The usage of bacteria *Streptococcus mutans* as rat’s incisors fragility agent

E Adliyah1 and S Priyambodo1*

1 Department of Plant Protection, Faculty of Agriculture, IPB University, Jl. Kamper, Kampus IPB Darmaga, Dramaga, Bogor 16680, West Java, Indonesia

*Email: swaspri@gmail.com

**Abstract.** Rat incisors keep growing along with their life since there is no hole at the base of the incisor. The elongation of rat incisors can be controlled by rodent through gnawing activity. It may lead to several disadvantages, such as crop and furniture damages and even transmit some diseases to human. Rat teeth are also strong, therefore it can destroy hard objects. This research aimed to test bacteria *Streptococcus mutans* as rat’s incisor fragility agent. *S. mutans* suspensions were made into 4 different concentrations: control, 3x10⁴, 3x10⁶ and 3x10⁸ cfu/mL. Each concentration was applied to the rats for 4 and 8 d, consecutively. The variables observed in this test were the hardness of the rat incisors and feed consumption. The result showed that the hardness of rat incisors and grain consumption were decreasing in all concentrations. The treatment indicated that the longer the bacteria infected rat incisors, the more fragile the incisors were. It affected their grain consumption as it was decreasing until 59.42% while their pellet consumption was increasing.

1. **Introduction**

Order Rodentia is introduced from the Latin word *rodere* which means to gnaw. They are characterized by the existence of 2 pairs of incisors that relatively large and long in the upper and lower jaw. There is a gap between the incisors and molars because they do not have canine/fangs and premolars. Incisors grow throughout their life and resulting rodents have to maintain its length by gnawing hard objects around them. Therefore, they become pest to some extent, both in rural and urban habitat/environment [1].

Rats in urban area could cause damage to buildings, reduce food storage, contamination of food ingredients and even transmit several pathogens to humans or pets [2]. Their ability to cut/gnaw hard objects is related to their teeth morphology, namely the size and hardness of the incisors. Rats are able to destruct or break hard materials up to 5.5 on the geological hardness scale. These materials include wood in buildings, aluminum sheets, poor quality concrete and asphalt [2]. Incisors rattling is one way to reduce their ability to cut hard materials, which causes a lot of damage and losses to human.

*Streptococcus mutans* has been known as the main bacterium in the formation of dental plaque and dental caries in human. Plaque is dextran that attaches bacteria, thus colonizing the teeth and forming a creamy sticky mass. Dental caries is shown by a decaying area inside the tooth, due to a process that gradually dissolves enamel and continues to develop inside the tooth. Both are caused demineralization
that triggers teeth brittleness. On the next stage, untreated dental caries will destroy the internal structure of the tooth and trigger tooth loss [3].

Minerals contained in the teeth can be decomposed by the demineralization process. The demineralization process occurs due to dissolution of tooth enamel in the acidic conditions. The interaction between \textit{S. mutans} and sugar are catalyzed by the glycosyltransferase and fructosyltransferase enzymes resulted from the fermentation in the mouth. \textit{S. mutans} is the only bacteria that can bind glucose polymers that are insoluble in saliva and able to colonize and form plaque on the teeth. Plaque attachment to these teeth triggers the dissolution of tooth enamel and causes fragility [4]. Decreasing of hardness level is one of the indicators of teeth brittleness.

The role of this bacterium can be studied to break down rat incisors. According to the previous study, the composition of rat teeth is similar to human’s, which 93% to 95% is composed of inorganic materials such as minerals [5]. Hence, tooth brushing using \textit{S. mutans} bacteria on rat incisors can have the same effect. This study aimed to observe the effect of \textit{S. mutans} on the hardness of rat incisors with different concentrations and durations of application by recording the consumption on hard and soft feeds. \textit{S. mutans} can be used as an alternative control of rat pest by reducing the hardness of its incisors which serve to cut hard feed.

2. Methods

2.1. Research location and time

The research was conducted at (a) Vertebrate Pest Laboratory, Department of Plant Protection, Faculty of Agriculture; (b) Clinical Microbiology Laboratory, Faculty of Veterinary Medicine; and (c) Food Processing Laboratory, Department of Food Science and Technology, Faculty of Agricultural Technology, IPB University, Bogor. The research was started from April to June 2013.

2.2. Bacterial ingredients preparation

Pure isolates of \textit{S. mutans} were obtained from Microbiology Laboratory, University of Indonesia, Jakarta. The pure isolates were then multiplied in the Clinical Microbiology Laboratory using scratch plate technique on Blood Agar (BA) media. Blood Agar media was made from 40 g of blood agar base/L of distilled water, then put it into a petri dish and sterilized at 121°C for 15 min. After that, the media was cooled down at 45 to 50 °C, then 7% of sterile blood was added. Petri dishes were placed into an anaerobic jar and candles were lit to create an anaerobic environment (N\textsubscript{2}, CO\textsubscript{2} and H\textsubscript{2} gasses were channeled), then anaerobic jar was closed and incubated for 2 x 24 hr [6].

According to previous study [7], the average amount of \textit{S. mutans} in saliva was 10\textsuperscript{7} cfu/mL, hence higher concentration of 3x10\textsuperscript{8} cfu/mL and lower concentrations of 3x10\textsuperscript{6} and 3x10\textsuperscript{4} cfu/mL were chosen in this research. \textit{S. mutans} suspension with concentration of 3x10\textsuperscript{8} cfu/mL was refered to McFarland 1 Solution [8]. Meanwhile, \textit{S. mutans} with concentration of 3x10\textsuperscript{4} and 3x10\textsuperscript{6} cfu/mL were made by using serial dilutions technique to get concentration of 3x10\textsuperscript{7} cfu/mL. As much as 1 mL of 3x10\textsuperscript{8} cfu/mL concentration was removed and then put it into 9 mL of 0.9% physiological NaCl. The process was performed continuously to achieve a concentration of 3x10\textsuperscript{4} cfu/mL. The final concentrations to be subjected in the test consisted of control (0.9% physiological NaCl) and concentrations of 3x10\textsuperscript{4}, 3x10\textsuperscript{6} and 3x10\textsuperscript{8} cfu/mL, respectively. They were kept in a closed container and put it into an ice box. These suspensions were immediately transferred to the refrigerator at the Vertebrate Pest Laboratory.

2.3. Test on rats

White rats (\textit{Rattus norvegicus albinous}) were used as the test animal in this study. The test was carried out at the Vertebrate Pest Laboratory. A test cage containing 2 small containers filled with feed and a glass of drinking water was prepared. The feed was grain and pellets as much as 20% of their body weight. Grain and pellets were used as the indicator of different feed hardness. Rat body weight ranged from 95 to 195 g or 40 to 60 d-old rat. Rat incisors are fully grown when they are more than 40 d-old [5, 9]. The first monitoring of rat body weight was recorded as their initial weight.
The study was consisted of 3 steps, namely before, during, and after application. At all stages, the rats were provided with feed (grain and pellets) and drinking water. Monitoring of feed consumption was performed by weighing the feed every 24 hr. The stage of before application was intended to determine the rat's eating preferences for grain and pellets and was carried out for 2 to 10 d depending on the initial weight of the rat. Different treatments only occurred at stage during application, in which the rats were treated by administering a suspension of bacteria. Bacterial suspension was taken from the refrigerator, then put it into a syringe of 1 mL. Furthermore, the prepared bacterial suspension was applied to rats by dripping it onto their incisors.

The enamel of incisors were only found on one side, namely the outward facing side [2]. The test on the incisors was intended so the bacterial suspension would go directly onto the rat enamel. Applications were carried out for 4 and 8 d, consecutively. Rodenticide testing was generally carried out with a maximum duration of application for 4 consecutive days. It was due to rodenticides were currently able to control rats in a short period of time [10]. The stage of after application was intended to determine the effect of the treatment at the stage during application (feed preference and hardness of rat incisors). Rats were killed using chloroform and then the final weight was recorded. Their teeth were pulled out using tweezers and cleaned, then they were collected into a sealed plastic.

2.4. Measurement of rat incisors and feed hardness

The measurements were carried out at the Food Processing Laboratory using the Kiya Hardness Tester [11]. Calculation of the rat feed consumption was based on formula below [10]:

\[
C = \frac{x}{m} \times 100
\]

C: rat feed consumption (g), x: average rat feed consumption (g), m: average rat body weight (g).

According to the previous study [12], the hardness unit of Kiya Hardness Tester is kgf, which referred as the force unit. Teeth hardness measurements for each rat were using 4 incisors, consisted of 1 pair of upper incisors and 1 pair of lower incisors. As much as 20 grains and pellets of each were also measured to record the average hardness.

2.5. Data analysis

The experimental design used in this study was Completely Randomized Design (CRD) with 4 replications. Data was analyzed by analysis of variance, then followed by Duncan's test at alpha 5%. Calculation of the rat feed consumption was processed using Microsoft Excel 2007. The converted data was analyzed by using the Statistical Analysis System (SAS) for Windows version 9.1.

3. Results and discussion

Tooth hardness represents the strength of the tooth in accepting load until the tooth is broken. The hardness of rat incisors decreased along with higher concentration of bacteria applications (Table 1). The incisors from the control group (without bacterial exposure) indicated that the hardness value was more than 20 kgf. This value was not measured accurately because the device had a maximum value of 20 kgf. The hardness of rat incisors was decreased at higher concentration. The hardness value for the concentration of 3x10^6 and 3x10^8 cfu/mL was lower than the concentration of 3x10^4 cfu/mL.
Table 1. Value of incisors hardness based on different concentrations of bacterial exposure for 4 and 8 d period.

| Treatment | Hardness value treatment (kgf)¹  |
|-----------|----------------------------------|
| Control   | > 20                             |
| 3x10⁴     | 17.85 ± 1.30 a                   |
| 3x10⁶     | 14.03 ± 2.60 b                   |
| 3x10⁸     | 13.14 ± 2.50 b                   |

Duration of application (days)

| 4 d  | 15.49 ± 2.84 a  |
| 8 d  | 14.71 ± 3.17 a  |

¹ Number followed by the same letter indicates values that are not significantly different at the 5% test level (Duncan's Multiple Range Test).

The duration of bacterial application had no effect on incisors hardness due to the time interval was relatively short. Plaque began to coalesce with teeth about 20 min after eating [3]. The process of demineralization occurred since dental plaque was formed, but caries in human teeth could take months or even years to develop. This caries triggered porous teeth, although the effect was small, incisors hardness was still decreasing. The results of this test showed that the higher concentration and duration of S. mutans bacteria decreased the hardness of rat incisors.

Table 2. Average of grain and pellets daily consumptions before application.

| Concentration of bacterial suspension (cfu/mL) | Feed consumption (g)²  |
|-----------------------------------------------|-------------------------|
| Control                                       | Grain: 7.34 ± 2.26 a, Pellet: 3.96 ± 3.44 a |
| 3 x 10⁴                                       | Grain: 6.82 ± 1.89 a, Pellet: 4.26 ± 1.77 a |
| 3 x 10⁶                                       | Grain: 6.95 ± 2.26 a, Pellet: 4.84 ± 3.13 a |
| 3 x 10⁸                                       | Grain: 6.90 ± 1.37 a, Pellet: 4.19 ± 1.92 a |

² Numbers in the same column followed by the same letter indicate values which are not significantly different at the 5% test level (Duncan's Multiple Range Test).

The content of grain is 75% carbohydrate and 8% protein, as well as fat, fiber and ash [13]. On the other hand, pellets comprises of 40% to 50% corn, 7 to 8% rice, and 30 to 35% grains. Other constituents are coconut oil, salt, mineral mixture, vitamin mixture, etc. [14]. Rats required some basic ingredients in their food, i.e 45% to 50% carbohydrates, 20% to 25% protein, 5% fat, 5% crude fiber, 4% to 5% ash and vitamins [9]. Based on the composition, the 2 feeds were in accordance with the basic needs of rats.
In the stages during and after application, the higher bacterial concentration resulted in the decrease of grain consumption (Table 3). The availability of grain as a carbohydrate containing sugar supported the fermentation process by the bacteria *S. mutans*. Rat’s feed consumption decreased sharply at concentration $3 \times 10^8$ cfu/mL. After application, grain consumption for the concentrations of $3 \times 10^6$ and $3 \times 10^8$ cfu/mL was lower compared to control. The same response was performed by the concentration of $3 \times 10^8$ cfu/mL which was lower compared to concentration of $3 \times 10^4$ cfu/mL.

This showed that the effect of bacteria on rat’s consumption to grain did not stop when the application was completed. Termination of bacterial application made the amount of bacteria in the mouth did not increase in high numbers, hence the duration of application had no effect on the consumption.

Table 3. Average of grain daily consumption during and after application.

| Treatment | Feed consumption (g)\(^a\) |
|-----------|---------------------------|
|           | During                    | After                    |
| Concentration of bacterial (cfu/mL) | | |
| Control   | 6.55 ± 1.42 a             | 6.37 ± 2.22 a            |
| $3 \times 10^4$ | 5.94 ± 2.29 a            | 5.38 ± 2.14 b            |
| $3 \times 10^6$ | 5.45 ± 2.26 a            | 4.46 ± 2.78 bc          |
| $3 \times 10^8$ | 3.67 ± 0.70 b            | 2.80 ± 0.83 c           |
| Duration of application (days) | | |
| 4 d       | 6.03 ± 2.28 a             | 5.40 ± 2.56 a            |
| 8 d       | 4.88 ± 1.60 b             | 4.23 ± 2.19 a            |

\(^a\) Numbers in the same column followed by the same letter indicate values which are not significantly different at the 5% test level (Duncan's Multiple Range Test).

Effect of concentration and duration of bacterial exposure on grain consumption during and after applications could be seen in Table 3. Grain consumption at a concentration of $3 \times 10^4$ cfu/mL for 8 d was lower than that of 4 d, whereas for other concentrations was not affected for longer duration of bacterial application. The lowest grain consumption occurred at concentration of $3 \times 10^8$ cfu/mL for 4 and 8 d. This was due to the need for rats to continue consumed grain. The average consumption to the grain of the highest concentration ($3 \times 10^8$ cfu/mL) before application was 6.90 g, while during application was only 3.67 g, and after application was 2.80 g.

There was no effect between concentrations in the duration for 4 d of application. Duration of 8 d, consumption of grain for concentrations of $3 \times 10^4$, $3 \times 10^6$, and $3 \times 10^8$ cfu/mL was lower than control, hence the duration of application and concentration gave an influence on the decrease in grain consumption. The higher concentration and duration of bacterial exposure were decreased grain consumption.

Pellet consumption during application was not influenced by the concentration of bacterial suspension but duration of application (Table 4). On the other hand, pellet consumption after application was influenced by bacterial concentration and duration of application. After application, consumption for $3 \times 10^6$ and $3 \times 10^8$ was higher compared to concentration of $3 \times 10^4$ and control. The longer application of bacteria, the higher their consumption. Pellet consumption was inappropriate at the stage during application because the main content of pellets was corn (secondary crops). Secondary crops is not suitable for rat’s metabolism [2].
Table 4. Average daily consumption of pellets during and after application.

| Treatment | Feed consumption (g)\(^a\) | During | After |
|-----------|-----------------------------|--------|-------|
| Concentration of bacterial (cfu/mL) | | | |
| Control  | 3.41 ± 2.01 a | 2.90 ± 1.17 b |
| 3 x 10^4 | 3.20 ± 1.23 a | 3.84 ± 1.64 b |
| 3 x 10^6 | 3.27 ± 1.61 a | 5.44 ± 1.44 a |
| 3 x 10^8 | 3.29 ± 1.83 a | 5.48 ± 1.32 a |
| Duration of application (days) | | | |
| 4 d | 4.24 ± 1.36 a | 3.65 ± 1.31 b |
| 8 d | 2.35 ± 1.28 b | 5.06 ± 1.87 a |

\(^a\) Numbers in the same column followed by the same letter indicate values that were not significantly different at the 5% test level (Duncan's Multiple Range Test).

Based on the previous study, the average hardness of ten rice varieties in Indonesia is 6.31 kgf [12]. The hardness value for several legumes, such as soybean is 12.81 kgf [15] and green beans are 12.95 kgf [16]. The average hardness of grain was 9.93 kgf, while pellet was 1.55 kgf. The average incisors hardness on the highest bacterial concentration was 13.14 kgf, while on 8 d of bacterial application was 14.71 kgf. This could reduce the rat’s consumption on grain from 6.90 g before application to 3.67 g during application and 2.80 g after application. The decrease in consumption was around 59.42%. On the other hand, rat’s consumption on pellet was increased from 4.19 g before application to 3.29 g during application and 5.48 g after application, with the increase in consumption was around 23.54%.

Rats can destruct objects with less than 5.5 geological scales [2]. The geological level of hardness was based on the scale of Mohs mineral hardness. Mohs hardness scale showed the ability of a sample from natural material to others. The sample material used was mineral [17]. The hardness of 1-2 Mohs scale is equivalent to finger nails, 2 to 5 equivalent to small iron blades, 5 to 6.5 equivalent to steel and 6.5 to 10 equivalent to diamonds. The hardness of 5.5 Mohs scale is equivalent to 60 kgf [18]. There was a potential decrease in the ability of rats to gnaw in accordance to the reduction on rat’s consumption of grain for the highest concentration and duration of \(S. \text{mutans}\) applications.

4. Conclusion
The hardness of rat’s incisors decreases along with the increase in the concentration of \(S. \text{mutans}\). The duration of bacterial application has no effect to the rat’s incisors hardness. Grain consumption decreases between treatments with the lowest consumption at a concentration of 3x10^8 cfu/mL. The higher concentration and duration of bacterial applications, the higher consumption to pellets after application. \(S. \text{mutans}\) can reduce the rat’s incisors hardness and consumption to grain. \(S. \text{mutans}\) concentration of 3x10^8 cfu/mL is the most effective treatment to reduce incisors hardness and rat’s consumption to grain.

Further research is required to study the similar aspect, but using different rat species and linked it with the hardness of rat incisors, rodent behavior, and the integration of its application to other control methods.

References
[1] Marbawati D and Ismanto H 2011 Balaba 7 46-48
[2] Priyambodo S 2003 Pengendalian Hama Tikus Terpadu (Jakarta: Penebar Swadaya) pp 135
[3] Houwink B 1993 Prevalensi penyakit gigi dan mulut Ilmu Kedokteran Gigi Pencegahan ed Maulana C (Yogyakarta: UGM Press) pp 12-19
[4] Veld H, Helderman V P and Dirks B 1993 Plak gigi Ilmu Kedokteran Gigi Pencegahan ed Maulana C (Yogyakarta: UGM Press) pp 58-103

[5] Hendrik Y C, Gunawan H A and Puspitawati R 2013 Pengaruh pemberian substrat ikan teri jengki (Stolephorus insularis) terhadap kekerasan mikro permukaan email gigi tikus Sprague dawley (in vivo) (Jakarta: available at www.lib.ui.ac.id)

[6] Volk W A and Wheeler M F 1993 Mikrobiologi Dasar Basic Microbiology 5th edition translator Adisoemarto S (Jakarta: Erlangga)

[7] Gronroos L 2000 Quantitative and qualitative characterization of Streptococcus mutans in saliva and in dentition (Helsinki: University of Helsinki)

[8] Sutton S 2011 Journal of GXP Compliance 15(3) 49-53

[9] Smith J B and Mangkoewidjojo S 1988 Pemeliharaan, pembiakan dan penggunaan hewan percobaan di daerah tropis The Care, Breeding and Management of Experimental Animals for Research in the Tropics translator Mangkoewidjojo S (Jakarta: UI Press)

[10] Priyambodo S 2012 Buku Praktikum Vertebrata Hama (Bogor: IPB Press) pp 88

[11] Webb B D, Pomeranz Y, Afework S, Lai F S and Bollich C N 1986 Cereal Chemistry 63(1) 27-30

[12] Argasasmita T U 2008 Karakterisasi sifat fisikokimia dan indeks glikemik varietas beras beramilosa rendah dan tinggi (Bogor (ID): IPB University)

[13] Haryadi 2008 Teknologi Pengolahan Beras (Yogyakarta: UGM Press)

[14] Lusiana E A 2008 Efektivitas penggunaan bungkil biji jarak pagar (Jatropha curcas Linn.) terdetoksifikasi dalam ransum dan adanya fase recovery terhadap performa ayam broiler (Bogor: IPB University)

[15] Ratnaningtyas A 2003 Tahu dari kaacang non kedelai; studi kasus kaacang komak (Bogor: IPB University)

[16] Sirojudin 1996 Mempelajari karakteristik fisikokimia produk teksturisasi kaacang hijau (Vigna radiata L. Wilcjeck) (Bogor: IPB University)

[17] Gerrard A J 1987 Quarterly Journal of Engineering Geology and Hydrogeology 20 99

[18] Railsback R B 2006 Some fundamental of mineralogy and geochemistry (Georgia (GE): University of Georgia)