EFFECT OF EGGSHELL FLOUR ON BLOOD CALCIUM LEVELS IN PREGNANT MICE

Ida Ratna Safitri, Supriyana, Bahiyatun

Postgraduate Midwifery Program, Semarang Health Polytechnic, Semarang, Indonesia

*Corresponding author:
Ida Ratna Safitri
Postgraduate Midwifery Program, Semarang Health Polytechnic
Jl. Tirto Agung, Pedalangan, Banyumanik Kota Semarang, Jawa Tengah, Indonesia (50268)
E-mail: ida.ratnasaff92@gmail.com

Abstract
Objective: To examine the effect of eggshell flour on blood calcium levels in pregnant mice.
Methods: This was a true experimental study with pretest and posttest control group design. The research was conducted at the Nutrition Laboratory of the Center for Food and Nutrition Studies, Gadjah Mada University on December 1, 2017 to December 31, 2017. There were 30 pregnant rats included using simple random sampling, which assigned in five groups. Spectrophotometer was used to measure blood calcium levels. Paired t-test and One-way ANOVA were used for data analysis.
Results: There were significant differences in blood calcium levels before and after treatment with eggshell flour dose 5.4 mg/dl (p= 0.000), 10.8mg/dl (p = 0.000), and 21.6 mg/dl (p = 0.000). There was also significant difference in blood calcium levels in control group with generic tablet of calcium powder of 21.6 mg/dl (p = 0.000).
Conclusion: There was a significant effect of given eggshell flour for 7 days with dose 5.4 mg/dl, 10.8 mg/dl, and 21.6 mg/l as well as generic tablet of calcium powder of 21.6 mg/dl in increasing blood calcium levels. Thus, eggshell can be used as alternative for calcium supplement.

Keywords: eggshell flour; blood calcium level; pregnant mice

INTRODUCTION
World Health Organization (WHO) indicated that in 2010 maternal mortality during pregnancy and childbirth amounted to 287,000 women, which was caused by maternal health complications such as hypertension in pregnancies occurred in 2-8% of pregnancies and associated with low birth weight and maternal mortality (World Health, 2013). According to Indonesia Demographic Survey (IDHS 2012), it shows that Maternal Mortality Rate (MMR) in Indonesia reaches 359 per 100,000 live births, as the highest number in ASEAN (MOH, 2014). Based on Department of Health of Central Java Province, MMR in 2013 was 118.62 per 100,000 live births, increased to 126.55 per 100,000 live births in 2014. Of those, 57.95% of maternal deaths occurred during childbirth, 27.00% at the time of pregnancy, and 15.05% at the time of delivery (Dinkes, 2014). Preeclampsia and eclampsia are considered as the second cause of maternal mortality after bleeding.

During pregnancy, a woman will experience changes in both physical and psychological, and nutritional intake determines the health of pregnant women and the fetus they contain. Nutritional needs during pregnancy will be increased by 15% compared to the needs of normal women. Increased nutrition is required for uterine growth, breast (mammae), blood volume, placenta, amniotic fluid and fetal growth. The food consumed by pregnant
women will be used for fetal growth of 40% and the remaining 60% is used for the mother's growth (Wiknjosastro, 2005). It could be said that pregnant women essentially need additional nutrients, however, most of them often have a deficiency in protein energy and some minerals such as iron and calcium (Guyton & Hall, 2012).

Calcium is a macro mineral that plays a very important role in the body. More than 99% of calcium is found in hard tissues of human bones and teeth in the form of calcium phosphate (Almatsier, 2002). Calcium intake during pregnancy serves to maintain the stability of the mother's condition. Calcium can help a number of physiological and biochemical processes that include the work of the blood clotting system, neuromuscular, and the activity of hormones and enzymes. A well-calculated need for calcium can also prevent the occurrence of hypertension during pregnancy, reducing the risk of death from preeclampsia and possibly premature birth. WHO suggests calcium supplementation in antenatal care programs primarily to prevent preeclampsia; whereas inadequate consumption may have maternal and fetal effects, including osteopenia, tremor, paresthesia, muscle cramps, tetanus, late growth, low birth weight, and poor fetal mineralization (World Health, 2013).

Literature indicated that calcium intake of 1500-2000 mg daily can reduce high blood pressure in pregnancy up to 70% and preeclampsia by 50% (Mason, 2012). More importantly, calcium also affects the future of infant health. Sarwono reported that women who were given calcium supplements during pregnancy have children who are adequately protected from the risk of hypertension (Prawirohardjo, 2006).

According to the Data of the Indonesian Food Security Statistics, egg consumption in Indonesia is increasing every year as well as egg production. In 2014, the egg production of layer chicken was 1764000 tons (Pangan, 2015). The increasing production of laying eggs raises the number of eggshell wastes produced. According to Thapon and Bourgeois, the eggshell is 11% of the total weight of the egg as a whole and the content contained in the eggshell. The eggshell can be a source of calcium carbonate (94%), calcium phosphate (1%), magnesium carbonate (1%) and organic (4%) (Than, Lawanprasert, & Jateleela, 2012). Calcium carbonate as a pharmaceutical preparation is mainly used as a diluent of solid form. It is also used as a basis for medicine and dental preparations, buffering and dissolution for soluble tablets, as well as food additives and calcium supplements (Murakami, Rodrigues, Campos, & Silva, 2007). However, literature stated that the generic brand might not meet the standard requirements for tablet-crushed time (Susanto, 2010). In other words, the generic brand is destroyed more slowly resulting in decreased effectiveness. Thus, this study aimed to examine the eggshell flour as a calcium supplementation to changes in blood calcium levels.

METHODS
Research design
This was a true experimental study with pretest and posttest control group design to determine the effect of eggshell flour on calcium levels in pregnant rats.

Settings
The research was conducted at the Nutrition Laboratory of the Center for Food and Nutrition Studies, Gadjah Mada University on December 1, 2017 to December 31, 2017.

Research subjects
There were 30 pregnant rats included in this study using simple random sampling, which assigned in five groups: 1) an experiment group given standard feed treatment, aquadest, and 5.4 mg of eggshell powder (K1); 2) An experiment group given standard feed treatment, aquadest, and 10.8 mg of eggshell powder (K2); 3) An experiment group given a standard feed treatment, aquadest, and 21.6 mg of eggshell powder (K3); 4) A positive control group with standard feed treatment, aquadest, and generic tablet of calcium powder of 21.6 mg/dl (K4) - The dose used to adjust to the maximum dose of humans is 1200 mg/dl converted in the dose of mice obtained a maximum dose of 21.6 mg/dl (1200 mg/dl x 0.018); and 5) A
negative control group only given standard feed treatment and aquadest.

**Intervention**
The eggshell used in this study used a chicken eggshell, in which each group was given treatment for 7 consecutive days from the 8th to the 14th day of pregnancy.

**Instrument**
The instrument used in this study to measure blood calcium levels was using a spectrophotometer, as a tool used to measure absorbance by passing light with a certain wavelength on a glass or quartz object called cuvet. Some of the light will be absorbed and the rest will be missed. The absorbance value of the light passed will be proportional to the concentration of the solution present in the cuvet. Normal blood calcium level is 8.9-10.1 mg/dl. Blood collection was done through the plexus retro-orbitalis on the eyes.

**Ethical consideration**
This study has been approved by the Health Research Commission of POLTEKES Kemenkes Semarang with number: 021/KEPK/Poltekkes-SMG/EC/2017. Researchers have confirmed that each respondent has signed an informed consent.

**Data analysis**
Paired t-test was used to see the changes of calcium in blood, and One-way ANOVA was used to see the difference among the groups.

**RESULTS**
Table 1 shows that the average weight of mice in the five groups ranged from 201.5 to 205.83 with p-value 0.516, which indicated that there was no significant difference of weight among the five groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>205.83</td>
<td>201</td>
<td>210</td>
<td>0.516</td>
</tr>
<tr>
<td>K2</td>
<td>201.5</td>
<td>195</td>
<td>207</td>
<td></td>
</tr>
<tr>
<td>K3</td>
<td>205.83</td>
<td>201</td>
<td>209</td>
<td></td>
</tr>
<tr>
<td>K4</td>
<td>209.83</td>
<td>203</td>
<td>214</td>
<td></td>
</tr>
<tr>
<td>K5</td>
<td>204</td>
<td>198</td>
<td>212</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Blood calcium level before and after given intervention in the experiment and control group using Paired t-test

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood calcium level</th>
<th>Mean</th>
<th>Median</th>
<th>Min – Max</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>Pretest</td>
<td>2.57</td>
<td>2.59</td>
<td>2.40 – 2.71</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Posttest</td>
<td>6.04</td>
<td>6.03</td>
<td>5.87 – 6.20</td>
<td></td>
</tr>
<tr>
<td>K2</td>
<td>Pretest</td>
<td>2.49</td>
<td>2.45</td>
<td>2.19 – 2.90</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Posttest</td>
<td>7.89</td>
<td>7.89</td>
<td>7.49 – 8.38</td>
<td></td>
</tr>
<tr>
<td>K3</td>
<td>Pretest</td>
<td>2.70</td>
<td>2.75</td>
<td>2.37 – 2.94</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Posttest</td>
<td>10.09</td>
<td>10.2</td>
<td>9.47 – 10.76</td>
<td></td>
</tr>
<tr>
<td>K4</td>
<td>Pretest</td>
<td>2.62</td>
<td>2.62</td>
<td>2.30 – 2.93</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Posttest</td>
<td>9.64</td>
<td>9.68</td>
<td>9.38 – 9.85</td>
<td></td>
</tr>
<tr>
<td>K5</td>
<td>Pretest</td>
<td>2.65</td>
<td>2.56</td>
<td>2.36 – 3.07</td>
<td>0.171</td>
</tr>
<tr>
<td></td>
<td>Posttest</td>
<td>2.79</td>
<td>2.84</td>
<td>2.55 – 2.96</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 shows that the mean of blood calcium level in the K1 group before intervention was 2.57 and then increased to 6.04 after given intervention, similar with the K2 group that has an increase of blood calcium level from 2.49 during pretest to 7.89 during posttest; K3 group shows the mean of blood calcium level of 2.70 before intervention and became 10.09 after intervention; and K4 group was also similar that there was an increase of blood calcium level to 9.64 after intervention from 2.62. P-value in these four groups was 0.000, which indicated that there were significant effects of eggshell flour with dose 5.4 mg/dl,
10.8 mg/dl, and 21.6 mg/dl, and generic tablet of calcium powder of 21.6 mg/dl in increasing blood calcium level in pregnant rats. However, there was no significant difference of blood calcium level in the K5 group with p-value 0.171.

Table 3 Blood calcium level differences among the groups using One-way ANOVA

<table>
<thead>
<tr>
<th>Group pair</th>
<th>p-value</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>K1 vs K2</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>K1 vs K3</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>K1 vs K4</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>K1 vs K5</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>K2 vs K3</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>K2 vs K4</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>K2 vs K5</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>K3 vs K4</td>
<td>1.000</td>
<td>No Significant</td>
</tr>
<tr>
<td>K3 vs K5</td>
<td>0.000</td>
<td>Significant</td>
</tr>
<tr>
<td>K4 vs K5</td>
<td>0.000</td>
<td>Significant</td>
</tr>
</tbody>
</table>

One way ANOVA as shown in the table 3 indicated that there was significant difference of blood calcium levels in all groups with p-value 0.000 (<0.05), except in K3 and K4 group with p-value 1.000 (>0.05), which indicated that there was no significant difference between K3 (eggsheel flour 21.6mg) with K4 (Calcium Generic 21.6).

DISCUSSION
The purpose of this study was to examine the effect of eggshell flour in increasing blood calcium levels in pregnant mice. Findings of this study revealed that there was a significant increase of blood calcium levels after given eggshell powder with dose 5.4 mg/dl, 10.8 mg/dl, and 21.6 mg/dl. These results proved that changes in blood calcium levels in mice are caused by the high content of calcium in the eggshell. The chemical composition (by weight) of the eggshell product is as follows: calcium carbonate (94%), magnesium carbonate (1%), calcium phosphate (1%) and other organic (4%) (Susanto, 2010). The main component of eggshells is calcium carbonate, can be used to replace calcium carbonate, which is used as a pharmaceutical preparation, in solid dosage forms. In addition, calcium carbonate from eggshells has an advantage because it does not contain elements of toxic substances such as calcium carbonate from oyster shells containing residual substances with the potential of toxic elements such as aluminum, cadmium, mercury and other substances (Neunzehn, Szuwart, & Wiesmann, 2015).

Eggshell flour proved able to increase blood calcium levels well after given consecutively for 7 days. However, increased blood calcium levels are influenced by several factors, one of which is the presence of hormones contained in the body serving to help the process of eggshell flavor absorption, then eggshell flour enters into the process of metabolism of the body and blood flow system. The hormone includes parathyroid hormone, calcitonin, and 25-hydroxycholecalciferol; where eggshell flour consumed by pregnant rats enter the body by 30% into the calcification process of bone, teeth and cells (Meikawati & Suyanto, 2015). Calcium carbonate that has been consumed begins with ingress into the gastrointestinal tract, then Ca binds protein, absorbed by the intestinal mucosa, transported in the cell cytoplasm, into the bloodstream system through the basolateral cells, causing changes in calcium levels in the blood, and others about 70% are directly processed and expelled through sweat, urine, and feces (Guyton & Hall, 2012).

In addition, eggshell weigh 9-12% of total egg weight containing 94% calcium carbonate, 1% potassium phosphate, and 1% magnesium carbonate. Calcium from eggshell is a perfect supplement for food and its calcium bioaccumacy is quite high, at 93.80% (Ray, Barman, Roy, & Singh, 2017). Many studies have sought ways to utilize eggshell waste,
for example, using eggshell powder as a stabilizing agent to improve soil properties, as a coating pigment for printing ink-jet paper, food additives and as a source of calcium in animal and human nutrition (Hacker, Fung, & King, 2012) (Neunzehn et al., 2015). During this time, some people consider that the eggshell is a waste that is not useful; whereas in eggshells there are many benefits for the health of the human body, animal health and for plant growth.

This study proves that eggshell can be used as an alternative substance of calcium supplements. However, this study also revealed that there was a significant effect of generic tablet of calcium powder of 21.6 mg/dl in increasing blood calcium level in pregnant rats. Thus, it provides the options whether to use generic tablet or eggshell, which has the same effect on blood calcium level.

CONCLUSION
It can be concluded that there were significant effects of eggshell flour with dose 5.4 mg/dl, 10.8 mg/dl, and 21.6 mg/l as well as generic tablet of calcium powder of 21.6 mg/dl in increasing blood calcium levels. Thus, eggshell can be used as alternative for calcium supplement. Further study is needed to examine the effect of eggshell in human.

Declaration of Conflicting Interest
None declared.

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Author Contribution
All authors contributed equally in this work.

References


