The Study of Blood Creatinin and Urea Concentration of Wistar Rats (*Rattus norvegicus*) due to Sodium Nitrite Induction

(Konsentrasi Kreatinin dan Urea dalam Darah Tikus Putih (*Rattus norvegicus*) Galur Wistar Akibat Pemberian Natrium Nitrit)

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Abstract: Sodium nitrite is one of common food addition in most meat product. This kind of food preservative is allowed by Permenkes No.722/Menkes/Per/IX/88 only in minimum doses due to its carcinogenic effect. The most targeted organ is kidney which is sensitive to chemical matter as nefrotoxin. When the kidney is damaged, the erytropoietin secretion to form erytrocite is disturbed. Physiological damage in kidney can be identified by the concentration of blood creatinin and urea. The objective of this research is to study about blood creatinin and urea concentration of Wistar rats which is induced by sodium nitrite. Two groups of Wistar rats were induced by two doses of sodium nitrite (11.25 and 22.50 mg/kg body weight, respectively) a day with one group of control. Blood serum of those three groups were then be analyzed for creatinin and urea concentration each week for three months. The result showed that creatinin concentration is fluctuitive during the day one to day ninety. The average of creatinin concentration for two treated groups is not significantly lower than control group. Generally, the urea concentration is increasing for day fourteen to seventy seven then decreasing in day eighty four to ninety. However, there are no significant differences among three groups.

Keywords: Creatinin, sodium nitrite, urea.

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INTRODUCTION

FOOD preservatives can be natural or synthetic which are added to increase shelf life and maintain quality of food by inhibit, retard, or arrest its fermentation, acidification, decomposition, and contamination(1). Meat product is one of the most kind of food which necessary to be preserved. The common preservative for meat product is sodium nitrite (NaNO₂) which inhibit growth of patogen Clostridium botulinum, defend the meat color(2,3), and provide specific meat flavor(4). Sodium nitrite is kind of sintetic food preservatives which is used in the form of free acid or salt(5). Sodium nitrite (NaNO₂) which is eaten will unravel to nitroxide (NO) and oxcide (O). Nitroxide (NO) bind erytrocye to form nitrosohemoglobin which cause decreasing of oxygen fixation ability(6).

Indonesian Ministry of Health decide the limit of sodium nitrite usage for food preservation is 125 mg/kg(7). Nitroso compounds in sodium nitrite has potential to yield nitrosamines which is carcinogenicic(8).

Sodium nitrite intake during preserved meat consumption influence several organ function, include kidney, liver, esophagus, stomach, pancreas, bladder, etc. The kidney has important role to release the toxins(9). Nitrite ions from sodium nitrite are soluble in water(2) and may cause some effect for kidney during its tubular reabsorption. There are some marked damage in kidney cells due to sodium nitrite doses of 60 and 75 mg/kg body weight consumption in rat. Intake of Sodium nitrite increase the activity of AST and ALT enzymes which trigger the formation of cytotoxic N-nitrosocompound. These compounds cause severe renal and hepatic necrosis( and cause kidney disfunction(11). The damage of kidney cells also can be shown by analyzed the creatinin and urea concentration in serum(12,13,14). This research want to study about creatinin and urea concentration of Wistar rats kidney which is induced by sodium nitrite .

MATERIAL AND METHOD

MATERIAL. Wistar rats (Rattus norvegicus) 2.5 m.o., sodium nitrit (NaNO₂), aquadest, standard woof, eter alcohol, ketamine, creatinin kit (Diasys; Catalog No:1 1711 99 10 021), NaCl 0.9%, wash reagent, sodium hydroxide, picric acid; urea kit (Diasys; Catalog No: 1 3101 99 10 021), Tris, 2-oxoglutarate, ADP, glutamate dehydrogenase, NADH, urea standard 50 mg/DL), cages, hematocrit, microtubes 1.5 mL, spectrophotometer, micropipet, centrifuge, vortex-mixer, and pipet tip.

METHODS. Dose Determination. The dose which use in T1 was refer to sodium nitrite safe dose, that is 125 mg/kg meat. While, dose for T2 was twice. Dose for T1 is 11.25 mg/kg body weight and T2 is 22.50 mg/kg body weight.

Animal Reserch Grouping. Twenty seven female Wistar rats (Rattus norvegicus) wa use in this research. These rats was 2.5 m.o. with body weight is 110-120 gram in average. Each group which are control (C), treatment 1 (T1), and treatment 2 (T2) consists of nine Wistar rats. Control group had no treatment, treatment 1 was treated with 11.25 mg/kg body weight sodium nitrite, and treatment 2 was treated with 22.50 mg/kg body weight. Each groups was treated for ninety days.

Sodium Nitrite Per Oral Treatment. Stock solution was made by adding 0.1125 g to 100 mL aquadest for T1 and 0.2250 mL in 100 mL aquadest for T2. Those stock solution were administrating per oral to Wistar rats during ninety days. Stock solution administration was adapted with Wistar rats body weight.

Serum Collection. Blood was collected each week for each rat by sinus orbitalis at LPPT UGM (Preclinical unit). Collected blood was store at microtube 1.5 mL without anticoagulant then be centrifuged at 5000 rpm for 5 minutes. Serum was transfered to other microtubes before analyzed.

Measurement of Creatinin Concentration. Fifty microliter of collected serum was added to 1000 μL monoreagent from Diasys creatinin measuring kit and incubated for 60 seconds. The absorbance of serum samples were read at λ 492 nm twice with interval 1 minute. The first absorbance of serum samples was averaged with the second absorbance and multiplied with multiplier which printed on Diasys creatinin measuring kit.

Measurement of Urea Concentration. The monoreagent (4 part from reagent I and 1 part from reagent II) were incubated at 15-25°C for 30 minutes then storage in dark bottle before use. Ten microliter of collected serum and 10 μl urea standard were added to 1000 μL monoreagent, each, and incubated for 60 seconds at 20-25°C. The absorbance of serum samples were read at λ 340 nm twice with interval 1 minute. The first absorbance of serum samples was averaged with the second absorbance and measured with formula:

$$\text{Urea concentration} \left( \frac{\text{mg}}{\text{dL}} \right) = \frac{\text{sample absorbance}}{\text{standard absorbance}} \times \text{standard concentration/cal}$$

Data Analysis. The data of creatinin and urea concentration were analyzed with one way ANOVA (Analysis of Variance) to know either signficancy.
RESULT AND DISCUSSION

Creatinin Concentration (mg/dL). This study perform 90 days per oral administration of sodium nitrite (NaNO₂) with two different doses. There are three groups of nine Wistar rats, each. Creatinin and urea concentration are one of parameters to identify the condition of kidney(13). Kidney damage can be marked by the increase of both concentration in the blood(15,14). Creatinin and urea in blood are the end products of nitrogenous protein metabolism and commonly filtered at glomerulus due to its small molecule size(15). Changes in Glomerular Filtration Rate (GFR) is responsible for the changes of serum creatinin and urea concentration(13).

Increase of creatinin concentration in blood indicate the early kidney damage. Creatinin concentration of Wistar rats which induced by sodium nitrite in this study show a very fluctuative results (Figure 1.). Each day of the test, creatinin concentration is always go up and down, in C, T₁, and T₂. At day 0, C and T₁ group showed same creatinin concentration average 0.4 mg/dL. While T₂ showed 0.57 mg/dL creatinin concentration average. Generally, the creatinin concentration was fluctuative during ninety days of test, with trend T₁ and T₂ is below C level.

At day fourteen, T₂ is higher than both C dan T₁. T₁ is the lowest one, and T₂ is the highest. But, those T₁ and T₂ result is lower than day 0. The result generally showed that creatinin concentration is fluctuative during day 0 to 90. In rats, creatinin will increase due to its muscle mass condition. The higher muscle mass, the higher creatinin level(16). But, in old rats, creatinin level will decrease due to its lower muscle mass than younger one. Level of creatinin concentration is depend on tubular secretion of serum creatinin. Factors which affect creatinin concentration in blood are age, sex, furrow, body habits, and diet(14).

All the Wistar rats in this study were feeded with standard woof. So, that is not one of the factor which affect level of creatinin concentration in this study.

Sodium nitrite stimulate thyroid and adrenal glands to blockade synthesis of protein, faster breakdown, increase free amino acids rate, and decrease protein turnover(12). So, creatinin forming is faster and stimulate the great storage in blood level. Sudden decrease of GFR are stimulated by trauma, haemorragiae, anesthesia, and bacteri infectio(17). Anesthesia before blood colection were reported increase creatinin concentration and trigger kidney damage(18).

Generally, creatinin concentration on day 90 is nearly same as its concentration on day 0. Kidney secrete toxic and waste component. Sodium nitrite is a polar matter so it may already release from body system. At early sodium nitrite treatment, Na ion is increase so renin is secreted to normalize Na concentration.
activity with high muscle mass, it may also have a high creatinin concentration\(^{(14,16,20)}\).

Based on ANOVA test, those three groups did not represent significant differences of creatinin concentration (Table 1.). Sodium nitrite treatment in \(T_1\) and \(T_2\) do not significantly affect the creatinin concentration compared with \(C\). Those fact imply that sodium nitrite treatment everyday during ninety days in both \(T_1\) and \(T_2\) doses give no significant subchronic effect to kidney function. In \(C\) group, there ia a significant creatinin concentration between day 63 and day 77. However, other than that, there are no significant differences in creatinin concentration among three groups. Creatinin concentration decrease at day 56 and 63, but it start to increase at day 70 (Figure 1.).

Those trend show that all the creatinin concentration may be influenced by body condition of Wistar rats, but not significantly been influenced by sodium nitrite treatment.

Creatinin concentration in this study indicate that 11.25 and 22.50 mg/kg bodyweight a day sodium nitrite is not high enough to increase creatinin concentration in blood. These fact conclude that if sodium nitrite is consumed in low doses, bellow 22.50 mg/kg body weight a day, there may not be a problem to kidney function at least in short term treatment.

However, the effect of this sodium nitrite may different if this chemical matter combine with another in one meat product. So, an appropriate use of sodium nitrite as food preservative is necessary to be controlled.

**Urea Concentration (mg/dL).** Beside creatinin, changes in kidney function is also can be detected by blood urea concentration. Urea is an excretion product from protein metabolism\(^{(14)}\) and its amount is affected by protein consumption. Formation of urea in body system is influenced by several factors, such as function of kidney, function of liver, protein intake, protein catabolism\(^{(13)}\), and hydration status\(^{(21)}\). Actually, urea nitrogen is normally found in blood as waste nitrogen product that comes from food protein breakdown\(^{(14)}\). But, the urea concentration in blood is increasing while there is a kidney failure occurs. Urea concentration has to combine with creatinin concentration in blood to indicate the right kidney condition\(^{(21)}\).

Same as creatinin concentration, blood urea nitrogen (BUN) is also varied among various age and between sex\(^{(15)}\). BUN increase in line with Wistar rats age (Figure 2.). In common, accumulation of

<table>
<thead>
<tr>
<th>Days</th>
<th>Creatinin concentration (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>(0.4 \pm 0.00^{ab})</td>
</tr>
<tr>
<td>7</td>
<td>(0.43 \pm 0.05^{ab})</td>
</tr>
<tr>
<td>14</td>
<td>(0.40 \pm 0.1^{ab})</td>
</tr>
<tr>
<td>21</td>
<td>(0.43 \pm 0.11^{ab})</td>
</tr>
<tr>
<td>28</td>
<td>(0.37 \pm 0.05^{ab})</td>
</tr>
<tr>
<td>35</td>
<td>(0.47 \pm 0.05^{ab})</td>
</tr>
<tr>
<td>42</td>
<td>(0.40 \pm 0.00^{ab})</td>
</tr>
<tr>
<td>49</td>
<td>(0.53 \pm 0.23^{ab})</td>
</tr>
<tr>
<td>56</td>
<td>(0.37 \pm 0.15^{ab})</td>
</tr>
<tr>
<td>63</td>
<td>(0.30 \pm 0.10^a)</td>
</tr>
<tr>
<td>70</td>
<td>(0.37 \pm 0.05^{ab})</td>
</tr>
<tr>
<td>77</td>
<td>(0.57 \pm 0.05^{ab})</td>
</tr>
<tr>
<td>84</td>
<td>(0.43 \pm 0.05^{ab})</td>
</tr>
<tr>
<td>90</td>
<td>(0.40 \pm 0.00^{ab})</td>
</tr>
</tbody>
</table>

Note: Number with different letter show significance creatinin concentration.
Kidney malfunction is also been indicated as a obesity effect(22). Older Wistar rats with higher body mass has higher urea concentration (Figure 2.) and increase the risk of kidney damage.

Based on the result of ANOVA test, all the enhancement of urea concentration level did not have significant differences among groups. Per oral administration in T₁ and T₂ did not affect the urea concentration level, significantly. Those two groups show that in doses 11.25 and 22.50 mg/kg body weight a day of sodium nitrite did not high enough to increase the risk of kidney failure. However, the effect will be different if sodium nitrite reacts with other food additives to form one marketable food. Different reaction of sodium nitrite with different food additives will show different effect in body function. So, this study show that no effect of the treatment is only happen when sodium nitrite is a single use at doses 11.25 and 22.50 mg/kg body weight a day.

Effect of sodium nitrite is different due to its mixture, either other chemical matter added or other concentration. Based on ANOVA test, blood urea concentration measured at day 0 is significantly different with day 49 and so on. Low filtration rate cause long storage of filtrat in kidney tubular before it release as urine. The longer filtrat storage time, the bigger urea reabsorption in blood. So, urine urea concentration is reduce, on the other hand, blood urea concentration is increase.

Blood urea concentration in T₂ is significantly different at day 14, 21, and 63 with day 70 and 84. But, all those concentration is not different from concentration at day 0. Those fact may be caused by sudden reduction of glomerular filtration rate. Beside all internal factors due to sodium nitrite induction, high creatinin and urea concentration in blood is also been affected by anesthesia(18) and trigger kidney mulfunction.

Blood urea increasing is started at day 21. Same as creatinin concentration, the urea concentration in C is also mostly higher than other two groups with treatment. Urea concentration in T₂ is mostly the lowest one. Those result may indicate that sodium nitrite treatment with two different doses is not high enough to affect blood urea concentration. The highest urea concentration is at day 77 and 84 for C, day 77 for T₁, and day 84 for T₂. All three groups show urea concentration increasing start at day 70 to 77, then decrease at day 84 and 90. Enhancement of urea concentration is depend of Wistar rats age level and its level of protein catabolism. Wistar rats may face metabolism problem so its protein breakdown is also disturbed then affect to urea concentration in blood.

Enhancement of urea concentration may be affected by excess of protein consumption(23). The excess protein is absorbed by small intestine and be changed to ammonia by colon microbes. Ammonia diffuse through blood stream to liver then enter the urea cycle. Sodium nitrite intake each day increase the nitrogen level. The excess nitrogen enter urea cycle to be changed to urea. Urea is excreted through kidney as urine. As the glomerular filtration rate (GFR) reduction, urea is pile up in the blood. So, the urea concentration is increase each seven days until day 77. Glomerular filtration rate can be decrease due to trauma, haemorrhaghi, anesthesia, and bacteri infection. If those occur, so the kidney will loose its function as filtration organ.

**Figure 2.** The average of blood urea concentration in Wistar rats induce with sodium nitrite during ninety days.
During this study, Wistar rats body weight is also measured. The data show that body weight is increase day after day in all three groups. Those indicate the growth of Wistar rats been going well. However, interval of body weight increase in T\textsubscript{1} and T\textsubscript{2} is not as much as C due to its sodium nitrite treatment. Sodium nitrite in Wistar rats ruin its body metabolism and disturb nutrient absorption, so its body weight is also smaller than C, even though those three groups consume relatively same woof ammount.

Table 2. The differences of urea concentration among three groups based on ANOVA test.

<table>
<thead>
<tr>
<th>Days</th>
<th>Blood urea concentration (mg/dL)</th>
<th>C</th>
<th>T\textsubscript{1}</th>
<th>T\textsubscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td>17.43 ± 4.06\textsuperscript{a}</td>
<td>19.97 ± 3.43\textsuperscript{a}</td>
<td>22.07 ± 6.26\textsuperscript{ab}</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>25.53 ± 4.12\textsuperscript{abc}</td>
<td>27.27 ± 4.90\textsuperscript{abc}</td>
<td>28.30 ± 3.84\textsuperscript{ab}</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>23.13 ± 1.90\textsuperscript{ab}</td>
<td>20.60 ± 1.15\textsuperscript{a}</td>
<td>19.87 ± 2.85\textsuperscript{a}</td>
</tr>
<tr>
<td>21</td>
<td></td>
<td>23.37 ± 2.56\textsuperscript{ab}</td>
<td>21.90 ± 4.68\textsuperscript{ab}</td>
<td>20.60 ± 4.16\textsuperscript{a}</td>
</tr>
<tr>
<td>28</td>
<td></td>
<td>25.33 ± 2.00\textsuperscript{abc}</td>
<td>25.93 ± 2.40\textsuperscript{abc}</td>
<td>23.90 ± 1.80\textsuperscript{ab}</td>
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<tr>
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<td></td>
<td>27.33 ± 4.44\textsuperscript{abc}</td>
<td>26.43 ± 2.61\textsuperscript{abc}</td>
<td>24.77 ± 3.38\textsuperscript{ab}</td>
</tr>
<tr>
<td>42</td>
<td></td>
<td>26.87 ± 2.72\textsuperscript{abc}</td>
<td>26.83 ± 1.07\textsuperscript{abc}</td>
<td>24.97 ± 3.32\textsuperscript{ab}</td>
</tr>
<tr>
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<td></td>
<td>35.97 ± 12.35\textsuperscript{bcd}</td>
<td>35.27 ± 10.75\textsuperscript{cd}</td>
<td>32.40 ± 10.30\textsuperscript{ab}</td>
</tr>
<tr>
<td>56</td>
<td></td>
<td>39.20 ± 8.27\textsuperscript{cd}</td>
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<td>35.53 ± 4.79\textsuperscript{ab}</td>
</tr>
<tr>
<td>63</td>
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<td>36.90 ± 5.61\textsuperscript{bcd}</td>
<td>37.37 ± 2.73\textsuperscript{cd}</td>
<td>30.75 ± 3.46\textsuperscript{a}</td>
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<td></td>
<td>39.67 ± 4.14\textsuperscript{cd}</td>
<td>43.27 ± 5.10\textsuperscript{d}</td>
<td>41.23 ± 6.71\textsuperscript{b}</td>
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<td>42.23 ± 4.85\textsuperscript{d}</td>
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<td>38.07 ± 11.93\textsuperscript{ab}</td>
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<tr>
<td>84</td>
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<td>41.77 ± 2.23\textsuperscript{d}</td>
<td>41.97 ± 4.85\textsuperscript{b}</td>
</tr>
<tr>
<td>90</td>
<td></td>
<td>38.93 ± 5.65\textsuperscript{cd}</td>
<td>33.63 ± 5.27\textsuperscript{bcd}</td>
<td>38.07 ± 4.16\textsuperscript{ab}</td>
</tr>
</tbody>
</table>

Note: Number with different letter show significance blood urea concentration.

CONCLUSION

Generally, in this study, sodium nitrite treatment is not influence the level of blood creatinin and urea concentration yet, in both T\textsubscript{1} and T\textsubscript{2}. Those result may be affected by small amount of sodium nitrite, so the dose is not high enough to disturb glomerular filtration rate. Even more, sodium nitrite in this study is only apply without other chemical added. Blood creatinin and urea concentration in three groups is not significantly different during 90 days of treatment. Blood creatinin and urea concentration in T\textsubscript{1} and T\textsubscript{2} is also not significantly different from C.

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