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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Potential of Pigmented Rice Enriched with Kappa-Carrageenan and Anthocyanin Extract For Glycaemic Property Improvements in Diabetic Rats

Nurhidajah[1], Mary Astuti[2], Sardjono[2], Agnes Murdiati[2]
[1]Food Technology Program, Muhammadiyah University of Semarang, Central Java, Indonesia
[2]Faculty of Agricultural Technology, Gadjah Mada University, Yogyakarta, Indonesia

Abstract: The objective of this study was to evaluate the glycaemic property of diabetic rats after consumption of enriched pigmented rice. During the experiment, thirty 2.5-month-old male Wistar rats with 175-250 g body weights were divided into 6 groups (n = 6) and fed Mandel Handayani pigmented rice enriched with 2% kappa-carrageenan and anthocyanin extract from black rice (5 ml/100 g pigmented rice) and standard feed AIN 93 for maintenance. After 5 days of adaptation, diabetes was induce using STZ and nicotinamide and treatment was started 5 days afterwards for 6 weeks. The observed parameters were feed remains and efficiency, body weight, blood glucose level, serum insulin assayed using a rat ELISA insulin kit and Homa IR and Homa β analyses. The results showed that both pigmented rice and kappa-carrageenan and anthocyanin-enriched pigmented rice improved the glycaemic properties of diabetic rats, as indicated by increases in rat body weight of between 10.14 and 11.94%, with feed efficiency ranging between 13.62 and 17.39%, reduced and stabilized blood glucose levels, increased insulin levels, reduced insulin resistance (HOMA IR) and improved pancreatic β cell function (HOMA β).

Key words: Anthocyanin extract, diabetic rats, kappa-carrageenan, pigmented rice

INTRODUCTION

According to the results of basic health research released by the Indonesia Ministry of Health in 2007, 10.2% of 24,417 respondents aged older than 15 years suffered from impaired glucose tolerance (blood glucose level of 140-200 mg/dl after 14 hours fasting and ingesting 75 g glucose). Another 1.5 and 4.2% were predicted to suffer from diagnosed and undiagnosed diabetes mellitus, respectively.

The high prevalence of diabetes mellitus in Indonesia is closely influenced by lifestyle, of which high carbohydrate-fat and low fibre diets are a critical part. Regarding this, rice (and white rice in particular) is the main staple of most Indonesian diets. Pigmented rice has the potential to replace white rice, particularly for diabetic prevention, because of its hypoglycaemic properties due to its anthocyanin content, higher level of fibre and other remaining components after milling. Qi Sun *et al.* (2010) reported that daily white rice consumption might increase type 2 diabetes risk, which is decreased by pigmented rice or replacement with other grains. The American Dietary Guidelines suggest that at least half the carbohydrate intake should come from grain consumption, including pigmented rice, to prevent type 2 diabetes.

Pigmented rice is usually unhulled or partially hulled rice with a red husk; therefore, the cooked rice has a hard or rough texture compared to dehulled rice, which is partly why it is less accepted. Kappa-carrageenan addition to pigmented rice is a method to increase its sensory acceptance, particularly to soften its cooked properties and it also serves as a source of fibre. Kappa-carrageenan is a potential dietary fibre source with 68% fibre content (Hardoko, 2006). It can maintain health due to its water-absorption and glucose-binding abilities, which allow blood glucose reduction. Bazzano (2004) and Astawan (2006) reported that dietary fibre delays postprandial carbohydrate absorption and decreases the insulin response towards carbohydrate intake, thereby lowering the risk of type 2 diabetes. Adequate dietary fibre contributes to carbohydrate-fibre complex formation, leading to reduced carbohydrate digestion that controls the blood glucose level (Santoso, 2011).

Anthocyanin extract added to pigmented rice can enhance the anthocyanin content, which is lost during processing. Kaneto *et al.* (1999) observed that black rice bran contains a bioactive anthocyanin, cyanidin-3-glucoside. This compound can improve hyperglycaemia and insulin resistance and acts as antioxidant to protect pancreatic β cells against reactive oxygen species (ROS). Rahimi *et al.* (2005) reported that oxidative stress in diabetic patients was reduced by anthocyanin, contributing to the prevention of diabetes mellitus complications. The objective of the present study was to evaluate the glycaemic properties of diabetic rats after the consumption of pigmented rice enriched with kappa-carrageenan and anthocyanin extract.

MATERIALS AND METHODS

Design, period and location: An *in vivo* experiment was performed using male Wistar rats and was a randomized post-test-only control group design. The

Corresponding Author: Nurhidajah, Food Technology Program, Muhammadiyah University of Semarang, Central Java, Indonesia

study occurred from March-April 2014 at the Food and Nutrition of Inter-university of Universitas Gadjah Mada Yogyakarta.

Number and experimental animal sampling: Thirty male albino Wistar (*Rattus norvegicus*) rats aged ±2.5 months old and weighing 175-250 g were randomly divided into 6 groups, with one normal control group (healthy rats) and five groups of diabetic rats induced with STZ-Na fed standard feed (DM), pigmented rice (RR), kappa-carrageenan-enriched pigmented rice (RRK), anthocyanin-enriched pigmented rice (RRA), or anthocyanin-kappa-carrageenan-enriched pigmented rice (RRAK) for 6 weeks. Treatment was administered after 5 days each of the pre- and post-adaptation periods.

Materials and instruments: The main material for the study was pigmented rice cv. Mandel from Gunung Kidul. Other materials were standard feed AIN 93, streptozotocin (STZ), nicotinamide, 70% ethanol, a rat ELISA insulin kit, kappa-carrageenan obtained from the Faculty of Natural Science UII Yogyakarta and anthocyanin extracted from black rice cv. Melik from Bantul. Kappa-carrageenan (2%, w/w) and black rice anthocyanin extract (5 ml/100 g pigmented rice, v/w) were added based on previous sensory measurements (Nurhidajah *et al*., 2013).

Instruments used during the experiments were animal welfare maintenance equipment, including individual cages (45 x 35.5 x 14.5 cm), water bottles, analytical balance, aluminium bowls and latex gloves. For biological measurements, disposable suits, 15 ml Falcon tubes, Eppendorf tubes, a centrifuge, a spectrophotometer, a vortex mixer and an ELISA reader were used. The feed composition is presented in Table 1.

Research stages: At the initial stage, albino Wistar rats were divided into six groups of five rats using a simple randomization sampling method. After the rats were weighed, five days of adaptation occurred prior to induction, in which standard feed AIN 93 and water were provided *ad libitum*. Induction using STZ-Na was performed for the DM, RR, RRK, RRA and RRKA groups and the rats underwent 5-day pre-treatment adaptation. The body weight was measured for each rat to ensure that there was no significant decline and that the rats remained in a stable condition.

Data processing and analysis: The results were statistically processed using SPSS Statistics 18 software. The normality of the data distribution was measured using the Kolmogorov-Smirnov and Shapiro-Wilk tests. When data were normally distributed, a one way ANOVA was applied, whereas the Kruskal-Wallis test was applied otherwise.

RESULTS AND DISCUSSION

Body weight gain and feed conversion ratio (FCR): Diabetes mellitus is often characterized by weight loss due to patient inability to acquire energy from glucose catabolism. According to Mayes and Bender (2003), energy is critically required by body cells. Therefore, the body will search for alternative energy supplies when a substrate is unavailable, during which fat breakdown from adipose tissue occurs. Furthermore, glucose generated in the body is discarded via urine when glycogen synthesis is inhibited, significantly reducing muscle and adipose tissue. Diabetes patient show significant weight loss despite increasing appetite (polyphagia) and normal or higher calorie uptake.

Changes in body weight in control group, showed a similiar pattern as changes in feed conversion ratio against body weight as presented in Fig. 2. Feed conversion ratio indicating the relationship between the consumed feed towards body weight gain. Standard feed for normal control group has a lower FCR value than the treatment groups which shows high level of effiency compared to the treatment groups. Diabetic control groups had a negative value of -26.33, indicating high intake of diet that can't be used as energy to increase body weight. In general, the addition of kappa-carrageenan in feed resulted in better efficiency than any other groups (Fig. 2).

Blood glucose level: Streptozotocin, a diabetogenic agent, combined with nicotinamide was used as the induction agent for the hyperglycaemic effect. Matthaei (2000) and Shukla *et al*. (2007) noted that the STZ-Na hyperglycaemic effect results from dephosphorylation of the insulin receptor located in the target cell membrane. Therefore, translocation of GLUT 4 (glucose transporter 4) to the cell membrane is impaired, reducing glucose uptake into the cells, which in turn leads to extracellular hyperglycaemia.

The blood glucose levels of rats fed various types of feed are presented in Fig. 3. The groups treated with pigmented rice and kappa-carrageenan-enriched pigmented rice had lower blood glucose levels similar to normal rats after 6 weeks of intervention. Statistical analysis gave a p = 0.000, indicating the effect of treated feed on blood glucose levels. A subsequent Duncan analysis showed differences among groups, except the normal group and the kappa-carrageenan-enriched pigmented rice (RRK) group and the anthocyanin-kappa-carrageenan-enriched pigmented rice (RRKA) group (Fig. 3).

According to Rahayu *et al*. (2006), carrageenan has a positive role in reducing rat blood glucose levels and can also improve pancreas functions initially damaged due to STZ induction. This was also supported by Hardoko (2006), who observed that kappa-carrageenan can reduce diabetic rat blood glucose levels in a dose-dependent manner.

Table 1: Composition of feed per 100 g according to treatment groups

	Groups					
Ingredients	1. Normal	2. DM	3. RR	4. RRK	5. RRA	6. RRKA
Corn starch	62.5	62.5	20	19.2	62.5	19.2
RR powder	0	0	62.7	0	0	0
RR powder+car	0	0	0	63.9	0	0
RR powder+ant	0	0	0	0	62.7	0
RR powder+k.car+ant	0	0	0	0	0	63.9
Casein	14	140	79.1	77.9	79.1	77.9
Sucrose	10	100	15	15	15	15
Soybean oil	4	40	27.22	26.97	27.22	26.97
Fiber	5	50	0	0	0	0
Mineral mix	3.5	35	3.5	3.5	3.5	3.5
Vitamin mix	1	10	1	1	1	1
L-Cystin	0.18	1.8	0.18	0.18	0.18	0.18
Choline bitartrate	0.25	0.25	0.25	0.25	0.25	0.25
Total (g)	100.4	100.4	99.73	100.02	99.73	100.02
Energy (Kkal)	361.9	361.9	361.4	361.8	361.4	361.8

Normal : Normal control group (-)/without STZ-NA induction, fed with standard feed
DM : Diabetic control (+)/STZ-NA induction, fed with standard feed
RR : Diabetic rats, fed with pigmented rice
RRK : Diabetic rats, fed with pigmented rice+kappa-carrageenan
RRA : Diabetic rats, fed with pigmented rice+anthocyanin extract
RRKA : Diabetic rats, fed with pigmented rice+kappa-carrageenan+anthocyanin

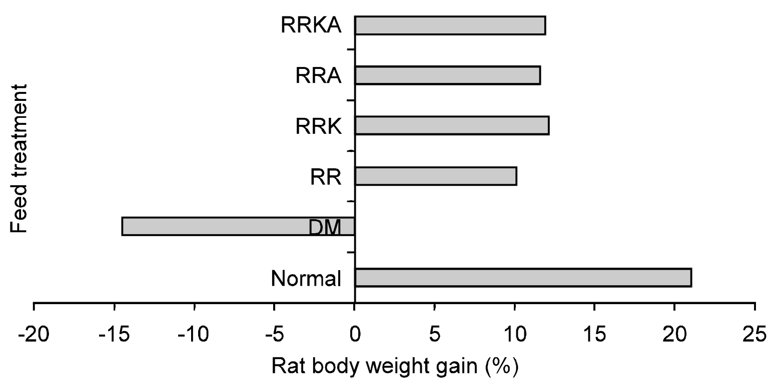

Fig. 1: Changes in rat body weights during treatment

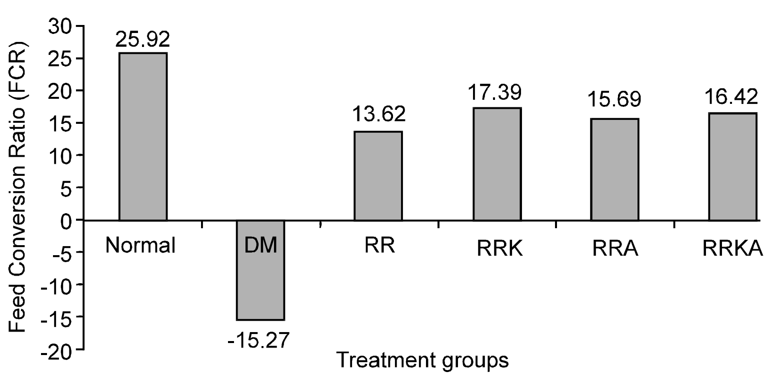

Fig. 2: Feed efficiency ratio versus body weight (%)

Insulin levels: Insulin secretion occurs as a response to increasing concentrations of blood plasma glucose. The insulin level of type 2 diabetes patients is initially higher (hyperinsulinaemia) due to excessive insulin secretion, followed by reduced insulin levels. Subsequent insulin insufficiency is also related to insulin resistance (tissue insensitivity to insulin) in target cells, such as muscle tissue, adipose tissue and hepatic tissue (Mealey and Ocampo, 2007). The insulin levels presented in Fig. 4 show that the diabetic group (DM) had the lowest insulin,

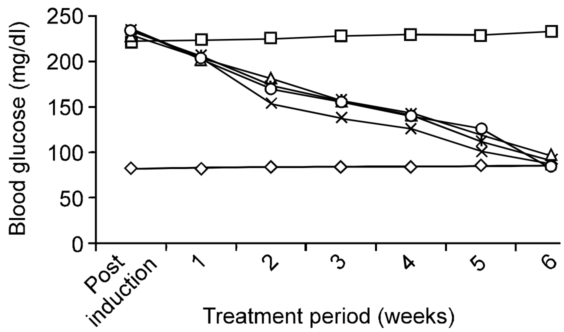

Fig. 3: Average blood glucose level (mg/dl) during 6 weeks of intervention

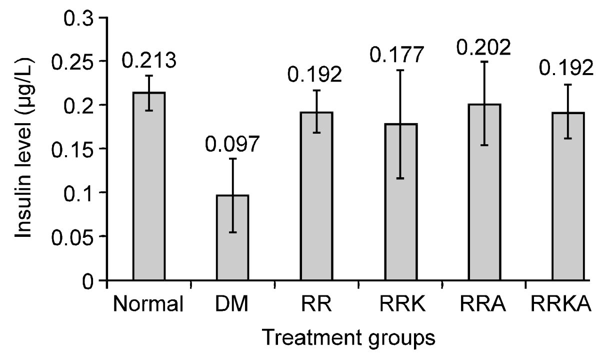

Fig. 4: Average of rats insulin level at various feed treatments

whereas the opposite result was obtained for the pigmented rice enriched with kappa-carrageenan and anthocyanin (RRKA). Statistical analysis showed no significant relationship ($p>0.05$) between the feed and insulin level. This was likely caused by an insufficient intervention period; therefore, the difference in insulin level was not yet apparent. However, it also indicated that consumption of pigmented rice enriched with kappa-carrageenan and anthocyanin extract increased the insulin level by 23% compared to the DM group (Fig. 4).

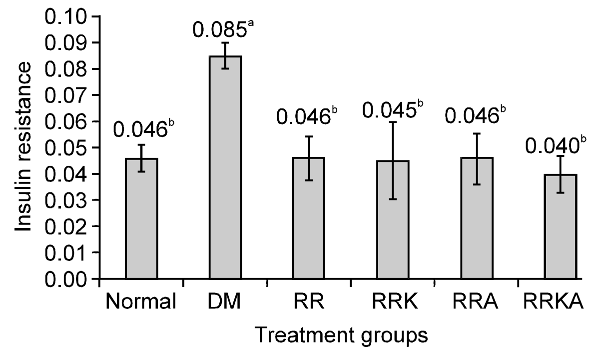

Fig. 5: Average insulin resistance levels (Homa IR) for various feed treatments

Insulin resistance: Insulin resistance is defined as clinical endogenous or exogenous degeneration of insulin function in cellular glucose uptake and utilization. An insulin resistance diagnosis can be obtained through direct or indirect measurements. Homeostasis model assessment (HOMA) analysis is widely used due to its practical and simple application. It can also be used to predict insulin sensitivity using a formula based on the fasting blood glucose and insulin concentration (Meier and Dan Grassner, 2004).

The results showed that the highest Homa IR value was obtained by the DM group compared to the normal group. Rats fed pigmented rice or enriched pigmented rice with either kappa-carrageenan or anthocyanin extract showed Homa IR values close to those of the normal group. This indicated that the insulin resistance level of the treated groups was lower or comparable to that of the normal group (Fig. 5). Sardesai (2003) demonstrated that higher intake of dietary fibre positively affected insulin resistance. Dietary fibre from cereals, nuts and vegetables has been shown to be beneficial for diabetes patients.

Statistical analysis indicated a highly significant effect ($p = 0.000$) between feed intake and insulin resistance. A Duncan post hoc analysis also showed the difference between the DM group and those in the normal group and the group that were fed pigmented rice. Consumption of pigmented rice and enriched pigmented rice reduced insulin resistance from 57.84% to 65.69%. Moharib and El-Batran (2008) observed that nearly all soluble fibre, such as guar gum, pectin, agar, carrageenan and β-glucan, can be rapidly and fully fermented and showed hypoglycaemic and hypolipidaemic properties due to their ability to reduce hepatic insulin resistance and oxidative stress in the endoplasmic reticulum.

HOMA β: The Homa β value is a parameter to measure β cell function strength and a higher value indicates better pancreatic β cell function in insulin production (Fig. 6). In this study, the highest Homa β value was obtained in the anthocyanin-kappa-carrageenan pigmented rice (RRKA) group. The DM group suffered from a reduction of β cell function by 88.36% compared to the normal group and intake of pigmented rice with added kappa-carrageenan and anthocyanin extract improved the function by 88.02%.

Statistical analysis showed a highly significant difference ($p = 0.000$) in the Homa β value caused by feed intake. The Duncan analysis indicated a difference between the DM group and the treated groups, with the results of the RRKA group comparable to those of the normal and RRK groups.

The effect of antioxidants on blood glucose reduction is closely related to oxidative stress prevention and improvement, leading to the inhibition of non-enzymatic

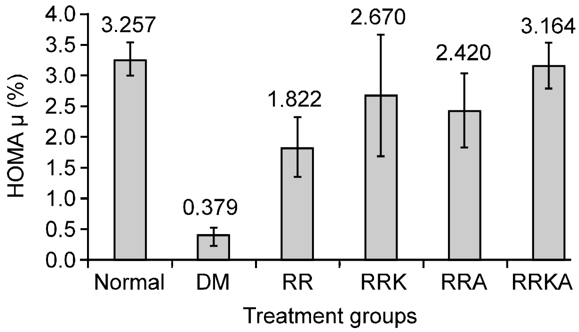

Fig. 6: Average HOMA β values for various feed treatments

glycation of plasma proteins. Moreover, antioxidants also contribute to β cell regeneration. Antioxidant compounds have beneficial effects on pancreatic β cell function in diabetes patients by protecting against oxidation. Therefore, cell dysfunction can be prevented or at least delayed (Kaneto *et al*., 1999).

Conclusion: Pigmented rice enriched with kappa-carrageenan and anthocyanin extract can improve the glycaemic properties of diabetic rats, as shown by an increased ratio of feed efficiency to body weight reduction, stabilized rat blood glucose levels in the normal range, a 23% increase in insulin levels, a 65.69% reduction in insulin resistance (HOMA IR) and an improvement of pancreatic β cell function (HOMA β) by 88.02%. It is concluded that pigmented rice enriched with kappa-carrageenan and anthocyanin extract can improve the glycaemic properties of diabetic rats.

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