Effect of Giving Nano Calcium Phosphate Diet on Mineral Content and Function Groups of Ovariectomy Tibia Rats

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ABSTRACT— Lack of calcium and phosphorus minerals in bones can cause decreased bone density, bone becomes brittle and at risk of osteoporosis. The purpose of this study was to analyze changes in mineral levels and functional groups in tibia osteoporosis because of ovariectomy and were treated with a nano-calcium phosphate diet. In this research, dietary formulations containing nano-calcium phosphate made. Diets given to animal models of osteoporosis rats caused by ovariectomy with the aim to balance the mineral content in the rats tibia. Dietary formulations were made of 3 types based on their calcium content, namely the first diet (D1), the second diet (D2) and the third diet (D3) each having a total calcium content of 1.0x, 1.5x and 2.0x normal body needs. Characterization and analysis of mineral content in the tibia bone using Atomic Absorption Spectroscopy (AAS) and Ultraviolet-Visible (UV-vis), while the characterization and analysis of functional groups with Fourier Transform Infrared Spectroscopy (FTIR). Based on the data obtained, ovariectomy rats showed signs of osteoporosis with decreased levels of calcium in the tibia and a decrease in phosphorus ion function groups at 9 weeks since ovariectomy. The results of dietary treatment showed that postovariectomy osteoporosis rats fed a diet with a total calcium content of 1.5x normal body needs (D2) and gave more effective and efficient results compared to diets containing 1.0x (D1) and 2.0x (D3) total calcium normal body needs.

Keywords— nano-calcium phosphate, ovariectomy, osteoporosis, tibia mineral, functional groups

1. INTRODUCTION

Osteoporosis is a metabolic disease that is widespread in the world [1,2,3,4,5]. Osteoporosis is an abnormality in bone in the form of a decrease in bone mass [6,7,8]. Osteoporosis can occur both in men and women especially those who have entered old age [9,10,11]. Women who enter the menopausal mass are more risky of osteoporosis because of an imbalance of the hormone estrogen [12,13,14,15].

In addition to age, hormonal problems can occur because of ovarian cysts or surgical removal of the uterus (ovariectomy). Several previous researchers examined the effects of ovariectomy (OVX), including causing morphometric and densitometric changes in the tibia bone [16], being overweight [17], and bone becomes porous [18]. Other researchers also have shown that OVX causes an increase in cortical bone blood vessel porosity and decreased bone mineral [19]. All things that arise because of OVX contribute to bone fragility during the process leading to osteoporosis. Although there have been many studies that discuss the effects of OVX, but no one has explained the time until the onset of osteoporosis. In this study observed and analyzed a decrease in minerals gradually every 2 weeks until the occurrence of osteoporosis. The observation time is chosen every 2 weeks because the estrous cycle period in rats lasted for 2 weeks [20].

Some techniques for treating osteoporosis include bisphosphonates [21,22,23], alendronate or odanacatib [24], parathyroid hormone and abaloparatide [25]. These drugs have recently been known causing digestive disorders, esophagus, hypocalcemia, inflammation of the eyes, bone and joint pain, femur fractures, hypercalciuria, malignant bone tumors and damage to the jawbone, so the use of these drugs is still pro and counter. In addition, there are also

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researchers who use herbal ingredients used in osteoporosis treatment. Some of them use sage, rosemary, thyme [26], roots and leaves of *Marantodes pumilum* [27]. However, the use of herbal ingredients has several limitations including requiring longer time to obtain medicinal properties, raw materials have not been standardized and are hygroscopic and voluminous, have not been clinically tested and are easily contaminated by various types of microorganisms. Therefore, in this study selected postovariectomy osteoporosis recovery therapy by giving nano-calcium phosphate diet. In accordance with the estrous cycle in rats, the effect of giving a nano-calcium phosphate diet is also observed periodically every 2 weeks. The aim is to find out the intensity of the mineral rise in the tibia bone, to obtain bone mineral levels as in normal bone conditions at the most optimal time.

The renewal of this research is the analysis during the stage towards osteoporosis and the recovery phase of osteoporosis carried out in stages. In addition, calcium phosphate is added to the nano-sized diet. Nano-sized particles are known to have a wider surface area, so they will be more easily absorbed by the body [28].

## 2. EXPERIMENTAL PROCEDURE

### 2.1 Preparation of Animal Model and Sample Collection

There were 110 white rats (*Rattus norvegicus*) kept from the age of 6 weeks, 90 were ovariectomized and the remaining (15) were not ovariectomized as controls. The use of animal models of rats and treatment of rats both ovariectomy and euthanasia had been approved by the Health Research Ethics Committee of the Faculty of Medicine, University of Indonesia, Cipto Mangunkusumo Hospital. The bone that is characterized and analyzed was the cortical portion of the tibia bone. The study was conducted in two stages, namely the first stage of ovariectomy rats and given a non-nano diet (standard diet), this stage is called the stage towards osteoporosis. The second stage was the rats that had already in the condition of osteoporosis obtained from the first stage, then given a nano-calcium phosphate diet, this stage is called the stage of diet treatment. The distribution of the use of rats was presented in Table 1.

### Table 1: Treatment groups

<table>
<thead>
<tr>
<th>Stage</th>
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<th>Description</th>
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<td></td>
<td>Abbreviation</td>
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<tr>
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<tr>
<td></td>
<td>12</td>
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</tr>
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<td></td>
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<tr>
<td>Towards</td>
<td>13</td>
<td>Collection of control tibia rats bone (non-OVX)</td>
<td>TK</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>15</td>
<td></td>
<td>TO3</td>
</tr>
<tr>
<td>(standard diet)</td>
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<td></td>
<td>19</td>
<td></td>
<td>TO7</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td></td>
<td>TO9</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>27</td>
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<td>TDA6, TDA8, TDA10, TDA12, TDA19</td>
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<tr>
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<td></td>
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<tr>
<td></td>
<td>31</td>
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<td>TDC6, TDC8, TDC10, TDC12, TDC19</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
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</tbody>
</table>

TK: Control rats tibia bone (not ovariectomy)
TO3-TO9: Tibia bone from 3-9 weeks rats since ovariectomy
TDA6-TDA19: Osteoporosis tibia bone given D1 diet in 6-19 weeks
TDB6-TDB19: Osteoporosis tibia bone given D2 diet in 6-19 weeks
TDC6-TDC19: Osteoporosis tibia bone given D3 diet in 6-19 weeks

### 2.2 Creation of Standard Diet and Nano-Calcium Phosphate Diet

A standard diet obtained from commercial products with dietary composition consisting of protein, fat, fiber, ash, calcium and phosphorus [29]. A standard diet was given to rats that were kept during the stages leading to osteoporosis. The nano-calcium phosphate diet was made with a diet modification formulated by Astuti [30]. The main ingredients for making nano-calcium phosphate diets were rice flour, casein, corn oil, glucose, DL-methionin, carboxymethyl cellulose
(CMC), calcium phosphate (Ca₃(PO₄)₂), vitamin mix, and NaCl. Calcium phosphate used in the manufacture of diets was a commercial calcium phosphate product from Merck with a catalog number 804604 and made into nano-size according to the modified Mulyaningish et al. [31] and Cabeza et al. [32] methods. Calcium phosphate that milled for 30 hours and measuring 8.17 nm was added to the main ingredient of the diet and made 3 types, namely the first diet (D1) containing 0.40% calcium (total calcium content of 1.0x normal body needs), the second diet (D2) containing 0.62% calcium content (total calcium content of 1.5x normal body needs) and the third diet (D3) containing calcium 0.99% (total calcium content of 2.0x normal body needs). Nano calcium phosphate diet was given to rats that were kept at the stage of dietary treatment.

2.3 Characterization and Analysis

Bone samples that had been cleaned then soaked with a solution of hydraziniumhydroxid (100% N₃H₃OH) for 1 week, continued soaking with 70% alcohol for 1 hour and rinsed with distilled water three times, and dried at 60 °C for 24 hours, then crushed until smooth and repeated soaking with a solution of hydraziniumhydroxid (100% N₃H₃OH) for 1 week, followed by soaking with 70% alcohol for 1 hour, and rinsed with distilled water three times, and dried again at 60 °C for 24 hours referring to the modified Oetama method [33]. The tibia bone sample was calculated for its calcium and magnesium levels using the atomic absorption spectrometry (AAS) method, while the phosphorus mineral using ultraviolet-visible (UV − vis) spectroscopy.

The procedure for testing bone mineral content using AAS is the dry ashing method based on the Association of Analytical Communities [34]. Bone samples were placed on a porcelain cup and then burnt on a furnace for 4 hours at a temperature of 700 °C. After that it was removed and cooled to the excitor. After the ashing was complete, it was weighed of 0.1 g on a porcelain cup. Next 10% HCl was added by 10 mL and heated on a hot plate until the volume decreased to 1/4 cup then 10% HCl was added as much as 10 mL to be heated again on the hot plate until the volume reduced to ¼ cup. Samples were transferred to a measuring flask and added distilled water to 50 mL, then filtered with whatman 41 paper. Clear samples were ready to be measured with AAS.

The procedure for the characterization of phosphorus minerals by UV − vis is bone samples, blanks and standards of 10 μL was put in a test tube then 1000 μL enzymes-1 in the form of solvents were then vortex for 10 seconds and incubated for 5 minutes, added 250 μL of enzyme-2. Then vortex again for 10 seconds was measured using a reagent kit and then read the absorbance at a wave length of 570-580 nm using a UV − vis spectrophotometer.

Fourier Transform Infrared Spectroscopy (FTIR) characterization was carried out to identify functional groups contained in tibia bone samples. In this study the use of FTIR is intended to identify the presence of various phases of calcium compounds by knowing the phosphate, carbonate and hydroxyl ion groups and crystallinity estimates. Bone samples to be tested were crushed with a mortar until smooth and then separated by 2.5 mg and added 250 mg of potassium bromide (KBr), then homogenized using mortar and pestle. After being homogeneous, the sample was molded into pellets with pressures up to 8 tons then analyzed. The range of infrared energy used in measurements was expressed in wavenumber from 400 - 4000 cm⁻¹. The analysis was done by looking at the shape of the spectrum by looking at specific peaks that indicated the type of functional group that is owned by bone. The sample function groups were determined based on the absorption of the detected wavenumbers [35,36]. The crystallinity index was calculated based on the splitting function (SF), by adding up the height of the two anti-symmetric phosphate peaks (range of wavenumbers 603 cm⁻¹ and 563 cm⁻¹) and dividing the value by the height of the trough between the two peaks. All heights measured above the baseline are drawn from wavenumbers around (780 - 495) cm⁻¹ [37]. The splitting function measurement technique is presented in Fig. 1.

![Figure 1: Splitting function (SF) calculation technique.](image-url)
2.4 Statistics Analysis

In this study there were 4 treatments and 5 replications in combination as follows:

DK: treatment of unovariectomized rats by administering a standard diet
D1: ovariectomy diet treatment by giving calcium phosphate diet 1.0x normal requirements
D2: ovariectomy diet treatment by giving calcium phosphate diet 1.5x normal requirements
D3: ovariectomy diet treatment by giving calcium phosphate diet 2.0x normal requirements

Analysis of the data used was an independent T test by comparing the results obtained from the data of DK with D1, DK with D2 and DK with D3 and it was also compared to dietary treatment data D1 with D2 and D2 with D3. P values <0.05 were considered statistically significant. Results were expressed as the mean ± standard deviation.

3. RESULTS AND DISCUSSION

3.1 Mineral Content in Tibia Bone

Minerals measured in the tibia were calcium (Ca), phosphorus (P) and magnesium (Mg). In the stage to osteoporosis, the mineral content of Ca and P in the tibia bone had a tendency to decrease (Fig. 2 and Fig. 3), except in the fifth week since ovariectomy, an increase in Ca and P minerals. The increase that occurred was not significant (P > 0.05), one of the factors causing the increase was the variety of rats used. The seventh and ninth weeks showed that Ca and P minerals were back down. The decrease in the mineral content of Ca and P in the ninth week was significantly different from the controls (P < 0.05). The linear regression equation for the reduction of Ca and P had a R² value of 0.87 (Fig. 2.b) and 0.82 (Fig. 3.b). Both of these values indicated that Ca and P decrease in time correlation.

The Mg content had a fluctuating value (Fig. 4). Three weeks after ovariectomy increased significantly (P < 0.05). Five weeks after ovariectomy returned. The seventh and ninth weeks were respectively up and down but not significant (P > 0.05). Therefore, the Mg content in the tibia bone cannot be predicted based on the time function. R² value = 0.13 (Fig. 4.b) showed that there was no strong relationship between Mg content and time. This happens because Mg can act as an inhibitor of bone.

Findings from rats had decreased mineral levels in their bones, especially Ca and P minerals. Minerals decreased gradually. Residual minerals in the minerals in osteoporotic bone occurred in the ninth week since ovariectomy. In the ninth week, Ca mineral content was 17.40% w/w and P 1.09% w/w. The results obtained support the results of previous studies stated that osteoporosis bone Ca mineral content caused by ovariectomy was 22.92% w/w, whereas osteoporosis bone phosphorus content was 10.26% w/w [38]. The results obtained confirm that ovariectomized rats with osteoporosis were seen based on the mineral content of Ca and P that occurred at the ninth week.

Nano-calcium phosphate diet treatment, on average can increase the three types of minerals in the tibia bone. The mineral content of Ca and P, the size returned to normal after giving a D2 or D3 diets for 19 weeks, but in the group of rats given the D1 diet and the value had not returned to normal as a control. Nano-calcium phosphate diet showed Ca increased with linear regression equation as in Fig. 2.c. The increase of Ca was correlated with time. Based on the linear regression equation, the administration of the D3 diet had the highest correlation with time compared to the D1 or D2 diets. Likewise, the effect of diet on the increase in P minerals showed a linear relationship with time (Fig. 3.c). During 19 weeks of administration of the nano-calcium phosphate diet, starting from the D1 to D3 diets showed a greater value of the mineral P deposited in the tibia bone compared to controls. This indicates the growth of bone crystals.

Mg in the tibia bone that had been treated with nano-calcium phosphate diet at the age of 40 weeks (19 weeks of dieting). It showed a higher value than the Mg content in the tibia control. This is in accordance with the nature of Mg, which helps the absorption of Mg in the body, thus making bones strong and dense [39]. This condition showed that even though the mineral content of Ca and P is as normal as bone, the strength had not yet returned to normal. It is necessary to carry out further tests such as mechanical tests in the form of tensile tests or bone morphological observations with Scanning Electron Microscopy (SEM) or Transmission Electron Microscopy (TEM).
**Figure 2:** Average tibia calcium mineral content: a) full display, b) osteoporosis step and c) diet treatment step.

**Figure 3:** Average mineral content of tibia phosphor mineral, a) full display, b) osteoporosis step and c) diet treatment step.
3.2 Typical Spectra of Phosphate Clusters

Phosphate groups in the tibia were observed in two regions, namely wavenumbers of 495-645 cm$^{-1}$ and 900-1200 cm$^{-1}$, respectively shown in Fig. 5 and Fig. 6. Phosphate groups in the range of wavenumbers 495-645 cm$^{-1}$ had two maximum peaks at wavenumbers 569 cm$^{-1}$ and 604 cm$^{-1}$ that were phosphate groups with bending vibrations ($v_4$). From the $v_4$ phosphate group with the splitting function (SF) value obtained as presented in Table 2.

Phosphate peak for groups of rats in the stage to osteoporosis showed a decrease in phosphate intensity (Fig. 5.a) and SF values (Table 2). From Fig. 5.a, it can be seen that the rats in the TO3 group to the TO9 group had almost the same pattern, namely the division of the ribbon in the phosphate group was getting shorter which indicated the presence of an amorphous phase. Amorphous phases that appeared indicated that the condition of the bones was more fragile. The shortened cleavage also indicates that the crystallinity index decreased, so that in the tibia there was a mixture of crystalline and amorphous phases, with the more dominating amorphous phase. This means that the tibia bone that had been ovariectomized had decreased strength. The biggest decrease occurred at the 9th week after ovariectomy, because in the 9th week the level of damage was higher than in the other groups. This level of damage is a sign of osteoporosis in the tibia.

In the group of rats fed the D1 diet (Fig. 5.b), the maximum peak of the phosphate group with fluctuating intensity was quantitatively supported by the fluctuating SF values and until 19 weeks of the diet the SF values were still under control. The maximum peak of the phosphate group of the group of rats fed the D2 and D3 diets for up to 19 weeks showed a more symmetrical peak shape (Fig. 5.c-d). It is supported also by the SF value that was increasing along with the longer time of giving the diet. The tibia bone treated with diet D2, the SF value exceeded the control value at week 19, and the tibia bone treated with diet D3 showed the same SF value as the control at week 12 giving the diet. In the 19th week, SF had greater value than the control value. The increasing value of SF showed the index of bone crystallinity increasing.

The phosphate group in wavenumber between 900-1200 cm$^{-1}$ in the rats group at the stage towards osteoporosis, qualitatively decreased the typical peak of the phosphate at wavenumber 962 cm$^{-1}$ that was a phosphate group with a vibrational stretching symmetry ($v_1$) mode. This phosphate group was an unstable phosphate group. In addition, there was also a typical peak of stable phosphate at wavenumber 1031 cm$^{-1}$ with asymmetric stretching vibration mode ($v_3$) (Fig. 6.a). In the treatment of D1, D2 and D3 diets showed no significant difference, but from week 6 to week 19 giving the...
diet, showing the direction of bone repair, seen from the growth of phosphate groups that were sharper. Unstable phosphate groups appeared to grow back. This supports the results of mineral analysis that indicates the presence of phosphorus deposited in bone because of the administration of nano-calcium phosphate diet.

### Table 2: Splitting function (SF) value of phosphate group $v_4$ FTIR spectra of tibia bone FTIR

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>SF = $\frac{(A1+A2)}{A3}$</th>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
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<td>2.89</td>
<td>2.78</td>
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<tr>
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<td>2.93</td>
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<tr>
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<td>2.53</td>
<td>2.14</td>
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</table>

### 3.3 Typical Spectra of Carbonate Cluster

The carbonate group was observed in two areas of wavenumbers, namely between 700-950 cm$^{-1}$ and 1300-1600 cm$^{-1}$, respectively shown in Fig. 7 and Fig. 8. In Fig. 7 there are two types of carbonate groups, namely carbonate $v_2$ in the range of wavenumber $873$ cm$^{-1}$ and carbonate $v_4$ in the range of wavenumber $719$ cm$^{-1}$. The carbonate group $v_2$ tended to be the same that was found for all groups. The $v_4$ carbonate group did not appear in all groups. In control rats $v_4$ carbonate groups were not found, whereas in the ovariectomized group of rats the presence of carbonate groups with higher intensity began from the 3$^{rd}$ week since ovariectomy. This indicated that in the ovariectomy tibia more phosphate groups were replaced by carbonate groups (Fig. 7.a).

Likewise for carbonate groups in wavenumbers between 700-950 cm$^{-1}$ for the diet treatment group (Fig. 7.b–d) showed that the carbonate group $v_2$ was present for each group and the carbonate group $v_4$ was increasingly disappearing. Nineteen weeks on the administration of the nano-calcium phosphate diet, the $v_4$ carbonate group again disappeared. The area of carbonate groups in this region was strongly influenced by HPO$_4^{2-}$ concentration [40]. No appearance of the carbonate group indicated that the phosphate group started to grow back.
Figure 5: Footage of FTIR results for the phosphate group from tibia at 495-645 cm\(^{-1}\) wavenumber.
Figure 6: Footage of FTIR results for the phosphate group from tibia at 900-1200 cm$^{-1}$ wavenumber.
Figure 7: Footage of FTIR results for the carbonate group from tibia at 700-950 cm$^{-1}$ wavenumber.

Fig. 8 shows a sample of the tibia bone FTIR results for the carbonate group at wavenumber between 1300-1600 cm$^{-1}$. The carbonate group at wavenumber between 1300-1600 cm$^{-1}$ consisted of several different peaks. The carbonate peak at wavenumber 1450-1500 cm$^{-1}$ was a type A apatite, which is a carbonate group that appears because it replaces the
hydroxyl group, and the carbonate peak at wavenumber of 1530-1550 cm\(^{-1}\) was a type B apatite, which is a carbonate group that replaces the phosphate position. Based on Fig. 8.a, it can be seen that for groups of rats 3 to 9 weeks since ovariectomy (TO3 to TO9), they had more type B apatite groups compared to type A apatite. In the TO9 group clearly the highest type B apatite was evident. This means that in the TO9 group more phosphates were replaced by carbonates. The carbonate peak was at wavenumber 1530-1550 cm\(^{-1}\) and showed a greater growth of type B apatite in the ovariectomy group of rats. In the TO9 group the carbonate group intensity was relatively the largest compared to the other groups. This means that the longer the time after the ovariectomy rats, the more the phosphate group was replaced by the carbonate group, and this study occurred in the 9th week since the rat was ovariectomized. This result was supported by the analysis of calcium and phosphorus minerals that are already under normal conditions.

Nano-calcium phosphate diet for osteoporosis rats is shown in Fig. 8.b-d. The figure shows that 19 weeks of dieting, apatite of type A and apatite of type B peaks are almost parallel as in normal bone spectra. The alignment of the two peaks indicates that the growth of phosphate groups was previously replaced by carbonates, so that the administration of nano-calcium phosphate diets for all types gives a better influence on the growth of phosphate in bones.

3.4 Typical Spectra of Hydroxyl cluster

Fig. 9–10 showed FTIR spectral samples for hydroxyl groups at wavenumber between 1600-1700 cm\(^{-1}\) and 2800-3400 cm\(^{-1}\). The hydroxyl group for wavenumber between 1600-1700 cm\(^{-1}\) was crystal water. The appearance of crystal water on osteoporosis bones, serves to maintain the shape of the bone so it did not shrink drastically. In groups of ovariectomized rats ranging from TO5 to TO9 (Fig. 9.a), the peak of crystal water at wavenumber 1631 cm\(^{-1}\) tended to be higher than TC and TO3. This showed that the ovariectomy of rats from the fifth week showed a decrease in bone quality. In the group of rats treated with diet, both groups of rats fed the D1, D2 or D3 diets showed curves that changed in a continuous and smooth direction. Fig. 9.b–c, FTIR spectra for crystalline water peaks began to lead continuously with a few low hydroxyl peaks occurring starting in the 12th week since dieting, while the group of rats fed the D3 diet (Fig. 9.d), the crystalline hydroxyl group had disappeared at the 10th week of dieting.

The hydroxyl cluster that appeared at wavenumber between 2800-3400 cm\(^{-1}\) consisted of 3 main peaks that were in the range of wavenumbers 2848 cm\(^{-1}\), 2918 cm\(^{-1}\), and 3319 cm\(^{-1}\), which are typical peaks of surface water (Fig. 10). The three peaks were found in the tibia of the TO3-T09 group as shown in Fig. 10.a. Giving D1, D2 and D3 diets tended to reduce surface water in the range of wavenumber 3319 cm\(^{-1}\). Surface water reduction began at 12 weeks of diet. After 19 weeks the surface water reduction diet was seen increasingly clearly as shown in Fig. 10.b–d. Two other hydroxyl clusters that were in the range of wavenumber 2848 cm\(^{-1}\) and 2918 cm\(^{-1}\) still appeared but the intensity was decreased. The biggest decrease occurred in the group of rats at given a diet for 19 weeks. This indicates, the diet provided an influence in bone repair.
Figure 8: Footage of FTIR results for the carbonate group from tibia at 1300-1600 cm\(^{-1}\) wavenumber.
Figure 9: Footage of FTIR results for the hydroxyl group from tibia at 1600-1700 cm$^{-1}$ wavenumber.
This research still has some limitations including the unknown effect of excess mineral consumption from the D3 diet, so it is necessary to measure mineral uptake and mineral measurements wasted through feces or urine.
4. CONCLUSION

Female white rat (Rattus norvegicus) experienced osteoporosis based on a significant decrease in the mineral content of calcium and phosphorus at 9th week since ovariectomy. This condition is supported by the decrease in phosphate group ν₄ that was replaced by carbonate group and the loss of unstable phosphate in vibrational mode of stretching symmetry ν₁, besides that there was an increase in crystal water as a result of decreased bone quality. This condition is also supported by the declining value of the splitting function which indicates the presence of an amorphous phase. Amorphous phases that appear indicates that the condition of the bones is more fragile.

Nano-calcium phosphate diet given to postovariectomy osteoporosis rats provided an optimal response to the improvement of tibial bone minerals, for nano-calcium phosphate that was added at least 1.5x the normal requirement (D2). Giving a diet of D2 and D3 for 19 weeks for postovariectomy osteoporosis rats, causing mineral calcium and phosphorus content was higher than normal rats, while magnesium content fluctuates. The increase in calcium and phosphorus minerals in tibia given the D2 diet were 2.72% w/w Ca and 12.95% w/w P respectively, while the increase in calcium and phosphorus minerals in the tibia fed the D3 diet was 22.92% w/w Ca and 29.11% w/w P respectively. Of the three types of diets given, the D2 diet was more recommended, because the D1 diet had not been able to meet the mineral needs of the bones, while the D3 diet was over doses of the mineral content.

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6. REFERENCES


