Improvement of antioxidant status on adults obesity after intervention antioxidant drinks

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Abstract. Obesity will increase the risk of oxidative stress that can lead to metabolic syndrome. Antioxidant foods have known benefits in improving the antioxidant activity and inhibiting oxidation reaction in the body. Tomato and rice bran are functional foods containing high antioxidant compounds specifically lycopene in tomato and oryzanol in rice bran. The purpose of study was to analyze the effect of the drinks of tomato extract, rice bran powder and rice bran oil emulsion to antioxidant activity. The design of this study used was quasi-experimental study with two groups pre and posttest. The 26 subjects were divided into 2 groups, each group has 13 subjects with purposive sampling. Intervention conducted for 28 days that each group was given 2 cups @220 ml tomato extract per day for 14 days and then continue as group A given 2 sachets (30 g) of flavored-rice bran powder and as group B given 2 cups @ 220 ml rice bran oil emulsion drink during 14 days. The analysis of activity antioxidant used was MDA and DPPH methods. The results of paired t test analyzed showed that intervention could decrease MDA levels significantly in group B (p = 0.03) and AEAC in group A and B (p = 0.00). Intervention products are improve level of antioxidant subjects.

1. Introduction
Obesity is a health and nutrition issue in both developed and developing countries. The prevalence of obesity in Indonesia increases from year to year. The prevalence of obesity based on Indonesian Basic Health Research (Riskesdas) data in 2007 (19.1%), 2010 (21.7%), and 2013 (26.3%) [1]. Obesity is a condition of excess fat accumulated in adipose tissue [2]. It can be indicated by using Body Mass Index (BMI). Individual with BMI ≥25.0- <27.0 kg / m² is categorized as overweight, while BMI ≥27.0 kg / m² is categories as obese [1].

Obese individual has a higher risk for type 2 diabetes mellitus, cardiovascular, hypertensive, dyslipidemia and cancer [3]. It also will increase the incidence of oxidative stress due to the imbalance number of oxidants and antioxidants, which consequently causes unpaired mitochondria and β oxidation, causing increased species of reactive oxygen. This will cause dysregulation of adipose tissue, i.e. increased production of adipocytokines and decreased adiponectin which lead to metabolic syndrome development [4].

Obesity is associated with an increase in lipid peroxidation, such as malondialdehyde (MDA). MDA is one of the parameters used to measure oxidative stress caused by free radicals. In individuals with obesity, the mean MDA was significantly higher than non obese [5]. High MDA concentrations indicate the presence of oxidation processes in cell membranes. In the case of oxidative stress, the ability to reduce normal oxidation is impaired and causes tissue oxidative damage [6].
Functional drinks that contain high antioxidant activity, such as tomatoes and rice bran, can improve the oxidative status. Tomato contains bioactive compounds in the form of lycopene, whereas in rice bran has tocopherol (vitamin E), tokoctrienol, oryzanol and pangamic acid [7]. Previous studies reveal that antioxidant compounds in tomatoes namely lycopene proved to increase blood plasma antioxidant levels [8].

Research conducted by Bub et al, in 2000 showed 40 mg lycopene has proven to have an effect on increasing blood plasma antioxidant. According to the USDA-NCC Carotenoid Database for US Foods, in a glass of tomato juice (240 mL) contains about 22.9 mg of lycopene [9].

A study conducted by Damayanthi et al in 2011 showed that the preserved rice bran is capable on inhibiting the proliferation of sustainable cancer cells, shrinking the size of breast cyst lesions, inhibiting modified LDL, and lowering total serum cholesterol levels. The amount of antioxidant activity on tomato juice and rice bran is 60.74% and 83.89% respectively. In other words, in 100 g of rice bran, the reduction ability (AEAC) is equal to 28.74 times while in tomato is equal to 1.87 times [10]. This research aimed to analyze the effect of tomato juice drink, rice bran powder drink, and rice bran emulsion oil drink to antioxidant activity in obese adults.

2. Method
The research design used quasi experimental two pre and post test groups. The research was conducted at the Bogor Agricultural Institute (IPB) Dramaga from July 2013 to September 2014. The study population is an employee of IPB drawn by purposive sampling technique (n = 26). The inclusion criteria to be met by the subjects include obesity category 1 and 2 (having IMT ≥ 25[^1^], aged 18-60 years, not undergoing treatment from a physician, not getting a similar antioxidant intervention, non-pregnancy or breast-feeding, not outside the city during intervention, willing / adherent to consume intervention products, and willing to fill informed consent.

Intervention was conducted for four weeks (28 days), subjects were divided into 2 groups (n=13). Group A (tomato-bran) was given 2 cup drinks per day @ 220 ml in the first two weeks then continued beverage powder bekatul two sachets per day @ 15 g during the next two weeks without any lag time. Group B (tomato-bran oil) is given tomato juice drink and bran emulsion oil beverage as much as 2 cup per day @ 220 ml by following the same time pattern.

The socio-economy characteristics data were collected at the beginning of the study with interviews using questionnaires. Body height data were collected once at the beginning of the study using microtoise, while body weight data were collected using a calibrated digital scales at three time points: during research screening, before intervention, and after intervention. Compliance was monitored and data were collected twice a week with interviews using questionnaires. Blood samples were collected at three time points: (1) start of study, week 0 before intervention, (2) second week after tomato intervention, (3) week 4 after intervention of bran powder drink and rice bran.

Blood serum samples used to analyze the antioxidant activity with MDA and DPPH method, which was done in Community Nutrition laboratory of IPB. Data analysis includes univariate and bivariate. Unpaired t-test is used to indicate the diversity of data between groups and paired t-test to determine the effect before and after intervention. Data were presented in tables and descriptive statistics.

3. Result
Subjects are consisted of 12 males and 14 females with 69.2% are at age range of 41–60 years. Age becomes one important factor that must be considered to expect similar metabolic processes in general, although there are variations in each individual. Most of subjects in group B (77%) have low level of education (elementary to high school graduate), while group A have higher level of education (Diplome or Undergraduate). As for income, 38.5% of both groups fell into category 0 - 4,000,000. Data is shown completely in table 1 below.
Table 1. Subject characteristics.

| Characteristics          | Group A | Group B | Total | Result of statistic test |
|--------------------------|---------|---------|-------|--------------------------|
|                         | n  | %     | N   | %     | N   | %     |
| Sex                      |    |       |     |       |     |       |
| Male                     | 7  | 53.8  | 5   | 38.5  | 12  | 46.1  | 0.452|
| Female                   | 6  | 46.2  | 8   | 61.5  | 14  | 53.9  |
| Total                    | 13 | 100   | 13  | 100   | 26  | 100   |
| Age                      |    |       |     |       |     |       |
| Young Adult (20-40)      | 4  | 30.8  | 4   | 30.8  | 8   | 30.8  | 0.801|
| Adult (41-60)            | 9  | 69.2  | 9   | 69.2  | 18  | 69.2  |
| Total                    | 13 | 100   | 13  | 100   | 26  | 100   |
| Education level          |    |       |     |       |     |       |
| Elementary-High School   | 7  | 53.8  | 10  | 77.0  | 17  | 65.4  | 0.156|
| Diploma - Undergraduate  | 6  | 46.2  | 3   | 23.0  | 9   | 34.6  |
| Total                    | 13 | 100   | 13  | 100   | 26  | 100   |
| Occupation               |    |       |     |       |     |       |
| Janitor                  | 1  | 7.7   | 6   | 46.2  | 7   | 26.9  | 0.007*|
| Education Staff          | 9  | 69.2  | 7   | 53.8  | 16  | 61.5  |
| Lecturer                 | 3  | 23.1  | 0   | 0     | 3   | 11.6  |
| Total                    | 13 | 100   | 13  | 100   | 26  | 100   |
| Income                   |    |       |     |       |     |       |
| 0 - Rp. 2.000.000        | 5  | 38.5  | 5   | 38.5  | 10  | 38.5  | 0.813|
| >Rp. 2.000.000 – Rp 4.000.000 | 5  | 38.5  | 5   | 38.5  | 10  | 38.5  |
| >Rp. 4.000.000 – Rp. 5.000.000 | 0  | 0     | 2   | 15.4  | 2   | 7.6   |
| >Rp. 5.000.000           | 3  | 23.0  | 1   | 7.6   | 4   | 15.4  |
| Total                    | 13 | 100   | 13  | 100   | 26  | 100   |
| BMI Category             |    |       |     |       |     |       |
| Overweight               | 3  | 23.1  | 3   | 23.1  | 6   | 23.1  | 1     |
| Obesity                  | 10 | 76.9  | 10  | 76.9  | 20  | 76.9  |
| Total                    | 13 | 100   | 13  | 100   | 26  | 100   |

Both group has similar percentage of overweight (23.1%) and obese (76.9%) This indicates that the metabolic variation between groups is not much different. Statistical analysis showed that there are no difference in gender, age, education, income and BMI. Therefore the characteristic of subject in each group is not much different.

The intervention compliance of tomato juice consumption for group A and B showed almost the same values 95.3% and 95.8% respectively. While for rice bran consumption, group A was higher (96.4%) than group B (88.4). However, there was no statistically difference between the two groups.
Table 2. The intervention complianceness of product intervention.

| Intervention Product       | Group A (%) | Group B (%) |
|----------------------------|-------------|-------------|
| Tomato juice               | 95.3        | 95.8        |
| Rice bran                  | 88.4        |             |
| Rice brain oil emulsion    |             | 96.4        |

Based on energy adequacy level, the average of group A is about 76% (medium-level deficit) and group B about 90% (enough level). The protein adequacy rate is significantly different, in which group A categorized as moderate deficiency level while group B categorized as excessive level. The average fat consumption in group B was slightly higher with 23% of RDA than group A with 21% od RDA (table 3).

Table 3. Adequacy level of energy and other nutrient.

| Nutrient Intake   | Group A       | Adequacy level (%) | Group B       | Adequacy level (%) | Result of statistic test |
|-------------------|---------------|--------------------|---------------|--------------------|----------------------------|
| Energy (kkal)     | 1746 ± 472    | 76                 | 2043 ± 718    | 90                 | 0.226                      |
| Carbohydrate (g)  | 398 ± 322     | 68                 | 357 ± 178     | 63                 | 0.626                      |
| Protein (g)       | 49 ± 22       | 79                 | 73 ± 33       | 121                | 0.038*                     |
| Fat (g)           | 53 ± 23       | 21                 | 58 ± 25       | 23                 | 0.599                      |

The result of unpaired t test of serum MDA level at week 0 (before intervention) showed no difference between group A and B (p> 0.05). Based on the percentage of decrease of MDA level, group B is bigger (25.3%) compared to group A (8.7%). Changes in MDA levels in the interval period showed that both intervention products proved to lower serum MDA levels in different intensities. The result of paired t test showed only in group B differ significantly (p <0.05) before and after the intervention (table 4). When we tested the antioxidant content in blood serum using DPPH method, there was no statistically different between group (p=0.40). However, change in Ascorbic Acid Equivalent Antioxidant Capacity (AEAC) activity levels before and after intervention showed a statistically significance (p <0.05) (table 5).

Table 4. Average MDA concentration (µmol/L).

| Week | Group A       | Group B       | Result of statistic test |
|------|---------------|---------------|----------------------------|
| 0    | 4.97±2.43     | 5.33±2.30     | 0.92                       |
| 2    | 4.84±1.90     | 4.85±1.82     | 0.69                       |
| 4    | 4.54±2.15     | 3.98±1.63     | 0.16                       |
| Result of statistic test | 0.347 | 0.031* |                     |
| Delta | 0.4          | 1.35          |                           |

Table 5. Average antioxidant activity AEAC (mg/mL).

| Week | Group A AEAC | Group B AEAC | Result of statistic test |
|------|--------------|--------------|----------------------------|
| 0    | 0.70±0.22    | 0.60±0.22    | 0.40                       |
| 2    | 0.74±0.22    | 0.66±0.22    | 0.93                       |
| 4    | 0.92±0.19    | 0.86±0.22    | 0.70                       |
| Result of statistic test | 0.00* | 0.00* |                     |
| Delta | 0.22          | 0.26          |                           |

* AEAC = Ascorbic Acid Equivalent Antioxidant Capacity
4. Discussion
Statistical analysis on subject characteristics showed no difference between groups, thus it does not become a confounding factor in the study. According to the subjects, they do not taste bitterness from rice bran emulsion oil beverage. It also found that the addition of artificial flavoring and sweetener are very helpful in increasing product acceptance. In other hand, the subjects who were given bran powder drink expressed bitterness from the drink which makes somewhat less comfortable to consume. The taste difference between rice bran emulsion drink and rice bran powder drink explains why the intervention compliance is different. It showed no significant difference (p <0.05). Overall, the percentage of all product compliance in the study was above 85%

Free radicals and oxidants play dual roles both as toxic and beneficial compounds because they can be a hazard or a benefit to the body. When over-produced, free radicals and oxidants produce a phenomenon called oxidative stress that damages cell membranes and other structures such as proteins, lipids, lipoproteins, and deoxyribonucleic acids (DNA). Oxidative stress can arise when cells unable to destroy the excess free radicals that are formed. This reaction leads to the formation of MDA and cytotoxic and mutagenic conjugated diene compounds [11]. The high levels of free radicals in the body can be shown by low antioxidant enzyme activity and high levels of MDA in plasma [6].

The decrease in MDA levels in group B (1.35) was greater than that of group A (0.4). This difference was made possible by the difference in compliance of the intervention product consumption, in addition to bioavailability of γ-oryzanol increased 200 times in the form of emulsions [12]. This proves that the decrease in MDA levels is due to product intervention. Previous study showed that consumption of 5–20 mg of lycopene from ketchup for 2 weeks can lower MDA levels by 10% [13]. The lycopene mechanism of action as an antioxidant is by capturing reactive oxygen species, increasing the overall antioxidant potential, thus reducing oxidative damage to lipids, proteins and DNA. All of these things will reduce oxidative stress. According to Amirkhizi, plasma MDA levels in normal adults are 1.4 ± 0.5 μmol / L [14].

Previous study by Damayanthi states that consumption of γ-oryzanol 31.45 mg/day can inhibit modified LDL [10]. The active components of bran are oryzanol, tocopherol and tocotrienol which present in the oil fraction [15]. These compounds prevent or inhibit LDL oxidation, by capturing free radicals during the propagation stage by donating hydrogen [16]. A study conducted by Faigayanti, stated that consumption of rice bran emulsion beverage drink for 15 days managed to reduce plasma MDA levels from 4.0 ± 1.2 μmol / L to 3.8 ± 1.3 μmol / L, but it is not statistically significant [17]. The γ-oryzanol component comprises a ferulic acid structure, the three main components consisting of the most influential 24-methylene cycroartanyl ferulate, cycroartanyl ferulate and campasteryl ferulate in increasing the antioxidant activity in bran oil. The three constituent components are 4 times more effective than the vitamin E component [15]. Things that can affect MDA levels include body antioxidant status, stress levels, physical activity, consumption and exposure to pollution [11].

Antioxidant activity is the ability of a substance that contains antioxidants in reducing free radical compounds. DPPH is a stable free radical compound that can be used as a determinant of total antioxidant activity, which expressed as AEAC (Ascorbic acid equivalent antioxidant capacity) using vitamin C as the standard. Factors that have an effect on total antioxidant activity are free radical levels in the body and foods that contain antioxidants [18]. A study conducted by Arza in 2010 showed that drinking 480 ml of tomato juice a day for 14 days, increased antioxidant activity by 11.8% from 0.810 mg / 100mL before the intervention to 0.906 mg / 100mL [19]. Cooked tomatoes with ripe red skin color has a lycopene content of 4600 μg / 100g. Abdurohman (2013) states that in 100 g of bran powder has oryzanol content of 330 mg / 100 g which can reduce DPPH free radical equivalent to 163.56 mg vitamin C [20]. Another research jelly drink made from green okra and strawberry, in 100 grams jelly has radical scavenging ability 1.056 times as vitamin C lower than rice bran drink [21].
5. Conclusion and Recommendation
The products of the intervention, equally improve the antioxidant activity of the subjects. Group B showed a significant decrease of MDA level before and after intervention (p = 0.03), but when using the DPPH method, the two groups are both significantly different for increasing the antioxidant activity (p = 0.00). Tomato and rice bran products intervention needs to be developed both in terms of products and education to the community considering the benefits for health.

References
[1] Balitbangkes 2013 Riset Kesehatan Dasar 2013 (Jakarta: Kementerian Kesehatan Republik Indonesia)
[2] WHO 2000 Preventing and managing the global epidemic. Report of WHO consultation (Switzerland: WHO)
[3] WHO. World Health Organization Fact Sheet for World Wide Prevalence of Obesity. Available online: http://www.who.int/mediacentre/factsheets/fs311/en/ (accessed on 1 November 2017)
[4] Furukawa S, Fujita T, Shimabukuro M, Masanori I, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I 2004 Increased oxidative stress in obesity and its impact on metabolic syndrome Journal of Clinical Investigation. 114 pp 1752–61
[5] Yesilburs D, Serdar Z, Serdar A, Sarac M, Coskun S, Jale C 2005 Lipid peroxides in obese patients and effect of weight loss with orlistat on lipid peroxides levels International Journal of Obesity. 29 pp 142–45
[6] Halliwell B 2006 Reactive Species and Antioxidants. Redox Biology Is a Fundamental Theme of Aerobic Life Plant Physiology. 141 pp 312–22
[7] Damayanti E, Muchtadi D, Zakaria F R, Syarief H, Wijaya C H, Damardjati D S Aktivitas antioksidan minyak bekatul awet dan fraksinya secara in vitro Jurnal Teknologi dan Industri Pangan. 15 pp 11–19
[8] Hadley C W, Clinton S K, Schwartz S J The consumption of processed tomato products enhances plasma lycopene concentrations in association with a reduced lipoprotein sensitivity to oxidative damage J Nutr. 133 pp 727–32
[9] Bub A, Watzl B, Abrahamse L, Delince H, Adam S, Weyer J, Muller H, Rechkemmer G 2000 Moderate intervention with carotenoids-rich vegetable products reduces lipid peroxidation in men J Nutr. 130 pp 2200–06
[10] Damayanti E, Kustiyah L, Kardinah Roosita K Efektivitas jus tomat dan minuman bekatul terhadap pengecian ukuran lembu kisti payudara Indonesian Journal of Cancer National Cancer Center 113 pp 25–30
[11] Droge W Free radicals in the physiological control of cell function Physiol Rev. 82 pp 47–95
[12] Hailat W 2004 Bioavailability and Pharmacokinetics Studies of Gamma Oryzanol (Malaysia: Universiti Putra Malaysia)
[13] Rao A, Shen H 2002 Effect of low dose lycopene intake on lycopene bioavailability and oxidative stress Nutr Res. 22 pp 1125–31
[14] Amirkhizi F, Siassi F, Minaie S, Djalali M, Rahimi A, Chamari M 2007 Is obesity associated with increased plasma lipid peroxidation and oxidative stress in women ARYA atheroslerosis Journal 2 pp 189–192
[15] Xu Z, Godber J 1999 Purification and identification of components of – oryzanol in rice bran oil J. Agric. Food Chem. 47 pp 2724–28
[16] Damayanti E, Muchtadi D, Syarief H, Wijaya C H, Damardjati D S 2004 Aktivitas antioksidan minyak bekatul awet dan fraksinya secara in vitro J. Teknologi dan Industri pangan. 15 (1). pp 11–19
[17] Damayanti E, Nirmala L C, Faigayanti A, Septiarini, Muharam N 2013 The use of chocolate in rice bran oil drink and its effect of health status on obese college students Proceeding IPIMA
[18] Nishizawa M, Kohno M, Nishimura M, Kitagawa A, Niwano Y 2005 Non-reductive scavenging
of 1,1-diphenyl-2-picrylhidrazyl (dpph) by peroxyradical: a useful method for quantitative analysis of peroxyradical Chem. Pharm. Bull. 53 pp 714–16

[19] Arza P 2010 Pengaruh Pemberian Minuman Jus Tomat dan Penyuluhan Gizi terhadap Status Antioksidan Likopen Plasma dan Gaya Hidup Penderita Kista Payudara (Bogor: Institut Pertanian Bogor)

[20] Abdurohman 2013 Pengembangan Minuman Fungsional Bubuk Bekatul Padi Siap Seduh dengan Berbagai Flavor untuk Pencegahan Penyakit Tidak Menular (Bogor: Institut Pertanian Bogor)

[21] Nuramalia D R, Damayanthi E 2017 Effect of Green Okra and Strawberry Ratio on Antioxidant Activity, Total Phenolic Content, and Organoleptic Properties of Jelly Drink. Proceeding IPIMA