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Original article

Impact of resistant starch: Absorption of dietary minerals, glycemic index and oxidative stress in healthy rats

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SUMMARY

Background & aims: Resistant starch (RS) is a prebiotic fiber that has been scientifically shown to control the development of obesity. Prebiotic role of RS has also seen to be very important as it helps gut bacteria to regulate fermentation and fatty acid production. This study aimed to check the different levels of RS on glycemic index, oxidative stress and mineral absorption rate in healthy rat models. To evaluate these objectives, the trial was conducted for 40 days of follow up; 10 days were the adjustment period and the collection period over 30 days.

Methods: Thirty-six healthy female Wistar rats were divided into 4 groups of (9 animals each) NC (Normal Control: without resistant starch), $RS_{0.20}$ (resistant starch: 0.20 g/kg body weight), $RS_{0.30}$ (resistant starch: 0.30 g/kg body weight), $RS_{0.40}$ (resistant starch: 0.40 g/kg body weight). All the diets were *isocaloric* and *isonitroginous*.

Results: The impact of different levels of RS on the dry-matter intake (DMI) presented statistically significant results ($p \leq 0.05$): DMI was reduced in RS $_{(0.02)}$ fed rats as compared to NC rats in first 3 weeks; and after 4th and 5th weeks, there was a DMI reduction of 28% in RS $_{(0.04)}$ fed rats. Moreover, there was no significant increase in the nutrient intake in all RS diets. The dry-matter (DM) digestibility was statistically significantly ($P \leq 0 \cdot 05$), which increased in all rats fed with different level of RS. The weight loss showed statistically significant results: RS $_{(0.04)}$ exhibited 19 g reduction in weight as compared with NC rats. Significant increase was observed in total oxidant status (TOS), in all the RS fed rats when compared with NC rats. The levels of Mg, Ca, Fe and Zn were shown to be decrease in feces analysis, which proves their better absorbance in gut. Statistically significant increase was observed in antioxidant capacity, whereas significant decrease was observed in the total weight of the animals, showing the role of RS in controlling obesity.

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Conclusions: Overall, significant results were found in all dosage level of RS but long term administration of the higher dosage level ($RS_{0.40}$) may need to be studied for enhanced results. RS can help improve insulin sensitivity in overweight adults.

Abbreviations

AH-HAS	Acid-hydrolysed high amylose corn starch
ANOVA	Analysis of variance
Ca	Calcium
CHO	Carbohydrates
CKD	Chronic kidney disease
CRD	Completely randomized design
CF	Crude fiber
CP	Crude protein
DM	Dry-matter
DMI	Dry-matter intake
EE	Ether extract
FCR	Feed conversion ratio
C7H14O2	Heptanoic acid
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
Fe	Iron
Mg	Magnesium
MBG	Mean blood glucose
MC	Moisture contents
NBS	native banana starch
NFE	Nitrogen free extract
NC	Normal Control: without resistant starch
RH	Relative humidity
RS	Resistant starch
$RS_{0.20}$	Resistant starch: 0.20 g/kg body weight
$RS_{0.30}$	Resistant starch: 0.30g/kg body weight
$RS_{0.40}$	Resistant starch: 0.40g/kg body weight
TBARS	Thiobarbituric acid reactive substances
TAC	Total antioxidant capacity
TOS	Total oxidant status
T2D	Type-2 diabetes
Zn	Zinc

1. Introduction

Dietary adjustments can prevent or mitigate many chronic health issues and improve health status of individuals. The increasing popularity of functional foods among health conscious customers are an emerging field in food and nutrition [1]. The objectives of functional foods include (i) the improvement of general health conditions, *i.e.* the role of pre- and probiotics in gut health, (ii) delaying the risk of many disease and (iii) the treatment of many illnesses [2].

Resistant starch (RS) is defined as the sum of starch and its degradation products that are not absorbed in the small intestine of healthy individuals. Much scientific and commercial interest has been given to RS as an explicit substrate of gut microbiota. The factors for this resistance in absorption might be caused due to some reasons like limitations in physical availability, by the structure of native starch which is granular, by the rate of retrogradation or by physical adaptation of starch contents [3,4]. Resistant starch is a complex carbohydrate made up of glucose polymers that is classified into many types but the most renown is only five: (i) physically inaccessible starches known as RS_1, (ii) granular starches with B- or C-polymorph, which are called RS_2 (the subject under study here), (iii) retrograded starches named as RS_3, (iv) chemically modified or commercial starches are RS_4, and (v) amylose-lipid complexes called as RS_5 [5].

Dietary fiber is gaining more importance in nutraceutical, pharmaceutical and agri-food processing industries because it can change the consistency of food, its texture, its rheological behavior and sensory characteristics especially the end products, it plays a significant role in the improvement of lower cholesterol level, metabolic process, lower chronic heart diseases, control the blood sugar, reduce hypertension, reduce the risk of chronic diseases by regulating oxidative stress, maintain bowel health, reduce the risk of stroke and combat the digestive enzymes of human gastrointestinal tract [6–10]. In this study, the resistant starch diets are formulated to observe their potential on weight gain, nutrient intake, digestibility, absorption of minerals, oxidative stress and glycemic index in healthy rats.

2. Materials and methods

A total of 36 healthy Wistar albino female rats – with 75 ± 5 day of age and initial body weight 100–150 g – were derived from Colony of Laboratory Animals of Department of Physiology, Government College University Faisalabad, Punjab, Pakistan. Rats were divided randomly according to completely randomized design (CRD). Three replicate groups of each treatment was placed in separate metabolic cages, which contained 3 animals each, to calculate the faecal output and food intake. All rats were kept under controlled conditions of (28 ± 1 °C) and 45–55% relative humidity (RH) under 12-h light 12-h dark cycle for 5 weeks.

All the treatments to the animals were done according to the principles of Laboratory Animal Care after the approval of the Ethical Committee of Animals of the Government College University, Faisalabad, Punjab, Pakistan. The rats were given free access to standard *ad libitum* diet and water, and each one weighed weekly throughout the trail. In this study, *isocaloric* and *isonitrogenous* diet was formulated with Hi–maize 260 resistant starch provided by Rafhan foods, Faisalabad, Punjab, Pakistan. Each group of rats were named according to diet, *viz.* NC (normal control), $RS_{(0.20)}$ (resistant starch: 0.20 g/kg body weight), $RS_{(0.30)}$ (resistant starch: 0.30 g/kg body weight), and $RS_{(0.40)}$ (resistant starch: 0.40 g/kg body weight) (Fig. 1). Control group was fed with a basal rat diet designed from

Fig. 1. All treated group of rats according to diet.

93-M protocol (Table 1). After the 5 weeks of experimental trail, the rats were anesthetized by CO_2 asphyxiation before killing.

2.1. Chemical composition and nutrient intake

The samples of raw-materials were characterized for determination of carbohydrates (CHO), crude protein (CP), fat, crude fiber (CF), moisture contents (MC), dry-matter intake (DMI), ether extract (EE), ash content and nitrogen free extract (NFE) according to standard method of AOAC [11] with minor modifications (Table 2).

2.2. Faeces collection

In this study, fresh samples of faecal from each rat were daily collected in last 7 days and kept at low temperature (−20 °C). After that, each sample of fresh faecal was collected in sterile flask containers and immediately dissolved in 3 volumes with standard solution of enanthic acid or heptanoic acid ($C_7H_{14}O_2$) under controlled conditions for further analysis [12].

2.3. Nutrient digestibility and weight changes

Nutrient digestibility (Equation (1)) was measured by using the methods 985.29 and 991.43. All diets and feces were analyzed to calculate DM, CP, CF and EE [13]. Body weight and feed conversation ratio (Equation (2)) were estimated for five weeks throughout this study.

$$Nutrient\ Digestibilty\ (\%) = \frac{Nutrient\ Intake - Nutrient\ in\ feces}{Nutrient\ Intake} \times 100 \quad (1)$$

Table 1
Composition of experimental diet (g/kg) with nutritional facts.

Ingredients	NC	Kcal	$RS_{(0.20)}$	Kcal	$RS_{(0.30)}$	Kcal	$RS_{(0.40)}$	kcal
Casein	140	560	140	560	140	560	140	560
Dextrose	155	620	155	620	155	620	155	620
Corn Starch	465.692	1862.768	415.692	1662.76	365.69	1462.76	315.69	1262.76
RS	–	–	100	160	150	240	200	320
Cellulose	50	–	–	–	–	–	–	–
Sucrose	100	400	100	400	100	400	100	400
Corn oil	40	360	40	360	40	360	40	360
TBHQ	0.008	–	0.008	–	0.008	–	0.008	–
Salt Mix #210050	35	–	35	–	35	–	35	–
Vit. Mix #310025	10	–	10	–	10	–	10	–
L-cysteine	1.80	–	1.80	–	1.80	–	1.80	–
Choline Bitrate	2.50	–	2.50	–	2.50	–	2.50	–
Total	1000	3802.768	1000	3762.76	1000	3642.76	1000	3522.76
Kcal	–	3803	–	3763	–	3643	–	3523

NC = Normal control (without resistant starch); $RS_{(0.20)}$ = Resistant starch: 0.20 g/kg body weight; $RS_{(0.30)}$ = Resistant starch: 0.30 g/kg body weight); $RS_{(0.40)}$ = Resistant starch: 0.40 g/kg body weight; RS = Resistant starch; TBHQ = Tert-butylhydroquinone.

Table 2
Chemical composition of experimental diet.

Nutrient	Grams per kg				Kcal			
	NC	$RS_{(0.20)}$	$RS_{(0.30)}$	$RS_{(0.40)}$	NC	$RS_{(0.20)}$	$RS_{(0.30)}$	$RS_{(0.40)}$
CHO	721	671	621	571	2883	2683	2483	2283
CP	140	140	140	140	560	560	560	560
Fat	40	40	40	40	360	360	360	360
CF	50	100	150	200	–	160	240	320
MC	60	45	35	25	–	–	–	–
Ash content	278.2	298.2	269.8	266.5	–	–	–	–

NC = Normal control (without resistant starch); $RS_{(0.20)}$ = Resistant starch: 0.20 g/kg body weight; $RS_{(0.30)}$ = Resistant starch: 0.30 g/kg body weight); $RS_{(0.40)}$ = Resistant starch: 0.40 g/kg body weight; RS = Resistant starch; CHO = Carbohydrates; CP = Crude protein; Fat; CF = Crude fiber; MC = Moisture content; Ash content.

$$FCR = \frac{\text{Food intake (g)}}{\textit{weight gain (g)}} \quad (2)$$

2.4. Blood collection

The high quality blood samples were collected from jugular vein of rats according to a modification of the method as described by Parasuraman et al. [14]. In this study, blood collection from rats was done by two well-trained technicians as shown in Fig. 2. The neck of the rats was shaved around the thoracic region and its head was raised without escalating the submandibular gland. Subsequently, petroleum jelly was applied to reveal the jugular vein (blue in color) that was found near the pectoral muscle. The jugular vein was punctured with a 25G syringe (Becton Dickinson, Punjab, Lahore, Pakistan) and blood was drawn in vessels slowly to prevent collapse the vein. After collection of blood, finger pressure was applied immediately to stop bleeding. Blood vessels were stored at refrigeration temperature of 2–6 °C for further analysis.

2.5. Biochemical analysis

The biochemical analysis glycemic response, oxidative stress and mineral absorption were assessed in the Colony of Laboratory Animals of Department of Physiology, Government College University Faisalabad, Punjab, Pakistan. The glycemic response to each diet samples was calculated using a previously reported procedure in the research work of McCleary et al. [15]. Oxidative stress was estimated by thiobarbituric acid reactive substances (TBARS) assay in the liver [16] and concentrations of nitric oxide using the standard method of Arnaiz et al. [17]. The biomarkers for oxidative

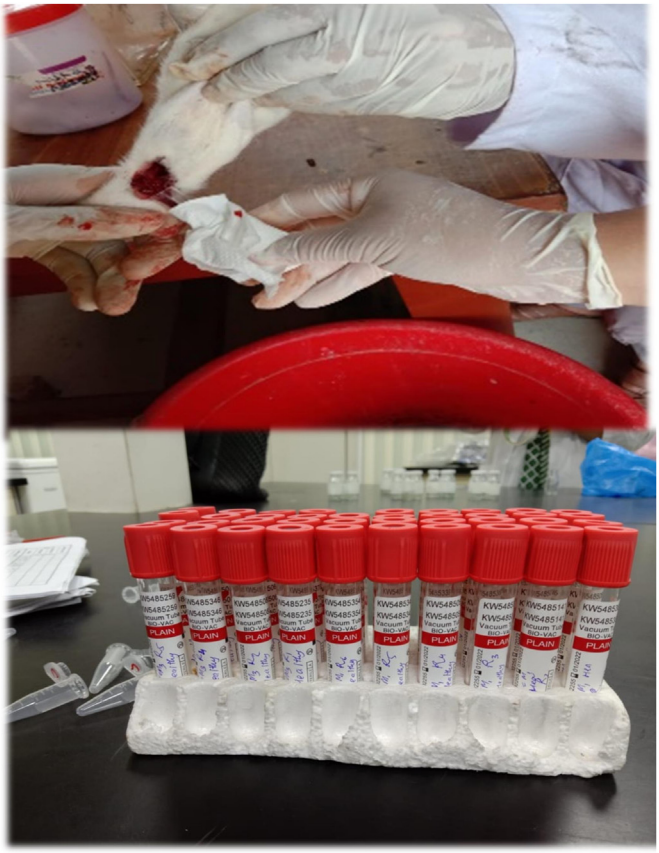

Fig. 2. Blood sample collection in rats.

stress were total antioxidant capacity (TAC) and total oxidant status (TOS) [18], which were analyzed by using the curve test. Furthermore, apparent mineral absorption was determined by feaces analysis.

Samples for analysis were prepared by wet digestion process [19] for inductively coupled plasma atomic emission spectroscopy (ICP-AES) [20]. In this process, 1 g feces sample was taken in a 100 ml beaker and subjected to wet digestion as described by Richards, [21]. Briefly, 10 ml of nitric acid was mixed with the sample, and the prepared solution was heated at (100–120) °C using the hot plate until the 2–3 ml solution remained. Then, 5 ml of perchloric acid was mixed and again heated at 100–120 °C until the solution became colorless. In the end, the left over solution was reconstituted with deionized water (25 ml), filtered and stored in clean screw capped containers at room temperature until mineral analysis. The estimation for zinc, magnesium, potassium and iron were concluded on ICP-AES. The final concentration was determined using a standard curve plotted from standards for each mineral against digested deionized water as a blank.

2.6. Statistical analysis

The data obtained from all experiments were analyzed with a completely randomized design (CRD) using the Statistics Version 8.1, and the results expressed as mean value ± standard deviation. Data were subjected to 1-way analysis of variance (1-way ANOVA), followed by the Tukey test at 0.05% level of significance for the impact of resistant starch on biochemical parameters of rats.

3. Results

3.1. Dry-matter intake

In this research work, the *isocaloric* and *isonitrogenous* diets ($RS_{(0.20)}$: (resistant starch: 0.20 g/kg body weight); $RS_{(0.30)}$: (resistant starch: 0.30 g/kg body weight) and $RS_{(0.40)}$: (resistant starch: 0.40 g/kg body weight) were offered to healthy rats for 5 weeks to estimate the biochemical analysis. The impact of different levels of RS on the dry-matter intake (DMI) is presented in Table 3. The results showed that during the period of adjustment the dry-matter intake increased ($P \leq 0{\cdot}05$) in rats fed with $RS_{(0.20)}$, when compared with those of the normal control (NC). Meanwhile, DMI was significantly ($P \leq 0.05$) increased with RS, compared with NC. This trend persisted until the first two weeks ended. In the 3rd week, DMI profile had changed and remained higher ($P \leq 0{\cdot}05$) in the NC fed rats compared to RS fed rats. It has been observed that higher dose of resistant starch ($RS_{(0.40)}$) lowered the intake of feed. After the 4th and 5th week, an approximately 28% reduction was unfolded in $RS_{(0.40)}$ in comparison to NC. DMI finally reached 17.33 ± 0.09 g in NC, while 12.24 ± 0.25 g in $RS_{(0.20)}$, 12.21 ± 0.59 g in $RS_{(0.30)}$ and 10.08 ± 0.09 g in $RS_{(0.40)}$.

3.2. Total nutrient intake

The impact of different level of resistant starch (RS) on the total nutrient intake is presented in Table 4. In this study, the daily intake of crude protein (CP) was significantly different in all treatments [but $RS_{(0.20)}$] compared to NC, due to equal amount of casein protein in their diets. After one week, the intake of CP was significantly lower in the RS fed rats [except $RS_{(0.20)}$] than that in normal control (NC) ($P \leq 0{\cdot}05$).

All RS diets had a significant impact on the daily intake of crude fiber (CF). The intake of crude fiber (CF) was increased with increasing the level of RS in the diet of rats ($P \leq 0{\cdot}05$) throughout one-week study trail due to high amylose in feed composition. On average nutrient intake per day, the overall CF intake remain almost constant in each treatment diet during the trial due to specific amount of fiber in each diet.

After one week, the intake of ether extract (EE) and ash contents showed that there was no significant ($P \leq 0{\cdot}05$) difference in rats fed with NC and RS diets. Furthermore, the nitrogen free extract (NFE) in the rats fed the RS diets were significantly lower when compared with the NC diet ($P \leq 0{\cdot}05$). Moreover, the higher NFE (2.24 ± 0.15) was observed in the rats fed with $RS_{(0.30)}$ diet when compared to the other RS levels and NC diets throughout one-week study trail.

3.3. Digestibility

The effect of different levels of high maize resistant starch (RS) on digestibility of healthy rats are showed in Table 5. In this research work, the results revealed that the drymatter (DM) digestibility was significantly ($P \leq 0{\cdot}05$) increased in all rats fed with various levels of RS ($RS_{(0.20)}$, $RS_{(0.30)}$, $RS_{(0.40)}$). The maximum CP digestibility was observed in the treatment level $RS_{(0.30)}$ (95.01 ± 0.15) when compared to other treatment levels $RS_{(0.20)}$ and $RS_{(0.40)}$. Furthermore, crude fiber (CF) digestibility trend was significantly decreased in all levels of RS but $RS_{(0.20)}$, which showed its resistance in the hindgut ($P \leq 0{\cdot}05$). The lowest value for CF digestibility was 56.77 ± 0.18 in $RS_{(0.40)}$, and significantly ($P \leq 0{\cdot}05$) increase in values of ether extract (EE) digestibility was observed in all rats fed different levels of RS when compared with NC.

3.4. Weight changes

In this research work, weight changes were assessed weekly to check the impact of resistant starch (RS) diets on the female rats (Table 6). After administration of RS in healthy female rats, a 19 g reduction in the final weight was observed in rats fed $RS_{(0.40)}$, in particular when compared to a 2 g reduction in rats fed NC. The weight loss related to the amount of RS consumed in the diet. Feed conversion ratio (FCR) decreased in all rats fed with various

Table 3
Mean values and standard deviations of weekly dry-matter intake (g/kg) in healthy rats fed with different levels of resistant starch.

DMI	NC	$RS_{(0.20)}$	$RS_{(0.30)}$	$RS_{(0.40)}$
Adjustment week	13.75 ± 0.02^{d}	19.10 ± 0.21^{a}	16.56 ± 0.12^{c}	17.50 ± 0.25^{b}
After 1st week	13.82 ± 0.24^{c}	17.86 ± 0.14^{a}	17.92 ± 0.25^{a}	15.47 ± 0.36^{b}
After 2nd week	13.73 ± 0.36^{c}	16.11 ± 0.25^{a}	15.40 ± 0.04^{a}	14.62 ± 0.14^{b}
After 3rd week	15.17 ± 0.52^{a}	13.86 ± 0.34^{c}	14.88 ± 0.01^{b}	12.88 ± 0.47^{d}
After 4th week	17.76 ± 0.45^{a}	12.91 ± 0.33^{c}	13.15 ± 0.78^{b}	11.26 ± 0.58^{d}
After 5th week	17.33 ± 0.09^{a}	12.24 ± 0.25^{b}	12.21 ± 0.59^{b}	10.08 ± 0.09^{c}

DMI = Dry-matter intake; RS = Resistant starch; NC = Normal control (without resistant starch); $RS_{(0.20)}$ = Resistant starch: 0.20 g/kg body weight; $RS_{(0.30)}$ = Resistant starch: 0.30 g/kg body weight); $RS_{(0.40)}$ = Resistant starch: 0.40 g/kg body weight. $^{a-d}$Mean values between different treatments within a row with different superscript letters are significantly different ($p \leq 0.05$).

Table 4
Mean values and standard deviations of total nutrient intake (g/kg) in healthy rats fed with different levels of resistant starch.

Average total nutrient intake (g/kg) per day				
Parameters	NC	$RS_{(0.20)}$	$RS_{(0.30)}$	$RS_{(0.40)}$
CP	1.07 ± 0.19^{a}	1.15 ± 0.21^{a}	0.90 ± 0.18^{b}	0.78 ± 0.16^{c}
CF	0.11 ± 0.13^{c}	0.22 ± 0.14^{b}	0.28 ± 0.15^{b}	0.41 ± 0.15^{a}
EE	0.09 ± 0.11^{a}	0.09 ± 0.11^{a}	0.08 ± 0.11^{a}	0.08 ± 0.11^{a}
Ash content	0.62 ± 0.17^{a}	0.61 ± 0.17^{a}	0.59 ± 0.17^{a}	0.61 ± 0.17^{a}
NFE	0.39 ± 0.15^{a}	0.21 ± 0.12^{a}	0.32 ± 0.15^{a}	0.20 ± 0.12^{a}
Average total nutrient intake (g/kg) per week				
Parameters	NC	$RS_{(0.20)}$	$RS_{(0.30)}$	$RS_{(0.40)}$
CP	7.49 ± 0.22^{b}	8.05 ± 0.24^{a}	6.3 ± 0.21^{c}	5.46 ± 0.20^{d}
CF	0.77 ± 0.10^{d}	1.54 ± 0.11^{c}	1.96 ± 0.12^{b}	2.87 ± 0.16^{a}
EE	0.63 ± 0.11^{a}	0.63 ± 0.11^{a}	0.56 ± 0.10^{a}	0.56 ± 0.10^{a}
Ash contents	4.34 ± 0.19^{a}	4.27 ± 0.19^{a}	4.13 ± 0.19^{a}	4.27 ± 0.19^{a}
NFE	2.73 ± 0.15^{a}	1.47 ± 0.14^{c}	2.24 ± 0.15^{b}	1.40 ± 0.14^{c}

RS = Resistant starch; CP = Crude protein (g/kg); CF = Crude fiber (g/kg); EE = Ether extract (g/kg); Ash content (g/kg); NFE = nitrogen free extract (g/kg); NC = Normal control (without resistant starch); $RS_{(0.20)}$ = Resistant starch: 0.20 g/kg body weight; $RS_{(0.30)}$ = Resistant starch: 0.30 g/kg body weight); $RS_{(0.40)}$ = Resistant starch: 0.40 g/kg body weight. $^{a-d}$Mean values between different treatments within a row with different superscript letters are significantly different ($p \leq 0.05$).

Table 5
Mean values and standard deviations of digestibility in healthy rats fed different levels of resistant starch.

Parameters	NC	$RS_{(0.20)}$	$RS_{(0.30)}$	$RS_{(0.40)}$
DM	66.60 ± 0.50^{c}	71.31 ± 0.92^{b}	74.70 ± 0.67^{a}	75.09 ± 0.29^{a}
CP	95.59 ± 0.01^{a}	92.21 ± 0.04^{c}	95.01 ± 0.15^{a}	94.77 ± 0.18^{b}
CF	68.42 ± 0.01^{a}	67.54 ± 0.04^{a}	57.51 ± 0.15^{b}	56.77 ± 0.18^{b}
EE	83.20 ± 0.01^{c}	89.24 ± 0.04^{b}	89.42 ± 0.15^{b}	93.71 ± 0.18^{a}

RS = Resistant starch; DM = Dry-matter; CP = Crude protein; CF = Crude fiber; EE = Ether extract; NC = Normal control (without resistant starch); $RS_{(0.20)}$ = Resistant starch: 0.20 g/kg body weight; $RS_{(0.30)}$ = Resistant starch: 0.30 g/kg body weight); $RS_{(0.40)}$ = Resistant starch: 0.40 g/kg body weight. $^{a-d}$Mean values between different treatments within a row with different superscript letters are significantly different ($p \leq 0.05$).

amounts of RS, with the highest drop recorded in RS (0.40) roughly at 25% when compared to NC.

3.5. Glycemic response

The results on the effects of different levels of high maize RS on glycemic response of healthy rats are showed in Table 7. The results revealed that no significant changes occurred in the glycemic response in blood glucose level of all rats treated with different diets of $RS_{(0.20)}$, $RS_{(0.30)}$ and $RS_{(0.40)}$, when compared to NC diet. At 90 min, all groups provided the best levels, which were 5.5 ± 0.34, 5.6 ± 0.34 and 5.8 ± 0.35 mmol/L and this gradual decrease confirms more resistant in gut − thus representing the role of fiber.

Table 6
Mean values and standard deviations of weight (g) changes in healthy rats fed different levels of resistant starch.

Parameters	NC	$RS_{(0.20)}$	$RS_{(0.30)}$	$RS_{(0.40)}$
Initial weight (g)	156 ± 0.83^{a}	157 ± 0.30^{a}	155 ± 0.80^{a}	155 ± 0.30^{a}
Weight after 1st week	158 ± 0.10^{a}	149 ± 0.16^{b}	150 ± 0.55^{b}	150 ± 0.83^{b}
Weight after 2nd week	158 ± 0.66^{a}	147 ± 0.60^{c}	151 ± 0.33^{b}	151 ± 0.01^{b}
Weight after 3rd week	155 ± 0.83^{a}	149 ± 0.90^{b}	154 ± 0.83^{a}	149 ± 0.66^{b}
Weight after 4th week	152 ± 0.16^{a}	145 ± 0.01^{b}	146 ± 0.66^{b}	142 ± 0.50^{c}
Final Weight (g)	154 ± 0.33^{a}	142 ± 0.30^{b}	139 ± 0.33^{c}	135 ± 0.60^{d}
Weight loss (g)	2 ± 0.50^{d}	15 ± 0.30^{b}	16 ± 0.47^{b}	19 ± 0.70^{a}
Feed Conversion Ratio	120 ± 0.50^{a}	22 ± 0.33^{c}	27 ± 0.93^{b}	28 ± 0.87^{b}

RS = Resistant starch; NC = Normal control (without resistant starch); $RS_{(0.20)}$ = Resistant starch: 0.20 g/kg body weight; $RS_{(0.30)}$ = Resistant starch: 0.30 g/kg body weight); $RS_{(0.40)}$ = Resistant starch: 0.40 g/kg body weight. $^{a-d}$Mean values between different treatments within a row with different superscript letters are significantly different ($p \leq 0.05$).

3.6. Oxidative stress

The effects of different levels of high maize resistant starch (RS) on oxidative stress of healthy rats (Table 8). The linear standard curve of total oxidant status (TOS) is represented by the concentration against absorbance level ($y = 15.832x - 2.396$). Significant increase was observed in all the rats fed with $RS_{(0.30)}$ and $RS_{(0.40)}$, when compared to NC. Maximum reduction was observed in the lowest level of $RS_{(0.20)}$, which was 2.00 ± 1.02 μmol H_2O_2 equivalent/L.

Furthermore, the linear standard curve of TAC is represented by the concentration against absorbance level ($y = -1.256x + 1.9595$). Significant increase was observed in all the groups $RS_{(0.20)}$, $RS_{(0.30)}$ and $RS_{(0.40)}$, when compared to NC.

Table 7
Mean values and standard deviations of glycemic response (mmol/L) in healthy rats fed different levels of resistant starch.

Parameters	NC	$RS_{(0.20)}$	$RS_{(0.30)}$	$RS_{(0.40)}$
0 min	7.6 ± 0.35^{b}	7.8 ± 0.36^{a}	7.7 ± 0.34^{a}	7.5 ± 0.35^{b}
30 min	7.4 ± 0.38^{a}	6.7 ± 0.32^{c}	6.7 ± 0.37^{c}	7.2 ± 0.40^{b}
60 min	7.2 ± 0.28^{a}	5.8 ± 0.37^{c}	6.9 ± 0.40^{b}	6.9 ± 0.39^{b}
90 min	6.9 ± 0.36^{a}	5.5 ± 0.34^{c}	5.6 ± 0.37^{c}	5.8 ± 0.35^{b}

RS = Resistant starch; NC = Normal control (without resistant starch); $RS_{(0.20)}$ = Resistant starch: 0.20 g/kg body weight; $RS_{(0.30)}$ = Resistant starch: 0.30 g/kg body weight); $RS_{(0.40)}$ = Resistant starch: 0.40 g/kg body weight. Mean values between different treatments within a row with different superscript letters are significantly different ($p \leq 0.05$).

Table 8
Mean values and standard deviations of oxidative stress in healthy rats fed different levels of resistant starch.

Parameters	NC	$RS_{(0.20)}$	$RS_{(0.30)}$	$RS_{(0.40)}$
TOS	2.38 ± 1.32^{c}	2.00 ± 1.02^{c}	4.36 ± 1.78^{a}	3.97 ± 1.23^{b}
TAC	0.32 ± 0.10^{b}	0.97 ± 0.60^{a}	0.93 ± 0.71^{a}	0.92 ± 0.52^{a}

RS = Resistant starch; TOS = Total oxidant status (H_2O_2 equivalent/L); TAS = Total antioxidant capacity (mmol Trolox equivalent/L); NC = Normal control (without resistant starch); $RS_{(0.20)}$ = Resistant starch: 0.20 g/kg body weight; $RS_{(0.30)}$ = Resistant starch: 0.30 g/kg body weight); $RS_{(0.40)}$ = Resistant starch: 0.40 g/kg body weight. $^{a-d}$Mean values between different treatments within a row with different superscript letters are significantly different ($p \leq 0.05$).

3.7. Mineral absorption

The results of the effects of different levels of high maize RS on mineral absorption of healthy rats are shown in Table 9. A significant increase was observed in the retention of all the minerals (Mg, Ca, Zn and Fe) by the administration of all levels of RS in comparison to NC. Retention in $RS_{0.40}$ group was 67% for Ca, 35% for Zn and 40% for FE, which were significantly higher than NC and other levels of RS. Additionally, iron loss in feces was higher than other minerals. Only Mg showed similar values in rats fed with different levels of RS.

4. Discussion

In the current study, the RS diets were designed to see how they affect the weight gain, nutritional intake and digestibility, mineral absorption, oxidative stress and the glycemic index in healthy rats. The result was that the dry-matter intake was higher with RS consumption as opposed to NC from adjustment to two weeks. According to Allen [22], the chemical and physical characteristics of diets impact dry-matter intake. It can be suggested that the diets provided to rats might have less filling, thus allowing greater dry-matter intake. According to Doo-Hong [23], dry-matter intake rises until it is no longer restricted by fill and declines when it is limited by an excess of metabolic fuels. Site of starch digestion affects the form of metabolic fuel absorbed [22]. Related findings by Da Silva et al. [24] revealed that pigs fed RS-containing diets increased feed intake per meal and meal duration. So, rats needed adequate time as they adjust to RS diets. The RS feed rats exhibited a decline in dry-matter intake after two weeks. The decline in dry-matter intake could be associated with excessive fermentation of RS and protein [22].

The intake of crude protein was not significantly different on the first day for the $RS_{(0.20)}$ with the exception for the high dosage at $RS_{(0.30)}$ and $RS_{(0.40)}$. This was probably due to equal amount of casein protein in their diets. Al-Mana & Robertson [25], found similar results when comparing an *ad libitum* meal to a placebo in overweight/obese males. There is positive association between crude protein and dry-matter intake [22]. The change observed in the crude protein after one week where it decreased with the intake of RS. The fermentation of RS in the colon has been proposed as the cause of the decrease in food intake [22]. While other nutrients showed no significant change, the nitrogen free extract in the rats fed the RS diets were significantly lower when compared with the NC diet. Moreover, the higher nitrogen free extract was observed in the rats fed with $RS_{(0.30)}$ diet as compared to the other RS levels, but these levels were lower than that of NC diets throughout one-week study trail.

The RS digestibility increased at elevated doses, most likely due to a rise in components that are more digestible. The results contradict the findings of Da Silva et al. [24] who stated that RS-diets reduced digestibility of dry-matter (DM). According to Lv et al. [26], the RS increases as amylose components increase. Higher amylose concentration favors the development of closely packed linear sequences, limiting heat treatment damage to starch structure and the rate of starch enzymatic hydrolysis. Dietary fibers, a part of the cell wall, could interact with digestible food molecules and alter its physicochemical and digestible characteristics [27]. The digestibility of crude protein showed was lower in RS diets when compared to NC diet. Factors like amylose-lipid interaction or hydrophobicity of protein, which resists alpha-amylolysis, are associated with reduced digestibility [28]. The digestibility of crude fiber reduced considerably across all levels of RS, indicating resistance in the hindgut. The reduction could link to the formation of starch−protein associations or Maillard reaction [29]. Cell surface proteins are involved in starch attachment which can be inhibited by pancreatin and low pH [4].

Weight loss was found in rats on RS diets, which is consistent with earlier reported findings [22,30]. According to Slavin [31], fiber consumption as RS is inversely associated with body weight and body fat as a result of reduced food intake. Likewise, because RS is difficult to break down into glucose, which is easily utilized by the human body, RS generates very little energy, accounting for just around 10% of digestible starch [32]. Our findings revealed loss in the weight related to the amount of RS consumed in the diet. Moreover, the reduced weight correlated well with a decrease in feed conversion ratio, especially at high RS doses.

Our results also found significant change in glycemic response in blood glucose level of all rats treated with different levels of diet with RS against NC diet. At 90 min, all groups provided the best levels (average of 5.6 mmol/L), and this progressive drop reveals more resistive in the gut, indicating the contribution of fiber. Jiménez-Domínguez et al. [33] found a similar finding in a cross-over study assessing the effects of acute intake of native banana starch (NBS) on glycemic profiles in obese and lean adults by continuous glucose monitoring. Arias-Córdova et al. [34] found the opposite, observing that RS did not cause a decrease in the 24 h mean blood glucose (MBG) levels.

On all RS diets, healthy rats demonstrated a reduction in oxidative stress, with the most significant decrease occurring at low dosage. A systematic review published by Lu et al. [35] discovered that RS supplementation increased antioxidant capability. They concluded that RS supplementation might considerably lower a

Table 9
Mean values and standard deviations of total minerals intake in healthy rats fed different levels of RS.

Parameters		NC	$RS_{(0.20)}$	$RS_{(0.30)}$	$RS_{(0.40)}$
Mg	Intake (mg/day)	12 ± 1.4^{a}	12 ± 1.4^{a}	12 ± 1.4^{a}	12 ± 1.4^{a}
	Apparent absorption (% of intake)	8 ± 2.0^{c}	10 ± 1.2^{b}	10 ± 1.5^{b}	11 ± 1.5^{a}
	% Retention	62 ± 06^{c}	64 ± 10^{b}	64 ± 8^{b}	65 ± 8^{a}
Ca	Intake (mg/day)	103 ± 2.11^{b}	103 ± 2.12^{b}	102 ± 2.10^{c}	105 ± 2.15^{a}
	Apparent absorption (% of intake)	63 ± 0.13^{d}	68 ± 0.14^{c}	74 ± 0.13^{b}	75 ± 0.15^{a}
	% Retention	59 ± 0.8^{d}	62 ± 0.7^{c}	64 ± 0.8^{b}	67 ± 0.6^{a}
Zn	Intake (mg/day)	0.76 ± 0.9^{a}	0.85 ± 0.1^{b}	0.85 ± 0.9^{b}	0.85 ± 0.6^{b}
	Apparent absorption (% of intake)	0.24 ± 0.11^{a}	0.31 ± 0.15^{a}	0.33 ± 0.09^{a}	0.38 ± 0.10^{a}
	% Retention	25 ± 0.12^{d}	29 ± 0.14^{c}	30 ± 0.13^{b}	35 ± 0.16^{a}
Fe	Intake (mg/day)	0.80 ± 0.1^{a}	0.87 ± 0.1^{a}	0.88 ± 0.09^{a}	0.94 ± 0.07^{a}
	Apparent absorption (% of intake)	0.31 ± 0.17^{a}	0.35 ± 0.11^{a}	0.38 ± 0.11^{a}	0.41 ± 0.12^{a}
	% Retention	30.0 ± 0.10^{d}	34.0 ± 0.10^{c}	35.0 ± 0.10^{b}	40.0 ± 0.11^{a}

RS = Resistant starch; Mg = Magnesium; Ca = Calcium; Zn = Zinc; Fe = Iron; NC = Normal control (without resistant starch); $RS_{(0.20)}$ = Resistant starch: 0.20 g/kg body weight; $RS_{(0.30)}$ = Resistant starch: 0.30 g/kg body weight); $RS_{(0.40)}$ = Resistant starch: 0.40 g/kg body weight. $^{a-d}$Mean values between different treatments within a row with different superscript letters are significantly different ($p \leq 0.05$).

few oxidative-stress and inflammation biomarkers, including malondialdehyde and C-reactive protein, especially in type-2 diabetes (T2D) patients. Chronic kidney disease (CKD) rats consuming RS significantly exhibited reduced creatinine clearance, interstitial fibrosis, inflammation, tubular damage, upregulation of pro-inflammatory, pro-oxidant and pro-fibrotic molecules, down-regulation of antioxidant enzymes, and disruption of colonic epithelial tight junction [36].

Minerals absorption increased in healthy rats on RS diets. Significant increase was observed in the retention of all the minerals (Mg, Ca, Zn and Fe) by the administration of all levels of RS in comparison to NC. This finding correspond well with previously report by Tousen et al. [37], who suggested that RS could have a positive effect on intestinal Ca and Mg absorption. RS tends to promote the microbiota, which can regulate bone metabolism. Tousen et al. [37] reported increase of *Bifidobacterium* spp. in faces and bone loss brought on by ovariectomy mitigated by acid-hydrolysed high amylose corn starch (AH-HAS) treatment that contained 12% of RS. In their review, Han et al. [32] stated that minerals, vitamins and other nutrient absorption and utilization are promoted by RS. The short-chain fatty acids produced by RS fermentation lower the pH in the colon, speeding the transformation of mineral elements into soluble ions that are easily absorbed.

5. Conclusions

Our study revealed that RS have potential role in weight management, improving feed conversion ratio, nutrient digestibility, mineral absorption and controlling oxidative stress. Significant results were observed in managing weight on weekly basis in all the treatment diets. However, no significant difference was observed in the glycemic response of different levels of RS. Although significant results were demonstrated at higher doses of RS, long-term administration of these levels may cause some intolerances. Therefore, long term study with high dosage level of RS is needed for further investigation.

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Author contributions

Conceptualization, M.U.N. and M.A.R.; methodology, M.U.N.; software, F.A.K. and I.H.; validation, F.A.K, L.M.K. and I.H.; formal analysis, J.M.R.; investigation, M.U.N.; resources, I.H.; data curation, M.A.R., F.A.; writing—original draft preparation, M.U.N.; writing—review and editing, A.A., E.B. and J.M.R.; visualization, M.A.R.; supervision, M.U.N.; funding acquisition, J.M.R. and F.A.

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Declaration of competing interest

The authors declare no conflict of interest.

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