Conference Paper

The Effect of Chicken Bone Powder Supplementation during Pregnancy and Lactation on the Calcium Level of Rat Pups' Teeth

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Abstract

Calcium is an important mineral needed during tooth and bone development. Additional calcium intake from the diet may help to maintain calcium level during pregnancy and lactation. Chicken bone contains high calcium so it can be used as a natural calcium source due to its bioavailability. This study was conducted to investigate the effect of chicken bone powder during pregnancy and lactation period on the calcium level of *Sprague Dawley* pups' teeth. Six female *Sprague Dawley* were divided into treatment and control group. The treatment group was given chicken bone powder supplementation from the first day of pregnancy up to the fifteenth day after the delivery of the pups while the control group was given CMC-Na suspension by intragastric administration method. On the 15 d after the delivery, the pups were decapitated and the teeth from both groups were collected. The calcium level then assayed by atomic absorption spectrophotometry (AAS) method and the data were analyzed with an independent t-test. The result from the study showed that the calcium level of the teeth from the treatment group was significantly higher than the control group (P = 0.003). This result suggests that the administration of chicken bone powder during pregnancy and lactation period of mother rat could increase the calcium level of *Sprague Dawley* pups' teeth.

Keywords: Chicken bone powder, Tooth calcium level, Pregnancy and lactation period, *Sprague Dawley,* Tooth development.

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1. Introduction

Calcium is one of the many nutrients needed during pregnancy and lactation period [1]. This mineral maintains the stability of the body during pregnancy and is needed to assist the growth and development of child's teeth [2–3]. During pregnancy, the unstable psychological condition can result in reduced appetite, so sometimes the optimal calcium intake is not fulfilled [4].

Calcium has an important role in the regulation of biological processes in the body [5]. Calcium intake which is given to women during pregnancy and lactation period will help to fulfill both mother and baby's calcium need with the active transport of calcium from mother through the placenta and breast milk (milk) after birth [6, 7]. This mineral has an important role in teeth formation during calcification [8]. Calcium obtained from food will be transported by blood and will be used in the formation of children's teeth [9]. An increase in calcium bioavailability is expected to support and optimize this process. On the other side, calcium deficiency can affect the growth and development of child's teeth, such as delayed tooth eruption, disorders of mineralization on enamel and dentin, and causing tooth structure abnormalities [10–13].

The intake of additional calcium can effectively maintain calcium level during pregnancy and lactation [14]. Some calcium sources that can be consumed during this period are vegetables, milk, and dairy products. The only problem with these calcium sources is that these foods contain substances like fiber, phytate, and oxalate that can inhibit calcium absorption. Chicken bone contains high minerals like calcium, phosphorus, and magnesium and does not contain substances that can inhibit calcium absorption so it can increase calcium bioavailability [15–16].

The chicken bone which is a side product from poultry industry can be an alternative source of calcium for women during pregnancy and lactation. Calcium supplementation during this period can help maintain calcium level during pregnancy and breastfeeding so it can optimize the process of tooth calcification in children [15, 17]. Calcium chicken bones consumed by pregnant and lactating women will be more easily absorbed and transferred to the fetus, to increase the level of calcium teeth during their formation [16].

2. Materials and Methods

2.1. Research materials

Sprague Dawley rats, chicken bone, standard feed rats (Comfeed brand), 2.5 % CMC-Na suspension, 100 mg · mL^{-1} Ketamine Hydrochloride (brand Ketamil®), alcohol 70 %, NaCl 0.9 % solution, $HNO_3$and $HClO_4$ solution, aquadest, and formaldehyde.

2.1.1. Research tools

Rat cage, grinding machine, minor surgery tools, oral sondasion tools, syringe, vortex mixer, small tube, digital scales, flask/beaker glass, Erlenmeyer flask, 50 mL and 100 mL

tube, micropipette, heater plate, and Atomic Absorption Spectrophotometry (AAS) machine.

2.2. Methods

2.2.1. Preparation of chicken bone powder and chicken bone powder suspension

Chicken bone was separated from the surrounding soft tissue, dried for 2 h, and ground with grinding machine. The suspension was made by adding 275 mg of chicken bone powder and 2.5 % CMC-Na suspension, and then the mixture was made homogeneous by vibrating above vortex mixer for 30 s. The dosage was determined based on measurement of rat's calcium need and measurement of calcium level in the chicken bone sample.

2.2.2. Animal experiment grouping

Amount six pregnant *Sprague Dawle*y rats aged 3 mo with a body weight of (200 to 250) g were divided into two groups: treatment and control group. Vaginal smears conducted to determine the pregnancy status [18].

2.2.3. Animal experiment treatment

The treatment group was given 2 mL of chicken bone powder suspension, whereas the control group treated with 2.5 % CMC-Na suspension by intragastric sondasion. Chicken bone powder supplementation was given since the first day of pregnancy to the fifteenth day after the birth of the pups. The pups were weighed every 5 d. At the fifteenth day, three rat pups with the most optimal condition from each mother are selected, with ideal body weight (20 to 30) g and without disabilities.

2.2.4. Calcium level examination

The pups were decapitated on the fifteenth day after the birth. At first, they were anesthetized with Ketamine Hydrochloride 100 mg $\cdot$ mL^{-1}. The teeth from upper and lower jaws were collected and cleaned from the surrounding soft tissue and the calcium levels were analyzed by AAS machine. The measurements were done using a spectrophotometer with a wavelength of 422.7 nm. Each 50 mL solution was taken to measure its

absorbance. Results of calcium level measurement in concentrations (%) were obtained after comparing the results with the standard curve.

2.3. Data analysis

The data obtained were analyzed using Shapiro-Wilk test for normality, Levene test for homogeneity, and followed by *independent t-test* with a confidence level of 95 %.

3. Result

TABLE 1: Calcium levels (%) *Sprague Dawley* rat pups' teeth in treatment and control group.

Group	*n*	Calcium level of rat pups' teeth (%) $\bar{x} \pm$ SD
Treatment	9	27.40 ± 2.07
Control	9	23.45 ± 2.77

Table 1 shows that there's a difference in the pups' teeth calcium levels in treatment and control group. The pups' teeth calcium level in the treatment group was higher than the control group. The data collected were analyzed using independent sample t-test.

TABLE 2: The t-test result of mean calcium levels *Sprague Dawley* rat pups' teeth in treatment and control group.

Group	T	P
Treatment – Control	3.428	0.003

The result of t-test shows that there are significant differences ($P < 0.05$) between the average of pups' teeth calcium level in the treatment and control group. Table 2 shows that chicken bone powder supplementation can significantly increase pups' teeth calcium level.

4. Discussion

The results showed that chicken bone powder supplementation on *Sprague Dawley* rats during pregnancy and lactation significantly increased the pups' teeth calcium levels. It supports the previous research which stated that maternal calcium supplementation could help the calcification process and may reduce the risk of childhood dental caries [19]. It also supports a statement which said that chicken bone powder supplementation on rats during pregnancy and lactation period could optimize the development of rat

pups' teeth, by causing an early eruption and early formation of rat pups' molar teeth [20].

The nutritional intake consumed during pregnancy and lactation plays an important role in the deposition of mineral, especially calcium in the teeth of children [3]. Calcium will actively be transferred from mother to fetus through the placenta during pregnancy. The amount of nutrition for the fetus depends on placental blood flow. The placenta can also produce $1.25(OH)_2D$ which can help calcium absorption. Therefore, nutritional needs must be fulfilled during pregnancy because it can affect fetus nutritional status as there's a transfer of nutrition from mother to child through placenta [21].

Chicken bone powder has the highest bioavailability when compared to some forms of calcium salts that are commonly available in the market. Calcium bioavailability of chicken bone powder with the same calcium content was higher than Calcium Carbonate (CaC), Calcium Lactate (CaL), Calcium Citrate (CaCi), Calcium Lactogluconate (CaLG), and significantly higher, 1.4 to 2 times, than milk powder. Calcium was more absorbable from chicken bone powder than from commercial salts. Chicken bones do not contain substances which can inhibit the absorption of calcium—such as fiber, phytate, and oxalate—and it makes calcium from chicken bone can be absorbed by the body easier [16]. It has been well recognized that calcium bioavailability is influenced by different types of calcium fortificants and other food components. Fiber can shorten the transit time of food in the gastrointestinal tract and can bind calcium into their structure so that will decrease calcium absorption [22]. Oxalic acid and calcium will form calcium oxalate which is insoluble and difficult to absorb [23]. Chicken bone contains high calcium and protein. Its calcium and phosphorus ratio is 2:1 and it will make the process of calcium absorption in the body easier [15]. Chicken bone powder with its high calcium content and good bioavailability on the body when administered to women during pregnancy and lactation period will increase the calcium level of children's' teeth.

Chicken bone powder was suitable for consumption by humans because microorganism content in the selected chicken bone powder was very low (< 10 CFU $\cdot$ g^{-1}). Microorganisms can grow in some food with water activity (A_w) level between 0.60 to 0.90 [6]. Water activity (A_w) in chicken bone powder was very low (0.152). It indicates that chicken bone powder is safe from the microorganism and can be kept for a long time [15].

Calcium supplementation during pregnancy can maintain the stability of pregnant women, and also plays an important role in the formation of children's teeth [2, 24]. Deciduous teeth calcification started from pregnancy and completed right before the eruption. Teeth which have been erupted and demineralized are less able to process

the remineralization. After the eruption in the oral cavity, only a little change can happen to teeth's mineral component, especially calcium. Calcium remineralization process is slow and occurs mostly in the dentin layer and only a little in enamel [23] This makes the children's needs of calcium must be fulfilled since intrauterine development. Teeth have calcium of about (27 to 28) % stored in enamel, dentin, and cementum. Under such circumstances, the teeth will have optimal hardness and grow normally without any abnormality [25]. This study shows that the calcium level of pups' teeth whom mother was given chicken bone powder supplement is 27.40 %. So, the chicken bone powder supplementation can meet the needs of calcium in teeth and helps the growth runs properly.

Baby's calcium needs after birth will be obtained from breast milk. Minerals found in it have better quality and will be easily absorbed than the minerals from formula milk. Breast milk contains nutrients that are needed for baby growth [26]. Calcium content of breast milk is not influenced by nutritional intake and status of the mother because the calcium needed will be taken from mother's bone. If the amounts of calcium fulfilled, the calcium taken from bone during the lactation period would be deposited back on the bone after the lactation period is over [7].

Calcium obtained from chicken bone powder supplements will undergo a series of metabolic processes in the body. Calcium consumption will be processed in the stomach into a liquid form with the help of gastric acid, in order to be absorbed. Calcium that has been converted into a liquid form then absorbed by the small intestine and will be directly circulated through the blood vessels through the process of diffusion when body absorption of calcium is high. When the body absorption of calcium is low, calcium metabolism will be helped by Vitamin D which will activate CaBP (Calcium Binding Protein) to transport calcium in order to pass through the cell membrane [9]. Calcium supplementation will provide calcium for calcification process during amelogenesis and odontogenesis. Ameloblasts and odontoblasts will take calcium circulated by the blood to replace the organic substances into the inorganic enamel and dentine. Dental calcium concentration increase during secretion stage and it will keep increasing until the final stage of tooth maturation [27].

In this study, rat pups' teeth were taken at 15 d after birth. The calcification process of incisors, first molars, and second molars of rat pups was completed on the fifteenth day after birth, characterized by the eruption of teeth in the oral cavity [28]. At this point, dental crowns have been formed, so it is possible for calcium level examination [29].

5. Conclusions

Based on the research, it can be concluded that chicken bone powder supplementation on the *Sprague Dawley* rats during pregnancy and lactation can increase the calcium levels of pups' teeth.

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