors' consent
All authors contributed in designing and writing the entire review article. All contributors have critically read this manuscript and agreed for publishing in IJAaEB.

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