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# Characteristics of Finger Millet (*Eleusine coracana* L.) Flour and Its Effect on Obese Rats: Biological and Applied Study

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OBESITY is one of the main causes of chronic inflammatory disorders and has an impact on the healthcare system by increasing healthcare costs. Replacing refined grains with whole grains like millets that are economical and available helps to reduce weight as well as improve the nutrient content of food. This study was conducted to investigate the positive effects of finger millet flour (FMF) as a replacement for wheat flour (WF) on obese rats fed a high-carbohydrate diet (HCD) as well as its effect on the sensory qualities of the resultant snack. The results revealed that the FMF is a good source of dietary fiber, B vitamins, particularly niacin (1.11 mg/100 g), minerals such as Ca, K, P, and Fe, as well as essential amino acids, mainly leucine, valine, and phenylalanine. The FMFalso showed high levels of phenols (2624 mg GAE/g), flavonoids (2.37 mg CE/g), and phytic acid (6.05 mg/g), which represent high antioxidant activity against DPPH radicals. In comparison to HCD alone, HCD supplemented with FMF reduced body weight gain, feed efficiency ratio, and body mass index of obese rats and improved the serum lipid profile, particularly by reducing triglycerides, cholesterol, and LDL-c compared to HCD alone. Additionally, HCD supplemented with FMF controlledinsulin, leptin, and cortisol hormones and improvedserum antioxidant biomarkers by reducing malondialdehyde (MDA) and raising glutathione peroxidase (GPX). The healthy snacks were more fragile, crispy, and palatable when WF was substituted with FMF up to 70 and 100%.

**Keywords:** Finger millet flour, Obese rats, Leptin, Cortisol, Lipidprofile, Oxidative stress, Healthy snacks

## Introduction

Obesity is widespread in developing and undeveloped countries. It may raise the risk of developing type 2 diabetes and cardiovascular diseases (Agrawal & Agrawal, 2015). Obesity-related metabolic disorders are endocrinology diseases that are linked to higher mortality and complications that have a negative impact on quality of life, productivity at work, and healthcare expenses (Kinlen et al., 2018). In 2016, 39% of adults (18 years old) and 13% of obese adults were overweight, but more importantly, 38 million children under the age of 5 were overweight or obese in 2019 (WHO, 2021). Shifts from eating traditional diets that are rich in whole grains to ones that are rich in processed grains, which contribute to excess calories, together with an enhanced sedentary lifestyle are the major contributing factors to this crisis (Forouhi et al., 2018). Several anti-obesity drugs approved by the Food and Drug Administration have been withdrawn from the market because of their unexpected adverse effects (Srivastava & Apovian, 2018). Natural products are promising alternatives because of their powerful biological activities and perhaps reduced negative effects (Fu et al., 2016). Consumption of whole grains decreases the risk of weight gain as well as the incidence of diabetes, cardiovascular disorders, and obesity. Whole grains are the grains that have the endosperm, bran, and germ from the plant intact. Replacing refined grains with whole grains is one of the most sustainable strategies for weight loss (Maki et al., 2019).

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Finger millet (*Eleusine coracana* L.), also known as ragi, is a tropical cereal crop that adapts to various agro-climatic conditions and is widely grown in some African countries, including Egypt. Finger millet (FM) is a small grain, even smaller than that of rice or sorghum, and the crop has a wide adaptability to local environments for its properties to be tolerant to drought and heat (Chamoliet al., 2018).It belongs to groups of small seeded species of cereal grains, which are annual plants (Ramashia et al., 2019). It has been called the ideal crop to be used as a stable food and a reserve for famine because of its high nutritional value and the possibility of its long storage without insect damage (DidaBulbula & Urga, 2018). It is a very useful cereal because of its high protein, fat, and carbohydrate content, which is comparable to wheat and higher than rice (Rathore et al., 2019). FM has high nutritional quality and adapts to different environmental conditions (Opole, 2019). It plays a great role in dietary needs and as a source of income for many rural households due to its richness in calcium, iron, zinc, phosphorus, and potassium (Zewdu et al., 2018; Asritha, 2021). Italso showed to have a good number of phenolic compounds that possess antioxidant and metal chelating properties (Chandrasekara & Shahidi, 2010). There is some evidence that foods from FM have a low glycemic index and are good for people with diabetes (Asritha, 2021). Because of what has been described about the health benefits of FM, as well as the fact that low-income people are affected by obesity, the current study was conducted to investigate the positive effect of finger millet flour (FMF) as a replacement for wheat flour (WF) on obese rats fed a high-carbohydrate diet(HCD), as well as the effect of partial or total replacement of WF by FMF on the sensory properties of snacks.

## Materials and Methods

*Materials*

*Raw materials*

Finger millet seeds (*Eleusinecoracana* L.) (FMS) were purchased from the local market in Cairo, Egypt. Ingredients for rat diets were obtained from El-Gomhoria Company, Cairo, Egypt. Ingredients for the healthy snacks: wheat flour samples (72% extraction), corn oil, and salt were purchased from the local market in Cairo, Egypt.

*Animals*

Adult male Sprague-Dawley rats (n = 36) weighing 125–135 g was purchased from Helwan Experimental Animals Farm, Egypt.

*Chemicals*

The 2,2-diphenyl - 1- (2,4,6-trinitrophenyl) - hydrazinyl(DPPH), and Folin-Ciocalteu reagent wereobtained from Sigma-Aldrich (St. Louis, MO, USA). Gallic acid and catechin were purchased from Sigma-Aldrich, St. Louis, MO, USA.All chemicals and solvents from different suppliers were of analytical grade.

*Methods*

*Preparation ofFMF and its extract*

The FMS were ground in a house blender and sieved toproduce the FMF and then packed in an airtight polyethylene bag for further use according to the procedure outlined by Fasogbon et al. (2021).

*Preparation of FMF extract*

The FMF was soaked in 70% methanol (1:7), kept in a dark glass bottle, and then shaken overnight using a rotary shaker at room temperature. The supernatant was centrifuged at 3000 xg for 10 min, filtered, and the residue was then re-extracted with the same amount of solvent. All extracts were combined and evaporated using a rotary vacuum evaporator under reduced pressure at 50 °C to complete the removal of solvent. The obtained extract was frozen, freeze-dried, and then stored at 4±2 °C until used.

*Chemical analysis of FMF*

*Chemical composition:*

The moisture, ash, crude protein, total fibre, and crude fat content of the FMF were determined according to themethods of AOAC (2012). The total carbohydrate content was calculated by the difference method as described by Manzi et al. (2004).Mineral content of FMF was determined using an Atomic Absorption Spectrophotometer (Perkin-Elmer Model 2380, USA) according to the method of AOAC (2012).

*B vitamin analysis:*

The HPLC analysis of the B vitamins:thiamin (B1), riboflavin (B2), and niacin (B3),was conducted using an Agilent 1260 series with the ZORBAX SB-C8 column (4.6 mm × 150 mm ID × 5 µm). Water (A) with 0.01% TFA (pH 2.9) and methanol (B) at a flow rate of 1.5 ml/min made up the mobile phase. The mobile phase was designed in this order: 0 min (90% A), 0–1 min (90–70% A), 1–4 min (70–50%A), 4–8 min (50–90% A), and 8–10 min (90% A). The injection volumes for phenolic compound and B-complex vitamin solutions were 10 and 5 µL, respectively. The multi-wavelength detector was monitored at 280 nm.

*Amino acids analysis:*

The amino acid composition of FMF was determined using the HPLC-Pico-Tag method, according to Cohen et al. (1987). The Pico-Tag method, which was developed commercially by Waters Associates, was an integrated technique for amino acid analysis. Phenylisothiocyanate (PITC, or Edman's reagent) was used for pre-column derivatization, while reversed-phase gradient elution HPLC separates the phenylthiocarbamyl (PTC) derivatives, which were detected by their UV absorbance. The sample corresponding to the protein ratio was weighted into a 25 × 150 mm hydrolyzed tube using 6 N HCl and placed in a 110 °C oven for 24 h, then the tube was removed from the oven and allowed to cool. The tube contents were quantitatively transferred to a volumetric flask and diluted with HPLC grade water. The diluted hydrolysate was filtered through a 0.45 um sample filter. Aliquots of hydrolysate, together with appropriate standards, were placed in disposable glass sample tubes from Waters Associates. The sample was placed into the Pico-Tag amino acids workstation (Waters, USA) for sample preparation (drying, re-drying, and derivatization) using Waters reagents. The chromatographic analysis was carried out using HPLC with the Pico-Tag amino acid method. The liquid chromatography apparatus was equipped with a 600 E multi-solvent delivery system and the following gradient of Pico-Tag solvents A and B (Waters eluent A and B) at 38 °C and a flow rate of 1 ml/min. Twenty microliters of sample were injected and loaded onto an amino acid column. Pico-Tag amino acids (150 x 3.9 mm, stainless steel) using linear gradient elution. UV absorption measurements at a fixed wavelength of 254 nm (2489 UV/Vis Detector) are used to detect PTC derivatives. Before injecting the sample, the illustrated was calibrated with two injections of the lysine standards.

*Phytochemical content and antioxidant activity:*

The phytic acid and tannin content were determined in the FMF according to the methods described by Haugh & Lantzech (1983) and Makkar et al. (1993), respectively. The total phenolic content was determined as gallic acid equivalents (µg GAE/g extract) using the Folin–Ciocalteu spectrophotometric procedure as described by Annisworth & Gillespie (2007). The total flavonoid content was determined as catechin equivalents (µg CE/g extract)according to the method of Sarikurkcu et al. (2009).The antioxidant activity of FMF extract was determined using the stable DPPH radical assay developed by Brand-Williams et al. (1995). Briefly, 100 µL of various concentrations (0.25–2.0mg/ml) of the tested extract were distributed into different test tubes and then 3.9 ml of a DPPH solution (25 mg/L methanol) was added to each tube. After incubation for 30 min in the dark at room temperature the absorbance ($A_1$) was recorded at 517 nm. A control solution, without a tested compound, was prepared in the same manner as the assay mixture ($A_0$). The DPPH radical-scavenging activity was calculated using the following formula: Radical scavenging activity (%) = $[(1 - A_1/A_0) \times 100]$. The radical scavenger activity was expressed in terms of the amount of antioxidants necessary to decrease the initial DPPH absorbance by 50%. The $IC_{50}$ value for each sample was determined graphically by plotting the percentage disappearance of DPPH as a function of the sample concentration.

*Biological study*

*Experimental design:*

After one week on a normal diet for acclimatization to the laboratory environment, thirty-six rats were randomly divided into the following six groups: Group 1 was fed a normal diet (Table 1) and kept as a negative control group. Group 2 included obese rats fedhigh-carbohydrate diets (HCD, Table 1), which served as the positive control. Obese rats also were fed a (HCD)supplemented with 100% wheat flour (WF) in group 3, while in groups 4, 5, and 6, WF was substituted with FMF at levels of 50, 70, and 100%, respectively. The flour was provided as a source of carbohydrates based on the diets' remaining daily carbohydrate intake. After 8 weeks, the rats were fasted overnight, and blood samples were collected from the medial throat of rat eyes via micro-glass tubes without any anticoagulant and centrifuged for 20 min at 3000 rpm to obtain serum, which was stored at -20°C until subsequent biochemical analysis.

**TABLE 1. Ingredients as a source of energy inboth normal and high-carbohydrate diets.**

| Ingredients | Normal diet (ND) (g/Kg) | High-carbohydrate diet (HCD) (g/Kg) |
|---|---|---|
| Protein (Casein) | 180 | 100 |
| Carbohydrates | | |
| - Corn starch | 564.69 | 644 |
| - Fiber | 10 | 10 |
| - Sucrose | 100 | 100 |
| Corn oil | 100 | 100 |
| Vitamins | 10 | 10 |
| Minerals | 35 | 35 |
| Methionine | 1 | 1 |

Source: Pugh et al. (1999) and Altunkaynak (2005).

*Biological evaluations:*

The amounts of food consumed and/or wasted were recorded every day. Total feed intake (FI) was calculated. Individual body weight (BW) of rats in each group was assessed per week. Body weight gain percentage(BWG%) and feed efficiency ratios (FER) were calculated according to Champman et al. (1959) using the following equations:

$$BWG\ (\%) = (FBW - IBW)/(IBW) \times 100$$

$$FER = BWG\ (g/day)/FI\ (g/day)$$

* Where: BWG, body weight gain; FBW, final body weight;IBW, initial body weight; FER, efficiency ratio; FI, feed intake

Body mass index (BMI) was calculated as body weight (g)/ [body length (cm)]$^2$. Body length (nose to anus) was measured every four weeks using a measuring tape under light anaesthesia (Novelli et al., 2007).

*Biochemical analysis:*

Serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-c), and very low-density lipoprotein cholesterol (VLDL-c), as well as oxidative stress biomarkers like malondialdehyde (MDA) and glutathione peroxidase (GPX) and hormones like insulin, leptin, and cortisol hormones, were measured using a biochemical blood analyzer (Alfa Wassermann Dignostic Technologyies, LLC, Ace, Alera, USA).

*Snacks making*

The snacks were made by combining WF and FMF in a series of proportions to choose the best mixtures that would produce healthy snacks with high-quality properties.Snacks made completely of wheat flour (100%) were used as a control. The total amount of WF in the control snack was replaced with 50, 70, and 100% FMF to create the different tested treatments.All ingredients were mixed, scaled, shaped, and proofed in accordance with Rehal et al. (2021). The ingredients and their percentages used in the making of snacks are presented in Table 2. Roll the dough out on a lightly floured surface to a thickness of 1/8 inch or less. Place the dough on an ungreased baking sheet and mark out squares with a knife, but don't cut through. Prick each piece with a fork a few times and sprinkle with salt. Bake for 15 to 20 min in the preheated oven at 190 °C, or until crisp and golden brown. Cool on the baking sheet, and then separate into individual snacks.

*Sensory evaluation*

Sensory evaluation of the resulting healthy snacks compared to the control snack was carried out by 20 panel members, according to Kong et al. (2008). Consumers were asked to assess each coded sample of the snacks using a nine-point hedonic scale ranging from 1 (extremely dislike) to 9 (extremely like) to verify their acceptability. The highest score of sensory likings is based on aroma, color, crispness, taste, appearance, and general acceptability.

*Statistical analysis*

The collected data were expressed as means ±SD. An analysis ofvariance (one way ANOVA) was performed, and the mean comparisonswere analyzed by Duncan's multiple range test. The statistical analyses were accomplished using a SPSS package (SPSS, 2011; Version 19.0 forWindows, SPSS Inc.).Significantdifferences were determined using Duncan's multiple range tests($p < 0.05$).

**TABLE 2. Ingredients used in snacks making with different percentage of wheat flourand/or finger millet flour.**

| Ingredient | Snacks treatments | | | |
|---|---|---|---|---|
| | Control | T1 | T2 | T3 |
| WF(g) | 500 | 250 | 150 | - |
| FMF (g). | - | 250 | 350 | 500 |
| Corn oil (ml). | 25 | 25 | 25 | 25 |
| Salt (g). | 5.0 | 5.0 | 5.0 | 5.0 |
| Water (ml). | 200 | 187.5 | 175 | |

WF, wheat flour; FMF, finger millet flour; Control, 100% WF; T1, 50% WF: 50% FMF; T2, 30% WF: 70%FMF; T3, 100% FMF

## Results and Discussion

*Chemical composition of FMF*

Table 3 shows the proximate composition, minerals, and B vitamin contents of finger millet flour (FMF).The contents of moisture, protein, fat, ash, total dietary fiber, and total carbohydrates were 7.3, 1.3, 3, 19.1, and 72.6%, respectively. According to Sharobaet al. (2013), FMF had similar percentages of carbohydrates and moisture but was lower in protein and higher in fiber and ash content when compared to wheat flour (WF).FMF was also rich in K, Ca, and P content, as major minerals, which represent 345.9, 333.6, and 249.6 mg/100g, respectively. Also, FMF has high contents of Fe (4.62 mg/100g), Mn (7.51 mg/100g), and Zn (1.83 mg/100g).Kulkarni et al. (2012) reported that the Ca, P, and Fe contents of WF were 18.0, 107, and 2.1 mg/100 g, which were lower when compared with those of FMF. The total dietary fiber of millet grains (19–22%) was reported to be relatively higher than that of wheat (Ambre et al., 2020) and the best source of micronutrients such as Ca, Fe, P, Zn, and K (Asritha, 2021; Nakarani et al., 2021).FMF contains sugars such as sucrose, glucose, fructose, and raffinose (Gupta and Nagar, 2010).FMF has high concentrations of B vitamins, including B1, B2, and B3, with values of 0.48, 0.12, and 1.1 mg/100 g, respectively. Compared to WF, these values were higher in FMF, according to Batifoulier et al. (2006), who reported that the B vitamin concentrations range from 0.260 to 0.613 mg/100 g DM and 0.048 to 0.106 mg/100 g DM for thiamine and riboflavin, respectively.

As shown in Table 4, FMF is also a rich source of the essential amino acids (EAAs). The highest EAAs concentration in FMF was 690 mg/g N for leucine, followed by isoleucine (400 mg/g N) and valine (480 mg/g N). The lowestessential AA that was found in FMF was tryptophan (100 mg/g N), followed by cystine (140 mg/g N). These results are in line with Jideani (2012),who mentioned that millets are rich in EAAs, especially sulfur-containing EAAs including methionine and cysteine, and also superior in fatty acids than maize, rice, and sorghum (Chethan & Malleshi, 2007) as well as carbohydrates, lysine-, threonine-, and valine-rich proteins, crude fibers, and minerals **(**Chandrashekar, 2010)

Phytochemical contents and antioxidant activity of FMF are presented in Table 5. Tannins and phytates are natural plant compounds that are found in all plant seeds but are most abundant in cereals and legumes. They have received considerable attention due to their effects on lowering the nutritional quality of foods. For these reasons, tannic and phytic acids are considered to be anti-nutrients (Febles et al., 2002). However, these anti- nutritional components are mainly found in the bran layer. The FMF contains a high amount of anti-nutrients such as phytic acid (6.05 mg/g) and tannic acid (2.27 mg/g). The phytic acid concentrations in FMF were within the range reported by Febles et al. (2002), who discovered that most phytic acid concentrations are between 2 and 4 mg/g for refined flours and 6-10 mg/g for whole flours. However, FMF had higher tannic acid content than that found by Asres et al. (2017). There exists a positive correlation between the presence of anti-nutrients and in vitro protein digestibility. Minimal processing methods reduce the anti-nutritional factors to significant levels (Léder, 2004). The presence of anti-nutrients in FM was reported to lower glycemic effect and reduces starch digestibility and absorption (Lakshmi Kumari & Sumathi, 2002). Moreover, FMF exhibited high polyphenol content as measured by its total phenolic and flavonoid contents, which showed a significant level of antioxidant activity against DPPH radicals($IC_{50}$, 1.68 µg/ml).The concentrations of total phenolic and flavonoid content in FMF were 2.62mg GAE/g and 2.37mg CE/g, respectively.Nassarawa & Sulaiman (2019)

found the presence of tannin, terpenoid, alkaloid, cardiac glycoside, saponin, oxalate, phytate, flavonoids, and total phenol in finger and pearl millet flours. Ferulic acid, vanillic acid, caffeic acid, syringic acid, and p-coumaric acid are some of the common phenolic acids identified in FMF grains (Chethan et al., 2008). Asritha (2021) reported that ancient food grains known as millets are rich in phenolic acids, flavonoids, and micro-minerals.

*Biological evaluation*

Diet diversification, including whole grains in the diet, consumption of dietary fiber, and antioxidant-rich diets are among the diet-based strategies proposed to control weight gain and associated metabolic diseases (Forouhi et al., 2018). Table 6 shows the effect of substituting WF for FMF in the diets of obese rats on the body weight parameters. Rats fed a HCD showed the highest BWG, FER, and BMI ($P<0.05$), followed by those fed a HCD supplemented with WF only. Also, rats fed a HCD had the highest FI compared to other animal groups. However, body weight parameters of obese rats fed a HCD supplemented with FMF significantly decreased as the supplement level increased ($P<0.05$). When 70% WF was substituted for FMF, the BWG, FER, and BMI of rats fed a HCD supplemented with WF (100%) decreased from 64.20, 0.035, and 0.59 to 30.11, 0.016, and 0.49, respectively. The BWG, FER, and BMI of the rats fed a HCD supplemented with 50% FMF were comparable to those of the rats fed a normal diet ($P>0.05$). Furthermore, there was no statistically significant difference in body weight parameters between rats fed a HC diet supplemented with 70 and 100% FMF. As a result, FMF had a clear inhibitory effect on rat BWG and BMI increases, as well as a slight reduction in appetite and a reduction in the FER, indicating strong anti-obesity effects. These findings are consistent with those of Jakobek (2015), who found that the high polyphenol content enhanced catabolic activity in adipose tissues and decreased fat absorption from the stomach. Flavonoids can inhibit WBG directly or through their biologically active metabolites via various potential pathways (Song et al., 2019).The beneficial effects of polyphenols on obesity and metabolic complications are attributed mainly to their anti-inflammatory and antioxidant properties (Finicelli et al., 2019). The polysaccharide present in FM also prevented adiposity in high-fat diet-induced rats (Sarma et al., 2018).The high Ca content of FMF (Table 3) may be another factor in lowering BWG. Increasing dietary Ca intake increased the Ca concentration in the intestine, which in turn induced the formation of insoluble fatty acids and bile acid soaps that were excreted through the faeces, thus decreasing the amount of dietary fat available for storage (Boon et al., 2005). According to Zemel et al. (2000), increasing dietary Ca intake resulted in a decrease in $Ca^{2+}$, which in turn increased lipolysis.

**TABLE 3. Chemical composition, minerals, and B vitamincontents of finger millet flour.**

| Items | Concentration |
|---|---|
| ***Major ingredient content*(g/100 g)** | |
| Moisture | 13.1 |
| Protein | 7.31 |
| Fat | 1.30 |
| Ash | 3.01 |
| Dietary Fiber | 19.1 |
| Carbohydrates by difference | 72.6 |
| ***Minerals content (mg/100 g)*** | |
| Ca | 333.6 |
| P | 249.4 |
| K | 345.9 |
| Na | 29.98 |
| Mg | 115.4 |
| Fe | 4.62 |
| Mn | 7.51 |
| Zn | 1.83 |
| ***B vitamin (mg/100 g)*** | |
| B1 (Thiamin) | 0.48 |
| B2 (Riboflavin) | 0.12 |
| B3 (Niacin) | 1.11 |

**TABLE 4. Concentration of essential amino acids in finger millet flour.**

| Essential amino acids | Concentration (mg/g N) |
|---|---|
| Arginine | 300 |
| Lysine | 220 |
| Tryptophan | 100 |
| Phenylalanine | 310 |
| Tyrosine | 220 |
| Methionine | 210 |
| Cystine | 140 |
| Threonine | 240 |
| Leucine | 690 |
| Isoleucine | 400 |
| Valine | 480 |

**TABLE 5. Phytochemicalscontentand DPPH radical scavenging activity ($IC_{50}$) of finger millet flour.**

| Phytochemicals | Concentration |
|---|---|
| Total phenolic content (mg GAE/g) | 2.62 |
| Total flavonoid content (mg CE/g) | 2.37 |
| Phytic acid (mg/g) | 6.05 |
| Tannic acid (mg/g) | 2.27 |
| DPPH radical scavenging activity ($IC_{50}$, mg/ml) | 1.68 |

GAE, Gallic acid equivalent; CE, Catechin equivalent .

**TABLE 6. Effect of substituting wheat flour with finger millet flour in obese rats' diets on body weight parameters.**

| Types of diets | BWG (%) | FI (g/d) | FER | BMI (g/cm$^2$) |
|---|---|---|---|---|
| Normal | 47.70 ±4.98$^{c}$ | 19.73±0.32$^{ab}$ | 2.41 ±0.25$^{c}$ | 0.54 ±0.03$^{c}$ |
| HCD | 85.20 ±3.21$^{a}$ | 20.10±1.03$^{a}$ | 4.23 ±0.15$^{a}$ | 0.68 ±0.01$^{a}$ |
| HCD with 100% WF | 64.20 ±5.59$^{b}$ | 18.33±0.90$^{ab}$ | 3.50 ±0.57$^{b}$ | 0.59 ±0.02$^{b}$ |
| HCD with 50% WF and 50% FMF | 46.15 ±5.01$^{c}$ | 19.26±0.85$^{ab}$ | 2.39 ±0.25$^{c}$ | 0.57 ±0.01$^{bc}$ |
| HCD with 30% WF and 70% FMF | 35.09 ±6.03$^{d}$ | 18.23±0.85$^{b}$ | 1.88 ±0.39$^{cd}$ | 0.46 ±0.01$^{d}$ |
| HCD with 100% FMF | 30.11 ±4.65$^{d}$ | 18.83±1.21$^{ab}$ | 1.59 ±0.24$^{d}$ | 0.49 ±0.01$^{d}$ |

Means (n=6 ±SD) with the same letters in the same column are not significantly different (P<0.05); HCD, high-carbohydrate diet;WF, wheat flour; FMF, finger millet flour; BWG, body weight gain; FI, feed intake; FER, feed efficiency ratio; BMI, body mass index

As shown in Table 7, the obese rats fed a HCD had the highest (P<0.05) mean value of serum TC, TG, LDL-c, and VLDL-c and the lowest serum HDL-c as compared to the control group. In general, replacing WF in the HCD with FMF enhances the blood lipid profile (P<0.05). In general, substituting WF with FMF in the HCD improves the serum lipid profile (P<0.05). Serum TC, TG, LDL-c, and VLDL-c decreased, and serum HDL-c increased; the changes were proportional to the addition of FMF. However, only the serum levels of TG and VLDL-c in rats fed a HCD were very close to those of rats fed a normal diet (P>0.05). So, FMF intake can significantly reverse the high TC, TG, and LDL-C levels caused by the HCD as well as the low HDL-C levels in a dose-dependent manner, which also contributes to its anti-obesity effects. The hypocholesterolaemic effect of FM seed coat matter has also been observed in streptozotocin-

induced diabetic rats (Shobana et al., 2010). The presence of dietary fiber in the seed coat of millet grain may influence several metabolic and digestive processes, such as glucose absorption and cholesterol levels (Upadhyaya et al., 2011). Murtaza et al. (2014) reported that the FMF whole grain supplementation (10%) prevented BWG, improved lipid profile and anti-inflammatory status, alleviated oxidative stress, regulated the expression levels of several obesity-related genes, and increased the abundance of beneficial gut bacteria. Polyphenol intake has been shown to alleviate obesity and associated metabolic alterations such as insulin resistance, hepatic steatosis, and cardiovascular complications (Finicelli et al., 2019; Jin et al., 2019; Shen et al., 2022).

The results presented in Table 8 showed that HCD-fed obese rats had higher MDA and lower GPX levels than the control group. MDA was reduced and the level of GPX was improved in obese rats fed aHCD supplemented with FMF; the changes were correlated with the addition of FMF. However, there were no significant differences in MDA or GPX between groups of rats fed aHCD supplemented with 50 and 70% FMF.The FMF had a greater effect at a replacement level of 100% WF.These results could be explained by the potent antioxidant activity of FMF's phenolic, flavonoid, and tannin compound content (Table 5). Such an effect was found by Shobana et al. (2010) when FM whole grain was added to a high-fat diet. Flavonoids, phenols, and tannins act as powerful free radical scavengers (Tapiero et al., 2002). Viswanath et al. (2009) reported that millets are known for their rich sources of bioactive compounds, including vitamins, phenolics, and flavonoids and their glucosides, folic acid, carotenoids, coumarins, and highly fermentable fiber with potential health-promoting properties. Moreover, 10% FM-bran supplementation reduced oxidative stress, regulated the expression of several genes associated to fat, and increased the number of beneficial gut bacteria (Murtaza et al., 2014).

**TABLE 7. Effect of substituting wheat flour with finger millet flourin obese rats' diets on serum lipid profile.**

| Types of diets | TC | TG | LDL-c | VLDL-c | HDL-c |
|---|---|---|---|---|---|
| | ---------- (mg/dl) ---------- | | | | |
| Normal | 181.30±4.49$^{e}$ | 114.22±5.26$^{d}$ | 97.58±4.90$^{e}$ | 22.84±1.05$^{d}$ | 60.86±2.29$^{a}$ |
| HCD | 276.72±5.67$^{a}$ | 195.97±5.00$^{a}$ | 195.89±5.89$^{a}$ | 39.19±1.01$^{a}$ | 41.63±2.31$^{e}$ |
| HCD with 100% WF | 257.61±4.39$^{b}$ | 157.88±4.34$^{b}$ | 176.49±6.19$^{b}$ | 31.57±2.06$^{b}$ | 49.54±2.43$^{cd}$ |
| HCD with 50% WF and 50% FMF | 237.29±3.37$^{c}$ | 148.10±5.52$^{b}$ | 159.26±6.14$^{c}$ | 29.62±1.12$^{b}$ | 48.40±2.88$^{d}$ |
| HCD with 30% WF and 70% FMF | 231.25±3.75$^{c}$ | 134.87±4.66$^{c}$ | 148.26±2.78$^{d}$ | 26.97±0.93$^{c}$ | 54.24±3.87$^{bc}$ |
| HCD with 100% FMF | 221.93±3.82$^{d}$ | 118.67±3.82$^{d}$ | 143.95±1.81$^{d}$ | 23.73±0.36$^{d}$ | 56.01±3.61$^{ab}$ |

Means (n=6 ±SD) with the same letters in the same column are not significantly different (P<0.05); HCD, high-carbohydrate diet; WF, wheat flour; FMF, finger millet flour; TC, total cholesterol; TG, triglycerides; LDL-c, low density lipoprotein; VLDL-c, very low-density lipoprotein; HDL-c, high density lipoprotein

**TABLE 8. Effect of substituting wheat flour with finger millet flourin obese rats' diets on serum antioxidant biomarkers.**

| Types of diets | MDA (nmol/ml) | GPX (U/ml) |
|---|---|---|
| Normal | 21.60±2.33$^{d}$ | 65.983.25±$^{a}$ |
| HCD | 40.58±2.09$^{a}$ | 85.270.99±$^{e}$ |
| HCD with 100% WF | 33.86±1.31$^{b}$ | 86.00±1.37$^{a}$ |
| HCD with 50% WF and 50% FMF | 29.392.15±$^{c}$ | 81.481.69±$^{d}$ |
| HCD with 30% WF and 70% FMF | 29.20±١.03$^{c}$ | 77.191.07±$^{c}$ |
| HCD with 100% FMF | 23.75±1.66$^{d}$ | 73.511.42±$^{b}$ |

Means (n=6 ±SD) with the same letters in the same column are not significantly different (P<0.05); HC, high-carbohydrate diet; WF, wheat flour; FMF, finger millet flour; MDA, malondialdehyde; GPX, glutathione peroxidase

Circulating leptin appears to be one of the best biological markers of obesity, and hyperleptinemia is closely associated with several metabolic risk factors related to insulin resistance in the diabetes syndrome (Velasquez et al., 2001). Leptin is the satiety hormone, providing negative feedback to the hypothalamus to control appetite and energy expenditure (Feng et al., 2013).The levels of insulin, leptin, and cortisol hormones were significantly higher in rats fed a HCD than those fed a control diet (Table 9). Several authors have suggested that obesity is also associated with a greater rate of cortisol metabolic removal (Rask et al., 2002; Morais et al., 2019). Additionally, leptin and insulin were highest in obese adolescents (Albers et al., 2014). When WF was substituted with FMF, insulin, leptin, and cortisol hormones significantly decreased compared to the HCD and the HCD supplemented with 100% WF. However, there was no significant difference (P>0.05) in the levels of all hormones between groups fed a diet containing 50 or 70% FMF.Finicelli et al. (2019) reported that polyphenol intake has been shown to alleviate obesity and associated metabolic alterations such as insulin resistance, hepatic steatosis, and cardiovascular complications.

*Sensory evaluation of snacks made with FMF*

The quality of food products is determined by physical properties, chemical composition, the level of contaminants, and sensory attributes. A product is evaluated using sensory methods to quickly determine if there are noticeable differences in flavor, texture, and appearance(Mihafu et al., 2020).As shown in Table 10, the addition of FMF to snacks as a substitute for wheat flour improved all sensory attributes including color, aroma, taste, crispness, and acceptability. The improvement was more pronounced in the color and taste of the resultant snacks (P<0.05).The snacks are characterized by a more appetizing flavor, a desirable golden color, and a crisp and crunchy texture. However, there was a feeling of roughness throughout the chewing process because whole grain flour made from millet seeds was used, and this feeling increased more severe as the replacement rate increased.

**TABLE 9. Effect of substituting wheat flour with finger millet flourin obese rats' diets on insulin,leptin and cortisol hormones levels of obese rats.**

| **Types of diets** | Insulin (miu/l) | Leptin (ng/ml) | Cortisol(ng/ml) |
|---|---|---|---|
| Normal | 48.79±1.32$^{e}$ | 11.37±0.13$^{e}$ | 201.60±2.03$^{e}$ |
| HCD | 66.71±1.44$^{a}$ | 13.58±0.25$^{a}$ | 225.71±1.38$^{a}$ |
| HCD with 100% WF | 62.14±1.20$^{b}$ | 13.02±0.16$^{b}$ | 219.92±1.63$^{b}$ |
| HCD with 50% WF and 50% FMF | 57.16±2.57$^{c}$ | 12.81±0.08$^{b}$ | 215.23±0.92$^{c}$ |
| HCD with 30% WF and 70% FMF | 53.78±1.67$^{d}$ | 12.51±0.10$^{c}$ | 213.83±2.20$^{c}$ |
| HCD with 100% FMF | 53.57±2.29$^{d}$ | 12.10±0.11$^{d}$ | 208.30±2.62$^{d}$ |

Means (n=6 ±SD) with the same letters in the same column are not significantly different (P<0.05); HCD, high-carbohydrate diet; WF, wheat flour; FMF, finger millet flour

**TABLE 10. Sensory evaluation of snacks made with different percentage of wheat flour and/or finger millet flour.**

| **Sensory attributes** | **Snacks treatments** | | | |
|---|---|---|---|---|
| | **Control** | **T1** | **T2** | **T3** |
| Aroma | 8.00±.00$^{c}$ | 8.50±.57$^{b}$ | 9.00±0.00$^{a}$ | 9.00±.00$^{a}$ |
| Color | 8.00±0.81$^{b}$ | 8.50±.57$^{ab}$ | 9.00±0.00$^{a}$ | 9.00±0.00$^{a}$ |
| Crispness | 8.25±.50$^{b}$ | 8.50±0.57$^{ab}$ | 9.00±0.00$^{a}$ | 9.00±0.00$^{a}$ |
| Taste | 8.00±0.81$^{b}$ | 8.50±.57$^{b}$ | 9.00±.00$^{ab}$ | 9.00±0.00$^{a}$ |
| Appearance | 8.00±0.82$^{b}$ | 8.50±.57$^{ab}$ | 9.00±.00$^{a}$ | 9.00±.00$^{a}$ |
| General acceptability | 8.00±0.81$^{b}$ | 8.50±0.58$^{ab}$ | 9.00±.00$^{a}$ | 9.00±.00$^{a}$ |

Means (n=20±SD) with the same letters in the same column are not significantly different (P<0.05); WF, wheat flour;

## Conclusion

Millets have a high nutritional value because their grains are rich in proteins, minerals, mainlycalcium and iron, vitamins, and phytochemicals, including flavonoids and polyphenols, which have powerful antioxidant properties. Substituting wheat flour with finger millet whole grains flour helps to reduce weight and improve lipid profile, as well as ameliorate leptin and insulin resistance, which contribute to the anti-obesity activity. Additionally, it can help reduce malnutrition, anemia, and calcium deficiency.Technologically, the sensory attributes of the resulting snacks can be preserved even when wheat flour is substituted with millet flour at a ratio of 70 and 100 % FMF.

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