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# Dyslipidemia Protective Effect of Resistant Starch-Based Dough Meals on Diabetes-Induced Rats

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All the authors contributed to the study's conception, design and execution. Author AAO analyzed and interpreted the haematological parameters and differential counts of the experimented rats. Their fasting blood glucose parameters were interpreted by Authors TNF and OSI. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** The adoption of functional foods as healthy diet therapy for the management of metabolic disorders such as diabetes is in vogue in the present world where many are no longer consuming carbohydrate or starchy foods. This study investigated the effect of resistant starch-based dough meals on dyslipidemia in rats with streptozotocin (STZ)-induced diabetes.
**Place and Duration:** Functional Foods Laboratory and Animal House of Department of Biochemistry, Federal University of Technology, Akure, from July 2022 to February 2023.
**Methodology:** Flour samples were prepared by dispersing the flour into boiling water and turning and being allowed to cook for 15 min to obtain dough meals: PLD – (100% Plantain flour), used as a control sample; PLPRD – plantain flour: pigeon pea flour: rice bran flour (73.08:15.15:11.76); PSPRD – native plantain starch: pigeon pea flour: rice bran flour (73.08:15.15:11.76); AP1SPRD –

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acetylated plantain starch: pigeon pea flour: rice bran flour (73.08:15.15:11.76); AP2SPRD – acetylated plantain starch: pigeon pea flour: rice bran flour (70.67:18.15:11.18). The weight gained, food intake, haematological parameters, fasting blood glucose and lipid profile of the resistant starch-based dough meal were compared with the commercial flour product, CRD (100% Cerolina flour: wheat and soybean) and the DM (untreated high-fat diet–STZ-induced diabetic rats) and NC (Normal control rats).

**Results:** The weight gained and food intake ranged from 50.98±0.53 g to 180.12±1.07 g in NC and 33.98±1.64 g to 140.87±1.80 g in DM. The feed conversion ratio to the feed efficiency ratio was 0.28:0.25 and 4.15:3.53 in NC and DM respectively, which attributed to the food product to weight loss. The values of the haematological parameters (PCV, RBC, HB, MCV, MCH and MCHC) confirmed that the experimental rats had no trace of infection due to the generation of antibodies by WBC and differential counts (neutrophils, eosinophils, basophils, monocytes and lymphocytes). Rats fed with DM+AP1SPRD showed a decrease in low-density lipoprotein cholesterol (LDL-C), total cholesterol and triglyceride, and an increase in high-density lipoprotein cholesterol (HLDL-C).

**Conclusion:** A resistant starch-based dough meal was significant for the production of short-chain fatty acids with lots of benefits on health. The intervention of resistant starch-based dough meals, AP1SPRD and AP2SPRD used in this study, almost normalized metabolic profiles in the experimental rats, similar to the control group by reducing the total cholesterol, total triglycerides and LDL-C while increasing HDL-C.

*Keywords: Dough meal; resistant starch; dyslipidemia; diabetes; induced rats.*

## 1. INTRODUCTION

"Resistant starch-based food is a helpful solution for managing metabolic disease. It cannot be digested and absorbed in the small intestine, but it can be fermented by the microbial flora in the colon. Resistant starch is a dietary fibre and a preferred ingredient in low-moisture food products. Resistant starch has gained much attention due to its potential health benefits and functional properties. It has been linked to several physiological effects that are beneficial for overall health" [1].

"Hypertension is a medical condition that refers to high blood pressure, caused by prolonged abnormal pressure in the main arteries. It is a global issue, and the number of individuals aged 30-79 years with hypertension has doubled from 1990 to 2019. In 1990, there were 331 million women (95% credible interval 306-359) and 317 million men (292-344) with hypertension, whereas in 2019, this number increased to 626 million women (584-668) and 652 million men (604-698). Despite a stable global age-standardized prevalence, hypertension continues to be a growing concern" [2]. "Hypertension is one of the main comorbidities associated with dyslipidemia" [3]. "Dyslipidemia is characterized by high levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), or low levels of serum high-density lipoprotein cholesterol (HDL-C). It is a known risk factor for cardiovascular disease" [4].

Efforts are being made to develop diets high in antioxidants and fibre that prevent high blood cholesterol and associated diseases without adverse effects [5–7]. Functional food ingredients and conventional foods are consumed, providing benefits beyond basic nutrition [8]. Consuming resistant starch can provide several physiological benefits for humans, going beyond basic nutritional functions and reducing the risk of chronic diseases [8]. These benefits include decreasing intestinal transit time, lowering postprandial blood glucose and insulin levels, increasing satiety, and reducing total and/or low-density lipoprotein cholesterol concentrations [9–11].

Studies on rats have indicated that consuming resistant starch can have a positive effect on lipid metabolism. This is marked by a decrease in various measures of lipid metabolism, including total lipids and total cholesterol [12], as well as different lipoprotein fractions in the blood [13,14]. Replacing 5.4% of overall dietary carbohydrates with resistant starch significantly increases lipid oxidation after meals, reducing long-term fat build-up [15,16].

Research has shown that unripe bananas and plantains are good sources of resistant starch [17,18]. The improvement of resistant starch in glycaemic and insulinaemic responses and their special functions in managing metabolic disorders such as diabetes and hyperlipidemia, as well as in preventing cardiovascular and colonic diseases have been reported [18]. This

highlights the need for diets rich in functional-resistant starch. Unripe plantain is a great source of carbohydrates, dietary fibre, iron, potassium and vitamins [17], and regular intake of plantain can help inhibit diabetes, hypertension and anaemia due to its high resistant starch content, and low glycemic index properties [19]. “Pigeon pea (*Cajanus cajan*) is an underutilized legume that is rich in protein, minerals, phytonutrients, antioxidants, resistant starch, and globulin which is good for building haemoglobin” [20]. “Rice bran (*Oryza sativa* L) is a by-product of rice milling, rich in γ-oryzanol an antioxidant, tocotrienol and can reduce and modulate total cholesterol and low-density lipoprotein (bad cholesterol). Hence, tocotrienol is used to prevent the risk of heart disease in humans” [21]. “Evidence has shown that blending rice bran into animal formulations reduces cholesterol levels” [22,23].

Several studies have developed dough meals from plantain, soybeans and rice bran [24,25], but there is a dearth of information on investigating the dyslipidemia protective effect of resistant starch-based dough meals from native starch, acetylated starch of unripe plantain, pigeon pea and rice bran in STZ-induced diabetes rats.

## 2. MATERIALS AND METHODS

### 2.1 Sources of Materials

Freshly harvested matured unripe plantains (*Musa ABB*) were obtained from a local farm in Sabongida-Ora, Owan-West LGA, dried pigeon pea grains were purchased at Jattu market, Etsako-West LGA, and rice bran was obtained from Pemos foods, Aviele, Etsako-West LGA, Edo State. The food materials were authenticated at the Department of Crop Soil and Pest Management, the Federal University of Technology, Akure, Nigeria. The male Wistar rats were obtained from the animal house, Department of Biochemistry, School of Science, and certificate of research ethical clearance from Centre for Research and Development (CERAD), the Federal University of Technology, Akure, Nigeria (FUTA/ETH/23/96). The chemicals for the analyses were of analytical grade and obtained from Sigma-Aldrich, London, United Kingdom.

### 2.2 Preparation of Flour Samples

The unripe plantain was processed into flour by manual peeling with a stainless knife in water to prevent a browning reaction, sliced with a manual plantain slicer (Art No: HOZG2-1) to 0.5 cm thickness, washed, and oven-dried at 60 °C for 24 h using a Lifecare Medical Limited USA Technological Lab Oven Model no DHG9023,500 W:220V/50 Hz. After drying, it was cooled in a desiccator, milled using a Rico MG 1803 Mixer Grinder, 1000 W, India, and sieved through a 200 µm mesh sieve. The resulting flour was then packaged in airtight polythene bags for analysis. Similarly, the pigeon peas were handpicked, oven-dried at 60 °C for 24 h using the same oven, cooled in a desiccator, milled using the same grinder, and sieved through a 200 µm mesh sieve before being packaged in airtight polythene bags. rice bran, it was washed with distilled water, drained, oven-dried at 60 °C for 24 h using the same oven, cooled in a desiccator, milled using the same grinder, and sieved through a 200 µm mesh sieve. The resulting rice bran was then packaged in airtight polythene bags for analysis [26].

### 2.3 Acetylation of Plantain Starch

To isolate the native starch of the plantain, the method described by Oladebeye et al*.* [27] was followed with slight modifications. First, the plantains were blended and then sieved through muslin cloth. The supernatant was decanted and the leftover cake was dried at 60 °C for 24 h in a Lifecare Medical Limited, USA Technological Lab Oven Model no DHG9023, 500W:220V/50Hz. After cooling, the dried cake was milled using a Rico MG 1803 Mixer Grinder (1000 W, India) and then passed through a 200 µm mesh sieve. The resulting powder was then packaged in an airtight polythene bag in preparation for acetylation. For the acetylation process, 100 g (dwb) of the native starch was dispersed in 200 mL of acetic anhydride in a reaction flask and stirred at 500 rpm with a mechanical stirrer for 5 min. A known NaOH solution (50 g NaOH/100 g water) was added as a catalyst and the mixture was stirred for 1 h at 100 °C. To precipitate the starch, 100 mL of ethyl alcohol solution (96%) was added followed by filtration by suction with a Buchner filter funnel (Whatman filter No. 4). The residue was washed with ethyl alcohol and then with distilled water until most of the the the acetic anhydride was removed. The resulting paste produced by these washes was dried in the same oven at 40 °C for 16 h until it reached approximately 9% moisture content [28,29]. Thus, two acetylated plantain starches were prepared from the native plantain starch.

## 2.4 Preparation of Resistant Starch-Based Flour Blends

The flour samples of plantain, pigeon pea and rice bran were blended with 14 g/day protein and 5 g/day fibre (i.e. 25% of recommended daily intakes of adult requirements), using Optimal Mixture Design Methodology. Five blended samples were prepared and coded as PLF – (100% Plantain flour); PLPRF – Plantain flour: Pigeon pea flour: Rice bran flour (73.08:15.15:11.76); PSPRF – Native Plantain starch: Pigeon pea flour: Rice bran flour (73.08:15.15:11.76); AP1SPRF – Acetylated Plantain starch: Pigeon pea flour: Rice bran flour (73.08:15.15:11.76); AP2SPRF – Acetylated Plantain starch: Pigeon pea flour: Rice bran flour (70.67:18.15:11.18). A positive control sample, CRF – 100% Cerolina flour (wheat and soybean produced by More Foods Lagos, Nigeria) was used for comparison with the flour samples formulated [26].

## 2.5 Preparation of Dough Meals

The flour samples were dispersed into boiling water, stirred and allowed to cook for 15 min to obtain dough meals [30]. The dough meals were coded as PLD – (100% Plantain flour), and were used as a control sample; PLPRD – plantain flour: pigeon pea flour: rice bran flour (73.08:15.15:11.76); PSPRD – native plantain starch: pigeon pea flour: rice bran flour (73.08:15.15:11.76); AP1SPRD – acetylated plantain starch: pigeon pea flour: rice bran flour (73.08:15.15:11.76); AP2SPRD – acetylated plantain starch: pigeon pea flour: rice bran flour (70.67:18.15:11.18). CRD (100% Cerolina flour: wheat and soybean produced by More Foods Lagos, Nigeria).

## 2.6 *In-vivo* Studies

Sixty-four (64) male Wistar Albino rats of weights ranging between 180 and 220 g were housed in clean cages and allowed to acclimatize for 7 days with free access to their normal commercial diets and water ad libitum (Ethical clearance, FUTA/ETH/23/96 of the Federal University of Technology, Akure, Nigeria). The animals were divided into eight groups of eight rats per cage. The animals were weighed three times a week and received the diet and water ad libitum. They were fed for 14 days to attain diet-induced obesity with a formulation of high-fat diets (HFD) in g/100g: skimmed milk (50 g), lard (25 g), cellulose (5 g), corn starch (15 g) and premix (5 g) [31].

## 2.7 Induction of Diabetes

The HFD-fed animals were subjected to an overnight fast prior to induction of diabetes. Freshly prepared Streptozotocin (STZ) in citrate buffer (0.01 M, pH 4.5) was administered intraperitoneally at a single dose of 35 mg/kg body weight. The blood glucose level was checked 72 h after induction with STZ via tail vein puncture, using an automatic auto-analyzer (ACCU-CHECK® Active Roche Diabetes Care GmbH Sandhofer Strasse 116 68305 Mannheim, Germany). Rats with blood sugar levels of ≥ 200 mg/dL after 72 h were considered to be diabetic and were used in this study, while non-diabetic animals that served as control received 1 mL citrate buffer intraperitoneally. The diabetic and the normal rats were then grouped.

### 2.7.1 Grouping and feeding of experimental diets on rats

The grouping was done in this order: Group 1: normal control rats (NC); Group 2: untreated high-fat diet–streptozotocin (STZ)-induced diabetic rats; Group 3: high-fat diet–streptozotocin-induced diabetic rats treated with 100% Cerolina dough meal (DM+CRD); Group 4: high-fat diet–streptozotocin-induced diabetic rats treated with 100% plantain dough meal (DM+PLD); Group 5: high-fat diet–streptozotocin-induced diabetic rats treated with plantain flour: pigeon pea flour: rice bran dough meal (73.08:15.15:11.76) (DM+PLPRD); Group 6: high-fat diet–streptozotocin-induced diabetic rats treated with native plantain starch: Pigeon pea flour: rice bran dough meal (73.08:15.15:11.76) (DM+PSPRD); Group 7: high fat-diet–streptozotocin-induced diabetic rats treated with acetylated plantain starch: pigeon pea flour: rice bran dough meal (73.08:15.15:11.76) (DM+AP1SPRD); and Group 8: high-fat diet–streptozotocin-induced diabetic rats treated with acetylated plantain starch: pigeon pea flour: rice bran dough meal (70.67:18.15:11.18) (DM+AP2SPRD). They were fed for 14 days and their blood sugar was monitored and recorded at 2-day intervals. Food consumption by the rats was determined by the food remnants method with the feeder being weighed before and after diet consumption. The animals were weighed three times a week and physical evaluation was performed by aligning one animal from each

group on the bench and measuring its length with a measuring tape.

#### 2.7.2 Serum collection

The animals were fasted overnight before being sacrificed by cervical dislocation. Blood samples were taken from the heart and 0.2 mL of blood was in EDTA coated for haematological studies and 1.0 mL of blood was dispensed in a plain sample tube, allowed to stand for 30 min at room temperature to clot and centrifuged to harvest serum. The sera obtained in each were aliquoted and stored at 20 $^{o}$C prior to analysis.

#### 2.7.3 Haematological parameters

Haematological parameters were analyzed by an automated haematologic analyzer (Sysmex KX-21, Sysmex Corporation, Japan). These are red blood cell (RBC), haemoglobin (Hb) concentration, packed cell volume (PCV), the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated from values obtained from PCV, RBC, and HBC content, then the white blood cell (WBC) count, and percentages of differential leukocyte count (lymphocytes (LYM), neutrophil (NEU), monocytes (MONO), basophils (BASO) and eosinophil (EOS) [32].

#### 2.7.4 Serum lipid profiles

The lipid profiles investigated were total cholesterol, total glycerides, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). Serum biochemical investigations were done using Span and Tulip diagnostics kits as per the manufacturer's guidelines using a UV-visible double-beam spectrophotometer. LDL and HDL cholesterol were calculated according to Friedeward's equation [33]. The electrolytes were estimated by using a flame photometer according to standard procedures [32].

### 2.8 Statistical Analysis

The statistical analysis of the data obtained was carried out with IBM SPSS 26.0 software for mean comparison, using Duncan's least significant test and one-way analysis of variance (ANOVA) at a 5% significance level. Graphpad statistical package (version 8.0.2) was used to analyze all the data and the significance level was accepted at $p < 05$ following Tukey's post hoc test using one-way analysis of variance (ANOVA). Results are expressed as mean ± standard error of the mean (SEM) of all animals per group in the lipid profile results.

## 3. RESULTS AND DISCUSSION

### 3.1 Food Intake and Body Weight

Table 1 depicts the food intake and changes in the body weight of the experimented rats. The weight gained and food intake range from 50.98±0.53 g and 180.12±1.07 g in normal control rats to 33.98±1.64 g and 140.87±1.80 g in the untreated HFD-STZ rats (DM) respectively. While the experimental samples show the same trend of increment in the body weight, it is however lower than the weight of the normal control rat. Unripe plantains and pigeon peas are loaded with resistant starch content and the rice bran fibre content helps to delay the stomach being full for a longer period thereby aiding weight loss [34,35]. The food conversion ratio is a mathematical representation of the quantities of feed provided to the weight gained by consuming it. The results show that the feed conversion ratio is higher than the feed efficiency ratios, 0.25±0.00 to 0.28 ±0.00 and 3.53±0.04 to 4.15±0.25 respectively, indicating that the food product can be attributed to weight loss. The lower values of the feed conversion ratio to the feed efficiency ratio help a farmer to know how to maximize the profit to provide the best indicator of the efficiency of a feeding strategy [36]. Resistant starch-based meal has major benefits such as dietary resistance to prevent overweight and obesity because they are not absorbed in the small intestine but are delayed and fermented in the colon [37,38].

### 3.2 Haematological Parameters

Table 2 shows the results of the haematology parameters, which indicate the nutritional status of rats fed with experimental resistant starch-based dough meals. The experimental resistant starch-based dough meal greatly improves the PCV, RBC, HB and MCHC compared with the normal control rats and the very low ranges observed in the untreated STZ-induced diabetic rat. This implies an amelioration of the transportation of oxygen and absorbed nutrients resulting in the primary and secondary rare disorders in which the bone marrow produces an abnormally large amount of blood cells [39].

**Table 1. Food intake and changes in body weight of experimented animals**

| Sample | Weight Gained (g) | Food Intake (g) | Food Efficiency Ratio | Food Conversion Ratio |
|---|---|---|---|---|
| **NC** | $50.98^{a}\pm0.53$ | $180.12^{a}\pm1.07$ | $0.28^{a}\pm0.00$ | $3.53^{e}\pm0.04$ |
| **DM** | $33.98^{e}\pm1.64$ | $140.87^{g}\pm1.80$ | $0.24^{c}\pm0.01$ | $4.15^{a}\pm0.25$ |
| **DM+CRD** | $45.27^{b}\pm0.02$ | $178.57^{b}\pm0.21$ | $0.25^{c}\pm0.01$ | $3.94^{b}\pm0.01$ |
| **DM+PLD** | $40.60^{d}\pm0.20$ | $150.80^{f}\pm0.10$ | $0.27^{b}\pm0.00$ | $3.71^{c}\pm0.02$ |
| **DM+PLPRD** | $40.50^{d}\pm0.10$ | $164.43^{d}\pm0.12$ | $0.25^{c}\pm0.00$ | $4.06^{ab}\pm0.01$ |
| **DM+PSPRD** | $44.04^{c}\pm0.02$ | $160.60^{e}\pm0.20$ | $0.27^{b}\pm0.00$ | $3.65^{cd}\pm0.01$ |
| **DM+AP1SPRD** | $44.24^{bc}\pm0.02$ | $180.40^{a}\pm0.20$ | $0.25^{c}\pm0.00$ | $4.08^{ab}\pm0.00$ |
| **DM+AP2SPRD** | $43.40^{c}\pm0.20$ | $176.20^{c}\pm0.20$ | $0.25^{c}\pm0.00$ | $4.06^{ab}\pm0.02$ |

*Mean±SD in the same column with different superscripts are significantly different at a 5% level. Mean separation is done by the Duncan Multiple Range Test. NC - Normal Control Rats; DM -Untreated high-fat diet–streptozotocin (STZ)-induced diabetic rats (STZ); DM+CRD - High-fat diet–streptozotocin-induced diabetic rats treated with 100% Cerolina dough meal; DM+PLD - High-fat diet–streptozotocin-induced diabetic rats treated with 100% plantain dough meal; DM+PLPRD - High-fat diet–streptozotocin-induced diabetic rats treated with Plantain flour: Pigeon pea flour: Rice bran dough meal (73.08:15.15:11.76); DM + PSPRD - High-fat diet–streptozotocin-induced diabetic rats treated with Native Plantain starch: Pigeon pea flour: Rice bran dough meal (73.08:15.15:11.76); DM+AP1SPRD - High fat-diet–streptozotocin-induced diabetic rats treated with Acetylated Plantain starch: Pigeon pea flour: Rice bran dough meal (73.08:15.15:11.76); DM+AP2SPRD - High-fat diet–streptozotocin-induced diabetic rats treated with Acetylated Plantain starch: Pigeon pea flour: Rice bran dough meal (70.67:18.15:11.18).*

**Table 2. Haematological parameters of rats fed with dough meal samples from the blends**

| Sample | PCV (%) | WBC× $10^3$ ($mm^{-3}$) | RBC× $10^3$ ($mm^{-3}$) | HB (g/dL) | MCHC (g/dL) | MCH (*p*g) | MCV (*f*L) |
|---|---|---|---|---|---|---|---|
| Normal Range | 32.60-46.20 | 3.70-5.80 | 6.10-8.50 | 11.80-16.20 | 32.70-36.20 | 17.70-20.00 | 5.10-10.20 |
| NC | $37.00^{ab}\pm1.41$ | $4.05^{ab}\pm0.71$ | $3.75^{b}\pm0.21$ | $14.45^{a}\pm1.63$ | $39.17^{a}\pm5.89$ | $38.72^{a}\pm6.53$ | $9.87^{ab}\pm0.18$ |
| DM | $31.50^{d}\pm0.71$ | $3.33^{b}\pm0.04$ | $3.05^{c}\pm0.07$ | $10.20^{c}\pm0.14$ | $32.39^{c}\pm0.28$ | $33.45^{ab}\pm0.31$ | $10.33^{a}\pm0.01$ |
| DM +CRD | $35.50^{ab}\pm0.71$ | $4.77^{a}\pm0.04$ | $4.60^{a}\pm0.28$ | $12.80^{b}\pm0.14$ | $36.07^{abc}\pm1.12$ | $27.89^{b}\pm2.02$ | $7.73^{d}\pm0.32$ |
| DM +PLD | $32.00^{cd}\pm0.00$ | $4.95^{a}\pm1.06$ | $3.60^{b}\pm0.28$ | $10.60^{c}\pm0.28$ | $33.13^{bc}\pm0.88$ | $29.57^{b}\pm3.11$ | $8.92^{bc}\pm0.70$ |
| DM +PLPRD | $34.50^{bc}\pm0.71$ | $4.65^{a}\pm0.21$ | $4.70^{a}\pm0.14$ | $13.26^{ab}\pm0.04$ | $38.43^{ab}\pm0.89$ | $28.22^{b}\pm0.93$ | $7.34^{d}\pm0.07$ |
| DM +PSPRD | $35.00^{b}\pm1.41$ | $4.50^{a}\pm0.14$ | $3.70^{b}\pm0.14$ | $12.40^{b}\pm0.28$ | $35.44^{abc}\pm0.62$ | $33.56^{ab}\pm2.04$ | $9.48^{ab}\pm0.74$ |
| DM +AP1SPRD | $38.00^{a}\pm1.41$ | $4.75^{a}\pm0.71$ | $4.74^{a}\pm0.16$ | $13.15^{ab}\pm0.01$ | $34.63^{abc}\pm1.33$ | $27.76^{b}\pm0.88$ | $8.03^{cd}\pm0.56$ |
| DM +AP2SPRD | $37.00^{ab}\pm1.41$ | $4.65^{a}\pm0.21$ | $4.80^{a}\pm0.14$ | $12.95^{b}\pm0.01$ | $35.03^{abc}\pm1.38$ | $26.99^{b}\pm0.82$ | $7.71^{d}\pm0.07$ |

*Mean±SD in the same column with different superscripts are significantly different at a 5% level. Mean separation is done by the Duncan Multiple Range Test*

**Table 3. Haematological differential count parameters of rats fed with dough meal samples from the blends**

| Sample | Neutrophils (%) | Lymphocytes (%) | Monocytes (%) | Eosinophils (%) | Basophils (%) |
|---|---|---|---|---|---|
| Normal Range | 23.40-40.50 | 54.90-65.30 | 0.00-7.70 | 0.00-3.40 | 0.00-1.00 |
| NC | 35.00$^{c}$±1.41 | 44.50$^{c}$±3.54 | 2.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| DM | 43.00$^{b}$±1.41 | 48.00$^{c}$±1.41 | 1.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| DM +CRD | 37.00$^{c}$±1.41 | 55.50$^{ab}$±2.12 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| DM +PLD | 41.00$^{b}$±1.41 | 54.00$^{b}$±1.41 | 2.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| DM +PLPRD | 37.00$^{c}$±1.41 | 54.00$^{b}$±2.83 | 2.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| DM +PSPRD | 35.00$^{c}$±1.41 | 57.00$^{ab}$±1.41 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| DM +AP1SPRD | 44.00$^{ab}$±1.41 | 60.45$^{a}$±0.04 | 2.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| DM +AP2SPRD | 47.50$^{a}$±2.12 | 57.65$^{ab}$±1.40 | 1.00±0.00 | 0.00±0.00 | 0.00±0.00 |

*Mean±SD in the same column with different superscripts are significantly different at a 5% level with. Mean separation is done by the Duncan Multiple Range Test*

The results of the PCV are all within the range except for the DM with 31.50±0.71%, which is lower than the normal range. A low level of the red blood cell count and/or haemoglobin is a symptom of anaemia, and this is subject to the red blood cell indices values (MCH, MCV and MCHC). The rate of increase in haemoglobin can be used to monitor the treatment of anaemia and determine the amount of blood required for transfusion [40]. A normal MCV is observed in all the experimental rats (77.10±0.07 to 98.7±0.18 *f*L), which indicates the presence of a normal range of red blood cells (RBC) (normocytic) while a high MCV is observed in the DM untreated high-fat diet streptozotocin (STZ) induced diabetic rat (103.30±0.01 *f*L) indicates the presence of large red blood cells (RBC) (macrocytosis), which is an indication of folate deficiency, liver disease, hypothyroidism and haemolytic anaemic. A normal (normochromic) MCHC indicates that the oxygen-carrying capacity of the red blood cells is normal [40].

## 3.3 Haematological Differential Count Parameters

Table 3 depicts the result of the white blood cells (WBC) or leukocytes comprise of five types of differential count cells: the neutrophils, the eosinophils, the basophils, the monocytes and the lymphocytes. WBC differential count of animals fed on experimental diets. The WBC and its differential counts play a very vital role in fighting infectious diseases and also preserve the development of antibodies for immunity [41].

The DM (Untreated High-fat diet- streptozotocin (STZ) induced diabetic rat) had its WBC to be very low (3.33±0.04×$10^{3}$ $mm^{-3}$), which is an indication of lymphoma, immune suppression-related diseases (HIV/AIDS), diseases of the liver or spleen and malnutrition [40] while the NC and other animals fed with the experimental resistant starch-based dough meals have their respective WBC within the normal range (4.05±0.71×$10^{3}$–4.95±1.06×$10^{3}$ $mm^{-3}$). This implies that DM animal with low WBC is exposed to a great risk of disease infection while the other animals treated with experimental resistant starch-based dough meals with high WBC count can generate antibodies in the course of a form of endocytosis in which a cell incorporates a particle by extending pseudopodia and drawing the particle into a vacuole of its cytoplasm to have high resistance to diseases [42]. The results also show that all the experimental rats fed on resistant starch-based dough meals are not infected with eosinophils and basophils with 0.00% as their values and neutrophils, lymphocytes and monocytes are within the acceptable range.

## 3.4 Fasting Blood Glucose

The results of the fasting blood glucose (FBG) and the lipid profile of the DM (untreated high-fat diet- streptozotocin (STZ) induced diabetic rats, the NC (normal control rat) and the treated experimental sample STZ induced diabetic rats are depicted in Figs. 1-5 respectively. The fasting blood glucose levels of the STZ-induced diabetic experimental rats are significantly higher than the NC rats at the beginning of the experiments, meanwhile, the NC rats remain steadily stable on the same range of fasting blood levels throughout the experimental days, the DM rats remain steadily high in blood glucose level during the experiment periods (340–380 mg/dL). The fasting blood glucose levels of the STZ-induced diabetes experimental rats fed with the resistant starch-based dough meals decrease within the experimental days [43].

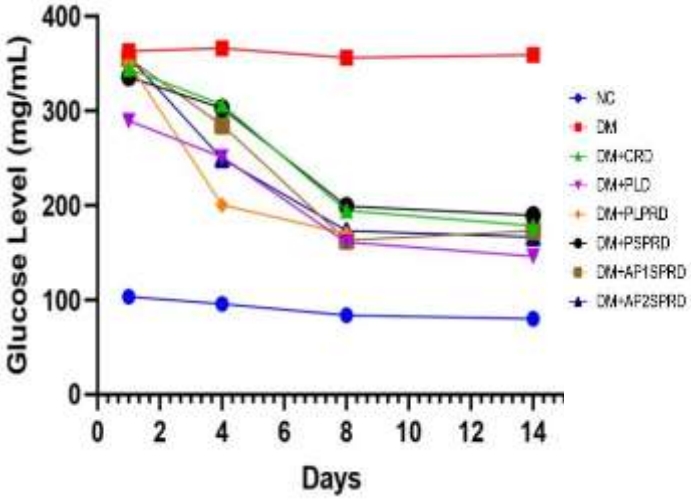


**Fig. 1. Glucose level per day for 14 days**

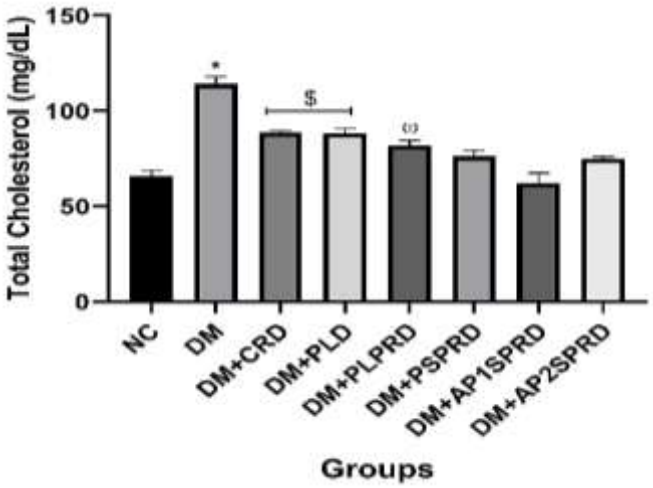


**Fig. 2. Total cholesterol**

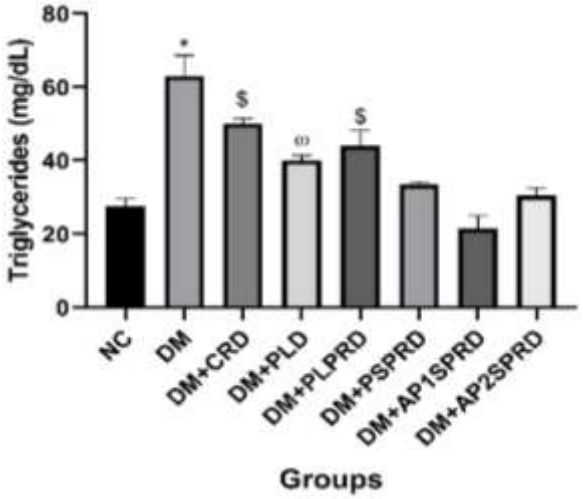


**Fig. 3. Total Triglycerides**

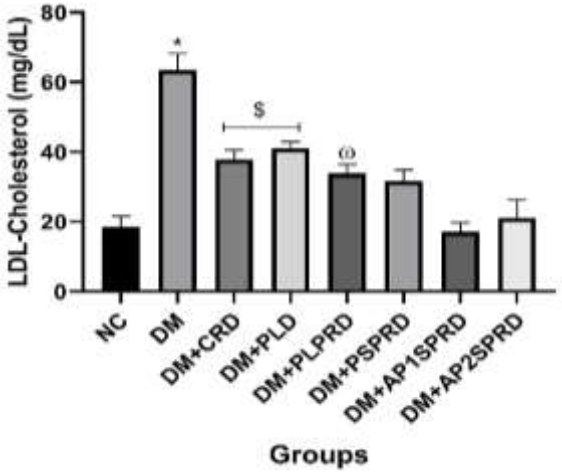


**Fig. 4. Low-Density Lipoprotein Concentration**

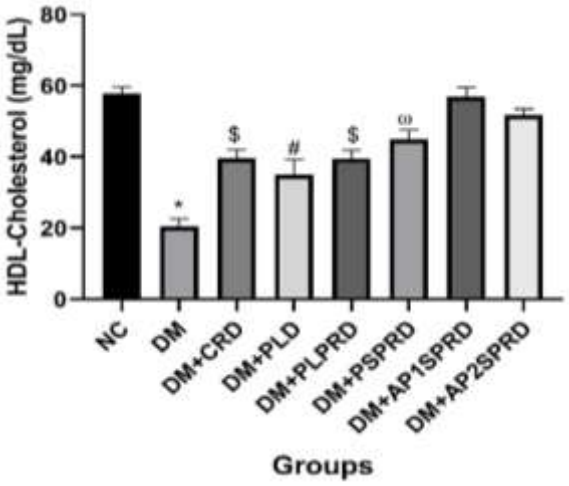


**Fig. 5. High-density lipoprotein concentration**

Health disorders in the human body can be caused by either increase or decrease in lipid levels. The health disorder can be through a high level of triglyceride and low-density lipoprotein (LDL) or an increase in both high and low levels of high-density lipoprotein (HDL). HDL is good cholesterol, which helps in removing bad cholesterol from the body [44]. The Low cholesterol level observed with the NC and another experimental STZ-induced rat (60-80 mg/dL) is within the mean reference of 60-100mg/dL [32] whereas the DM has a higher cholesterol level than the mean reference range with 120mg/dL. The Low triglyceride level observed with the NC and other treated and untreated experimental-induced rats (30-64 mg/dL) is within the mean reference of 32-78 mg/dL [32]. The Low-density lipoprotein level observed shows that the NC and DM+AP1SPRD are lower than 20 mg/dL whereas another experimental STZ-induced rat (20-40 mg/dL) is within the mean reference of 15-35 mg/dL [32] while the DM has a higher LDL than the mean reference range of 60 mg/dL. The increased level of HDL observed with the NC and another experimental STZ-induced rat (48-58 mg/dL) is within the mean reference of 36-54 mg/dL while the DM has a very low HDL level than the mean reference range of 18 mg/dL. The decrease in total triglycerides, total cholesterol, and low-density lipoprotein (LDL) with an increased level of high-density lipoprotein is observed in the experimented STZ-induced diabetes rats fed with resistant starch-based dough meals in this study. Thus, NC, AP1SPRD and AP2SPRD can be considered beneficial to the diabetic patients.

Cholesterol is a fatty substance that circulates in the blood. When the levels of cholesterol are high, there is a greater risk of developing cardiovascular diseases, such as heart disease or stroke. However, the good news is that cholesterol can be absorbed by high-density lipoprotein (HDL) and carried to the liver, where it can be flushed out of the body. Therefore, it is important to control the levels of total glyceride, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol to prevent such diseases and ensure better health outcomes for individuals. This has become a major goal for treatments and prevention in recent times [45–47].

## 4. CONCLUSION

A resistant starch-based meal is significant for the production of short-chain fatty acids, which have many health benefits. The study found that a meal made with resistant starch-based dough affects lipid metabolism by reducing total cholesterol, total triglyceride, and low-density lipoprotein while increasing high-density lipoprotein. The meal almost restored the experimental rats to the same health levels as the normal control rats. The experimental diets in this study did not affect weight reduction in NC and other experimental rats fed on the resistant starch-based meal but had little effect on the DM untreated rat. The haematological parameters recorded no trace of infection in all the experimented rats, and the WBC count was able to generate antibodies to this effect. The PCV, RBC, HB, MCV, MCH, and MCHC all indicated that the oxygen-carrying capacity of the red blood cells is normal. The interventions of samples AP1SPRD and AP2SPRD may serve as dietary functional meals for the management of metabolic diseases such as diabetes.

## ETHICAL APPROVAL

The male Wistar rats were obtained from the animal house, Department of Biochemistry, School of Science, and certificate of research ethical clearance from Centre for Research and Development (CERAD), the Federal University of Technology, Akure, Nigeria (FUTA/ETH/23/96).

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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