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The Effect of Haruan (Channa striata) Fish Bone Powder Supplementation during Pregnancy and Lactation on the Mineral Levels of Jaws and Teeth of Rat Offspring

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ABSTRACT

Background: Haruan fish (Channa striata) is an endemic fish of Kalimantan that is widely used by the people in South Kalimantan Province. Haruan fish bone waste can be used for additional nutrition in pregnant women and can support the process of bone and teeth remineralization. Haruan fish bones contain minerals in the form of calcium, phosphorus, fluoride and magnesium.

Purpose: this study was conducted investigate the effect of Haruan (Channa striata) fish bone powder during pregnancy and lactation on the mineral levels of jaws and teeth of Wistar rat (Rattus norvegicus) offspring.

Methods: This study used a true experimental design with a Post Test Only Control Group Design. Group 1 (treatment) were given Haruan fish bone powder (Channa striata) and group 2 (control) without haruan fish bone powder (Channa striata) from the fist pregnancy up to 22 days after the delivery. The calcium, phosphorus, fluoride and magnesium level of the jaws and teeth of the pups from both groups were assayed and analyzed.

Results: Statistic test result showed a difference in mineral levels in rat pups the jaw and teeth between the control and the treatment group.

Conclusion: There was an effect of giving Haruan fish bone powder (Channa striata) during pregnancy and lactation period on the mineral levels of jaws and teeth in Wistar rat pups (Rattus norvegicus).

Keywords: *Fish bone powder; Haruan fish; jaws and teeth development; mineralization*

INTRODUCTION

Nutrition during pregnancy is very important for the development of baby teeth. Tooth development begins during fetal development, typically between 6-8 weeks of gestation. The histological differentiation of tooh begins at around the 11th week of embryonic development. This stage is known as the cap stage, during with the enamel organ develops. The bell stage begins at approximately the end of the 10th week of embryonic development. Amelogenesis and dentinogenesis occur during the bell stage and continue into later stages of tooth development. At the end of the 5th month,of prenatal development, the hard tissue of the tooth begin to form through a process called mineralization. This is when calcium and other minerals are deposited onto the organic matrix that has been secreted by the enamel organ and dental papilla. The matrix apposition process occurs during this time, where the organic matrix is gradully mineralized, resulting in the formation of hard tissues. Any stressful events during pregnancy and childbirth can have impact on the enamel formation, leading to the development of clinical enamel defects.[1,2]

A balanced and nutritious diet during pregnancy is crucial for the health and development of both the mother and the growing fetus. Pregnancy and lactation require additional energy, nutrients the following minerals : iron (Fe), zinc (Zn), copper (Cu), calcium (Ca), phosphorus (P), magnesium (Mg), iodine, selenium and vitamins : A, B_1, B_2, niacin, choline, panothenic acid, B_6, B_{12}, C, E and folates.[3,4]

Calcium is indeed essential for the development of teeth and bones, particularly during pregnancy and lactation. It is needed for formation and mineralization of the tooth enamel and dentin, as well as for the growth and development of the fetal skeleton.[5] Magnesium is also an essential mineral for bone development and mineralization, along with calcium and other nutrients. It plays crucial role by stimulating the activity of osteoblasts, which are resposible for bone formation. Magnesium also activates enzymes from the phosphatase group, which are involved in the bone formation process.[6] Phosphorus is indeed most abundant mineral, after calcium, and is fundamental component of bone tissue and teeth. Phosphorus helps to regulate the activity of osteoblasts and osteocytes, which are involved in the process of matrix mineralization.[7] Fluorides, the ionic form of fluorine, in trace amounts play a vital role in bone and teeth development by incorporated into the hydroxyapatite crystal in the bone and teeth, making them more resistant to demineralization, which helps to prevent tooth decay and maintain healthy bones. They stimulate the proliferation of osteoblasts, the cells responsible for bone formation, and inhibit the activity of osteoclasts, the cells responsible for bone resorption, thus leading to an increase in bone mass and improved bone strength.[8]

A mineral intake can be fulfilled with one of the mineral-rich Haruan fish bones. Haruan fish bone is a form of waste generated from the fish processing industries. Fish bones are good source of calcium and phosporus, which are important minerals for bone and tooth development and maintenance. Additionally, fish bones contain other minerals such as magnesium, fluoride and trace elements like zinc and copper, which are important for various physiological function in the body, especially during pregnancy and lactation. Fish bones are rich in hydroxyapatite which is the main mineral component of bones and teeth.[9,10]

MATERIALS AND METHODS

This study was conducted in vitro with a post-test control group design and was approved by the Health Research Ethics Commission of the Faculty of Dentistry, Lambung Mangkurat University No. 024/KEPKG-FKGULM/EC/II/2021.

Thirty Wistar rats (10 males and 20 females) aged 2.5-3 months and weighing 200-250g were kept in an iron cage for 1 week and received standard food and drink ad libitum. The light was adjusted so that the rats were in conditions of 12 hours of light and 12 hours of darkness, with a temperature of 20-25°C. The rats were then housed with female rats with a ratio of male rats: female rats, namely 1:2.

Wistar rats (Rattus norvegicus) were divided into two groups. Group 1 (treatment) consists of Wistar rats (Rattus norvegicus) were given BR2 food and standard drink distilled water ad libitum and Haruan fish bone powder (Channa striata) at a dose of 0.27 g/day. 0.27 g Haruan fish bone powder which has been mixed with 2.5% CMC-

Na suspension was given as much as 2 ml per day given orally using a gastric tube. Group 2 (Control) consists of Wistar rats (Rattus norvegicus) fed BR2 without Haruan (Channa striata) fish bone powder.

On the 22nd day after the lactation period was completed, the pups with the most optimal conditions were anesthetized using ether inhalation anesthesia. After the rats were totally unconscious, the pups were decapitated. The jaws and teeth were taken and cleaned of the surrounding soft tissuem then dried in an oven. The dried jaws and teeth were then ground using a mortar and pestle until become a fine powder.[11]

Measurement of calcium levels using the Titrimetry method. The prepared tooth powder was put in a 250 ml beaker glass, added 100 ml aquades and 3 drops of red metal indicator into the solution. The solution was heated to boiling. A solution of 0.75 gram of NH4-oxalate was added in 12.5 ml of distilled water slowly, heated at a temperature of 70-80°C for 15 minutes. 3 drops of ammonia solution (1:1) were added while stirring and leave the solution hot for 1 hour. The precipitate was filtered using filter paper, washed with distilled water until free from oxalate. The filter paper was perforated using a stirrer. The precipitate was rinsed with sulfuric acid solution (1:8) into another Erlenmeyer, washed with distilled water and squeezed to a volume of 50 ml, titrated with 0.1 N KMN04 solution until the color turned clear. Calculation of the weight of Ca with the formula = 0.7056 x vol. KMNO4 x 2.8 mg CaO.

The jaws dan teeth that had been mashed were put into a platinum dish to measure dental phosphorus levels. After the sample was prepared, the measurement of phosphorus levels used the Uv-Vis Spectrophotometry method with a specific wavelength of 422.7 nm and a sensitivity level of ≥0.001 ppm. The measurement was carried out by the energy combustion process of atomizing the sample into free atoms at a temperature of 2300ºC, using a mixture of acetylene-air oxidant gas. Prior testing, the Uv-Vis Spectrophotometry was calibrated using standard phosphorus solutions. The absorption value of the standard solution was recorded and used to calculate the calibration curve, which was then applied to determine the concentration of phosphorus.

To measure fluoride levels, crushed dry samples were taken. Prior to the inspection, the measuring instrument was calibrated using standard NaF solutions (1000, 100 and 10 ppm). The sample was then diluted using 10 ml of aquadest and mixed with 2 ml of SPADNS to showed color change in the sample solution which was then examined for its absorbance. Fluorine levels was determined through UV-Vis spectrophotometry method. The absorption values of the standard solutions were recorded and used to calculate the calibration curve, which was then applied to determine the fluorine concentration.

Measurement of magnesium levels using the Titrimetry method. Approximately 350 mg of the sample is weighed accurately, then dissolved in dilute hydrochloric acid. Add to the solution with sodium hydroxide until a steady mist forms then add 5 ml of ammonia buffer. Titrate with dinatrium edetat 0.05M uses eriochrome black to blue indicators. The following reactions occur at this measurement:

$Zn^{++} + (H_2Y)^{=} > (ZnY)^{=} = 2H^{+}$

$HlIn^{=} + Zn^{++} > ZnIn^{-} + H^{+}$

$ZnIn^{-} + (H_2Y)^{=} > (ZnY)^{=} + HIn^{-}$

RESULTS

Mineral levels of the jaws and teeth of Wistar rat offspring is showed on Figure 1. The results showed levels of calcium, phosphorus, fluorine, and magnesium in the jaws and teeth of Wistar rat offspring (Ratus norvegicus) was increase. There were differences in the rat pups' jaws and teeth mineral including calcium, phosphorus, fluorine and magnesium levels in treatment and control group. The pups' jaws and teeth mineral level in the treatment group was higher than the control group.

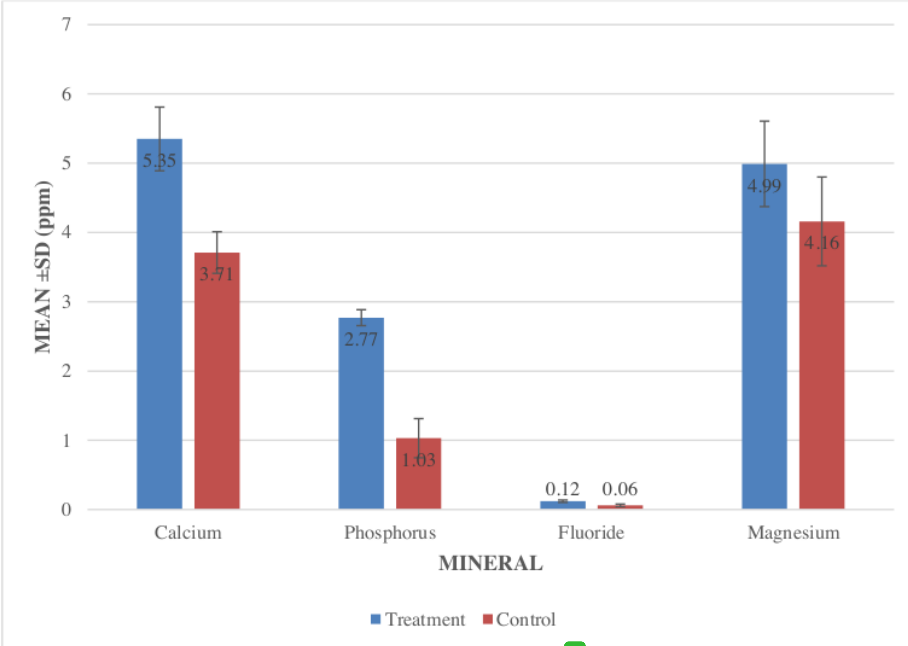

FIGURE 1. Mineral level of treatment and control group

The data collected were analyzed using Independent Sample T-test for calcium, phosphorus and fluorine level because the normality test was. The result of T-test showed that there were significant differences (p< 0.05) in the average of pups' teeth calcium (p = 0.000), phosphorus (p = 0.000) and fluoride (p = 0.000) level between the treatment and control group. Magnesium level was analyzed using Mann Whitney test because the normality test result was p >0.05. The result of Mann Whitney test showed that there was a significant difference (p < 0.05) between the treatment and control group (p = 0.002). It can be concluded that Haruan (Channa striata) fish bone powder supplementation can significantly increase mineral levels of pups' teeth.

TABLE 1. The results of statistical tests of mineral

Mineral	Group	N	Signification value (p)
Calcium	Treatment	17	0.000*
	Control	17	
Phosphorus	Treatment	17	0.000*
	Control	17	
Fluoride	Treatment	17	0.000*
	Control	17	
Magnesium	Treatment	17	0.002*
	Control	17	

(*) significant p<0.05

The results of this study is in accordance with previous research which stated that maternal mineral supplementation could increase the mineralization process and may reduce the risk of childhood dental caries. It is important for pregnant and breastfeeding women to consume a balanced diet for the growth and development of the bones and teeth. This includes an adequate intake of minerals and vitamins. Unlike vitamins, minerals could not be synthesized by the body and must be obtained through the diet or supplements.11,12 Adequate intake of essential minerals from Haruan fish bones can meet mineral needs during pregnancy and lactation.

Haruan fish bone is one of the raw materials that can be used to fulfill nutrition during pregnancy and breastfeeding because it has organic and inorganic components. In addition, the bones of the Haruan fish contain a lot of mineral salts such as calcium phosphate and creatine phosphate. The main mineral content found in Haruan fish bones consists of calcium, natrium, phosphorus, magnesium, fluoride, iron, zink, pottasium and copper.13,14

Formation and mineralization of primary teeth begins during fetal development. The process of tooth development involves the formation of dental tissue, which includes both the enamel and the dentin. Once the tooth buds have formed, the cells within them begin to differentiate and multiply, forming the various layers of dental tissue. Mineralization, which is the process of depositing minerals such as calcium and phosphate onto the dental tissue to make it hard and strong, also begins during fetal development and continues after birth as teeth erupt into the mouth. The mineralization process is a complex and tightly regulated process, and any disruptions or imbalances in the availability of minerals or the activity of the mineralizing cells can result in developmental defects or abnormalities in the teeth. Therefore the nutritional status of the mother during pregnancy and lactation play an important role in the development, formation, and mineralization of teeth.15–17

During fetal development, there is an exchange of nutriets between the mother and the fetus, which allows for the formation and mineralization of the developing of the jaws and teeth. Minerals such as calcium, phosphorus, magnesium and fluoride, which are important for tooh development, are circulated in the bloodstream of the fetus and are taken up by specialized cells called ameloblasts and odontoblasts. These cells are responsible for the formation of the enamel and dentin, respectively, and they use the circulating minerals to create the hard, mineralized tissue of the teeth.[18] Tricalcium phosphate is a key componet of this mineralization process, and it eventually coverts to hydroxyapatite crystals, which are very hard and durable.[19]

Calcium, sodium, magnesium, phosphorus, and potassium are considered major minerals due to their abundance in the body.[20] Calcium is an essential mineral for the growth and development of teeth, just as it is for bones. Enamel, which is the outermost layer of the tooth and the hardness tissue in the body, is primarily composed of calcium and phosphate. Calcium are deposited onto the organic matrix of enamel by ameloblasts during the tooth development process. Calcium is also an important component of dentin, which makes up the bulk of the tooth and provides it with structural support. Dentin is also mineralized with calcium and phosphate. [21,22,23]

Calcium absorbtion is particularly important during pregnancy for the development of the fetal skeleton, includes the bone and teeth, and during pregnancy, the demand for calcium increases as the fetal skeleton develops. The mother has mechanisms to increase calcium absorbtion from the diet to meet the increased demand during pregnancy. For example, the hormone estrogen, which is produced in higer level during pregnancy, increases calcium absorbtion from the intestines. The body produces more of an active form of vitamin D during pregnancy to help with calcium absorbtion and utilization. The recommendaed daily intake of calcium varies between countries and organizations, but generally falls within the range of 900-1200 mg/day for adults. the World Health Organization (FAQ) of the United Nations specifically recommend a dietary intake of 1000 mg/day of calcium for non-pregnant adults between ages 19-50 and 1200 mg/day for pregnant women.[24,25]

Phosporus is another important mineral for the bones and teeth development. Phosporus plays a key role in the formation and mineralization of bone, and it is required for the development of the fetal skeleton, including bones and teeth. It is primarily found in the form of phospate in the body. Within bone and teeth, phosporus and calcium work together to form hydroxyapatite crystal. In the human body, approximately 85% of phosporus is stored in the form of phosphoproteins and hydroxyapatite crystals in bones and teeth.[26] Phosphorus is regulated by the hormones PTH, 1,25(OH)$_2$ D$_3$, and FGF23. PTH increases the release of phosporus from bone, while 1,25(OH)$_2$ D$_3$ increases intestinal absorbtion of phosporus and FGF23 decreses renal reabsorption of phosporus. Inorganic phosphorus, in the form of phosphate ions, is an essential component for the formation of hydroxyapatite crystal in the bone matrix.[27]

The recommended daily intake of phosphorus for pregnant and lactating women is higher than that for non-pregnant adults. The "Recommended Dietary Allowances" or RDA data determine the recommendations for daily micronutrient intake for a pregnant woman. The recommended daily intake of phosphorus for pregnant and lactating woman is 700 per day.[12,28]

Fluorides, in trace amounts, is considered essential for proper bone and tooth development.[8] Fluoride stimulates osteoblasts, the cells responsible for bone formation, and helps to increase bone density. Fluoride can also inhibit the activity of osteoclasts, cells involved in bone resorption, which can help maintain bone mass.[29] Fluoride ions can be incorporated into the hydroxyapatite crystals in bone and teeth, making them more resistant to acid attact dan decay.[30] Fluoride has been shown to stimulate bone formation by increasing the activity of osteoblasts, the cells responsible for synthesizing new bone tissue. At the same time, fluoride also decreases bone resorption by inhibiting the activity of osteoclasts, the cells responsible for breaking down old bone tissue. This results in a net increase in bone mass and density, which can reduce the risk of fractures and other bone-related problems. Identification of osteoclast osteoblast.[8,31,32] In mineralized tissues, fluoride can be incorporated into apatite crystals through a process called ion exchange. This process leads to the formation of fluoroapatite.[8]

Fluoride may be supplemented during pregnancy until dental formation is completed. During pregnancy and breastfeeding, mothers should take 1 mg fluoride a day. Even though fluoride is an important mineral in the development of teeth and bone, excess intake of fluoride during pregnancy can lead to fluorosis in the developing teeth of the fetus. therefore, it is important for individuals to maintain a balanced fluoride intake and follow recommended guidelines to ensure the health and strength of their teeth, while also minimizing the risk of unwanted side effects.[33]

Magnesium (Mg) is not a major component of bone tissue like calcium and phosphorus, but it is still essential for proper bone development. Magnesium is mainly stored in bones and muscle. It is also found smaller amounts in soft tissues and extracellular fluids. Magnesium is involved in the formation and maintenance bones, as well as in the contraction and relaxation of muscles, including those involved in tooth movement.[34] Vitamin D and the parathyroid hormone (PTH) influence the absorbtion of magnesium. Vitamin D increases the expression of magnesium transporters in the intestine, which leads absorbtion of magnesium. On the other hand, parathyroid hormone increases magnesium absorbtion by stimulating the production of active vitamin D in the kidneys, which in turn promotes magnesium absorbtion in the gut.[35,36]

Magnesium ions play a crucial role in enamel formation by acting as a cofactor for enzymes involved in the process of mineralization. Magnesium also contributes to the structural integrity of enamel and can affect its hardness and resistance to decay by affect the morphology and structure of hydroxyapatite crystals. In addition to enamel, magnesium is also present in the dentin and cementum, the other hard tissues that make up the tooth structure.[4,22,37]

Intake of magnesium plays an important role in calcium and bone metabolism by increasing calcium absorption. Magnesium also plays a role in converting vitamin D into an active form that can increase calcium absorption. If calcium absorption in the intestine decreases, parathyroid hormone will increase calcium reabsorption in the kidneys which, if it continues to occur, can cause a decrease in bone density.[38] The recommended daily intake of magnesium during pregnancy is around 350-400 mg/day.[36]

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