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Research Article

Therapeutic Effects of BRC Functional Food from Indonesian Black Rice on Body Weight and Haematological Parameters in Obese Rats

Fajar Sofyantoro¹, Adi Mazdi Syam¹, Baik Aisyah Adania¹, Muhammad Fikri Almunawar¹, Nurlita Putri Bela Nasution¹, Rheina Faticha Asyamsa Hidayat¹, Made Bagus Auriva Mataram², Chesa Ekani Maharesi¹, Septika Nurhidayah¹, Yekti Asih Purwestri^{1,3}, Ardaning Nuriliani¹, Lisna Hidayati¹, Rarastoeti Pratiwi^{1,3*}

1)Department of Tropical Biology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia 55281

2)Pre-clinical and Experimental Animal Unit (Unit IV), Integrated Research and Testing Laboratory, Universitas Gadjah Mada, Yogyakarta, Indonesia, 55281

3)Research Center for Biotechnology, Universitas Gadjah Mada, Yogyakarta, Indonesia 55281

* Corresponding author, email: rarastp@ugm.ac.id

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ABSTRACT

Obesity increases the risk of various diseases. Black rice, renowned for its high anthocyanin content, is considered a potential functional food for preventing metabolic disorders. The current study investigated the effects of black rice crunch (BRC) on body weight and haematological profiles in obese rats. Rats were fed with high-fat diet to induce obesity and supplemented with different concentrations of BRC for 4 and 8 weeks. The results showed that high-fat diet effectively induced obesity, as evidenced by significant increase in body weight. Importantly, 75% BRC supplementation resulted in significant weight reduction in obese rats. Further analysis revealed an increase in erythrocyte numbers in obese groups supplemented with 75% BRC, but no significant changes in haemoglobin concentration or haematocrit percentage. Further investigation showed that 75% BRC led to a decrease in mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and mean corpuscular volume (MCV), potentially affecting the size and concentration of haemoglobin within erythrocytes. The total leucocytes count increased with the high-fat diet, while BRC supplementation alone did not have significant impact. Lymphocyte percentage remained stable across the groups, indicating minimal influence of the dietary interventions. Neutrophil percentage varied initially but was not specific to BRC or the high-fat diet. Platelet count and distribution width were not significantly influenced, but mean platelet volume (MPV) increased after 8 weeks of BRC treatment, suggesting larger platelet sizes associated with obesity. Overall, the study provides important insights into the effects of BRC supplementation on body weight and haematological parameters related to obesity.

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INTRODUCTION

Overweight and obesity are triggered by accumulation of excess or abnormal fat (Lin & Li 2021). The accumulation of fat may heighten the chances of developing cardiovascular disease and lead to a range of metabolic disorders (Després & Lemieux 2006; Després 2012). Genetic and non-genetic factors can lead to the accumulation of body fat that ultimately results in obesity. The monogenic disorders in the leptin and melanocortin signalling pathways have been reported to cause obesity (Brandl et al. 2012; Ren et al. 2019). Meanwhile, an imbalance in caloric intake and lack of physical activity are the main non-genetic factors that trigger obesity (Romieu et al. 2017).

The phenolic and anthocyanin contents of black rice are higher than white rice (Zhang et al. 2010). Phenolic compounds in black rice inhibit the activity of α -glucosidase and α -amylase in vitro, thus reducing the glycaemic index (An et al. 2016). In addition, anthocyanins significantly inhibit the pancreatic lipase activity and help to regulate lipid metabolism (Fabroni et al. 2016). Therefore, black rice contains various active compounds with health benefits and holds immense promise for becoming a functional food.

Indonesia boasts abundant biodiversity, encompassing a multitude of indigenous black rice cultivars (Kristamtini et al. 2012). Rukmana et al. (2017) demonstrated cytotoxic activity of black rice cultivars from Indonesia against cancer cells. A noteworthy variety hails from Yogyakarta, Indonesia, referred to as '*Cempo Ireng*' (Kristamtini et al. 2012). The anthocyanins, especially cyanidin 3-glucoside and peonidin 3-glucoside, in '*Cempo Ireng*' black rice is reported to activate the apoptosis pathways in human cervical cancer cells (Pratiwi et al. 2016). Similarly, extract from '*Cempo Ireng*' black rice bran also induces cell cycle arrest in breast cancer cells (Pratiwi et al. 2019). Sa'adah and Pratiwi (2016) reported that '*Cempo Ireng*' black rice and rice bran reduce cholesterol levels and atherogenic index in hyperlipidaemic rats. In addition, '*Cempo Ireng*' black rice effectively repairs liver function by reducing serum glutamic pyruvate transaminase (SGPT) levels in hyperlipidaemia rats (Chasanah & Pratiwi 2019).

In recent years, the development of black rice-containing functional food has been gaining significant attention. Black Rice Crunch (BRC), patent number IDP000081011, is a black rice-containing product which has been manufactured as functional food with various health benefits (Purwestri et al. 2022). The BRC products contain "Sembada Hitam" black rice cultivar from Sleman, Yogyakarta, Indonesia. In this study, BRC preclinical research was conducted using rats (*Rattus norvegicus*) as animal models. Obesity in rats can be induced by genetic modification or high-calorie feeding. High-calorie feeding is the simplest and most relevant method for obesity in humans (Von Diemen et al. 2006). Feeding with 30-85% of calories of lipids induce obesity and increase insulin resistance in animal models (Buettner et al. 2007; Hariri et al. 2010). The success of obesity induction in animal models can be determined by observing the increase in body weight and fat percentage (Hariri et al. 2010).

Feeding *R. norvegicus* a high-fat diet that induces obesity has been found to result in an elevation of white blood cell (WBC) levels (Shabbir et al. 2015; Monteomo et al. 2018). However, it has been observed that the administration of antioxidants can effectively reduce the WBC count in obese rats (Shabbir et al. 2015), bringing the levels closer to those observed in the control group comprising non-obese rats. Moreover, the administration of black rice extract has also been shown to have a reducing effect on WBC count (Park et al. 2020). In addition to leucocytes, platelet distribution width (PDW) and mean platelet volume (MPV) are commonly utilized as indicators of inflammation in obesity (Aktas et al. 2018). Furthermore, in a diet-induced obesity (DIO) rat model, increased platelet counts in obese rats was reported (Barrachina et al. 2020). These

findings highlight the association between obesity and alterations in immune cell and platelet parameters, suggesting a potential link between obesity-induced inflammation and haematological changes.

The evidence to support the benefits of black rice-containing products in promoting health and preventing metabolic diseases remains limited. Therefore, it is necessary to carry out preclinical studies to investigate the potential of black rice-containing products in preventing overweight and obesity by examining anthropometric, physiological, chemical, and biomolecular parameters. Body weight is one of the anthropometric parameters commonly used to identify obesity. In addition, haematological data is one of the basic parameters in preclinical tests, which might help to show the physiological response and changes in body metabolism. This research was conducted to determine the benefits of BRC, a black rice-containing product, in suppressing weight gain in obese rats and its safety by observing the haematological profiles. The use of animal models, such as *R. norvegicus*, may provide valuable insights into the potential benefits of BRC in mitigating obesity-associated diseases.

MATERIALS AND METHODS

Materials

The study design was approved by the Ethics Commission of the Integrated Research and Testing Laboratory, Universitas Gadjah Mada (UGM), Indonesia (Certificate No. 00023/04/LPPT/VIII/2022). A total of 25 male Wistar rats (*Rattus norvegicus* Berkenhout, 1769), 12 weeks of age, and weighing 130-170 g were used. The standard feed Rat Bio was purchased from PT. Citra Ina Freedmill, East Jakarta. The obesity and BRC feeds were manufactured as in Tsalissavrina et al. (2013) at the Centre for Food and Nutrition Studies, UGM, Indonesia.

The rats were divided into 5 groups, with each group consisting of 5 individuals. The control group (NO) and the placebo (BRC0) group were provided with standard feed and obesity feed, respectively. The treatment groups were fed with obesity feeds for 5 weeks (pre-BRC) followed by obesity and BRC feeds for 8 weeks (post-BRC), consisting of BRC 1 (25% BRC: 75% obesity feed), BRC2 (50% BRC: 50% obesity feed), and BRC3 (75% BRC: 25% obesity feed) (Nasution & Pratiwi 2023; Syam & Pratiwi 2023). The rats were weighed every two days.

Methods

Blood Sample Collection

Prior to blood sampling, the rats were anesthetized using a ketamine:xylazine cocktail (10:1 ratio) administered via the intramuscular route, with a dosage of 0.1 mL per 100 g of body weight. Blood collection was performed using the microhematocrit method through the retro -orbital plexus route, obtaining 2-5 drops of blood. The blood samples were then collected into a 1.5 mL microtube coated with EDTA.

Haematological Profile Analysis

Red Blood Cells (RBC) and White Blood Cells (WBC) analysis were performed using a haematology analyser (Sysmex KX-21). Parameters of RBC including RBC count, haemoglobin level, haematocrits percentage, and other erythrocyte indices such as mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), and mean corpuscular volume (MCV). The parameters measured for WBC and platelets include WBC count, lymphocyte percentage, neutrophil percentage, mean platelet volume (MPV), platelet distribution width (PDW), and platelet count (PLT).

Data Analysis

The data were presented as mean \pm SEM and tested using one-way ANOVA ($\alpha = 0.05$) followed by a Tukey test. Data analysis was performed using GraphPad Prism 8. The results were presented in a bar chart constructed in Microsoft Excel.

RESULTS AND DISCUSSION

BRC contributes to the suppression of weight gain in obese rats

At present, there is limited scientific evidence supporting the potential health advantages of incorporating black rice into food products to enhance overall well-being and prevent metabolic disorders. Hence, it is imperative to conduct preclinical studies to delve into the possibilities of these products in averting weight gain and obesity. These studies should encompass a range of parameters, including measurements of body size and shape, analysis of physiological responses, examination of chemical compositions, and evaluation of biomolecular profiles. Among the anthropometric parameters commonly used to assess obesity, body weight serves as a significant indicator.

Figure 1 shows that a significant increase in body weight was observed in obese-induced groups (BRC0, BRC1, BRC2, and BRC3). The results obtained provide clear evidence that the administration of an obesity-inducing diet over a period of 5 weeks effectively led to the development of obesity. Figure 1 also demonstrates that after 4 and 8 weeks of BRC treatment, there was a notable and statistically significant difference in body weight between the control group (NO) and the groups receiving BRC at various concentrations (BRC0, BRC1, BRC2, and BRC3). However, it is worth noting that the BRC0, BRC1, and BRC2 groups exhibited significant differences when compared to the BRC3 group. These findings suggest that 25% and 50% BRC were unable to effectively inhibit weight gain, as evidenced by the results observed in the BRC1 and BRC2 groups, respectively. Conversely, the obese rats receiving 75% BRC demonstrated a significant reduction in body weight, indicating its potential as an effective intervention in managing obesity.

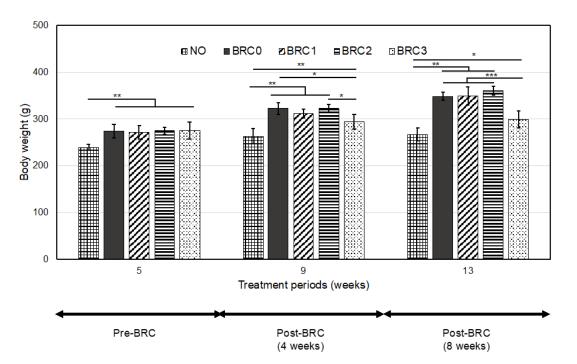


Figure 1. Body weight variations following the administration of high-fat diets and supplementation with Black Rice Crunch (BRC). The data is expressed as mean \pm SD. Statistical significance between groups is denoted by asterisks (* for P < 0.05, ** for P < 0.01, and *** for P < 0.001).

The findings of this study highlight the potential benefits of incorporating BRC into the diet to combat weight gain associated with a highfat diet. The presence of anthocyanins, which are natural pigments responsible for the dark colour of black rice, is believed to possess antioxidant and anti-inflammatory properties that can positively influence lipid metabolism and ultimately contribute to weight management. These results align with previous research that has demonstrated the potential health-promoting effects of anthocyanins in various metabolic disorders (Zhang et al. 2010; Fabroni et al. 2016; Lim et al. 2016). Further investigations are warranted to elucidate the specific mechanisms by which anthocyanins from black rice exert their effects on lipid metabolism and to explore their potential as therapeutic agents for the prevention and treatment of obesity-related conditions.

BRC supplementation modulates erythrocyte parameters

Figure 2A depicted the comparison of mean erythrocyte numbers among the groups at different time points. Initially, at the pre-BRC stage and after 4 weeks of BRC treatment, no significant differences were observed in the mean erythrocyte count between the groups. However, at the 8weeks post-BRC, the BRC3 group exhibited a significantly higher mean erythrocyte count compared to both the NO and BRC1 groups. These findings suggest that supplementation of 75% BRC for 8 weeks led to an increase in erythrocyte numbers, indicating that BRC may have a positive impact on erythropoiesis, the process of erythrocyte production. Importantly, it is noteworthy that despite this increase, the erythrocyte counts for all groups remained within the normal range according to de Kort et al. (2020), indicating that the observed changes were not indicative of any pathological conditions.

As indicated in Figures 2B and C, no statistically significant differences were observed in the haemoglobin concentration and haematocrit percentage among the various groups at the pre-BRC stage, as well as after 4 and 8 weeks of BRC treatment. These findings suggest that both the obesity-inducing feeds and BRC consumption did not have a significant impact on the haemoglobin concentration or haematocrit percentage. Importantly, the haemoglobin concentration and haematocrit percentage values for all groups remained within the normal range as defined by de Kort et al. (2020), indicating that there were no abnormal deviations from the expected values. Thus, it can be concluded that the observed changes in the erythrocyte profile were not associated with alterations in the haemoglobin concentration or haematocrit percentage, highlighting the stability of these parameters during the study period.

To gain a comprehensive understanding of the erythrocyte profile, further investigation of mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), and mean corpuscular volume (MCV) were performed. These additional measurements may provide valuable insights into the size, content, and concentration of haemoglobin within the erythrocytes, shedding light on potential variations that may contribute to the observed changes in the erythrocyte profile.

According to the findings presented in Figure 3A, it is evident that the MCV value of the BRC3 group at both 4 and 8 weeks post-BRC was significantly lower compared to the NO, BRC0, BRC1, and BRC2 groups. These MCV values fall below the range considered normal according to de Kort et al. (2020), suggesting that the administration of 75% BRC leads to a reduction in the MCV value. The MCV is a crucial parameter used to assess the size of erythrocytes and is an important indicator of red blood cell health and functionality. Values lower than the normal range may indicate the presence of underlying conditions such as micro-

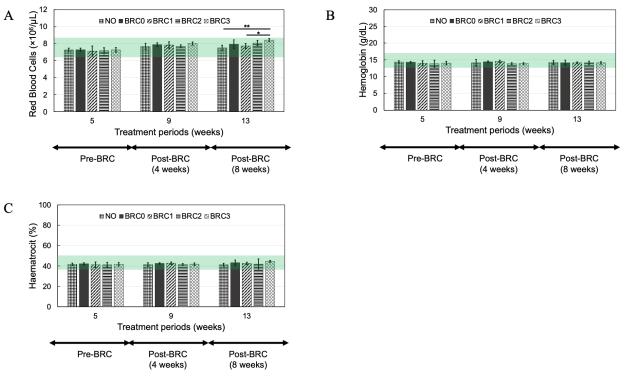


Figure 2. Mean values of erythrocyte numbers (A), haemoglobin concentration (B), and haematocrit values (C) following the administration of high-fat diets and supplementation with Black Rice Crunch (BRC). The data is presented as mean \pm standard deviation (SD). Significant differences between groups are denoted by asterisks (* for P < 0.05, ** for P < 0.01, and *** for P < 0.001). The normal range of values, as defined by de Kort et al. (2020), is indicated by the green box.

cytosis, which is characterized by smaller-than-normal red blood cells. The observed decrease in MCV in the BRC3 group suggests that the consumption of 75% BRC has an impact on the size of erythrocytes, potentially influencing their physiological functions. This reduction in size could potentially be attributed to the bioactive components present in BRC, which may modulate erythrocyte maturation or turnover. Further investigations are necessary to explore the underlying mechanisms behind this decrease in MCV and to determine whether it is a direct consequence of BRC consumption or an indirect effect resulting from other factors.

The data presented in Figure 3B demonstrate notable observations regarding the average MCH values in response to BRC treatment. Specifically, it is evident that at 4 weeks post-BRC, the BRC3 group displayed a significantly lower average MCH value compared to the NO group, as well as the BRC0 and BRC1 groups. Likewise, after 8 weeks of BRC treatment, the average MCH value in the BRC3 group remained significantly lower than all other groups. These findings provide compelling evidence that the inclusion of 75% BRC in the diet leads to a decrease in MCH values. The MCH value serves as a key haematological parameter that reflects the average amount of haemoglobin contained within individual red blood cells. Thus, the significant decline in MCH observed in the BRC3 group after both 4 and 8 weeks of post-BRC treatment suggests that the consumption of BRC has an impact on the haemoglobin content within the red blood cells. Moreover, it is noteworthy that the MCH values of the BRC3 group at 4 and 8 weeks post-BRC were found to be lower than the reference values provided by de Kort et al. (2020). Further research efforts are needed to unravel the underlying mechanisms responsible for the decrease in MCH values following BRC consumption.

The results presented in Figure 3C provide insights into the mean MCHC values among the different groups at various time points. It is noteworthy that there were no significant differences in the mean MCHC values between the pre-BRC and the 4-week post-BRC phase across all groups. This suggests that the initial introduction of BRC did not have a substantial impact on the MCHC values. However, an interesting observation arises when examining the mean MCHC values at the 8-week post -BRC stage. Notably, the mean MCHC values of the BRC3 group were found to be significantly lower than those of the NO group, as well as the BRC0 and BRC1 groups. Thus, the lower mean MCHC values observed in the BRC3 group after 8 weeks of post-BRC treatment suggest a potential alteration in the haemoglobin concentration within the red blood cells due to the consumption of 75% BRC. However, it is important to note that despite the observed differences in the mean MCHC values, all groups throughout the course of the experiments maintained MCHC values within the acceptable range as established by de Kort et al. (2020). Hence, while there may be differences between groups, the MCHC values of all groups remained within the acceptable range, suggesting that the overall haemoglobin concentration within the red blood cells was still within normal limits.

Collectively, these results demonstrate that the lower concentration of haemoglobin within each erythrocyte observed in the BRC3 group is compensated by a higher number of erythrocyte counts, despite their smaller size. This phenomenon suggests a potential adaptation or response to BRC3 supplementation, where the body aims to maintain or optimize oxygen-carrying capacity. This observation highlights the complex interplay between erythrocyte count and haemoglobin concentration in the context of BRC3 supplementation. Further investigations are nec-

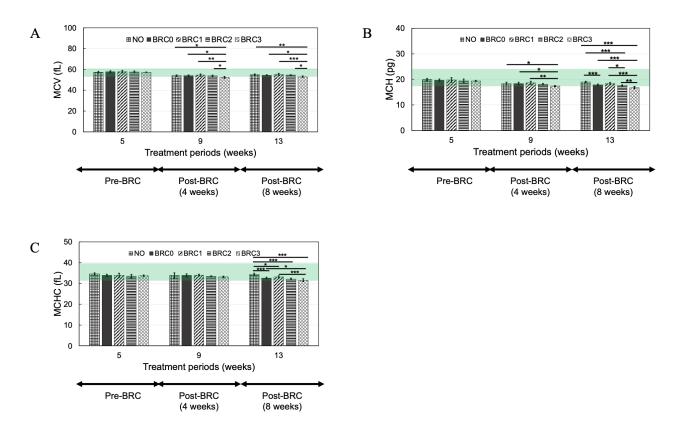


Figure 3. Mean Corpuscular Volume (MCV) (A), Mean Corpuscular Haemoglobin (MCH) (B), and Mean Corpuscular Haemoglobin Concentration (MCHC) (C) following the administration of high-fat diets and supplementation with Black Rice Crunch (BRC). The data is expressed as mean \pm standard deviation (SD). Significant differences between groups are denoted by asterisks (* for P < 0.05, ** for P < 0.01, and *** for P < 0.001). The normal range of values, as defined by de Kort et al. (2020), is indicated by the green box.

essary to elucidate the underlying mechanisms that regulate these changes and to assess the physiological implications of this combined effect on erythrocyte parameters.

High-fat diet increases leucocyte counts

Figure 4A depicts the comparison of total leucocyte counts between the NO and BRC groups at different time points. Following a 4-week period of BRC treatment, no statistically significant differences were observed in the total leucocyte count between the BRC groups and the NO group. However, at the 8-week post-BRC supplementation stage, a significant increase in the total leucocyte count was observed in the BRC0 group compared to the NO group. These findings indicate that a significant increase in leucocytes was only evident after 13 weeks of high-fat diet administration. Additionally, following 8 weeks of BRC supplementation, there was no significant reduction in total leucocyte count observed in the BRC 1, 2, or 3 groups compared to the BRC0 group. This indicates that BRC supplementation alone is inadequate in decreasing leucocyte levels in obese rats.

To further elucidate the specific types of leucocytes that undergo changes in response to high-fat diets and BRC supplementation, a differential leucocyte count was performed. This analysis may provide valuable insights into the cellular components of the immune system that are influenced by BRC supplementation in the context of obesity. Analysis of the data presented in Figure 4B revealed that there were no statistically significant differences observed in the percentage of lymphocytes between the NO and BRC groups after 4 and 8 weeks of BRC treatment. Furthermore, even after a 13-week period of consuming a high-fat diet, there were no notable effects on the percentage of lymphocytes. These findings indicate that the dietary intervention of BRC supplementation and the duration of high-fat diet feeding did not result in significant alterations in the percentage of lymphocytes. It is important to note that lymphocytes play a critical role in the immune system and their levels are known to be influenced by various factors. However, in the context of the current study, the percentages of lymphocytes remained relatively stable across the different treatment groups, suggesting that these specific dietary conditions did not have a substantial impact on lymphocyte populations.

Meanwhile, as depicted in Figure 4C, notable differences in neutrophil percentage were observed in the pre-BRC phase, wherein the BRC3 group exhibited significant difference from the NO, BRC1, and BRC2 groups. However, no significant differences in neutrophil percentage were observed between the NO group and all BRC groups, suggesting that the increased neutrophil percentage may not be attributed to the consumption of a high-fat diet. The initial variation in neutrophil percentage observed in the pre-BRC phase, specifically with a higher percentage in the BRC3 group compared to other groups, suggests the possibility of underlying factors influencing neutrophil levels independent of the dietary interventions. These factors may include individual variations in immune response, genetic factors, or other environmental factors unrelated to the BRC or high-fat diet. Furthermore, during the 4-week post -BRC phase, all experimental groups displayed neutrophil percentages below the established reference range (Said & Abiola 2014). However, in the 8-week post-BRC phase, the neutrophil percentages of all groups returned to the normal range, indicating that this fluctuation is not specific to BRC supplementation or the high-fat diet. It is important to consider that neutrophils play a crucial role in the immune response and their lev-

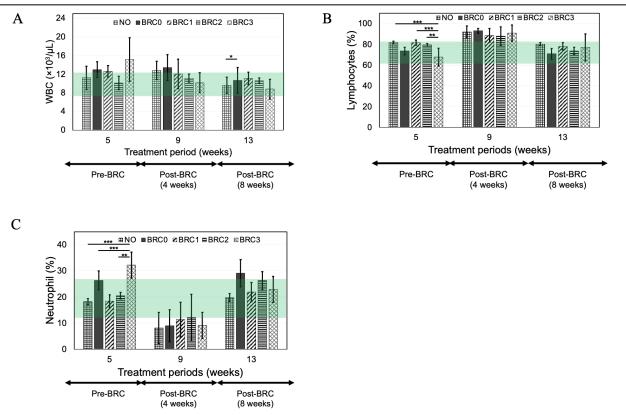


Figure 4. Mean values of White Blood Cells (WBC) numbers (A), percentage of lymphocytes (B), and percentage of neutrophils (C) following the administration of high-fat diets and supplementation with Black Rice Crunch (BRC). The data is expressed as mean \pm standard deviation (SD). Significant differences between groups are denoted by asterisks (* for P < 0.05, ** for P < 0.01, and *** for P < 0.001). The normal range of leucocyte numbers (Said & Abiola 2014) and percentage of lymphocytes or neutrophils (Sharp & Villano 2012) are indicated by the green box.

els can be influenced by various physiological and external factors. Therefore, it is necessary to explore and understand the underlying mechanisms contributing to the observed fluctuations in neutrophil percentage. These findings underscore the importance of carefully interpreting and contextualizing neutrophil percentage changes in the context of BRC supplementation and high-fat diet studies. Further investigations are warranted to unravel the potential factors contributing to the initial variation and subsequent normalization of neutrophil percentages, as well as to elucidate the specific mechanisms through which BRC and high -fat diet might influence neutrophil function and overall immune response.

The mixed cell percentage (MXD) value, which encompasses the collective presence of monocytes, basophils, and eosinophils in a blood sample, serves as an indicator of their cumulative accumulation. In our current study, however, we observed a remarkably low MXD value implying that the organism under scrutiny is not undergoing an allergic reaction or being affected by pathogenic infections (data not shown).

High-fat diet affects the average size of platelets

As shown in Figure 5A, the platelet count (PLT) values at various time points do not exhibit any notable differences between the NO group and the BRC groups. Furthermore, it is worth noting that all groups, across all time points, fall within the range considered normal based on the reference values established by Cox et al. (2011), indicating that BRC supplementation and high-fat diet have minimal influence on platelet count. Figure 5B showed that the Platelet Distribution Width (PDW) values at all time points also demonstrate no significant differences between the NO group and the BRC groups. However, after a 4-week period of BRC administration, the PDW values were observed to be higher than the normal range, as indicated by the reference values provided by (Mulvati et al. 2019). Elevated PDW values suggest an increased diversity in platelet size, potentially reflecting altered platelet activation or physiological conditions. Nonetheless, it is noteworthy that after an 8-week duration of BRC administration, the PDW values return to the normal range. This fluctuation in PDW values was observed across all groups, suggesting that it may occur independently of both the high fat diet and BRC supplementation. Taken together, these findings provide valuable insights into the platelet-related parameters in response to BRC supplementation and high-fat diet. The consistent platelet counts within the normal range suggest that BRC supplementation does not exert a significant impact on platelet number. Similarly, the lack of significant differences in PDW values between the NO and BRC groups indicates that platelet size heterogeneity remains unaffected by BRC supplementation.

According to the data presented in Figure 5C, the control group exhibits significant differences in mean platelet volume (MPV) when compared to both the BRC0 and BRC3 groups during the 8-week post-BRC treatment. These results suggest that a 13-week consumption of a high-fat diet leads to a notable increase in MPV among obese rats. Elevated MPV values are indicative of larger platelet sizes than those considered normal. This notable alteration in platelet morphology, as reflected by elevated MPV values, may have implications for platelet function and overall hemostatic processes. Larger platelet sizes, as indicated by high MPV values, can be associated with various pathological conditions, including cardiovascular diseases and inflammatory states. Such alterations in platelet size may affect platelet function, aggregation, and thrombotic events.

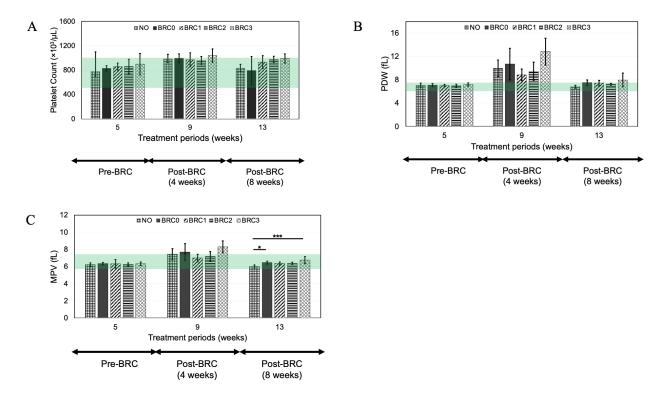


Figure 5. Platelet count (A), Platelet Distribution Width (PDW) (B), and Mean Platelet Volume (MPV) (C) following the administration of high-fat diets and supplementation with Black Rice Crunch (BRC). The data is expressed as mean \pm standard deviation (SD). Significant differences between groups are denoted by asterisks (* for P < 0.05, ** for P < 0.01, and *** for P < 0.001). The normal range of platelet counts (Cox et al. 2011), PDW (Mulyati et al. 2019), and MPV (Said & Abiola 2014) are indicated by the green box.

CONCLUSION

In conclusion, this study demonstrates that administering an obesityinducing diet for 5 weeks led to significant weight gain and obesity in rats. Importantly, supplementation with Black Rice Crunch (BRC) resulted in notable weight reduction in obese rats. Moreover, BRC consumption influenced erythrocyte numbers and mean corpuscular parameters. Interestingly, while the total leucocyte counts increased due to the highfat diet, BRC had no significant impact on this parameter. Additionally, lymphocyte percentage remained stable across groups, indicating minimal influence of dietary interventions. Notably, mean platelet volume (MPV) increased after 8 weeks of BRC treatment, seemingly associated with obesity. Future research should investigate the molecular mechanisms behind BRC's effects on haematological parameters. Exploring the impact of BRC impact on inflammatory markers and adipose tissue could also provide further insights into its anti-obesity properties. Additionally, long-term studies on the safety and efficacy of BRC in human subjects are warranted to assess its potential as a functional food for obesity management.

AUTHORS CONTRIBUTION

R.P., Y.A.P, A.N., and F.S. designed the research and supervised all the processes; A.M.A., B.A.A., M.F.A., N.P.B.N., R.F.A.H., M.B.A.M., C.E.M., and S.N. collected and analysed the data, F.S., Y.A.P, A.N., and R.P. analysed the data and wrote the manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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