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Effects of Dietary Brown Rice on Carcass Composition and Nitric Oxide (NO_x) Metabolite Levels in High-Fat High-Fructose Diet-induced Sprague Dawley Rats as Obesity Model

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Abstract. Obesity is characterized by excessive accumulation of fat in the body, which causes changes in body composition and endothelial dysfunction due to impaired production of nitric oxide (NO). The common means of managing obesity is through functional food, such as brown rice, which has high fiber and antioxidant content. Therefore, the purpose of this study was to determine the effect of adding brown rice to feed on carcass composition and NO_x levels in obese rats. This was an experimental study involving male Sprague Dawley rats placed in 5 groups, in which 4 were allowed to turn to obese rats with the remaining one group as control. These 4 groups were allowed to later feed on high-fat high-fructose (HFHF) diet while the control fed on the normal AIN-93 diet, followed by 8 weeks of HFHF intervention and brown rice in feed with 3 different doses; 12.43%; 24.86%; and 37.29%. The serum NO_x levels were analyzed by the Griess Reaction method, while the percentage of protein and fat was analyzed through Gravimetric Extraction. The results showed that the addition of brown rice resulted in changes in the Lee index ($p = 0.000$) and abdominal circumference ($p = 0.000$). Also, a correlation was observed between the Lee index and abdominal circumference ($p = 0.001$; $r = 0.558$) of rats. However, there was no significant difference between NO_x and carcass protein levels, and there was an increase in carcass fat levels due to an increase in mitochondrial energy efficiency and resistance to protein synthesis in obese rats.

Keywords: Abdominal Circumference, Brown Rice, Carcass Composition, Lee Index, NO_x

1. Introduction

There is sharp rise in the cases of obesity in Indonesia. Based on the 2018 Basic Health Research, the prevalence of obesity in adults was 21.8% which was initially at 14.8% in 2013 [1]. This condition could lead to various health problems, such as coronary heart disease (CHD), gastrointestinal diseases, type 2 diabetes mellitus, joint and muscle diseases, respiratory issues, and psychological problems [2].

Obesity is characterized by excessive accumulation of fat in the body which causes changes in body composition and metabolism, such as endothelial dysfunction. The measurement of body composition is based on the atomic, molecular, cellular, organ and tissue components, as well as the water, protein, and fat contents [3]. Also, one of the recommended methods for analyzing body composition of experimental animals is carcass examination [4].

Endothelial dysfunction due to obesity could occur in blood vessels due to disruption in the production of nitric oxide (NO), in which a decrease in its availability could trigger hypertension, atherosclerosis, and angiogenesis related disorders [5]. This NO is a chemical compound which plays an important role in the regulation of cells and tissues functions, one of which is endothelial derived relaxing factor (EDRF) with an important role in regulating blood pressure [6]. The half-life of NO compounds in the blood is very short, hence, its levels is measured through the results of its metabolites, such as nitrates and nitrites (NOx) [7].

Obesity could be managed through lifestyle changes with a dietary approach with functional food, such as the use of brown rice [8]. This type of rice goes through grinding process without being unpolished, therefore its epidermis or alueron are still intact. Brown rice has a lower glycemic index compared with the white type and contains γ -orizanol bioactive components, vitamins, minerals, and antioxidants in the form of phenolic acids, tocotrienols, and GABA which help in reducing the body's ROS levels [9-11]. Therefore, the purpose of this study was to investigate the effect of adding brown rice to the feed of obese rats on the carcass composition and NOx levels.

2. Methods

2.1 Research Design

This is an in vivo laboratory experimental study on Sprague Dawley rats. It was conducted using a Post Test Only Controlled Group Design while the simple random sampling method was used to select and group the research objects for each treatment. The research was conducted at the Biosciences Institute of Brawijaya University, while the permission to conduct it was granted by the Ethics Committee, Faculty of Medicine, Brawijaya University (No. 210/ EC/KEPK – S3/09/2018).

2.2 Research Subject

The research subjects were made up of 35 male Sprague Dawley rats (*Rattus norvegicus*), aged 8 weeks. These rats were divided into 5 groups based on the number of brown rice substitutions in their feed ingredients mixture, as shown in the following table.

Table 1. Animal Grouping

Group	Treatment
Control	Normal diet (AIN 93)
HFHF	High-fat diet + 30% fructose solution
HFHF + BR I	High-fat diet + brown rice 12.43% of feed + 30% fructose solution
HFHF + BR II	High-fat diet + brown rice 24.86% of feed + 30% fructose solution
HFHF + BR III	High-fat diet + brown rice 37.29% of feed + 30% fructose solution

2.3 Data Collection Technique

The study was conducted for 22 weeks, which involved the formation of obese rats in 4 groups at an initial period of 14 weeks. Then, the intervention was carried out in the remaining 8 weeks, according to the treatment group shown in the table above.

The food intake was measured by weighing and recording the remainder of the feed and fructose solution. Also, anthropometric measurement was carried out by weighing, and measuring the body length, abdominal circumference and carcass composition.

These rats were dissected with ketamine + xylazine anesthetics after the 8 weeks of intervention. Then, the NOx levels of rat serum were analyzed at the Biomedical Central Laboratory, Faculty of Medicine, Brawijaya University with the QuantiChrom™ NO Assay Kit from the Bioassay System (Catalog Number: D2NO-100). Also, about 5 grams of the rat carcass was taken for protein and fat levels testing at the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Brawijaya University using the Gravimetric Extraction method.

2.4 Data Analysis Technique

Data obtained were presented in the form of Mean ± SD. Also, the data on feed intake (g), intake of fiber, protein, fructose calories, and total calories were normally distributed and homogeneous, hence, were subjected to One Way ANOVA to know the difference among the groups. However, data such as fat intake, rat weight, weight gain, abdominal circumference, Lee index, and carcass composition were not normally distributed, hence, analyzed using the Kruskal Wallis test. Then, the data on NOx levels were analyzed using One Way ANOVA after transformation. The Spearman test was conducted to determine the relationship between the abdominal circumference and Lee index, as well as NOx levels and carcass composition. In general, there is a significant difference or correlation when the value of $p < 0.05$. Additionally, the analysis was carried out using IBM SPSS Statistics 26 for Windows.

3. Results

After the 22 weeks, 3 rats died remaining 32 which were still a representative number of subjects needed for this experimental study. Then, the test results showed significant differences among the mean of feed intake, energy and nutrients. This was further subjected to Post Hoc Test, where significant differences among the groups were indicated by the letter notations. Also, there was a significant difference between changes in abdominal circumference and the final lee index. However, there was no significant difference between the NOx level and percentage of carcass protein between groups, while the percentage of carcass fat increased with the intervention of brown rice.

Table 2. Intake Characteristic of Intervention Period

Variable	Group					p value
	Control	HFHF	HFHF + BR I	HFHF + BR II	HFHF + BR III	
Feed intake (g)	13.6 ± 2.8 ^a	11.3 ± 1.2 ^b	10.1 ± 1.3 ^b	8.3 ± 0.9 ^c	7.9 ± 0.7 ^c	0.000*
Energy intake/day (cal)	55.6 ± 11.3 ^a	80.4 ± 7.7 ^{bc}	86.8 ± 7.0 ^b	78.0 ± 5.2 ^{bc}	76.2 ± 5.0 ^c	0.002**
Protein intake/day (g)	3.3 ± 0.6 ^a	2.2 ± 0.2 ^b	2.0 ± 0.2 ^b	1.6 ± 0.2 ^c	1.6 ± 0.1 ^c	0.000**

Fat intake/day (g)	2.9 ± 0.6 ^a	5.8 ± 0.6 ^b	5.3 ± 0.7 ^{bc}	4.4 ± 0.5 ^{cd}	4.2 ± 0.3 ^d	0.000*
Fructose intake/day (cal)	0.0 ± 0.0 ^a	23.0 ± 3.8 ^b	33.9 ± 3.5 ^c	33.7 ± 4.1 ^c	33.7 ± 3.3 ^c	0,000*
Brown rice intake/day (g)	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	1.2 ± 0.1 ^b	2.0 ± 0.2 ^c	2.9 ± 0.2 ^d	0.000**
Fiber intake/day (g)	3.8 ± 0.8 ^a	2.7 ± 0.2 ^b	2.8 ± 0.3 ^{bc}	2.9 ± 0.3 ^{bc}	3.1 ± 0.3 ^c	0.001**

*One Way Anova Test

**Kruskal Wallis Test

A significant difference if $p < 0.05$ Post Hoc Test showed a significant difference between groups ($p < 0.05$) marked by different annotation

Control : Normal-diet group

HFHF : High Fat High Fructose-diet group

HFHF + BR I : High Fat High Fructose-diet group with brown rice substitution 12.43% of feed

HFHF + BR II : High Fat High Fructose-diet group with brown rice substitution 24.86% of feed

HFHF + BR III : High Fat High Fructose-diet group with brown rice substitution 37.29% of feed

Source: [12]

Table 3. Anthropometry Characteristic, NOx Levels, and Carcass Composition

Variable	Group					p value
	Control	HFHF	HFHF + BR I	HFHF + BR II	HFHF + BR III	
Weight gain (g)	7.43 ± 12.03	31.83 ± 29.66	26.90 ± 21.26	20.14 ± 27.65	14.07 ± 11.42	NS*
Abdominal circumference (cm)	15.57 ± 0.53 ^a	17.97 ± 0.71 ^b	17.40 ± 2.10 ^b	17.29 ± 1.19 ^b	17.07 ± 1.02 ^b	0.006**
Change of abdominal circumference (cm)	0.29 ± 0.49 ^a	1.22 ± 0.79 ^c	-0.20 ± 0.57 ^{ab}	-0.64 ± 0.48 ^b	-0.71 ± 0.57 ^b	0.000**
Lee Index	287.42 ± 5.03 ^a	319.93 ± 12.86 ^b	303.91 ± 12.67 ^c	299.41 ± 7.10 ^c	302.47 ± 5.75 ^c	0.000**
Change of Lee Index	1.64 ± 5.46 ^c	4.51 ± 9.57 ^{bc}	-10.77 ± 7.77 ^{ab}	-16.62 ± 8.42 ^a	-17.29 ± 12.39 ^a	0.000*
NOx Levels (µmol)	38.82 ± 11.50	48.77 ± 21.90	51.83 ± 33.43	51.38 ± 21.32	43.59 ± 12.64	NS**
Carcass protein levels (%)	12.90 ± 0.36	12.87 ± 1.04	14.77 ± 3.51	15.63 ± 3.50	15.51 ± 3.45	NS**
Carcass fat levels (%)	2.23 ± 0.13 ^a	2.83 ± 0.67 ^{ab}	2.90 ± 0.22 ^b	2.95 ± 0.55 ^b	3.15 ± 0.72 ^b	0.045**

*One Way Anova Test

**Kruskal Wallis Test

NS: Non Significant

A significant difference if $p < 0.05$

Post Hoc Test showed a significant difference between groups ($p < 0.05$) marked by different annotation

Control : Normal-diet group

HFHF : High Fat High Fructose-diet group

HFHF + BR I : High Fat High Fructose-diet group with brown rice substitution 12.43% of feed

HFHF + BR II : High Fat High Fructose-diet group with brown rice substitution 24.86% of feed

HFHF + BR III : High Fat High Fructose-diet group with brown rice substitution 37.29% of feed

The Spearman test results showed a correlation between the final abdominal circumference and final Lee index in the rats ($p = 0.001$; $r = 0.558$), where the low of the rats' abdominal circumference is directly proportional to the low of Lee index. In addition, there was a trend of the relationship between NOx levels and Lee index Lee ($p = 0.065$; $r = 0.330$). However, there was no correlation between carcass protein levels and the Lee index in this study ($p = 0.379$). Similarly, the Pearson test showed no correlation between the carcass fat levels and Lee index ($p = 0.437$).

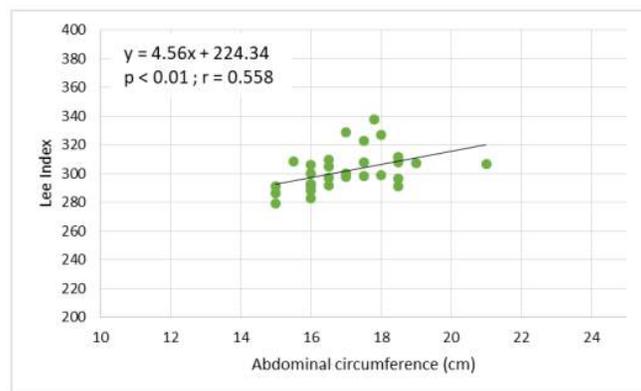


Figure 1. The correlation between abdominal circumference and Lee Index

4. Discussion

4.1 Intervention Period Intake Characteristics

The food intake in rats fed with HFHF diet was lower compared with the rats on normal diet, but this resulted in higher total energy and fat due to the high fructose intake. The provision of fructose solutions changed the food consumption patterns through appetite suppression, where fructose content could interfere neuropeptides ghrelin and leptin production. Additionally, leptin secreted by white adipose tissue usually result in a decrease in energy usage thereby increasing the body weight and fat storage in rats [13, 14].

Also, the amount of fiber intake in both the normal group and the HFHF with brown rice was higher than the HFHF group alone. The fiber source in the treatment group, especially those derived from brown rice, have the capacity of increasing the viscosity of the food consumed, therefore, taking longer time in either the mechanical process or chemical digestion in GIT [15, 16]. Furthermore, the fibers in brown rice were fermented in the colon to produce SCFA which could increase the energy absorption effectiveness and as L-cell receptor secreted PYY and GLP-1, the hormone suppressed appetite and food intake [16].

4.2 Anthropometric Characteristics

The body weight increase from the highest were HFHF, HFHF + BR I, HFHF + BR II, HFHF + BR III, and the normal group, although not significantly different. Also, the HFHF group had the highest fat and energy intake compared with other groups, where the high fat consumed was not oxidized to full energy, but stored in the adipose tissue [17]. Then, the increase in the weight loss from the brown rice intervention group became smaller with higher levels of brown rice. In general, brown rice has a dietary fiber content of 22.04 g / 100 g, including 4.9% beta glucan content, and fiber consumption is known to reduce food intake, thereby controlling weight gain [17-20].

The results of this study showed a correlation between the abdominal circumference and Lee index in rats. The higher the abdominal circumference size, the higher the value of Lee index. When the rats experienced weight loss, there was a decrease in the Lee index value and a decrease in visceral fat reserves marked by a diminished size of the abdominal circumference [21]. Greater changes in the Lee index were observed with increase in the consumption of brown rice, meaning that the fiber intake was also higher in the rats. Also, the decrease in the abdominal circumference and Lee index occurred due to the brown rice contents, namely vitamin E, phytosterol, phenol, triclin, and γ -oryzanol which could improve the hypothalamus function in controlling food intake [22-24]. The decrease in Lee index in the brown rice intervention group occurred due to the presence of ferulic acid. According to Wang *et al* (2018), brown rice reduces the storage body fat and increase protein accumulation [25].

4.3 NOx Levels

The NOx levels in the normal group were lower compared with rats with HFHF, although not significantly different. This result is in line with the research conducted by Fujita *et al* (2011) that NOx levels increased in subjects with obesity, and NOx levels were positively correlated with subject abdominal circumference [26]. However, another study showed that obesity reduced the NO synthase enzyme expression in rats and humans, thereby causing a decrease in the synthesis of NO, therefore triggering hypertension [6].

The highest increase in the abdominal circumference was recorded in the HFHF group and the lowest in the group given brown rice. These results also showed that the Body Fat Index (BFI) and White Adipose Tissue (WAT) of rats in HFHF group were higher compared with the normal group [12]. In addition, there was a decrease in BFI and WAT as the dose of brown rice increased. Also, the adipose tissue, especially the visceral type releases NO synthase and produces NO due to obesity-related hormones stimulation such as insulin, leptin, and angiotensin II. This increase in NO production by visceral fat contributes significantly to increase serum NO and NO metabolites (NOx) levels in obese subjects [26].

There is in addition an uncoupling eNOS event, as well as increase in asymmetric dimethylarginine (ADMA), an NO endogenous inhibitor, where eNOS which is supposed to produce NO now produces superoxide. This superoxide further converts NO into peroxynitrite, then the isomerization of peroxynitrite becomes nitrate and nitrite (NO_x) [27].

Based on the results of this study, a decrease in the levels of NO (NO_x) metabolites was observed first in the HFHF + BR III group, which is the group with the highest consumption of brown rice, although with no significant difference. This was due to the fact that the ferulic and fumaric acid antioxidant activity in brown rice are still insufficient to improve the NO levels in the body. Antioxidants play a vital role of binding ROS, thereby increasing the eNOS expression, as well as its activity and function in preventing the uncoupling eNOS reaction, and then the NO levels in the body back to normal [11, 28, 29].

4.4 Carcass Composition

There was no significant difference in the protein content in the carcass of the experimental rats, which is in line with some existing studies which stated that there was no change in carcass protein of obese rats [30]. However, an increase in the fat and protein carcass levels were observed in rats on the HFHF diet with brown rice. Changes in the level of protein in the rats carcasses were influenced by the obese rats feeding on high protein feed [31]. Leaving the epidermis intact in brown rice makes it to still contain a high protein content, of about 8.4% compared with rice without epidermis [20, 32]. Furthermore, brown rice contains lysine and other amino acids which are higher, compared with other types of rice. Protein and amino acids combination, especially lysine, causes energy absorption decrease in the muscle resulting body weight decrease and an increase in protein levels of the carcass [31-33].

Generally, protein levels in animals muscles (fish, chickens, bulls, cats, dogs, rabbits taken randomly without any intervention) and humans have muscles protein value in the region of $\pm 16\%$ [34]. Then, the highest protein value in carcasses of rats given brown rice approached that value, which was 15.3%. In this study, the brown rice provided was not effectively enough to increase the level of carcass protein because the rats were already in the obese state before the intervention, which resist the anabolism of muscle protein compared with normal weight conditions. It is associated with fat accumulation in the muscle, as well as systemic inflammation in obese condition [35].

This study showed fat content in rat carcasses given the HFHF diet was higher than rats given normal diet. The fat in the normal and HFHF groups differed from HFHF group intervened by brown rice (HFHF + BR I, HFHF + BR II, and HFHF + BR III). These results are in line with previous studies using DXA scans showing that the body fat percentage in rats with 25% feed fat levels was higher than rats with 15% feed fat levels [36].

In addition, long term high fat diets could also increase triglyceride fat levels in muscle tissue, especially in the gastrocnemius part. In obese rats, there is impaired adiponectin function or adiponectin resistance, with an increase in AdipoR1 protein levels and a decrease in the p-AMPK / AMPK ratio. This regulatory disturbance triggers changes in fatty acid metabolism, thereby causing increase in fat accumulation in carcasses or skeletal muscle tissue [37]. Moreover, the HFHF and HFHF + BR groups also consumed 30% fructose solution thereby contributing to high energy intake despite lower feed weight. The study results also showed that giving feed high in fat and fructose without hyperphagia in the rats, increased the fat level in muscle tissue [38].

This is caused as a result in the mitochondrial energy efficiency, with a decrease in the substrates number producing ATP, while an increase in NEFA and triglyceride levels resulted in an increase in lipid substrates flow in muscle tissue. Fat accumulation in muscle tissue further triggers insulin resistance [38, 39]. Then, apart from diets combination such as the functional foods use, deeper consideration towards intervention in cases of obesity, especially in relation to carcass and body composition, requires physical activity strategies to improve muscle protein synthesis and body anabolism signaling [35].

Furthermore, this study showed no correlation between percentage of carcass fat and Lee index. The percentage of carcass fat was analyzed from muscle tissue, while the Lee index count rat weight, including fat content normally stored in the visceral and subcutaneous sections. Visceral and subcutaneous fat is found at the bottom skin and could be seen directly by pinching or using a skinfold caliper [10, 40]. Experimental carcass fat measurement differ from the BFI, which is often associated with BMI in humans, or the Lee index in experimental animals. According to Akindele (2016), BFI in the population studied was directly proportional to BMI [41]. This is in line with Sulistyowati's research in 2020, which showed that the value of BFI is directly proportional to the Lee index of experimental animals, and the lower the BFI, the higher the Lee index in the experimental animals [12].

5. Conclusion

The addition of brown rice to the feed resulted in changes to the carcass fat composition, Lee index and rat abdominal circumference. Also, there was a correlation between the Lee index and abdominal circumference in obese rats, but no significant difference between the levels of NO_x and carcass protein composition due to increased mitochondrial efficiency and the presence of protein synthesis resistance in obese rats. However, there is need for further analysis on effective doses of brown rice needed to improve the antioxidant status and the synthesis of muscle protein.

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