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The Intervention of Anchovy (*Stokphorus commrsouli*) Calcium Extract Could Elevate Calcium Levels during Pregnancy and Give Birth and Promote Bone Development: An *in vivo* Study

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ABSTRACT

Background: During pregnancy, calcium deficiency has become a frequent phenomenon. Calcium intake is essential for supporting maternal and fetal health to maintain calcium homeostasis and promotes bone formation. Anchovy is a high calcium source from marine. The anchovy diet impact on fetal development during pregnancy is inconclusive. Therefore, *Anchovy Calcium Extract* (ACE) effect on calcium homeostasis and bone formation was studied.

Methods: Female Sprague Dawley rats were grouped into three: without ACE administration, with ACE administration prior to mating until two weeks post-labor and with ACE administration after pregnancy confirmation until two weeks post-labor. Blood calcium levels and bone hardness were measured.

Result: ACE improved maternal's and offspring's calcium levels. ACE also provided adequate blood calcium levels during pregnancy, labor and breastfeeding. In addition, ACE improve maternal bone firmness but has no significant effect on its offspring. In conclusion, ACE has excellent potential as a dietary supplement to promote adequate calcium requirements for maternal and infant health.

Key words: Anchovy's calcium extract, Blood calcium levels, Bone hardness, Pregnancy.

INTRODUCTION

Calcium is essential for humans to maintain their bone mass and several physiological activities. Calcium requirement in women will increase during the pregnancy and breastfeeding (Hacker *et al.,* 2012; Kumar and Kaur, 2017). The absorption of calcium in the intestine and excretion in the urine is higher during the pregnancy and breastfeeding than during conception and delivery (Prentice, 2000). Calcium deficiency during pregnancy leads to osteoporosis in pregnant women and impaired growth of the skeleton and teeth in the fetus (Mahadevan *et al.,* 2012). Thus, the intervention of calcium supplementation during pregnancy is important to maintain the maternal body's physiological balance and provide adequate materials for fetal development.

Milk is the main food source for calcium, providing 49% of global nutrient availability (Smith *et al.,* 2022). However, the habit of dairy product consumption is relatively low, particularly in developing countries (Pettifor, 2014). Also, dairy product intolerance has become a concern for calcium supplementation (Ugidos-Rodríguez *et al.,* 2018). One of the marine products with high calcium content is Anchovy fish. The calcium and phosphorus content of anchovies amounted to 1200 mg and 1500 mg in 100 g by edible weight, respectively (Swastawati *et al.,* 2020). Previous studies revealed that anchovy consumption could strengthen jawbone density and dentin formation (Astrina *et al.,* 2018; Ningtyas and Dharmayanti, 2014). Nevertheless, the influence of Anchovy consumption on calcium levels and bone development in pregnancy and maternal labor period and fetal development remains unknown. This study aimed to determine the effect of *Anchovy Calcium Extract* (ACE) supplementation during pregnancy, labor and breastfeeding in maternal and bone development of both maternal and fetal *in vivo*.

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MATERIALS AND METHODS

Preparation of extract and dose determination

The aqueous ACE was extracted by submerging 500 grams of Anchovy powder in 2500 ml distilled water for 24 hours. ACE was then diluted with distilled water before animal treatment. 21.6 mg/day of ACE was administered to the animal model. The dose was determined based on the conversion for calcium requirement in pregnant women (Hacker *et al.*, 2012).

Animal model and ethics

Two months of Sprague Dawley rats (250-280 g) were acclimatized for a week prior to treatment. Female and male rats were put in certain cages for mating. The observation of vaginal plaque in female rats was done every day. If there is a vaginal plaque identified, then the female rats were immediately separated from the male rats. This research has passed the ethical clearance from the Health Research Ethics Commission of the Ministry of Health, Semarang, No. 169/EA/KEPK/2019.

Animal treatments

Twenty-four female rats were divided into three groups: (1) Control group, consisting of pregnant rats but no ACE administration; (2) Intervention 1 with ACE administration starts from prior mating until two weeks after giving birth; and (3) Intervention 2 with ACE administration starts from first confirmed pregnant until two weeks after giving birth. Maternal blood serum (3 cc) was collected weekly during pregnancy until delivery for biochemical examination via infraorbital route. Collected blood was then tested for calcium level. The experiment was lasted for 48 days.

Calcium levels measurement

Blood serum was dissolved in a solution of Lanthanum (chloride acid) in the ratio of 1:50 (0.1% w/v). Lanthanum functions to control other chemicals, including phosphate. Next, a standard solution of calcium (100 mg/L) was prepared and added with a diluent solution until homogeneous. The serial working solutions of calcium were prepared as follows: 0.0 mg/L, 1.0 mg/L, 2.0 mg/L, 3.0 mg/L and 4.0 mg/L. The test was carried out on *Atomic Absorption Spectroscopy* (AAS) Perkin Elmer 3110 machine at 422.7 nm wavelength.

Calcium levels in the blood serum of both maternal (initial, pregnancy and labor) and newborn were measured. The collected blood sample from the infraorbital route was centrifuged (252 × g for 20 minutes) to obtain the blood serum. Calcium level was then measured from the blood serum using AAS. In addition, calcium levels in the bone were measured using the bone tissue of the femur and mandible of the maternal and offspring after euthanasia. Both bone tissues were separated from the soft tissue and then crushed to form a powder for further examination with the AAS.

Bone hardness examination

Bone hardness examinations were measured from the mandibular bone, femur and incisors of maternal and offspring of rats. After euthanasia, the femur and mandibular bone tissue were removed and separated from the soft tissue. The mandible was cut at the midpoint between the right and left incisors. Incisor teeth were obtained by cutting a tooth from the mandible. The bone tissue and teeth were aerated to dry and an impact test was immediately carried out using Vickers Hardness (VH) testing method.

Data analysis

Maternal calcium levels at different stages were analyzed using multivariate analysis. On the other hand, the calcium level of maternal among treatment groups, the calcium level of offspring and bone hardness were analyzed using one-way ANOVA. Duncan post-hoc analysis determined significant differences among experimental groups with p-value<0.05.

RESULTS AND DISCUSSION

Blood calcium levels

Maternal calcium requirements increase during gestation due to high absorption and excretion rates to maintain maternal physiological balance and fetal development (Hacker *et al.*, 2012; Kumar and Kaur, 2017). Inadequate calcium levels escalate the risk of adverse events in pregnancy, such as hypertension, maternal mortality and the risk of premature birth (Kumar and Kaur, 2017). Anchovy

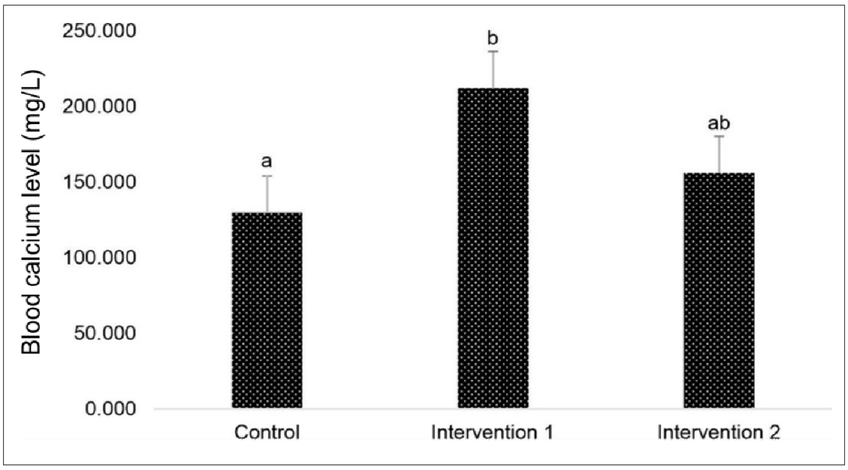

Fig 1: ACE administration improves calcium levels in maternal.

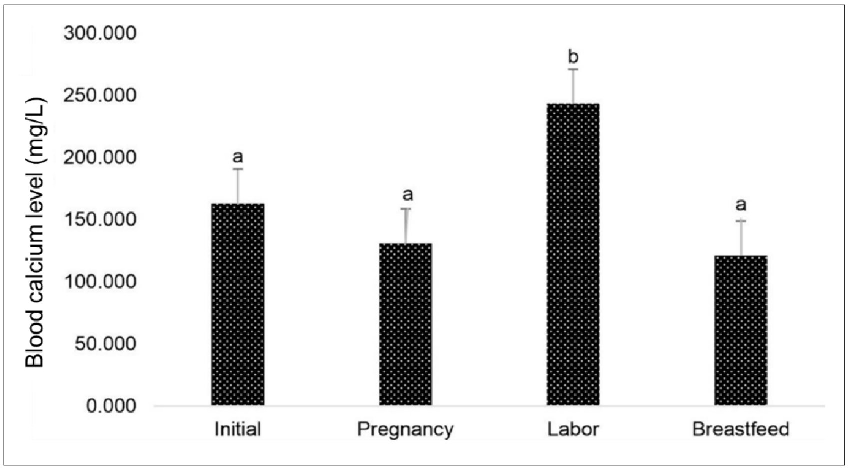

Fig 2: ACE assists in keeping calcium levels during pregnancy, labor and breastfeeding.

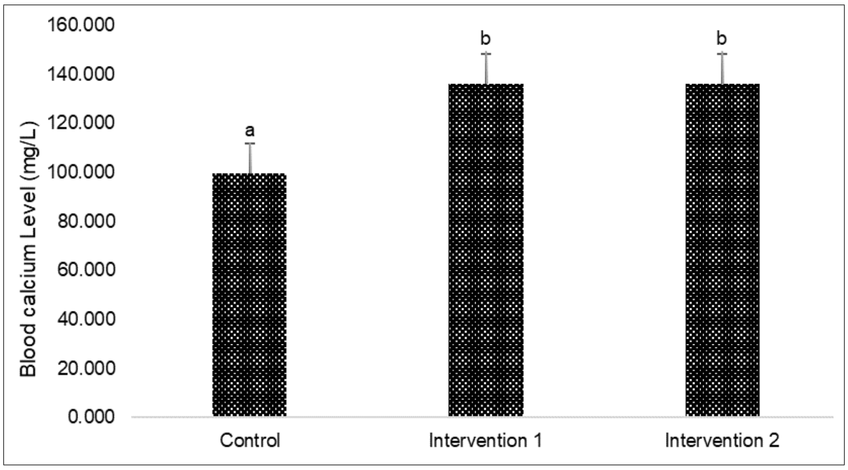

Fig 3: The augmentation of calcium level due to ACE administration also occurs in offspring

could serve as a potential calcium supplement to overcome calcium necessity (Swastawati *et al.,* 2020). Administration of ACE could elevate blood calcium levels both in maternal and offspring (Fig 1). The duration of administration significantly augments blood calcium levels, as shown by the level of calcium in the intervention group was higher than control ($p<0.05$). However, ACE administration after confirmed pregnancy insignificantly increases calcium levels ($p>0.05$). This result explains Anchovy's potential to fulfill maternal calcium requirements during pregnancy.

For exploring the calcium level during pregnancy and breastfeeding, the result of the intervention 1 group was analyzed (Fig 2). It shows that ACE supplementation could keep calcium levels before and during pregnancy. However, it became higher during labor and became lower after reaching the breastfeeding stage ($p<0.05$). It was also stated in a previous study that in labor, blood calcium is maintained at a higher level than in the maternal because of the increase of the ionized calcium level (Kovacs and Kronenberg, 1997). The escalation of calcium levels may occur due to the initiation of giving birth since calcium has a significant role in inducing uterus contraction for vaginal delivery (Papandreou *et al.,* 2004). The rise of blood calcium levels was also found in offspring with ACE administration compared to the control group (Fig 3). Maternal calcium intake also influences fetal development (Hacker *et al.,* 2012; Kumar and Kaur, 2017). These results suggest that ACE has good potential as a calcium supplement both in maternal and offspring.

Bone hardness

Supplementation of ACE contributes to the hardness of the maternal's bone but not to the offspring. Notably, the hardness of the intervention 2 group is higher than the intervention 1 group, implying that the time of ACE administration did not correspond to the hardness of bone formation. Yet, the hardness of intervention 2 and 1 in maternal bone hardness appears insignificantly different ($p>0.05$) (Fig 4). However, the bone density is also influenced by the amount of calcium and vitamin D consumed by the mother during pregnancy (Kumar and Kaur, 2017). Therefore, to further explain this result, vitamin D consumption should be determined. Even though the ACE administration did not influence the hardness of the bone of

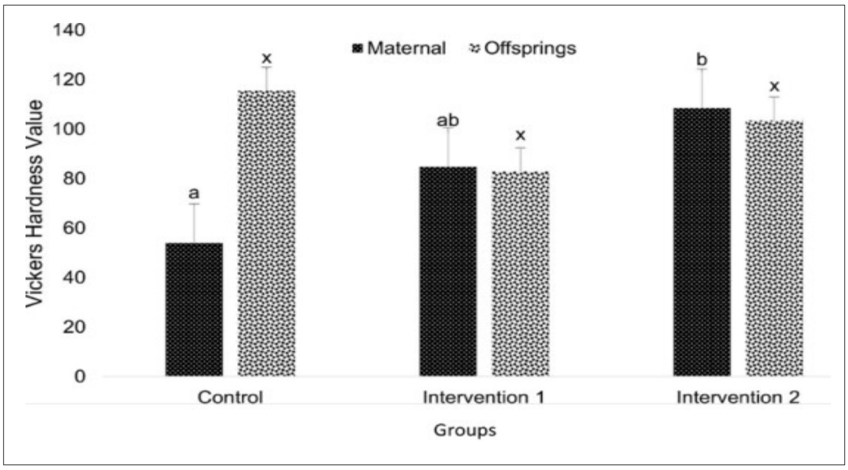

Fig 4: Calcium supplementation from ACE increases bone development in maternal but no direct effect on offspring.

the offspring, but this result also showed no bone loss during pregnancy. The high calcium absorption condition during pregnancy and breastfeeding makes the mother more susceptible to bone resorption and it can trigger osteoporosis, causing bones to become weak and brittle (Salari and Abdollahi, 2014). From these results, it is assumed that ACE has a constructive role in maintaining maternal bone mineralization to avoid osteoporosis and other bone diseases.

CONCLUSION

Administration of ACE showed physiological benefits in maintaining blood calcium levels in maternal and fetal. Moreover, ACE also significantly increased the bone hardness of the maternal rats compared to the control group, but there was no influence on fetal bone hardness. Thus, ACE has an admirable potential as calcium supplementation to preserve blood calcium supplies. Further clinical trials are required to determine ACE's effectiveness in maintaining calcium levels in pregnant women.

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Conflict of interest

Authors declare that there is no potential conflict of interest.

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