Effect of Administration of CHAGURO Made of Chayote (Sechium edule) and Tuna (Thunnus sp.) on Rats Induced with Streptozotocin-Nicotinamide and a High-Fat Diet

TOTO SUDARGO1*, BIANDA AULIA1, ATIKA ANIF PRAMESWARI1, ALIM ISNANSETYO2, INDUN DEWI PUSPITA2, SITI ARI BUDIYANTI2, RAHADYANA MUSLICHAH1, TIRA ARISTASARI1, SHEILA ROSMALA PUTRI2 and KHUSNUL ALFIONITA2

1Department of Nutrition and Health, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia.
2Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Abstract
This study was conducted to develop Chaguro, a low-cost supplementary food made of chayote (Sechium edule (Jacq.) Swartz) and tuna fish (Thunnus sp.), for diabetes and dyslipidemia diet therapy. In order to find a formula with effective hypoglycaemic and antidyslipidemic properties, dried tuna and chayote were mixed at different ratios: F1 (75% tuna, 25% chayote), F2 (50% tuna, 50% chayote), and F3 (25% tuna, 75% chayote). Thirty male Sprague Dawley rats were assigned into healthy control group or groups induced with streptozotocin-nicotinamide and a high-fat diet. Chaguro was administered 2.7 g/kgBW/ day using a gavage for 28 days. The administration of all Chaguro formulas improved blood markers compared to the negative control group (p < 0.001). Chaguro F1 lowered fasting blood glucose (97.07±1.18 vs 266.31±5.31), total cholesterol (113.59±2.22 vs 208.78±4.31), triglycerides (89.93±2.51 vs 142.35±2.83), LDL-c (33.87±1.87 vs 87.85±3.34) and increased HDL-c (69.08±1.85 vs 23.91±1.64) level the most compared to the negative control group (p < 0.001). Streptozotocin-induced weight loss was also prevented in all diabetic rats fed with Chaguro, with the body weight being similar to that of healthy controls at the end of the intervention (p < 0.001). This study found that Chaguro may be a potential food product to help lower blood glucose and improve lipid profile in diabetes and dyslipidemia.

CONTACT
Toto Sudargo toto_sudargo@yahoo.co.id Department of Nutrition and Health, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia.

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Introduction

Diabetes is an increasing metabolic problem that contributes to almost 4.2 million of global deaths in 2019.\(^1\) The worldwide prevalence of diabetes was predicted to be 10.2% in 2030.\(^2\) In 2018, 2.0% of adults were diagnosed with diabetes in Indonesia.\(^3\) In addition, around 19.7% of the population were estimated to have impaired glucose tolerance according to 2018 Indonesia’s Basic Health Research (Riskesdas).\(^3\)

Type 2 diabetes mellitus (DM) has been widely studied as a major risk factor for cardiovascular diseases (CVD), which is currently the number one cause of mortality worldwide.\(^4\) In 2018, the prevalence of CVD in Indonesia was 1.5%.\(^3\) CVD accounts for 52% of deaths in Type 2 DM and 44% in Type 1 DM.\(^5\) Adults with type 2 diabetes mellitus are more than twice as likely to develop CVD than adults without diabetes mellitus.\(^6\) It is estimated that more than 70% of individuals with diabetes mellitus will die from CVD.\(^6\) Among many predictors, lipid markers are the most commonly used indicator of CVD risk.\(^7\)

In terms of diet therapy for chronic disease and diabetes management, there is a growing trend in the use of natural compound or food with bioactive properties to help improve metabolic conditions.\(^8\) Functional food refers to food with basic nutritional impact, which also has beneficial effects on one or more functions of the human organism; it either improves the general and physical conditions and/or decreases the risk of the evolution of diseases.\(^9\)

Chayote or *Sechium edule*, a plant from the Cucurbitaceae family, is known for its economic value for trade and for its culinary applications in many countries due to its nutritional content.\(^10\) Chayote is also used for medicine and complementary treatment, such as for hypertension or dissolving kidney stones.\(^11\) The administration of chayote extract was reported to lower blood glucose and improved lipid profile in diabetic rats.\(^12,13\) Chayote juice has also been reported to significantly reduce starch-induced postprandial glycemic load even in the absence of carbohydrate digestive enzymes’ inhibitory activities.\(^14\) Feeding rats with chayote juice for 3 months along with sucrose or fructose-sweetened beverages not only decreased the progression of impaired glucose tolerance but also significantly prevented development of oxidative stress by maintaining total antioxidant potentials in the blood.\(^15\)

Fish consumption reduces chronic non-communicable diseases such as cardiovascular diseases, rheumatoid arthritis, and several cancer types.\(^16\) Diversification of protein sources may modulate insulin resistance in different manners. Based on studies in rats and human subjects, the intake of fish proteins was believed to have favorable effects on glucose tolerance and insulin sensitivity.\(^17,18\) Tuna fish (*Thunnus* sp.) is a high-protein food known for its bioactive compounds such as omega-3 fatty acids which exhibit beneficial effects, including its hypocholesterolemic, antiatherosclerosis, and anticancer properties.\(^19,20\)

Previous investigations have shown the health benefits of chayote and tuna independently, but the findings were mostly limited to the potential of isolated bioactive compounds or extract.\(^12–15,20–22\) In this study, we aimed to combine tuna and chayote to develop *Chaguro*, a food product that may improve blood glucose and lipid profile in diabetes and dyslipidemia. This animal study was conducted to determine the most effective *Chaguro* formula to be developed in further study.

Methods

Preparation of *Chaguro*

Fresh raw tuna fish (*Thunnus* sp.) was obtained from a local market in Yogyakarta. The flesh was separated from the bone and cooked for 25 minutes using a steamer, after which it was shredded before oven-dried for 6 hours using a temperature of 65°C. The dried meat was then blended into smaller particles of around 0.1 mm in diameter. *Sechium edule* (chayote) obtained from a local market in Yogyakarta was washed, peeled, and cut into slices of around 0.3 cm thick. The drying process was performed using an oven with a temperature of 55°C for 4 hours. Afterward, the dried slices were blended into small flakes.

The dried products of tuna and chayote were mixed together with different ratios for the intervention: 1) *Chaguro* F1 (75% tuna, 25% chayote), *Chaguro* F2 (50% tuna, 50% chayote), *Chaguro* F3 (25% tuna, 75% chayote).
Analysis of Macronutrient and Mineral Content

Analysis of macronutrients and mineral content were carried out in Chemix Pratama Laboratory, Yogyakarta, Indonesia. Dry ashing method was used to determine total ash content; samples were combusted using a muffle furnace at a temperature of 550°C.23 Total protein content of samples was estimated using Micro-Kjeldahl method.24 Total fat content was determined by the Soxhlet extraction method.23 Carbohydrate content was estimated by difference (subtracting the measured protein, fat, and ash from the total weight). Crude fiber was determined by boiling the sample with 1.25% dilute H2SO4, washing it with water, and boiling it further with 1.25% dilute NaOH. The remaining residue after digestion was the crude fiber. This method followed the guidelines described by Association of Official Analytical Chemists (AOAC).23 Calorie value (kcal per 100 g) was estimated by converting macronutrient content (protein, fat, carbohydrate) into energy using conversion factors of 4 kcal/g for protein and carbohydrate, and 9 kcal/g for total fat.25 Mineral content (Ca, Mg, Mn, Zn) was estimated using atomic absorption spectroscopy (Perkin-Elmer Model 3110, Germany) according to the manufacturer's manual. All measurements were repeated twice.

Experimental Animals

Thirty male Sprague Dawley rats (160—180 g) aged 8 weeks were obtained from the Experimental Animal Laboratory of Food and Nutrition Postgraduate Research Center, Universitas Gadjah Mada. All rats underwent an acclimatization period of seven days before the intervention. They were given the AIN-93 M standard diet (formulated by American Institute of Nutrition in 1993 for adult rodents) and water ad libitum. Throughout the experiment, all rats were kept inside a stainless-steel cage in a humidity-controlled room with a temperature of 20-24°C and received a 12/12-h dark-light cycle per day.

After the adaptation period, the rats were randomly divided into five groups with six rats each: 1) Healthy control: healthy rats that received no supplementation, 2) Negative control: rats induced with diabetes and dyslipidemia that received no supplementation, 3) F1: Diabetic and dyslipidemic rats that received Chaguro F1 (25% chayote, 75% tuna), 4) F2: Diabetic and dyslipidemic rats that received Chaguro F2 (50% chayote, 50% tuna), and 5) F3: Diabetic and dyslipidemic rats that received Chaguro F3 (75% chayote, 25% tuna).

The induction of dyslipidemia to negative control, F1, F2, and F3 groups was performed by daily oral administration of 3 ml high-fat food (a mix of beef fat, egg yolk, and water) for 2 consecutive weeks. After that, diabetes was induced with an Intraperitoneal injection of 45 mg/kg streptozotocin (STZ) prepared in 0.1 mol/L citrate buffer (pH 4.5), followed by an injection of 110 mg/kg nicotinamide (NA) diluted in saline solution.26

Intervention period with Chaguro was conducted for 28 days. The dosage for Chaguro administration was 2.7 g/kgBW/day. As this was a preliminary study, the dose was based on the assumption that the planned supplementation of Chaguro for human is 30 g per day. The dose for rat was calculated with a conversion factor of 0.018 from a human weighing 70 kg.27 All Chaguro formulas were blended and diluted in water for administration using an oral gavage to rats.

Blood collection was performed after diabetes and dyslipidemia induction and after the intervention ended. Blood was collected from retro-orbital plexus and allowed to coagulate for 30 minutes. Serum was obtained by centrifugation of the coagulated blood at 1400×g for 15 min and then stored at−20°C for measurements. Ethical approval for this animal study was obtained from the Ethical Committee of Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada (KE/1234/10/2019).

Serum Glucose and Lipid Profile Measurement

The serum levels of glucose, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured by enzymatic colorimetric methods (Diasys Diagnostics, Germany). All procedures were performed according to the manufacturer’s instructions. All measurements were repeated twice.

Statistical Analysis

Data are presented as mean ± standard deviation. A normality test was performed before performing comparative analysis. One-way ANOVA followed by post-hoc Tukey HSD test was used to Compare blood glucose, lipid profile, and body weight between
groups at different measurement times. A two-sided p-value of < 0.05 was considered statistically significant.

Results

Nutrient Content

Table 1 shows the nutrient content of all Chaguro formulas. The highest calorie content was found in Chaguro F1 (373.32 kcal), followed by F2 (341.03 kcal), and F3 (244.91 kcal). F1 formula contained the highest percentage of dried tuna and had the highest protein content (66.98%). The highest crude fiber and carbohydrate content were found in Chaguro F3, the formula with 75% dried chayote. The calcium content of the formulas ranged from 146.14 to 157.56 mg per 100 g. Chaguro F3 had the highest magnesium (149.44 mg/100 g), while F1 had the highest manganese content (4.70 mg/100 g). The zinc content of the formulas ranged from 1.59 to 1.73 mg/100 g product.

| Table 1: Macronutrient and mineral content of Chaguro formulas per 100 gram |
|--------------------------------------------------|----------------|----------------|
| Chaguro F1 (75% tuna, 25% chayote) | Chaguro F2 (50% tuna, 50% chayote) | Chaguro F3 (25% tuna, 75% chayote) |
| Calories (kcal) | 373.32±0.31 | 341.03±1.58 | 244.91±0.99 |
| Protein (g) | 66.98±0.06 | 52.52±0.08 | 28.75±0.07 |
| Fat (g) | 8.07±0.01 | 9.69±0.18 | 6.31±0.11 |
| Carbohydrate (g) | 19.42±0.10 | 30.39±0.30 | 59.80±0.24 |
| Crude fiber (g) | 7.73±0.02 | 9.81±0.26 | 10.74±0.10 |
| Ash (%) | 5.53±0.16 | 7.40±0.05 | 5.14±0.06 |
| Calcium (mg) | 146.14±3.40 | 157.56±2.46 | 146.97±1.18 |
| Magnesium (mg) | 116.74±0.55 | 107.34±0.59 | 149.44±0.57 |
| Manganese (mg) | 4.70±0.07 | 1.10±0.07 | 1.12±0.10 |
| Zinc (mg) | 1.59±0.01 | 1.60±0.03 | 1.73±0.03 |

Values are the means from two measurements ± standard deviation.

| Table 2: Fasting blood glucose (FBG) of rats before and after supplementation with Chaguro |
|----------------------------------|----------------|
| Group | Pre-test | Post-test |
| Healthy control (n=6) | 69.92±2.11<sup>a</sup> | 71.19±1.96<sup>a</sup> |
| Negative control (n=6) | 262.62±5.77<sup>b</sup> | 266.31±5.31<sup>b</sup> |
| F1 (n=6) | 260.35±6.58<sup>c</sup> | 97.07±1.18<sup>c</sup> |
| F2 (n=6) | 262.01±6.04<sup>d</sup> | 109.34±1.53<sup>d</sup> |
| F3 (n=6) | 261.88±3.33<sup>e</sup> | 129.21±3.25<sup>e</sup> |
| p | <0.001 | <0.001 |

FBG, fasting blood glucose; F1: 75% tuna, 25% chayote; F2: 50% tuna, 50% chayote; F3: 25% tuna, 75% chayote. Data are presented as mean ± SD; In each column, data with different superscripts are statistically significant at p < 0.05 (one-way ANOVA and Tukey HSD).
Blood Glucose
Before the treatment, all rats induced with diabetes had a higher fasting blood glucose level compared to that in the healthy control group (261.7 ± 5.3 vs 69.9 ± 2.2) (Table 2). This level of blood glucose was within 200 mg/dL-450 mg/dL range, indicating a Stage 1 Diabetes in rats.28 At the end of the treatment, all treated group had significantly lower blood glucose compared to the negative control group (p < 0.001). Diabetic rats treated with Chaguro F1 showed the greatest reduction in blood sugar level, even though it is still higher compared to that in normal control (p < 0.001).

Lipid Profile
The effect of treatment on serum lipids of rats is presented in Table 3. There was a significant difference between groups after Chaguro administration (p <0.001). A greater reduction in total cholesterol was observed in the F1 group. In accordance, the greatest improvement for other lipid profile components (TG, LDL-c, HDL-c) was also seen in the F1 group compared to other formulas (Chaguro 2 and 3). The posthoc Tukey HSD test showed that none of the lipid profile levels in treatment groups were statistically similar to that of healthy control at the end of the intervention.

Bodyweight
Figure 1 shows the change in body weight throughout the intervention. The body weight (mean ±SD) of each group of rats (n = 6 per group) was recorded weekly.

Discussion
Several studies have highlighted the health benefits of tuna and chayote including its potential in improving metabolic disturbance.12,22,29,30 However, this study is the first to develop a food product from these resources and evaluate the combined effect on blood glucose and lipid profile. Our study found that all Chaguro formulas improved the condition of diabetic and hyperlipidemic rats, even though it went through drying processes which might reduce its nutritional value.11,31

Regarding effectiveness, Chaguro F1 exhibited the most significant improvement of all blood parameters. Omega-3 fatty acids found in tuna, which has been widely studied as a cardiovascular protective nutrient,32 might be responsible for lowering TC, TG, LDL-c, as well as increasing HDL-c level in diabetic rats. One of the direct modulatory effects of omega-3 in repairing lipid metabolism is through the activation of peroxisome proliferator-activated receptor (PPAR) which subsequently reduces the secretion of TG, increased fatty acid oxidation, and improved insulin sensitivity.33 In this study, the formula with the highest ratio of tuna increased the HDL-c level by 181%. It was likely that the omega-3 polyunsaturated fatty acids played a direct role in
modifying HDL composition and function which results in increased HDL maturation.\textsuperscript{34,35}

Various cooking processes affected omega-3 in tuna differently. A study found that cooking tuna by boiling at 100°C had a minimal effect (1% reduction) on its omega-3 composition, but frying destroyed the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) abundantly.\textsuperscript{31} In this study, despite the use of lower temperature to dry the tuna, the long cooking time might lead to a higher degradation of fatty acid content.

Even though \textit{Chaguro} F1 was more effective than F2 and F3, when compared to the negative control, administration of F3 (25% tuna, 75% chayote) still resulted in a marked reduction of blood glucose level by 51%. This is supported by a previous study that showed decreased blood glucose and improved lipid profile in diabetic rats after oral administration of 100 and 200 mg/kg BW ethanolic extract of chayote fruit.\textsuperscript{12} In our study, however, we dried the fruit and subjected it to 55°C exposure of heat. Loizzo \textit{et al}. reported that cooked chayote pulp had approximately half the total phenols content of the raw version, while these phytochemical components were known to positively correlate with inhibitory activities of carbohydrate-hydrolyzing enzymes.\textsuperscript{11} None the less when compared to other cooking processes, roasted pulp showed the highest antioxidant activity according to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test.\textsuperscript{11} It can be implied that the improvement of metabolic abnormalities in this study might also be attributed to the phytochemical content of \textit{S. edule}. 

Table 3: Lipid profile among groups before and after supplementation with Chaguro

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Cholesterol (mg/dL) Pre-test</th>
<th>Total Cholesterol (mg/dL) Post-test</th>
<th>Triglyceride (mg/dL) Pre-test</th>
<th>Triglyceride (mg/dL) Post-test</th>
<th>HDL-C (mg/dL) Pre-test</th>
<th>HDL-C (mg/dL) Post-test</th>
<th>LDL-C (mg/dL) Pre-test</th>
<th>LDL-C (mg/dL) Post-test</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control</td>
<td>88.88±3.05\textsuperscript{a}</td>
<td>92.05±4.00\textsuperscript{a}</td>
<td>78.22±2.08\textsuperscript{a}</td>
<td>60.13±2.73\textsuperscript{a}</td>
<td>86.64±1.28\textsuperscript{a}</td>
<td>84.60±1.76\textsuperscript{a}</td>
<td>23.24±1.73\textsuperscript{a}</td>
<td>24.96±1.87\textsuperscript{a}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Negative control</td>
<td>206.41±4.00\textsuperscript{b}</td>
<td>208.78±4.31\textsuperscript{b}</td>
<td>140.02±2.41\textsuperscript{b}</td>
<td>142.35±2.83\textsuperscript{b}</td>
<td>25.00±1.28\textsuperscript{b}</td>
<td>25.00±1.28\textsuperscript{b}</td>
<td>142.91±1.64\textsuperscript{b}</td>
<td>23.91±1.64\textsuperscript{b}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>F1 (n=6)</td>
<td>210.19±6.32\textsuperscript{c}</td>
<td>113.69±2.23\textsuperscript{c}</td>
<td>141.60±3.63\textsuperscript{c}</td>
<td>89.03±2.51\textsuperscript{c}</td>
<td>24.54±0.90\textsuperscript{c}</td>
<td>24.42±1.71\textsuperscript{c}</td>
<td>63.46±1.80\textsuperscript{c}</td>
<td>56.54±2.01\textsuperscript{c}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>F2 (n=6)</td>
<td>214.08±5.64\textsuperscript{d}</td>
<td>123.70±1.01\textsuperscript{d}</td>
<td>144.03±1.57\textsuperscript{d}</td>
<td>88.63±3.27\textsuperscript{d}</td>
<td>24.20±1.82\textsuperscript{d}</td>
<td>24.20±1.82\textsuperscript{d}</td>
<td>62.46±1.80\textsuperscript{d}</td>
<td>56.54±2.01\textsuperscript{d}</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>F3 (n=6)</td>
<td>212.60±6.52\textsuperscript{e}</td>
<td>137.78±2.51\textsuperscript{e}</td>
<td>143.18±3.24\textsuperscript{e}</td>
<td>111.89±2.19\textsuperscript{e}</td>
<td>24.54±0.80\textsuperscript{e}</td>
<td>24.54±0.80\textsuperscript{e}</td>
<td>69.08±1.85\textsuperscript{e}</td>
<td>33.87±1.86\textsuperscript{e}</td>
<td>&lt;0.001</td>
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<td>p</td>
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<td>0.001</td>
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</table>

HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol
F1: 75% tuna, 25% chayote; F2: 50% tuna, 50% chayote; F3: 25% tuna, 75% chayote
Data are presented as mean ± SD; In each column, data with different superscripts are statistically significant at p <0.05 (one-way ANOVA and Tukey HSD)
STZ induction causes pancreatic B-cell damage which leads to inhibition of proinsulin synthesis. The severe loss of body weight in diabetic rat model might result from protein wasting because of the significant reduction of glucose uptake. Oral administration of three different Chaguro formulas for 28 days maintained the bodyweight of diabetic rats to a level similar to healthy controls. This might be due to the additional calorie and protein intake from Chaguro supplementation which contributed to the reversal effect of STZ-induced weight loss. It has also been discovered in a prior study that S. edule fruit extract could prevent bodyweight loss after diabetes induction.

This study is among those that attempt to administer the near-final form of a food product, not the extracted bioactive compounds, to animal models and showed positive outcomes. Our findings provide evidence for future research in functional food development, particularly about the potential of tuna and chayote as low-cost food resources and the use of simple drying methods to prolong shelf-life. This preclinical study also serves as a baseline for subsequent research in human subjects. Nevertheless, further development in food processing may be required to better preserve the nutritional quality and bioactive compounds of the product and to ensure sensory acceptability among target populations.

Conclusion
Our results indicate that the administration of Chaguro F1 (75% Tuna, 25% Chayote) produced the closest FBG, TC, TG, HDL-c, and LDL-c to that of normal control at the end of the intervention. We therefore suggest the combination of 75% dried tuna and 25% dried chayote as an effective supplemental food to reduce the risks of metabolic disease and help manage diabetes and dyslipidemia.

Acknowledgments
Author Contributions: T.S., A.I, I.D.P, S.A.B, and R.M conceived and designed the study; T.S., T.A., S.R.P, and K.A. performed the experiments and collected the data; T.S., B.A, and A.A.P analyzed the data; T.S., A.I, I.D.P, and S.A.B provided materials; T.S., B.A, and A.A.P wrote the manuscript. All authors read and approved the manuscript.

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Conflict of Interest
The authors declare no Conflict of interest.

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265


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