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Evaluating the Health Effects of RS2 Supplemented Diet in Healthy Rats

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Abstract: Resistant Starch (RS2) gets more attention nowadays because of its physiological characteristic like dietary fiber. Recent research was conducted to check the hyperglycemic, hyperlipidemic, satiety hormone, and oxidative biomarkers in healthy male rats. All rats were divided into four groups (9 rats per group) and named according to diets and treatments. The healthy rats in the negative control (NC) received a basal diet, the rats in the other three groups were receiving RS2 with different levels in their diets, 0.20g/kg body weight, 0.30g/kg body weight, 0.40g/kg body weight were named $HM_{0.20}$, $HM_{0.30}$, $HM_{0.40}$ respectively. In the case of lipid profile, significant ($P<0.05$) reductionin cholesterol, low-density lipoprotein (LDL), triglycerides, and increase in high-density lipoprotein (HDL) with HM0.40 level of RS2 were found. Blood glucose, insulin, and leptin level indicate a similar reduction trend with the last level of diet. In the hormonal profile, insulin and leptinshowed a significant ($P<0.05$) reduction; improvement in total antioxidants and reduction in total oxidants were observed with the highest level of RS2 supplemented diet.

Keywords: Resistant Starch, Blood Glucose, Lipid Profile, Hormones, Oxidative Stress.

评估抗性淀粉补充饮食对健康大鼠的健康影响

摘要：抗性淀粉因其具有膳食纤维等生理特性而受到越来越多的关注。最近的研究旨在检查健康雄性大鼠的高血糖、高血脂、饱腹感激素和氧化生物标志物。将所有大鼠分为四组（每组 9只）并根据饮食和治疗命名。阴性对照组的健康大鼠给予基础饲料，其余三组大鼠给予不同水平的抗性淀粉，0.20g/kg体重、0.30g/kg体重、0.40g/kg体重分别命名为$HM_{0.20}$、$HM_{0.30}$、$HM_{0.40}$。在血脂方面，发现抗性淀粉 HM0.40水平显着降低胆固醇、低密度脂蛋白、甘油三酯和高密度脂蛋白（$P<0.05$）。血糖、胰岛素和瘦素水平表明与最后一个饮食水平相似的降低趋势。在激素方面，胰岛素和瘦素显着降低（$P<0.05$）；在最高水平的抗性淀粉补充饮食中观察到总抗氧化剂的改善和总氧化剂的减少。

关键词：抗性淀粉、血糖、血脂、激素、氧化应激。

1. Introduction

For nutritional purposes, starch present in many foods is categorized into three types according to their digestibility rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) [1]. RS is defined as a portion of starches that resist digestion in the small intestine of a healthy person when fermented in the lower gut; it behaves as a dietary fiber by the standard American Association of Cereal Chemists method (AOAC).

Several nutritional studies have shown the health benefits of RS and helpful for improving body weight, blood glucose, lipid profile, gastrointestinal hormones, constipation, oxidative stress, and type 2 diabetes mellitus [2]. Based on RS botanical source and processing technique it is classified into four different types, RS1, RS2, RS3, and RS4 [3]. The nutritional

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implication of RS_2 in foods has a great interest because it has similar biological nature with dietary fiber to lower the chance of cancer in the digestive tract, increase laxation, reduce blood glucose level, lipid profile, RS_2 includes a specific part of starch which repels the digestion by pancreatic amylase when it comes in human colon [4].

RS2provides less caloric value like 7kJ/g when compared with other carbohydrates that give 16kJ/g [5]. However, it can be used in some food items without any effect on the physicochemical or organolepticcharacteristicsof the food product [6].

It is a non-digestible foodstuff that provides beneficial effects to host bacteria in the gastrointestinal tract to motivate the growth and activity of such microbes for providing health stimulating results [7]. Overall, RS2 possesses positive properties as a healthy food component.

1.1. Abbreviations

RS: resistant starch; CRD: Completely randomized design; LDL: Low-density lipoprotein; HDL: High-density lipoprotein intraperitoneally; LDL: Low-density lipoprotein; TAC: Total oxidative stress; TOS: Total oxidative stress; SCFA: Short-chain fatty acid; RDS: Rapidly Digestible Starch; SDS: Slowly Digestible Starch.

2. Materials and Methods

2.1. Research Area

This study was conducted at the Institute of Home and Food Science, Government College University, Faisalabad, and Institute of Animal & Dairy Sciences, University of Agriculture, Faisalabad, Pakistan.

2.2. Product Used

Resistant starch RS_2 (Hi Maize 260) provided by Rafhan Maize products company private limited, Faisalabad, Pakistan.

2.3. Procuring Rats and their Management

The thirty-sixWistar albino male rats (75±5) with the weight of 150±10 gram were obtained from the National Institute of Health (NIH) Islamabad. All animals were kept and treated according to the Principles of Laboratory Animal Care (NIH). The experimental procedure was approved by the Animal Ethical Committee. A diet was *isocaloric* and *i*sonitroginous. All rats were *ad libitum* access to clean drinking water and feed. Twelve-hour light-dark cycle with lights on from 07:00 hour and ambient temperature was set at 22±2 °C for rats.

2.4. Experimental Protocol

All rats were divided into two Completely Randomized Design (CRD) of four equal groups (9 rats in each group) and named according to diets and treatments. The healthy rats in the negative control (NC) received a basal diet. The rats in the other three groups were receiving RS2 with different levels in their diets, 0.20g/kg body weight, 0.30g/kg body weight, 0.40g/kg body weight were named HM0.20, HM0.30, HM0.40 respectively. End of the trial, a 5cc blood sample was taken from each rat and then separate serum for hormone and lipid analysis.

2.5. Data Assembling

Weekly blood glucose data was collected during this experiment.

2.6. Biochemical Analysis

Lipid Profile was checked by microplate reader URIT660. Cholesterol was determined with a kit method byusing Biosystem cholesterol kit, REF. 11505 (Barcelona, Spain). Triglyceride was estimated by Triglyceridesliquiform mono reagent kit (Paris, France). LDL and HDL were determined by the Wiener kit having REF. 1220229 and REF 1220114 respectively (Rosario, Argentina). Bloodglucose was determined by glucose glucometer AccuchekActive®. The concentration of serum insulin was determined using a commercially available Calbiotech Insulin ELISA® kit by ELISA (enzyme-linked immunosorbent assay. Serum leptin was determined by using a rat-LEP ELISA kit (E-EL-R0582). For the determination of total antioxidant capacity were used Erel Method [8] and total oxidative stress through a microplate spectrophotometer (Bio-lab 310).

2.7. Statistical Analysis

Data obtained from results were statistically analyzed by using SPSS (Version 17). Duncan Multiple Range Test (DMR) was used for mean comparison between groups [9].

3. Results and Discussion

3.1. Effect of RS_2 in Glucose Metabolism

Results of blood glucose levels in rats from 1-10 weeks of trial by feeding RS_2 are mentioned in table 1. The results of this study were similar to the results obtained by de Angelis-Pereira [10] who conducted an RS2 study that had the same consequences as RS2 (4g/kg/body/weight) in rats for 50 days significantly lower blood glucose level in the treatments group compared with NC. The best result of lower blood glucose was observed in the 10^{th} week of study in $HM_{(0.40)}$ diets. After the consumption of RS_2 its metabolism occurs in 5 to 6 hours, but many starches which are normally cooked immediately digested after their intake by reducing the postprandial level of blood glucose and increasing the satiety period [11]. Intakes of 14% RS2 from total starch intake improve blood glucose levels [12]. In another study, daily intake of supplemented bread with RS2 for six weeks decreases

significantlyblood glucose level [13]. Due to its low glycemic property, it is used as an ingredient in a different food product to lower the glycemic load (GI) value of that food, especially when it is replaced with a readily absorbed form of carbohydrates. Gradually it becomes an attractive food ingredient for most of food product manufacturers like in bread, cake, or similar product having higher Gl value.

Table 1 Effect RS2 with a different level on weekly blood glucose (mg/dL) in healthy rats

Weeks	Treatments			
	NC	$HM_{0.20}$	$HM_{0.30}$	$HM_{0.40}$
1st	117.15±1.16[a]	116.32±0.43[a]	115.32±0.34[a]	113.67±0.70[a]
2nd	116.57±1.49[a]	112.03±1.65[a]	111.61±1.23[a]	109.45±1.32[a]
3rd	116.78±1.35[a]	112.76±0.71[a]	110.71±0.54[a]	108.23±0.54[a]
4th	114.60±1.53[a]	111.65±1.53[a]	107.09±1.11[a]	102.45±0.56[a]
5th	114.58±1.47[b]	108.31±0.87[a]	107.51±0.78[a]	107.01±0.87[a]
6th	118.13±1.04[b]	111.54±1.34[a]	110.07±1.43[a]	109.45±0.91[a]
7th	113.83±1.48[b]	108.08±1.21[a]	106.53±1.62[a]	105.54±1.54[a]
8th	115.33±1.45[b]	105.21±1.53[a]	104.61±0.61[a]	103.21±1.56[a]
9th	117.44±1.09[c]	110.65±1.32[bc]	105.71±0.87[ab]	99.25±0.67[a]
10th	116.52±1.75[c]	109.87±0.93[bc]	102.51±1.51[ab]	97.08±0.71[a]

*NC (Negative control), $HM_{0.20}$ (RS2: 0.20g/kg body weight), $HM_{0.30}$ (RS2: 0.30g/kg body weight)$HM_{0.40}$(RS2: 0.40g/kg body weight)

3.2. Effect of RS2 on Lipid Profile

The statistical results regarding cholesterol, TG, HDL, and LDL levels of rats fed NC, $HM_{0.20}$, $HM_{0.30,}$ and $HM_{0.40}$diets have been shown in Table 2. They showed that rats fed different levels of RS2 supplemented diete xhibited significant ($P<0.05$) decrease in cholesterol, triglycerides, LDL levels (136.34±0.56), (86.01±1.21), (129.76±1.23) and increase in HDL(54.56±1.54) in $HM_{0.40}$groups as compared to NC. The results of this study were similar to the study of Jyoshna & Hymavathi [14], who conducted an RS2 study that had the same consequences as RS2 (3g/kg/body/weight) in rats significantly reduced triglycerides and increase HDL concentration in the experimental group compared with NC. Our results matched with Astina & Sapwarobol [15], who observed a decrease in TC and HDL in subjects with metabolic or without metabolic syndrome with 12 week consumption of flour fortified with RS_2 (25.5g/100g in flour). RS2 particularly affects the metabolism of lipids that reduce cardiovascular diseases [16]. Dyslipidemia is characterized by an increase in serum low-density lipoprotein (LDL) and lower high-density lipoprotein (HDL) concentration [17]. The mechanism by which RS2 improves lipid profile is due to its soluble fiber characteristics to bind dietary cholesterol and prevent its absorption, which causes an increase in the elimination of cholesterol through feces. RS2 also relates to bile acids uptake to lower the reabsorption of cholesterol. Due to its lipid-lowering effect through increased production of SCFA, they prevent cholesterol and triglycerides (TG) synthesis [18].

The mechanism for the reduction in the cholesterol level is due to the production of propionic acid after RS2 fermentation in the gut and this short-chain fatty acid (SCFA) having an effect in lower cholesterol synthesis [19] RS2 raise the activity of those enzymes that regulate cholesterol synthesis and lower the expression of fatty acid synthase gene. It is reported that RS2 helps to decrease the absorption of cholesterol [20].

Table 2 Effect of different levels of RS2 on lipid profile (mg/dL) in healthy rats

Parameters	Treatments			
	NC	$HM_{0.20}$	$HM_{0.30}$	$HM_{0.40}$
Cholesterol	144.0±0.79[c]	143.54±1.32[bc]	141.32±0.34[b]	136.34±0.56[a]
Triglycerides	93.9±1.98[bc]	93.38±1.67[bc]	90.08±1.45[b]	86.01±1.21[a]
HDL	50.1±0.79[c]	51.68±1.65[bc]	53.61±0.76[ab]	54.56±1.54[a]
LDL	139.70±0.46[d]	136.32±1.21[c]	133.45±0.21[b]	129.76±1.23[a]

*NC (Negative control), $HM_{0.20}$ (RS2: 0.20g/kg body weight), $HM_{0.30}$ (RS2: 0.30g/kg body weight)$HM_{0.40}$(RS2: 0.40g/kg body weight)

3.3. Effect of RS2 with a Different Level on Oxidative Stress

The results showing the effects of different levels of RS2 on total antioxidant capacity (TAC) and total oxidative stress (TOS) of healthy rat data are presented in table 3. Significant ($p < 0.05$) decrease in TOS value was observed in rats fed with ($HM_{0.40}$) 2.01±0.02 µmol H_2O_2 equiv/L and significant ($p < 0.05$) increase in TAC value was observed in (HI-$M_{0.40}$) 0.98 ±0.36 group when compared to NC.

Results of this study match with the finding of Dainty et al. [21], consumption of hi-maize containing RS_2 has shown some improvement in glycemic status and oxidative biomarkers. Moreover, another study of

maize amylose starch (HAM-RS2) tested diet has been compared to low fiber diet which demonstrated very positive results in the reduction of local and systematic inflammation. This is done by the restoration of colonic epithelial tight junction, improvement in microbial dysbiosis, and reversal of metabolic endotoxemia [22]. The mechanism through which RS2 affects inflammation is due to the production of SCFA, especially butyrate that can control the activity of macrophage and nuclear factor-kappa. It is the key regulator of the immune system and inflammation that increases the expression of cytokines [23]. Consumption of RS2 increases the production of SCFA in the colon, which plays an important role in the protection of the colonic epithelial barrier and the anti-inflammatory capacity of the body [24]. In another trial, the supplementation of a high dose of RS_2 has decreased postprandial glucose concentration without affecting the sensitivity of insulin [25]. The results of some studies are not matched with our results, supplementation of RS2 does not affect the marker of oxidative stress in healthy rats. In the study of Amoako & Awika [26] results indicate that oxidative markers like TAC and SOD levels were decreased after 8 weeks of consumption of RS2.

Table 3 Effect of RS2with a different level on oxidative stress in healthy rats

Parameters	NC	$HM_{(0.20)}$	$HM_{(0.30)}$	$HM_{(0.40)}$
TOS	18.92 ± 1.32^{bc}	18.69 ± 1.03^{c}	18.20 ± 1.76^{b}	17.85 ± 1.02^{a}
TAC	3.83 ± 1.10^{c}	3.86 ± 0.56^{b}	3.91 ± 0.64^{a}	3.95 ± 0.36^{a}

*NC (Negative control), $HM_{0.20}$ (RS2: 0.20g/kg body weight), $HM_{0.30}$ (RS2: 0.30g/kg body weight)$HM_{0.40}$(RS2: 0.40g/kg body weight)

3.4. Effect of RS2 with a different level on Leptin and Insulin

The results regarding insulin and leptin significant ($p < 0.05$) reduction was shown in all diets groups supplemented with RS2, shown in table 4. Maximum decrease showed by $HM_{0.40}$diet in insulin and leptin level (6.15 ± 0.49), (11.72 ± 0.71). Jamar et al. [27] showed that leptin level was significantly lower after consumption of RS2 for five weeks. Results of this research are parallel to [28] regarding glucose balance after the consumption of 20g from high amylose maize RS2 for ten weeks in normal-weight adults show a significant decline in insulin.

That may be due to the fermentation of RS2 by microbes in the lower portion of the gastrointestinal tract. In the intestinal gluconeogenesis propionate and butyrate are used as a substrate and the synthesis of glucose from the intestine lowers the hepatic gluconeogenesis by portal vein [29]. Leptin plays a vital role in regulating food intake and immune function. It is mainly secreted from adipocytes and acts centrally to lower appetite and raise energy expenditure [30].

The study of Gentile et al. [31] shows that the effects of the RS2 supplemented diet on body composition and appetite are multifactorial. For example, RS2 increases in the diet that reduces the energy mass of that food. Increasing RS2 adjusts intestinal and gastric motility, increases distal metabolism. These effects could modify the release of gut hormones involved in controlling food intake. Intake of RS2 also reduces GI value which is recognized to alter insulin response that is a possible mechanism for appetite reduction [32, 33]

Table 4 Effect of RS2 with a different level onLeptin and Insulin in healthy rats

Parameters	Treatments			
	NC	$HM_{0.20}$	$HM_{0.30}$	$HM_{0.40}$
Insulin (uIU/mL)	7.43 ± 0.72^{c}	7.13 ± 0.54^{bc}	6.62 ± 0.52^{ab}	6.15 ± 0.49^{a}
Leptin, ng/mL	15.16 ± 0.65^{c}	13.42 ± 0.81^{bc}	12.83 ± 0.76^{ab}	11.72 ± 0.71^{a}

*Negative control (NC), RS2: 0.20g/kg body weight ($HM_{0.20}$), RS2: 0.30g/kg body weight ($HM_{0.30}$), RS2: 0.40g/kg body weight($HM_{0.}$40)

Our primary aim was to examine changes in glucose homeostasis after consuming 30 g HAM-RS2 for 6 weeks in overweight adults. It was also measured the plasma bio- markers (GLP-1, PYY, and leptin) and subjective satiety which could alter dietary intake and body composition.

We found significant reductions in AUC glucose and AUC leptin in the HAM-RS2 group, although differences between groups did not occur. In addition, a significant increase in fasting PYY occurred within the HAM-RS2 group after consuming the treatment muffins for 6 weeks.

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4. Conclusions

In conclusion, this paper significantly contributed to the knowledge of RS2 (Hi-maize 260) exerts health

benefits and a positive impact on improving blood glucose, lipid profile, total oxidant, and hormones. Adding RS2 to the diet can improve fiber intake to enhance overall diet quality.

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