

## Antihypertensive effect of functional rice analogue containing corn, mocaf, pigeon pea and seaweed on rats

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### Abstract

The ingredients of rice analogue used in this research are corn (*Zea mays*), lebei or pigeon pea (*Cajanus cajan* (L.) Millsp.), mocaf (fermented cassava) and seaweed (*Euchema cottonii*) which was formulated in different compositions of amylose and amylopectin namely formula F30 (contain 30% amylose) and F31 (contain 31% amylose) (F30 and F31 Formula). Nutritive and bioactive compounds in the ingredients are supposed to have a role in the activity of blood pressure reduction. Therefore, this research aimed to determine whether the rice analogue in the two formulas can reduce blood pressure in Wistar rats, both in normotensive and hypertensive conditions. The rice analogue was prepared with a composition as the formula, followed by chemical content analysis and *in vivo* experiment. In the *in vivo* experiment, rats were divided into two major groups. One was the normotensive group, and the other was the hypertensive group (induced by dexamethasone). Blood pressure measurements were carried out using the CODA® Non-Invasive Blood Pressure tool. Blood pressure measurement data were analyzed using the Wilcoxon Signed-Rank and the Kruskal Wallis Test statistical tests, followed by the Mann-Whitney Test using a 95% confidence level. The results showed that changes in blood pressure, both in normotensive and hypertensive conditions treated with the rice analogue diet, did not differ significantly ( $p > 0.05$ ) when compared to standard feeding. The percentage changes in systolic blood pressure in the normotensive group treated F30 and F31 rice analogue were  $-0.29 \pm 1.5\%$  and  $1.14 \pm 1.8\%$ , respectively, while for diastolic blood pressure were  $-0.43 \pm 1.8\%$  and  $8.3 \pm 8.0\%$ . Whereas in the hypertensive group, the percentage changes in systolic blood pressure in the group treated F30 and F31 rice analogue were  $3 \pm 0.09\%$  and  $6 \pm 0.08\%$ , respectively, while for diastolic blood pressure were  $-2 \pm 0.15\%$  and  $6 \pm 0.15\%$ . Nutritive and bioactive compounds in the rice analogue might have a role in the regulation of blood pressure, however, the amount of the ingredients in the rice analogue with F30 and F31 treated in the rats might not be able to reduce blood pressure both in normotensive and hypertensive rats.

### 1. Introduction

Hypertension is a risk factor for several heart diseases, such as myocardial infarction, left ventricular hypertrophy, congestive heart failure, aneurysms, stroke, chronic kidney disease (hypertensive nephropathy) and hypertensive retinopathy (Sawicka *et al.*, 2011). The global epidemiology of hypertension is increasing in number mainly in low- and middle-income countries. One of the risk factors that can cause hypertension is an

unhealthy lifestyle and food consumption patterns that can lead to other various diseases, such as diabetes mellitus and heart disease (Mills *et al.*, 2020). In addition to consuming medicines, practising a healthy lifestyle for people with hypertension might provide many health benefits. Foods with low fats and glycemic index values can be chosen for diet management. Examples of these foods are corn, cassava, and seaweed (Noviasari *et al.*, 2015; Firdausia *et al.*, 2021).

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Rice analogue is a processed rice product that can be made from a portion or all non-rice ingredients and is prepared using extrusion technology (Mishra *et al.*, 2012; Budijanto *et al.*, 2016). The production of rice analogue in previous studies has been done with a range of raw materials as the ingredients. The rice analogue with a composition of sorghum, white corn and soy flour has been produced by Noviasari *et al.* (2015). Other rice analogues have also been produced and the ingredients were sago flour, corn flour, soy flour, rice bran, and cassava (Wahjuningsih *et al.*, 2020). Rice analogue enriched with bioactive compounds has been formulated to contain phytosterol (Sadek, 2017), black tea extract (Yahya, 2013), tuna protein (Franciska *et al.*, 2015); peas and hanjeli flour (*Coix lacryma-jobi* L.) (Sumardiono *et al.*, 2014) and rice bran (Kusnandar *et al.*, 2021). The rice analogue has been reported to have some bioactivities such as inhibition of colon cancer in Balb/c mice (Sadek, 2017) and hypocholesterolemic effects (Kusnandar *et al.*, 2021).

In this research, the rice analogue in this study is a combination of corn, mocaf, pigeon pea and seaweed. Another research using the same sample (F31) that studied the effect of the F31 diet on TCF7L2 gene expression in rats has been done (Widyaswara *et al.*, 2021). The study reported that the gene which has a role in insulin secretion can increase its expression by the F31 diet. Some bioactive compounds contained in the ingredients of F31 such as scopoletin in mocaf (Buschmann *et al.*, 2000; Armenia *et al.*, 2019; Youchahou *et al.*, 2020); daidzein, genistein, alkaloid and tannin in pigeon pea (Primani *et al.*, 2019; Setyaningrum *et al.*, 2020) and an angiotensin-converting enzyme inhibitory in corn (Guo *et al.*, 2015), are supposed to have a role in lowering blood pressure. Scopoletin (dose 10 mg/kg) has been known to have an activity in lowering pressure on oxidative stress-associated hypertensive rats and the activity may be related to their antioxidant activity (Armenia *et al.*, 2019). Isoflavones in legumes are reported to modulate the renin-angiotensin system (RAS) in spontaneously hypertensive rats (Jeong *et al.*, 2021). The study aimed to determine the blood-lowering effect of the rice analogue (F30 and F31) diet on both normotensive and hypertensive rats.

## 2. Materials and methods

### 2.1 Raw materials and equipment

Rice analogue was prepared from Lebui or pigeon pea flour (*Cajanus cajan* (L.) Millsp), corn, seaweed and mocaf (fermented cassava). All the raw materials were obtained from East Lombok, West Nusa Tenggara, Indonesia. Dexamethasone (PT. Sanbe Farma,

Indonesia), standard feed of Broiler-1 (BR1) obtained from PT. Japfa Comfeed Indonesia, saline solution (PT. Widatra Bhakti, Indonesia), and the male Wistar rats (bodyweight 160-270 g) were obtained from the Laboratory of Pharmacology and Toxicology, Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada.

The equipment of EXTD-01SC single screw extruder was obtained from PT. Barata, Serpong, Indonesia and CODA™ Non-Invasive Blood Pressure was from Kent Scientific.

### 2.2 Animal ethics

Ethical clearance for the experiment has been approved by The Institutional Animal Ethics Committee (IAEC) of Integrated Research and Testing Laboratory, Universitas Gadjah Mada. The numbers of the ethical clearance were 00163/04/LPPT/III/2018 and 00164/04/LPPT/III/2018

### 2.3 Rice analogue preparation

The F30 was made of 57% corn, 40% mocaf, 2% pigeon pea, 1% seaweed and the F31 was made of 21% corn, 71% mocaf, 7% pigeon pea, and 1% seaweed. The composition of the ingredients was weighted and then mixed with 30% water and 0.5% binder (CMC Na (Sodium Carboxymethyl cellulose) of the total mixture. The mixture was steamed for 15 mins to make a pregelatinized dough which was then moulded into rice using the extrusion process (EXTD-01SC single screw extruder machine (Serpong, Tangerang). The rice analogue was then dried (50°C) for 12 hrs until the moisture content reached less than 10%. These methods were performed following Budijanto and Yuliana (2015) with some modifications.

### 2.4 Proximate content analysis

Proximate content analysis conducted were water (thermogravimetry), protein (Kjeldahl), fat (soxhlet), ash (oven), and carbohydrate (by difference, defined as total carbohydrate [g/100 g] = dry matter [g/100 g] - protein [g/100 g] - lipids [g/100 g] - ash [g/100 g]). They were analyzed by following methods of AOAC (AOAC, 2005).

### 2.5 Antihypertensive assay *in vitro*

The experimental study was a simple random sampling design as performed according to a previous study (Nugroho *et al.*, 2013; Harwoko *et al.*, 2014). Wistar rats were divided into two major groups, namely the normotensive and hypertensive animal groups, consisting of 18 and 24 animals, respectively. Furthermore, the normotensive animal group was again

divided into three subgroups (control group fed with BR1, F30 and F31 diet group). The hypertension group was divided into four subgroups; the non-dexamethasone-induced group (normal, fed with BR1), the dexamethasone-induced group fed with BR1, the dexamethasone-induced group fed with F30, and the dexamethasone-induced group fed with F31, with each subgroup consisting of six animals.

### 2.5 The stipulation of dexamethasone dosage and the amount of rice analogue

The dose of dexamethasone used was 0.1 mg/kg BW (Nagaoka et al., 1979). The concentration of dexamethasone solution prepared was 0.05 mg/mL by dissolving the dexamethasone injection at a level of 5 mg/mL in normal saline injection up to 100 mL. The rice analogue given to the mice in this study was 10% of the rat body weight (~20 g).

### 2.6 Preparation of hypertension subjects

Hypertension conditions in rats were carried out by inducing a dexamethasone injection solution (Dubey et al., 2016). The concentration of dexamethasone solution made was 0.05 mg/mL by dissolving dexamethasone injection in normal saline injection. The dexamethasone solution was given subcutaneously for two weeks before giving the rice analogue. Dexamethasone induction causes systolic blood pressure to increase up to 147.4 mmHg or 1.23 times the normal systolic blood pressure in rat test animals.

### 2.7 Monitoring of rats' body weight

Rats' body weight monitoring was done every week, starting from the beginning until the end of treatment. The data on the body weight of rats was obtained to see the effect of dexamethasone induction and the rice analogue treatment on the body weight of rats.

### 2.8 Measurement of food and water consumption

The rats' consumption measurement was carried out every day. Their daily consumption was calculated by reducing the amount of food and water given each day with the remaining food and water (Rumiyati et al., 2016; Wigati et al., 2017). The food provided was as much as 10% of the rat's body weight or approximately 20 g, while water given per day was as much as 80 mL. This measurement was to obtain an overview of the rats' amount of food and water intake, as well as to see the comparisons of food and water consumption of each group. After that, the feeding efficiency was calculated from the food intake data and changes in body weight.

$$\text{Feeding efficiency} = \frac{\% \text{ body weight changes}}{\text{food consumption (\%)}} \times 100\%$$

### 2.9 Testing the effect of the rice analogue diet on rat's blood pressure

Blood pressure measurements used the non-invasive method with the CODA™ Non-Invasive Blood Pressure measuring device. The CODA™ Non-Invasive Blood Pressure device uses a type of volume pressure recording (VPR) sensor technology that can simultaneously measure six parameters, namely systolic blood pressure, diastolic blood pressure, average blood pressure, heart rate, blood volume, and tail blood flow. The parameters to be analyzed in this study are systolic blood pressure (SBP) and diastolic blood pressure (DBP). In the normotensive group, blood pressure was measured four times, particularly at week 0, week 2, and week 4. Whereas in the hypertensive group, blood pressure was observed at week 0, week 2, week 4, and week 6.

### 2.10 Data analysis

The data obtained from the rat's blood pressure measurement was systolic and diastolic blood pressure (mmHg). The average blood pressure before and after treatment, the difference and the percentage change in blood pressure were calculated and analyzed statistically. The percentage change in blood pressure (BP) was calculated based on the following equation:

Difference in BP (mmHg) = BP after treatment - BP before treatment

$$\text{Percentage change in BP} = \frac{\text{BP difference}}{\text{BP before treatment}} \times 100\%$$

The normality and homogeneity analysis of data were used in the Saphiro-Wilk and Lavene's Test. If the requirements for normality and homogeneity of data variance are met, the data is analyzed using parametric tests, using the Paired Sample T Test and One Way Analysis of Variance (ANOVA), followed by the Post Hoc Tukey HSD test. If the requirements for normality and homogeneity are not met, then the data was tested using non-parametric tests, using the Wilcoxon Signed Rank Test and the Kruskal Wallis Test, followed by the Mann-Whitney Test. The statistical confidence level was 95%. The significance of differences was met when  $p < 0.05$ .

## 3. Results and discussion

### 3.1 Monitoring of body weight of normotensive and hypertensive rats

The body weight of normotensive and hypertensive rats was measured every week to evaluate the effect of the rice analogue treatments. The data obtained can be seen in Figures 1 and 2.

As shown in a group of normotensive rats (Figure 1)

there are significant ( $p < 0.05$ ) differences in body weight development between the BR1 fed group (control) and the group treated with the rice analogue diet, both F30 and F31. In the control group, there was an increase in body weight continuously every week, while in the treated group with the F30 and F31 rice analogue diet, the rats experienced an increase in body weight during week one, and then there was a continuous weight loss. This weight loss could be caused by the rats' consumption of rice analogue rice which tended to be less than the normal BR1 fed.

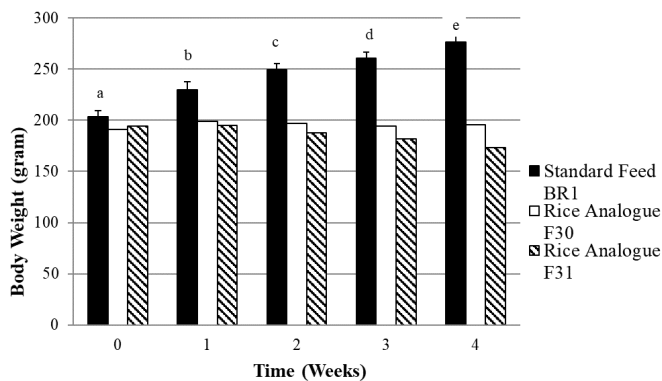


Figure 1. Histogram of body weight (g) of each group of normotensive rats during treatments. Body weight are presented as mean $\pm$ SE. There is a statistically significant ( $p < 0.05$ ) difference in the body weight between the BR1 fed group (control) and the group treated with the rice analogue diet, both F30 and F31. The control group experienced an increase in body weight every week. The treatment group with the F30 and F31 rice analogue experienced an increase in body weight at week one, then subsequently experienced a decrease.

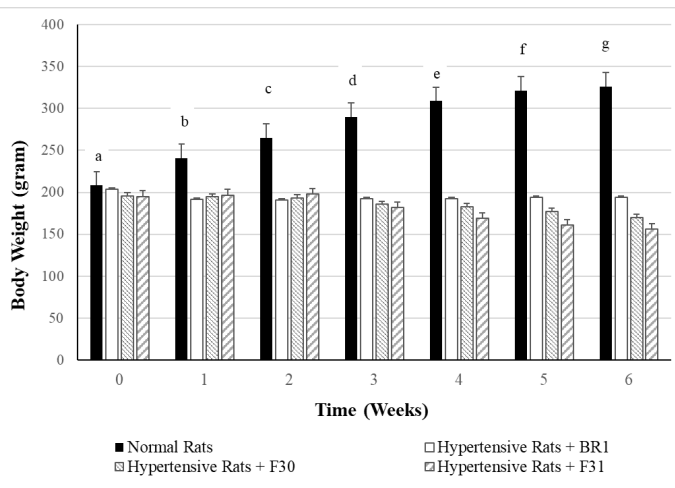


Figure 2. Histogram of the correlation between body weight (g) and treatment time for each group of hypertensive rats. Body weight are presented as mean $\pm$ SE. There is a statistically significant ( $p < 0.05$ ) difference between the body weight of normal rats and rats that had the dexamethasone induction. The normal group experienced an enhancement in body weight every week, and the dexamethasone-induced treatment group experienced a weight loss every week.

Furthermore, in the hypertensive rats' group, there are significant ( $p < 0.05$ ) differences between the body

weight of normal rats and rats that had the dexamethasone induction (hypertensive group) (Figure 2). In the normal group, there was an increase in the rats' body weight every week, starting from week 0 to week 6. Meanwhile, in the dexamethasone-induced group, the average weight loss occurred every week after being induced by dexamethasone. This shows that dexamethasone induction affects rats' body weight. Weight loss in rats due to dexamethasone induction can increase the synthesis and release leptin in adipose tissue (Lee *et al.*, 2007). Plasma leptin levels are known to regulate food intake. Leptin distribution suppresses dietary intake and increases energy expenditure resulting in weight loss in rodents (Pelley *et al.*, 1995; Schwartz *et al.*, 1996). Based on research from Jahng *et al.* (2008) providing dexamethasone repeatedly at a dose of 0.1 mg/kg/day or 1 mg/kg/day can suppress food intake and gain weight.

### 3.2 Measurement of food and water consumption of normotensive and hypertensive rats

The food and water consumption of the rats were also evaluated every day. The daily consumption is calculated by reducing the amount of food and water given each day with the remaining food and water. The handed food was 10% of the rat's body weight or approximately 20 g, while the drink given per day was as much as 80 mL. The purpose of the evaluation of food and water consumption is to obtain an overview of the amount of food and water intake of the rats, as well as to see the ratio of food and water consumption of each group.

Based on Figure 3, in normotensive rats, there are significant ( $p < 0.05$ ) differences in food and water consumption with the control with the rice analogue treatment groups, both F30 and F31. Whereas, in the group of rats that consumed the F30 and F31 rice analogue, there was no significant ( $p > 0.05$ ) difference in food and water consumption between the groups.

The results of the measurement of food and water consumption of the hypertensive rats group can be seen in Figure 4. Based on the statistical analysis, food and water consumption in the non-dexamethasone-induced group was significantly different ( $p < 0.05$ ) from the dexamethasone-induced group. The consumption of food and water in the group given the rice analogue was lower than the group given the standard food (BR1). In the group of rats given the F30 and F31 rice analogue, there was no difference ( $p > 0.05$ ) in food and water consumption between both groups. This is in accordance with the previous studies that dexamethasone can increase the synthesis and release of leptin in adipose tissue, thus suppressing food intake (Lee *et al.*, 2007;

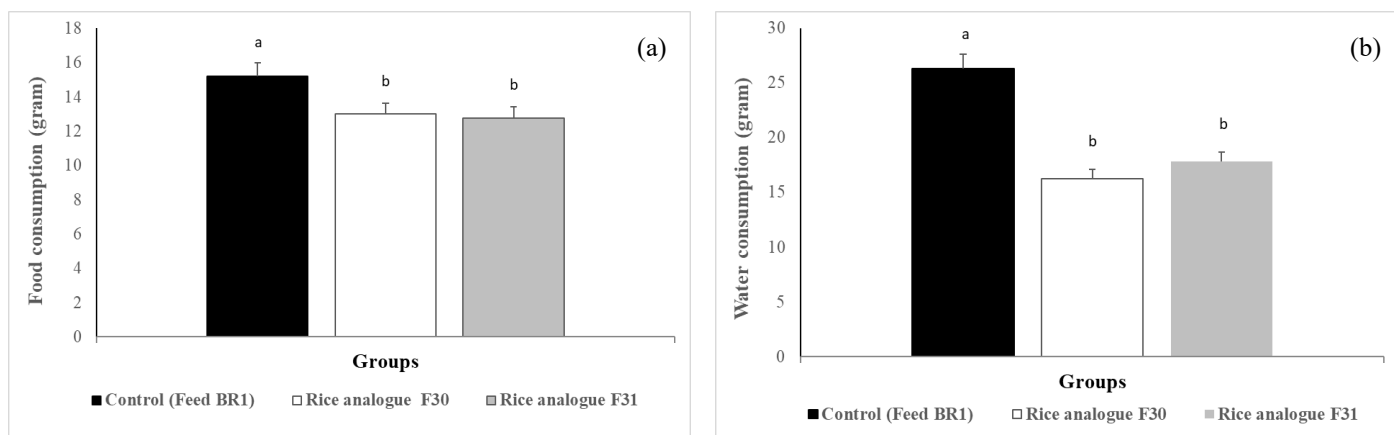


Figure 3. Histogram of the average food (g) and water (mL) consumption of the normotensive rats in all treatment groups. The amount of food (a) and amount to drink (b) are the average amounts of food and water consumption per rat  $\pm$  SE. There is a statistically significant difference ( $p < 0.05$ ) in food and water consumption between the control and the rice analogue treatment groups, both for F30 and F31.

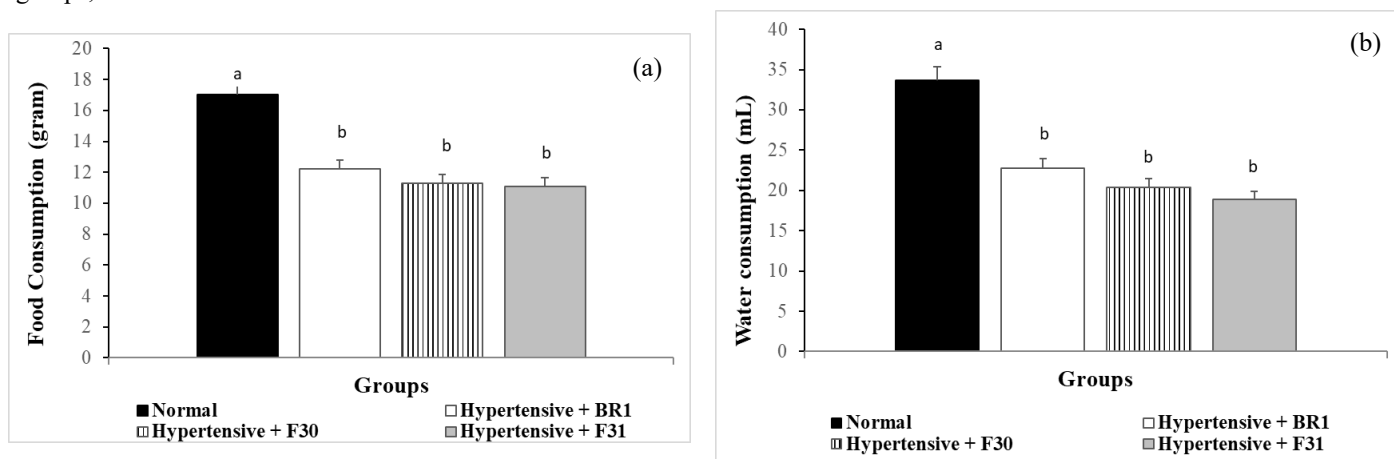


Figure 4. Histograms regarding the amount of food (g) and drink (mL) of the hypertensive rats in all treatment groups. The amount of food (a) and amount to drink (b) are the average amounts of food and water consumption per rat  $\pm$  SE. There is a statistically significant difference ( $p < 0.05$ ) in food and water consumption between the normal and the groups given BR1, F30 and F31.

Pelleymounter *et al.*, 1995; Schwartz *et al.*, 1996; Pico *et al.*, 2022).

### 3.3 The effect of lowering blood pressure in normotensive rats

The mean value of the systolic and diastolic blood pressure during treatment (week 0 to week 4), the difference in blood pressure, and their percentage change for each group are shown in Tables 1 and 2. As shown in the tables, the percentage change in blood pressure that is negative indicates a decrease in blood pressure, while positive values indicate an increase in blood pressure.

Data from Tables 1 and 2, showed that the control group (given BR1) has a systolic blood pressure (SBP) reduction of  $4.15 \pm 4.7$  and a diastolic increase of  $4.0 \pm 3.2$  mmHg, while the group given the F30 rice analogue diet had a decrease in systolic blood pressure of  $0.32 \pm 1.7$  and diastolic blood pressure (DBP) of  $0.43 \pm 1.3$  mmHg. On the other hand, the group treated with the F31 rice analogue diet had an increase in SBP by  $1.18 \pm 1.9$  and DBP of  $4.63 \pm 4.8$  mmHg, respectively. The results of the statistical analysis showed that the control group, the F30

rice analogue diet group, and the F31 rice analogue diet group did not have significant changes ( $p > 0.05$ ) in both SBP and DBP. The percentage change in SBP and DBP between groups was not significantly different ( $p > 0.05$ ).

### 3.4 The effect of lowering blood pressure in dexamethasone-induced hypertensive rats

Tables 3 and 4 show changes in SBP and DBP and their percentage changes for all groups. In the normal group, SBP value at week six was  $104.8 \pm 8.6$  and the DBP was  $72.8 \pm 9.3$ . Therefore, this group was a normotensive group because their blood pressure are still in normal conditions.

The dexamethasone-induced group with the regular feeding (BR1) showed an increase in SBP and DBP at week two after the induction which was  $143.2 \pm 8.8$  and  $97.8 \pm 4.9$ , respectively. In other groups, in the group given F30 and F31 rice analogue, there was a rise in blood pressure at week two as well. The group treated with the F30 rice analogue had an increase in blood pressure with SBP was  $143.4 \pm 3.9$  and DBP was  $101.2$

Table 1. Changes in systolic blood pressure in normotensive rats.

Group	Systolic Blood Pressure (Mean±SE mmHg)				
	Week 0	Week 2	Week 4	Mean of difference in	% change in
Control (BR1 feed)	116.3±9.9	109.9±4.9	112.2±6.5	-4.15±4.7 <sup>a</sup>	-2.39±3.7 <sup>b</sup>
rice analogue F30	107.4±1.2	107.6±3.0	107.1±2.0	-0.32±1.7 <sup>a</sup>	-0.29±1.5 <sup>b</sup>
rice analogue F31	114.3±7.3	107.3±2.3	115.5±7.3	1.18±1.9 <sup>a</sup>	1.14±1.8 <sup>b</sup>

Values are presented as mean±SE. Values with different superscripts within the same column are statistically significantly different ( $p < 0.05$ ).

Table 2. Changes in diastolic blood pressure in normotensive rats.

Group	Diastolic Blood Pressure (Mean±SE mmHg)				
	Week 0	Week 2	Week 4	Mean of difference in	% change in
Control (BR1 feed)	80.0±8.9	78.8±4.6	84.0±8.1	4.0±3.2 <sup>a</sup>	6.1±4.5 <sup>b</sup>
rice analogue F30	73.4±1.8	77.3±4.3	72.9±1.1	-0.43±1.3 <sup>a</sup>	-0.43±1.8 <sup>b</sup>
rice analogue F31	76.6±7.5	71.6±2.2	81.2±6.5	4.63±4.8 <sup>a</sup>	8.3±8.0 <sup>b</sup>

Values are presented as mean±SE. Values with different superscripts within the same column are statistically significantly different ( $p < 0.05$ ).

±9.9. While, in the group treated with the F31, their blood pressure increased with SBP was 147.4±14.1 and DBP was 103.0±12.8. Therefore, the results indicated that in the second week in all treated groups rats were considered to be in a hypertensive condition after induction of the dexamethasone because their systolic blood pressure was more than 120 mmHg (Guyton and Hall, 2016).

After treatment at week six, based on Tables 3 and 4, there was no significant difference ( $p > 0.05$ ) in SBP and DBP in the groups fed the F30 and F31 compared to the group fed with the regular diet (BR1). The group given the F30 had a percentage change in SBP of 3% and DBP of -2%, while the group given the F31 rice analogue had an increase in SBP of 6% and DBP of 6%. The statistical analysis showed that the percentage change in blood pressure in the groups given the rice analogue was not different from the group given the regular food (BR1).

Feeding rice analogue to hypertensive rats could not lower their blood pressure. That might be because the

active compounds contained in the ingredients for producing rice analogue were small. Hence, the levels of active substances in the blood were insufficient to cause an effect to lower blood pressure (Cahyani, 2018; Sani, 2018). The processing of ingredients into the flour and rice analogue can also affect the product compounds. An example is that cassava tubers have scopoletin compounds (10mg/kg) that can promote lower blood pressure (Armenia *et al.*, 2019). However, the scopoletin compounds in mocaf that have been processed through fermentation and flouring processes are yet known for their stability (Frediansyah, 2018). The consumption of rice analogue per day was also less than the optimal amount (10% of the rat body weight (20 g)) which may affect changes in the blood pressure. In addition, the diet time of 28 days might also affect the results. Several studies using the diet method have different periods. Research from Buassi (1998) conducted a high calcium diet in rats for 30 days to see the effect of lowering blood pressure, whereas Bosse *et al.* (2013) conducted a study on a low carbohydrate/high-fat diet in hypertensive rats for ten weeks to see the effect in blood pressure

Table 3. Changes in systolic blood pressure in hypertensive rats.

Group	Systolic blood pressure (Mean±SE mmHg)					
	Week 0	Week 2	Week 4	Week 6	Mean of difference in	% change in
Dexa + BR1	108.9±17.5	143.2±8.8	154.7±19.6	145.8±5.1	2.6±10.6 <sup>a</sup>	2.0±0.007 <sup>b</sup>
Dexa + F30	119.0±26.6	143.4±3.9	151.8±6.9	147.1±10.5	3.7±13.1 <sup>a</sup>	3.0±0.09 <sup>b</sup>
Dexa + F31	109.4±12.0	147.4±14.1	145.0±12.5	155.9±13.7	8.5±10.8 <sup>a</sup>	6.0±0.07 <sup>b</sup>

Values are presented as mean±SE. Values with different superscripts within the same column are statistically significantly different ( $p < 0.05$ ).

Table 4. Changes in diastolic blood pressure in hypertensive rats.

Group	Systolic blood pressure (Mean±SE mmHg)					
	Week 0	Week 2	Week 4	Week 6	Mean of difference in	% change in
Dexa + BR1	76.6±19.8	97.8±4.9	113.7±18.2	102.9±3.6	5.1±7.9 <sup>a</sup>	6.0 ± 0.08 <sup>b</sup>
Dexa + F30	86.8±22.3	101.2±9.9	114.1±6.3	98.6±7.6	-2.6±14.9 <sup>a</sup>	-2.0 ± 0.15 <sup>b</sup>
Dexa + F31	74.2±11.1	103.0±12.8	101.7±11.9	108.4±12.9	5.4±14.8 <sup>a</sup>	6.0 ± 0.15 <sup>b</sup>

Values are presented as mean±SE. Values with different superscripts within the same column are statistically significantly different ( $p < 0.05$ ).

reduction.

### 3.5 Analysis of chemical, nutritive and bioactive compounds in the raw materials of the rice analogue

Analysis of the chemical and nutritive content of the raw materials are included proximate level (water content, ash, fat, protein, and carbohydrate), starch, amylose and amylopectin. That data can be shown in Table 5. Based on the result of the analysis, mocaf has the highest water and carbohydrate content which is 13,69% and 98,35% compared to other materials. Lebui pea had the lowest water content at 10.44% and the highest protein content at 23.81%, while corn had the highest fat content (4.88%).

The highest ash content was in seaweed (20.38%), while the lowest (0.68%) was in mocaf. The highest starch (89.4%) was in mocaf and the lowest in seaweed (20-14%). Seaweed also had the lowest amylose content (0.38%) and amylopectin content (19.76%) while mocaf had the highest amylose (33.36%) and amylopectin content (56.13%).

The result showed that yellow corn, mocaf are a good source of carbohydrate, starch, amylose and amylopectin, Lebui pea is known as a protein source because it has the highest protein content of 23.81% which was in agreement with Fasoyiro *et al.* (2010) that the protein content was 23.33%. Seaweed is a source of ash as much as 20.38% and it is higher than previous research which is 14.81% (Agusman *et al.*, 2014).

In addition to the analysis, evaluation of the bioactive compounds content and their bioactivities in the ingredients has been done by some other researchers. Setyaningrum *et al.* (2020) reported that ethyl acetate extract of the pigeon pea flour (*Cajanus cajan* (L.) Millsp) in a concentration of 10 mg/mL had bioactive compounds such as flavonoid, alkaloid, terpenoid and tannin. The tannin is supposed to be responsible to  $\alpha$ -amylase inhibitory activity by *in vitro* assay. In another research by Aliyah (2018), it has been shown that flavonoids in pea flour have a medium potency as antioxidants in an *in vitro* assay. Other ingredients, scopoletin (10mg/kg) from cassava have also been reported to have antihypertensive on hypertensive rats (Armenia *et al.*, 2019). The bioactive contents in the rice analogue might have a role in hypertension prevention.

Table 5. Chemical and nutritive contents of raw material (% dry basis/db)

No	Sample	Content (% db)							
		Water	Protein	Fat	Ash	Carbohydrate	Starch	Amylose	Amylopectin
1	Yellow corn	11.42	8.51	4.88	1.58	85.03	65.04	28.54	36.5
2	Mocaf	13.69	0.46	0.50	0.68	98.35	89.49	33.36	56.13
3	Lebui (pigeon) pea	10.44	23.81	2.13	4.50	69.56	48.83	18.29	30.54
4	Seaweed	12.70	5.13	0.63	20.38	73.86	20.14	0.38	19.76

## 4. Conclusion

The percentage change in SBP in the normotensive group given the F30 and F31 rice analogue were  $-0.29 \pm 1.5\%$  and  $1.14 \pm 1.8\%$ , while for DBP were  $-0.43 \pm 1.8\%$  and  $8.3 \pm 8.0\%$ . Whereas in the hypertensive group, the percentage change in SBP in the group given the F30 and F31 rice analogue were  $3 \pm 0.09\%$  and  $6 \pm 0.08\%$ , respectively, while for DBP was  $-2 \pm 0.15\%$  and  $6 \pm 0.15\%$ . Changes in blood pressure, both in normotensive and hypertensive conditions of the rats given the rice analogue diet were not significantly different ( $p > 0.05$ ) when compared to standard feeding. To further develop F30 and F31 rice analogue which is effective as an antihypertensive, the amount of ingredients in the formula may need to be evaluated.

## Conflict of interest

The authors declare no conflict of interest.

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